PARA-HYDROXYBENZYLISOTHIOCYANATE
(CONSTITUENT OF WHITE MUSTARD) #123

Para-Hydroxybenzylisothicyante (Constituent of White Mustard) #123

MONOGRAPH

ON

p-HYDROXYBENZYL

ISOTHIOCYANATE

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p-HYDROXYBENZYL ISOTHIOCYANATE

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p-HYDROXYBENZYL ISOTHIOCYANATE

Summary

p-Hydroxybenzyl isothiocyanate is the constituent responsible for both flavor and aroma in white mustard. This isothiocyanate is not present in the free state as such but is produced as a result of hydrolysis of sinalbin present in the mustard. The presence of sinalbin in the protein meal of white mustard has restricted the use of the meal as an animal feed because of the toxic properties of its hydrolysed products (28). In an introduction to a report, Holmes (19) mentioned that p-hydroxybenzyl isothiocyanate is capable of causing acute gastroenteritis and salivation in farm livestock.

p-HYDROXYBENZYL ISOTHIOCYANATE

Chemical Information

I. Nomenclature

A. Common Names

None

B. Chemical Names

1. p-Hydroxylbenzyl Isothiocyanate

C. Trade Names

None

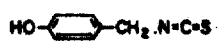
D. Chemical Abstracts Number

None

II. Empirical Formula

C_8H_7ONS

III. Structural Formula



IV. Molecular Weight

165.2

V. Specifications

No Information Available

VI. Description

A. General Characteristics

p-Hydroxybenzyl isothiocyanate is mainly responsible for the characteristic sharp, hot pungency of mustard. It crystallizes out of light petroleum as colorless needles at -10 degrees C.

B. Physical Properties

mp 42 degrees C

C. Stability

p-Hydroxybenzyl isothiocyanate decomposes when heated above 50 degrees C and is preferably stored under light petroleum in the ice box. In alkali rapid and complete decomposition of the compound to p-hydroxybenzyl-alcohol and thiocyanate ions occurs (2).

VII. Analytical Methods

Josefsson developed a method for the quantitative determination of p-hydroxybenzyl isothiocyanate in enzymic digests of seed meal of white mustard (Sinapis alba L.). The isothiocyanate was treated with sodium hydroxide and the released thiocyanate was determined by use of ferric nitrate reagent. A sample in which the ferric thiocyanate complex was destroyed by addition of mercuric chloride was used as a control for the colorimetric determinations. A relative standard deviation of 0.9% was found when the method was applied to micro-quantities (21).

A titrimetric method for the determination of p-hydroxybenzyl isothiocyanate has been developed by Raghavan et al. which is based on the quantitative conversion of p-hydroxybenzyl isothiocyanate into thiocyanate. The thiocyanate is then determined iodometrically after conversion to cyanogen bromide. The method is applicable to pure sinalbin, white mustard and also to mixed mustard flour consisting of black and white mustard (28).

In the method developed by Shankaranarayana, et al., the p-hydroxybenzyl isothiocyanate is reacted with piperidine to form p-hydroxybenzyl piperidyl thiourea. The excess unreacted piperidine is back titrated using standard sulphuric acid and the amount of p-hydroxybenzyl isothiocyanate calculated (32).

VIII. Occurrence

A. Plants

p-Hydroxybenzyl isothiocyanate is produced by the enzymatic hydrolysis of sinalbin, which occurs in mustard.

B. Animals

None

C. Synthetics

None

D. Natural Inorganic Sources

None

Biological Data

In a discussion of the safety of mustard as a forage crop for farm livestock, Holmes mentioned in passing that mustard contains p-hydroxybenzyl isothiocyanate and that this compound is capable of causing acute gastroenteritis and salivation (19).

Raghaven, et al. mentioned that the protein meal of white mustard has been restricted in use as an animal feed because of the toxic properties of p-hydroxybenzyl isothiocyanate, which occurs as a hydrolysis product of the sinalbin contained in the meal (28).

I. Acute Toxicity

No Information Available

II. Short-Term Studies

No Information Available

III. Long-Term Studies

No Information Available

IV. Special Studies

No Information Available

Biochemical Aspects

I. Breakdown

No Information Available

II. Absorption-Distribution

No Information Available

III. Metabolism and Excretion

No Information Available

IV. Effects on Enzymes and Other Biochemical Parameters

No Information Available

V. Drug Interaction

No Information Available

VI. Consumer Exposure

No Information Available

Addendum

Raghaven's metion of the toxicity of p-hydroxybenzyl isothiocyanate was based on the document by Barothy. However, examination of this document and of the references cited by Barothy revealed no direct information on p-hydroxybenzyl isothiocyanate (however, not all of the references have jet been obtained). Rather, Barothy had drawn his conclusions from work on related compounds. The document by Barothy and the pertinent references cited in it (those available) are reproduced at the end of this monograph.

p-HYDROXYBENZYL ISOTHIOCYANATE

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Synthesis of *p*-Hydroxybenzyl-Isothiocyanate and its Isolation from White Mustard Seeds

By J. Barothy and H. Neukom

Department of Agricultural Chemistry, Swiss Federal Institute of Technology, Zurich, Switzerland

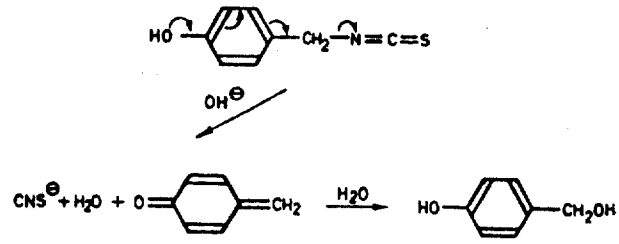
The isothiocyanate[1] of the white mustard seeds (*Sinapis alba L.*) has been identified by Salkowski[2] and recently by Kjaer and Rubinstein[3] as *p*-hydroxybenzyl-isothiocyanate by way of thiourea derivatives. To our knowledge *p*-hydroxybenzyl-isothiocyanate has however never been obtained in the pure state either by synthesis or by isolation from white mustard.

Taking into account the known instability of this isothiocyanate in aqueous and especially in alkaline systems,[4] a new attempt was made to synthesise and isolate *p*-hydroxybenzyl-isothiocyanate in a pure form. As a result the isothiocyanate could be obtained as a white crystalline material.

p-Hydroxybenzyl-isothiocyanate was synthesised by the method of Slotta and Dressler.[5] The final benzene solution was evaporated to dryness, the isothiocyanate dissolved in warm light petroleum and the solution concentrated until turbid. *p*-Hydroxybenzyl-isothiocyanate crystallised at −10°c. as long colourless needles, m.p. 42°c. after two recrystallisations from light petroleum. The compound decomposes when heated above 50°c. and is preferably stored under light petroleum in the ice box. The yield was only 14% based on the ammonium salt. Thin layer chromatography gave one single spot with a R_F-value of 0·50-0·53 (Kieselgel G, solvent: n-heptane-benzene-dioxan, 2:3:2; spraying agent: 2,6-dichloro-*p*-benzoquinone-4-chlorimine, 1% in alcohol, followed by 2N sodium carbonate solution). Reaction with aniline gave *N*-*p*-hydroxybenzyl-*N'*-phenyl-thiourea which was identical with the derivative obtained from *p*-hydroxybenzylamine and phenylisothiocyanate.[3]

For the isolation of the isothiocyanate from *sinapis alba* the seed meal was mixed with water and after one hour, the paste was extracted with light petroleum or chloroform. The emulsions were separated by centrifugation and the extracts dried over sodium sulphate. The solutions were separated by chromatography on a silica gel column with the same solvent as used for thin layer chromatography. The fractions containing the isothiocyanate were combined and the solvents distilled off. The *p*-hydroxybenzyl-isothiocyanate was dissolved in warm light petroleum and crystallised at −10°c. as colourless needles, m.p. 42°c. (yields: 370 mg. (11%) and 545 mg. (16%) from 100 g. of white mustard flour using respectively light petroleum and chloroform as solvent). The isothiocyanate was identical with the synthetic product (UV and IR-spectra, thiourea derivative).

p-Hydroxybenzyl-isothiocyanate is an unusually unstable mustard oil in contrast to the closely related *p*-hydroxyphenyl- and benzyl-isothiocyanate which are stable compounds. In alkali rapid and complete decomposition to *p*-hydroxybenzyl-alcohol (identified by thin layer chromatography) and thiocyanate ions occurs. The latter can be determined easily as ferric complex and the quantitative thiocyanate formation has in fact been used as an assay for *p*-hydroxybenzyl-isothiocyanate.[6] The instability is owing to the *p*-hydroxybenzyl grouping which permits formation of a quinone methide as an unstable intermediate:



In this respect *p*-hydroxybenzyl-isothiocyanate resembles the hypothetical 3-indolylmethyl-isothiocyanate formed during hydrolysis of glucobrassicin and which seems to be even more labile.[7]

We thank J. & J. Colman Ltd., Norwich, for financial support of these investigations.

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A Case of Suspected Poisoning of Dairy Cows by White Mustard Seeds (*Sinapis alba*)

BY

R. G. HOLMES

Veterinary Investigation Centre, Norwich

SUMMARY.—A case of suspected mustard seed poisoning is described. Eight of 54 cows died within 2½ days of having access to 35 lb. of white mustard seeds which had been deposited in a field where they were grazing.

Introduction

POISONOUS plants continue to present a hazard to farm livestock and the present case record shows the danger of ignorance of the poisonous properties of certain plants and their products and the need, when the danger is appreciated, of preventing stock having access to such material.

The "mustard" group of plants comprises white mustard (*Sinapis alba*), black mustard (*Sinapis nigra*), and wild mustard or charlock (*Sinapis arvensis*). White mustard is grown either as a forage crop for the folding of sheep, as a green crop for ploughing in, or for use in the commercial manufacture of mustard. It should be grazed six to eight weeks after sowing (Worden, Sellers & Tribe, 1963) before the seeds are formed because these contain glycosides from which the toxic principles p. hydroxybenzyl isothiocyanate and a trace of isopropyl isothiocyanate are released by enzymic decomposition. These active principles are capable of causing acute gastro-enteritis and salivation. Black mustard is much more toxic than white and is not therefore used as grazing but only for the production of seed for use as a condiment.

Forsyth (1954) described losses in sheep that grazed a crop in which the pods had formed. Eaton (1941) reported an outbreak of suspected poisoning in which three 3½-year-old bullocks died five days after being turned out on to a white mustard stubble. Sheep had grazed this same field without ill effect five weeks previously when the plant was in flower. Wild mustard, or charlock, has been reported to cause mortality in lambs which grazed it when the seeds were formed (Gallie & Paterson, 1945). Similar losses have been described in cattle (Kutuzov, 1958).

Case History

On Thursday, November 7th, 1963, approximately 35 lb. of white mustard seeds were swept up from the barn floor of a farm growing white mustard for manufacturing purposes and deposited on a pasture where the herd of 54 dairy cows was grazing. At 2.30 p.m. on Friday one cow was observed to stagger backwards and walk in circles. It became recumbent and showed opisthotonos and profuse salivation. No diarrhoea was evident. Later in the day another animal developed similar symptoms and both these cows together with two others were found dead on the Saturday morning. At this stage the mustard seeds remaining in the field were swept up and carried away. Three other cows developed signs of illness during the night and died on Saturday morning, while one died later the same day. A bull and two other cows appeared ill but recovered and altogether eight of the 54 cows at risk died within 30 hours of the first cow appearing ill.

(*Concluded at foot of col.* 1 *opposite*)

The Carriage of Pathogenic Staphylococci by Sheep. —*Concluded.*

presence or absence of infection in the ewes and in their lambs. A similar lack of correlation has been noted previously by Foggie (1947).

If more detailed studies of the source of infection in lambs with pyaemia are to be made it will be necessary to develop a phage-typing system designed for the examination of sheep strains of *S. aureus*.

Acknowledgments.—I wish to record my thanks to the flock owners who supplied these lambs and to their veterinary surgeons for allowing me free access to material from these flocks. The sheep at the Veterinary Investigation Centre, Leeds, were managed by Mr. R. Parker and the bacteriological examinations were performed by Miss I. M. Bewley, Mr. R. Bellhouse and Mr. A. Westwood. I am grateful to Dr. M. P. Jevons and Dr. M. T. Parker, Central Public Health Laboratory, Colindale, for typing the staphylococcal isolates. Dr. M. P. Vessey of Rothamsted Experimental Station kindly advised on the presentation of the results. My thanks are due to Mr. L. E. Hughes and Mr. W. B. V. Sinclair for their help and interest.

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A Case of Suspected Poisoning of Dairy Cows by White Mustard Seeds *(Sinapis alba)*.—*Concluded*.

Post-mortem Examination

Post-mortem examinations were carried out by the owner's veterinary surgeon and material from one cow was submitted to Norwich Veterinary Investigation Centre. The significant finding was the presence of abomasitis and enteritis, the intestinal contents being pink in colour. The kidneys showed patchy areas of congestion and cystitis was also evident. The rumen was well filled with fibrous material and also contained a considerable quantity of mustard seeds. Biochemical examination showed the lead content of the kidney to be normal and the liver contained less than 1 p.p.m. As_2O_3. Examination of the mustard seeds and rumen contents for cyanide gave negative results.

Conclusion

The *post-mortem* findings, together with the history and the negative results of biochemical examinations, offer sufficient evidence to warrant a diagnosis of suspected mustard seed poisoning.

Acknowledgments.—I wish to thank Miss G. Lewis of the Biochemistry Department, Central Veterinary Laboratory, Weybridge, for biochemical analyses and Messrs. Swann and Barrowman, MS.R.C.V.S., of King's Lynn, Norfolk, with whom this investigation was made.

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METHOD FOR QUANTITATIVE DETERMINATION OF *p*-HYDROXYBENZYL ISOTHIOCYANATE IN DIGESTS OF SEED MEAL OF *SINAPIS ALBA* L.

By E. JOSEFSSON

A method has been developed for the quantitative determination of *p*-hydroxybenzyl isothiocyanate in enzymic digests of seed meal of white mustard (*Sinapis alba* L.). The isothiocyanate was treated with sodium hydroxide and the released thiocyanate was determined by use of ferric nitrate reagent. A sample in which the ferric thiocyanate complex was destroyed by addition of mercuric chloride was used as a control for the colorimetric determinations. The yields of isothiocyanate obtained after hydrolysis of *p*-hydroxybenzylglucosinolate at different pH have been studied. A relative standard deviation of 0·9% was found when the method was applied to micro- quantitities. The simplicity and rapidity of the method and its applicability to small samples make it well suited for use in connexion with plant breeding.

Introduction

Seed meal of white mustard (*Sinapis alba* L.) contains *p*-hydroxybenzylglucosinolate, which upon enzymatic hydrolysis yields glucose, hydrogen sulphate and *p*-hydroxybenzyl isothiocyanate.[1,2] The toxic properties of the latter substance limit the use of white mustard seed meal as animal feed. In efforts to select plant material with a lower *p*-hydroxybenzylglucosinolate content, a method for quantitative determination of the isothiocyanate was sought. In order to be applicable to plant breeding, the method should be suitable for rapid serial analysis of small quantities of meal.

For estimating *p*-hydroxybenzyl isothiocyanate Mühlenfeld[3] suggested adding ammonia and silver nitrate. The silver nitrate was thought to react with the sulphur of the thiourea derivative of the isothiocyanate and the surplus silver could be titrated with ammonium thiocyanate. The same principle was used by Beer *et al.*[4] It has been shown, however, that when *p*-hydroxybenzyl isothiocyanate is treated with alkali it is rapidly and quantitatively split to *p*-hydroxybenzylalcohol and thiocyanate ion.[5–7]

Scharrer *et al.*[8] and Ettlinger & Thompson[9] determined the thiocyanate ion released from *p*-hydroxybenzyl isothiocyanate by treatment with alkali. The methods described, however, do not seem to be well adapted for analysing a large number of samples of plant breeding material.

Bauer & Holle[10] and Terry & Corran[11] made estimations of *p*-hydroxybenzylglucosinolate content by determination of released sulphate. Barothy[7] extracted the glucosinolate with methanol, evaporated the solvent, incubated with myrosinase and titrated the hydrogen ion released. In this laboratory a rapid serial method based on determination of sulphate was tested but it lacked precision when applied to quantities of meal lower than 30 mg.

Since colorimetric determinations are very suitable for serial analysis, and estimation of thiocyanate ions with ferric nitrate reagent is a very sensitive method, this principle was used in the present study.

Bowler[12] investigated the optimum conditions for determination of thiocyanate in blood serum with ferric nitrate reagent. He found that a large excess of reagent should be used. His method was adapted by the Central Veterinary Laboratory, Weybridge,[13] for use in analysis of fresh kale. In such material as well as in seed meal of white mustard, the ferric nitrate reagent reacts with phenolic compounds in the sample, giving rise to a yellowish coloration interfering with the colour of the ferric thiocyanate complex. This problem was overcome by preparing duplicate aliquots of each sample and adding to one set a little mercuric chloride which destroys the ferric thiocyanate and provides a control.[13] In the present studies it was found that when the absorption spectrum in the interval 380–550 nm was measured against the control it was the same as that of a sample of pure thiocyanate.

Experimental and Results

Materials

Seed material was obtained from the Oil Crops Division at the Swedish Seed Association, Svalöf.

Reagents were of analytical reagent grade.

Myrosinase was prepared using the method outlined by Wrede.[14,15]

p-Hydroxybenzylglucosinolate was obtained as the tetramethylammonium salt from Calbiochem, Los Angeles.

Conditions for optimum release of *p*-hydroxybenzyl isothiocyanate and thiocyanate ions

In studies of analysis of isothiocyanates of *Brassica* seed meals, it was found that the yield of isothiocyanates was highly dependent on the pH at which enzymatic hydrolysis of the glucosinolates was performed.[16] At pH 7 there were considerable losses, which could, however, be avoided by heating the seed and treating the meal with hot buffer.[15,17] Fig. 1 shows that when incubated at pH > 5 there are losses in yield of thiocyanate from white mustard. These losses could be avoided either by applying the treatments used for *Brassica* seed meals or by the use of hot buffer treatment alone (Table I). Since a pH of 4·5 gives an optimum yield without heat treatment, this pH was used in further studies.

After studying the incubation times needed for the complete enzymic release of isothiocyanate from *p*-hydroxybenzylglucosinolate and the release of thiocyanate ions after addition of different quantities of sodium hydroxide, the following analytical method was found to be suitable.

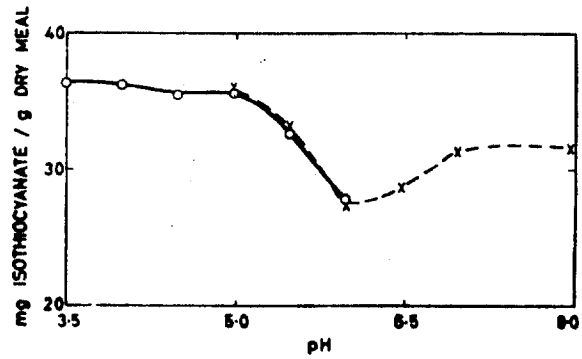

FIG. 1. *Effect of pH on amount of* p-*hydroxybenzyl-isothiocyanate obtained from digests of Seco white mustard meal*

(o) acetate buffer, (x) phosphate buffer

The samples were incubated with myrosinase at room temperature for 90 min and treated with sodium hydroxide for 15 min

TABLE I

Effect of heat treatments of a single batch of seed and seed meal of white mustard, cv. Seco, on the amount of *p*-hydroxybenzyl isothiocyanate obtained

The samples were incubated with myrosinase at room temperature for 90 min and treated with sodium hydroxide for 15 min

Exp. No.	Seed treatment	Meal treatment	Incubated in buffer	mg *p*-hydroxybenzyl isothiocyanate per g dry meal
1	None	None	Phosphate, pH 6·0, 0·05 N	$M_2 = 27·5$
2	Kept at 90°c for 15 min in a closed vessel	None	Phosphate, pH 6·0, 0·05 N	$M_2 = 30·5$
3	None	Treated with hot buffer, pH 6·0	Phosphate, pH 6·0, 0·05 N	$M_2 = 36·0$
4	Kept at 90°c for 15 min in a closed vessel	Treated with hot buffer, pH 6·0	Phosphate, pH 6·0, 0·05 N	$M_2 = 35·0$
5	None	None	Phosphate, pH 7·0, 0·05 N	$M_4 = 31·3$
6	Kept at 90°c for 15 min in a closed vessel	Treated with hot buffer, pH 7·0	Phosphate, pH 7·0, 0·05 N	$M_2 = 35·0$
7	None	None	Phosphate, pH 8·0, 0·1 N	$M_2 = 31·5$
8	Kept at 90°c for 15 min in a closed vessel	Treated with hot buffer, pH 8·0	Phosphate, pH 8·0, 0·1 N	$M_2 = 35·1$

Method

150 mg of defatted seed meal is placed in a 250 ml Erlenmeyer flask and 100 ml 0·1 M acetate buffer, pH 4·5, is added together with 1 ml 0·5% aqueous myrosinase solution. The flask is stoppered with a greased glass stopper and shaken for 15 min at room temperature. Then 12 ml 1 N sodium hydroxide are added and the sample is shaken for a further 5 min. The sample is filtered and 15 ml of the filtrate are treated with 15 ml 10% trichloroacetic acid. The precipitate is filtered off. From each filtrate are taken two 5 ml aliquots, and 5 ml 0·4 M ferric nitrate in 1 N nitric acid is added to each in artificial light. To one are added 2 drops of 5% mercuric chloride solution. This sample is used as a control and the colorimetric measurement is made at 460 nm in 1 cm cuvettes. In the present experiments the measurements were made in a Zeiss PMQ II spectrophotometer.

The method has been successfully adapted to analysis of microquantities. In this case about 15 mg of meal are incubated in centrifuge tubes with 10 ml acetate buffer and 0·1 ml myrosinase solution. During incubation, the tubes are shaken.

Precision and accuracy of the method

Studies of the precision of the method were made using the micro-modification. From Table II the standard deviation of ±0·3 is less than 1% of the average value.

TABLE II

Determination of *p*-hydroxybenzyl isothiocyanate in digests of a single seed meal of Seco white mustard according to different methods

Method	mg *p*-hydroxybenzyl isothiocyanate per g dry meal Means ± S.D.
Gravimetric determination of enzymatically released sulphate	$m_4 = 31·7 \pm 0·7$
Ettlinger & Thompson[9]	$m_6 = 35·8 \pm 0·7$
Present, micro- modification	$m_{10} = 36·8 \pm 0·3$

The accuracy of the method has been tested by comparing it with gravimetric determination of enzymically released sulphate, with the method of Ettlinger & Thompson,[9] and by observing the recovery upon addition of known amounts of potassium thiocyanate or *p*-hydroxybenzylglucosinolate. Table II shows that the present method gives a significantly ($P < 0·001$) higher isothiocyanate value than the gravimetric sulphate determination. The gravimetric method is, however, rather complicated and its standard deviation relatively large. The method of Ettlinger & Thompson[9] gave slightly lower values than the present one. The difference in values between the two methods (2·6%) was significant at the 1% level. As the method of Ettlinger & Thompson includes several extractions and washings, it seems probable that as a result of minor losses, low rather than high values would be obtained.

Addition of potassium thiocyanate to the meal gave a recovery of 95·7% (mean value of three determinations). Recovery tests were also made with *p*-hydroxybenzylglucosinolate added to the meal as the tetramethylammonium salt. The mean value of five determinations was 87·9% recovery if the glucosinolate added was regarded as 100% pure. It is, however, probable that its purity was lower. Thiocyanate analysis of the glucosinolate by itself gave a mean value (five determinations) of 89·9% of the theoretical. Calculated from 89·9% purity of the glucosinolate, the recovery with meal was 97·8%.

Discussion

The reason for the lower values for *p*-hydroxybenzylisothiocyanate when unheated meal is analysed at pH > 5·0 has not been clearly established. The minimum of estimated isothiocyanate at pH 6·0 may indicate the existence of an isothiocyanate-destroying enzyme with a pH optimum at that pH and sensitive to a low pH. André & Carbouères[18] report that an enzyme which destroys isothiocyanates exists in seeds of black mustard, rape and turnip rape but not in white mustard seed. At pH > 5 isothiocyanates react with proteins,[19,20] but this reaction would be supposed to proceed more quickly at pH 7·0 than at pH 6·0. It has been found, however, that at pH 7 considerable amounts of thiocyanate will be formed from *p*-hydroxybenzyl isothiocyanate.[6] Since thiocyanate ion is inert to protein this reaction will reduce the consumption of isothiocyanate by protein at higher pH.

As the analyses are carried out in test tubes and the operations are simple, the present method is very well adapted for rapid serial analysis. By still further reducing the volumes used in the analysis, it would be possible to analyse samples of 2–3 mg of white mustard meal.

Acknowledgments

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Volumetric Determination of *p*-Hydroxybenzyl Isothiocyanate in Sinalbin* (*p*-hydroxybenzylglucosinolate) and in White Mustard Seed (*Sinapis alba* L)

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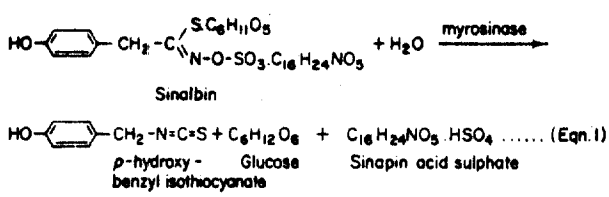
p-Hydroxybenzyl isothiocyanate is the constituent responsible for both flavour and aroma in white mustard. This isothiocyanate is not present in the free state as such but is produced as a result of hydrolysis of sinalbin present in the mustard. A survey of the existing methods showed the lack of a specific, quick and a reliable method for the estimation of p-hydroxybenzyl isothiocyanate. A titrimetric method has been developed which is based on the quantitative conversion of p-hydroxybenzyl isothiocyanate into thiocyanate. The thiocyanate is then determined iodometrically after conversion to cyanogen bromide. The method is applicable to pure sinalbin, white mustard and also to mixed mustard flour consisting of black and white mustard.

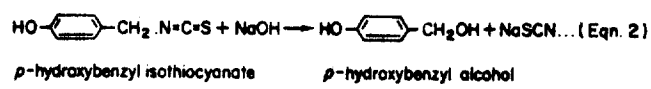
Introduction

WHITE mustard is one of the important spices used for both flavour and aroma. The whole seeds are used in pickles and in salads while the prepared mustard, which is a mixture of ground mustard with salt, vinegar, spices and other condiments, is used in sandwiches, frankfurter and gravies. Mustard flour finds a place in the preparation of meats, fish and sauces, and in medicine it is used as mustard plasters for giving relief in respiratory illnesses. White mustard has been found to be a valuable source of protein and edible oil. The condimental property of the white mustard is mainly due to the presence of *p*-hydroxybenzyl isothiocyanate which is responsible for the characteristic sharp, hot pungency. The *p*-hydroxybenzyl isothiocyanate does not exist as such in mustard but is produced as a result of hydrolysis of sinalbin (*p*-hydroxybenzylglucosinolate) with an enzyme myrosin. The presence of sinalbin in the protein meal of white mustard has restricted the use of the meal as an animal feed because of the toxic properties of its hydrolysed products.[1] Since the *p*-hydroxybenzyl isothiocyanate is not steam-volatile, the methods normally employed for the determination of allyl isothiocyanate in black mustard are not applicable for the white mustard.

A survey of the existing analytical methods described in literature for the determination of sinalbin shows that the initial step in all these consists of releasing the *p*-hydroxybenzyl isothiocyanate, glucose and sinapin acid sulphate by enzymic hydrolysis[2] (Eqn. 1).



Bauer & Holle[3] have determined the sinalbin in the whole seeds by converting the sulphate as barium sulphate and estimating it gravimetrically. It was, however, observed by Terry & Corran[4] that this method gave higher values due to the co-precipitation of proteins. Therefore, they modified the procedure by precipitating the sulphate as benzidine sulphate and determining it volumetrically. In the colorimetric method of Scharrer *et al.*[5], *p*-hydroxybenzyl isothiocyanate, in fat-free seeds, is converted into sodium thiocyanate which reacts with ferric chloride giving a red coloured ferric thiocyanate. Josefsson[6] modified the above method by replacing ferric chloride with ferric nitrate. In the methods of Bauer & Holle[3] and Terry & Corran,[4] the sulphate content is determined by indirect procedures and both these methods are time-consuming. The colorimetric methods of Scharrer *et al.*[5] and Josefsson[6] are limited to seed meal only and not applicable to the whole mustard seed which contains oil and colouring matter. Further, the instability of the red ferric thiocyanate in the presence of light is well known and also the intensity of the colour is dependent on the ferric concentration.[7] Therefore, there is a need for a quick and a reliable method for the estimation of *p*-hydroxybenzyl isothiocyanate in the products of white mustard. The present method is based on the determination of *p*-hydroxybenzyl isothiocyanate iodometrically. The isothiocyanate is reacted with sodium hydroxide to give sodium thiocyanate[8] (Eqn. 2)



which is determined by Schulek's method.[9] According to this method, thiocyanate reacts quantitatively with bromine to form cyanogen bromide (Eqn. 3). The cyanogen bromide is then allowed to react with potassium iodide to liberate iodine (Eqn. 4) which is titrated against standard sodium thiosulphate solution (Eqn. 5). The reactions involved are represented below:

$$SCN^- + 4\,Br_2 + 4\,H_2O \rightarrow CNBr + 7\,Br^- + SO_4^{--} + 8\,H^+ \quad \text{.... (Eqn. 3)}$$

$$CNBr + 2I^- \rightarrow CN^- + Br^- + I_2 \quad \text{.... (Eqn. 4)}$$

$$I_2 + 2Na_2S_2O_3 \rightarrow Na_2S_4O_6 + 2\,NaI \quad \text{.... (Eqn. 5)}$$

The overall reaction shows that one mole of *p*-hydroxybenzy isothiocyanate consumes two moles of sodium thiosulphate.

* Sinapin-*p*-hydroxybenzylglucosinolate

Experimental

Reagents and materials

Sodium hydroxide solution

An approximately 1 N solution was prepared by dissolving 4 g of sodium hydroxide (*pro analysi*, E. Merck) in 100 ml of distilled water.

Trichloroacetic acid

A 10% aqueous solution (w/v) of trichloroacetic acid (E. Merck) was prepared.

Liquid bromine

A.R. quality was used.

Ferrous ammonium sulphate crystals

(A.R.).

Orthophosphoric acid solution

1 vol. of 88% orthophosphoric acid (Laboratory chemical) was diluted with 3 vol. of water.

Potassium iodide (*pro analysi*, E. Merck).

Sodium thiosulphate

0·025 N.

White mustard seed

A sample available in the local market was finely ground and used. A Swedish variety of white mustard, kindly supplied by Dr. E. Josefsson, Swedish Seed Association, Svalof, Sweden, was also used.

Mixed mustard flour

This was prepared by mixing white (Swedish) and black (Indian) mustard powders in different proportions as shown in Table III.

Sinalbin

The pure compound, isolated from the white mustard, was obtained as a gift sample by the courtesy of Prof. Kjaer, Copenhagen.

Enzyme myrosin

This was prepared according to the procedure described by Appelqvist & Josefsson.[10] A 0·5% solution of this was prepared in water.

Estimation of sinalbin

An accurately weighed quantity of sinalbin (100–200 mg) was transferred into a 100 ml Erlenmeyer flask. About 10 ml of myrosinase solution were added to the flask followed by the addition of 30 ml of hot water (65°c). The mixture was kept on a water-bath maintaining the temperature of the solution at 65°c for about 1 h. It was cooled for 15 min and 10 ml of sodium hydroxide solution were added to this and shaken well. After 10 min the mixture was acidified using trichloroacetic acid bringing the pH to around 4–5 and the solution filtered through a filter paper. The filtrate was reacted with the vapours of bromine in a fume cupboard till the solution turned a light yellow colour, stable for at least 5 min. In order to remove the excess bromine, a few crystals of ferrous ammonium sulphate were added to this till the disappearance of the yellow colour. It was made acidic by the addition of 5 ml of phosphoric acid solution. About 2 g of potassium iodide crystals and then a little sodium hydrogen carbonate were added to this and stoppered. It was shaken well and kept in darkness for about 5 min. The liberated iodine was then titrated against standard sodium thiosulphate solution using freshly prepared starch as the indicator. The amount of sinalbin was calculated from the titre using the factor:

1 ml of N-sodium thiosulphate ≡ 0·4125 g of sinalbin. The results of analysis are given in Table I.

Determination of *p*-hydroxybenzyl isothiocyanate in white mustard seed and mixed mustard flour

Weighed quantities (2–5 g) of the powdered white mustard and mixed mustard flour were transferred into two different Erlenmeyer flasks. About 30 ml of hot water (65°c) were added to each of these and the mixture was kept on a water-bath, maintaining the temperature of the solution at 65°c for about 1 h. The *p*-hydroxybenzyl isothiocyanate liberated was determined by the procedure as described above.

The *p*-hydroxybenzyl isothiocyanate was calculated using the factor:

1 ml of N-sodium thiosulphate ≡ 0·0825 g of *p*-hydroxybenzyl isothiocyanate. The results of analysis are given in Tables II and III.

TABLE I
Results of analysis of sinalbin

Sample no.	Sinalbin taken mg	Sinalbin found	
		mg	%
1	100	97	97·00
2	150	143	95·32
3	175	170	97·14
4	200	196	98·00

TABLE II
Results of analysis of white mustard

Sample no.	Variety	Weight of white mustard taken g	*p*-Hydroxybenzyl isothiocyanate found	
			g	%
1	Local (Indian)	2·04	0·0241	1·18
		2·09	0·0226	1·08
		3·01	0·0361	1·20
		3·05	0·0366	1·20
		3·51	0·0428	1·21
2	Swedish	1·51	0·0515	3·41
		1·54	0·0524	3·40
		2·01	0·0683	3·39
		2·03	0·0686	3·38
		2·54	0·0858	3·38

TABLE III
Results of analysis of mixed mustard flour

Sample no.	Flour composition		*p*-Hydroxybenzyl isothiocyanate	
	Swedish white mustard %	Indian black mustard %	found %	Expected %
1	100	0	3·22	3·22
			3·24	3·22
			3·27	3·22
			3·17	3·22
2	71	29	2·24	2·28
			2·20	2·28
3	63	37	1·97	2·02
			2·00	2·02
4	55	45	1·74	1·77
			1·72	1·77
5	50	50	1·64	1·61
			1·65	1·61
6	45	55	1·40	1·44
			1·40	1·44
7	29	71	0·94	0·93
			0·93	0·93

Discussion

It can be seen from Tables I, II and III that the sinalbin and *p*-hydroxybenzyl isothiocyanate can be determined with a high degree of accuracy.

In Terry & Corran's method, the total sulphate released from sinigrin and sinalbin had been estimated first. Later, the allyl isothiocyanate in the mixture is determined by the argentometric method and the amount of sinigrin calculated. The theoretical sulphate contribution by sinigrin is calculated and deducted from the total sulphate content. The difference is then taken as the sulphate contribution by sinalbin. Thus, in an indirect way sinigrin and sinalbin in the mixed flour have been computed. Whereas the present method is specific for the *p*-hydroxybenzyl isothiocyanate unlike the gravimetric[3] and volumetric[4] methods. The allyl and *p*-hydroxybenzyl isothiocyanates in the mixed flour can be directly determined by the piperidine[11] (or argentometric method) and the present method respectively.

The use of phosphoric acid in the present method has two advantages. Since this acid is a weak acid the cyanogen bromide formed is stable in the solution. Second, it combines with the yellow ferric ions forming ferric phosphate rendering the reaction mixture white and clear, thus facilitating an easy detection in the end point of the titration.

The study shows that the local variety of white mustard contains about 1·2% whereas the Swedish variety contains about 3·4% of *p*-hydroxybenzyl isothiocyanate. According to Terry & Corran[4] the values of the percentage of the *p*-hydroxybenzyl isothiocyanate in the different varieties of white mustard range between 3·0–3·7. In view of the wide variation in the content of the *p*-hydroxybenzyl isothiocyanate in the different varieties of white mustard it may not be feasible to deduce exactly the percentage of white mustard in a given blend of the flour. On the other hand, if the variety and the variation in *p*-hydroxybenzyl isothiocyanate is known it may be possible to get a close picture of the white mustard content in the flour. It is tempting to suggest that the local variety of white mustard seems to be a promising one for plant breeding experiments to evolve varieties with low content of sinalbin.

Acknowledgment

The authors wish to thank Dr. H. A. B. Parpia, Director of the Institute, for his keen interest in the work.

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[Agr. Biol. Chem., Vol. 35, No. 6, p. 959~961, 1971]

Note

A Titrimetric Method of Determination of *p*-Hydroxybenzyl Isothiocyanate in White Mustard (*Sinapis alba* L.)

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White mustard is an important spice used in food flavourings. The condimental value of this spice is due to the *p*-hydroxybenzyl isothiocyanate which is obtained as a result of enzymic hydrolysis[1] (Eq. 1) of the naturally occurring thioglucosinolate (Sinalbin) present in it. Terry and Corran[2] have reported a value of 15~18% sinalbin in white mustard. In recent years the white mustard has been found to be a valuable source of protein meal[3~5] and edible oil. Since the *p*-hydroxybenzyl isothiocyanate is toxic[3] and harmful in larger concentrations, the protein meal has found a limited use as a feed. However, work is in progress in evolving a variety of sinalbin-free white mustard[6] and in the detoxification of the protein meal. Recently, Josefsson[5,7] has reviewed the various analytical methods of estimation of *p*-hydroxybenzyl isothiocyanate and pointed out their merits and demerits. Although the colorimetric method of Josefsson[7] is applicable to the seed meal of white mustard it was, however, found not applicable to the whole seeds. Since the *p*-hydroxybenzyl isothiocyanate is not steam distillable, the methods used for the determination of allyl isothiocyanate in black mustard are not applicable for the white mustard. It was, therefore, thought that the volumetric procedure developed for the estimation of allyl isothiocyanate[8] in black mustard could suitably be modified for the determination of *p*-hydroxybenzyl isothiocyanate in white mustard. In the present method the *p*-hydroxybenzyl isothiocyanate is reacted with piperidine to form *p*-hydroxybenzyl piperidyl thiourea (Eq. 2). The excess unreacted piperidine is back titrated using standard sulphuric acid (Eq. 3) and the amount of *p*-hydroxybenzyl isothiocyanate calculated. This method is simpler and compares favourably with the iodometric method.[9]

EXPERIMENTAL

Reagents and materials. Standard sulphuric acid solution (0.05 N) was prepared using A. R. sulphuric acid and distilled water.

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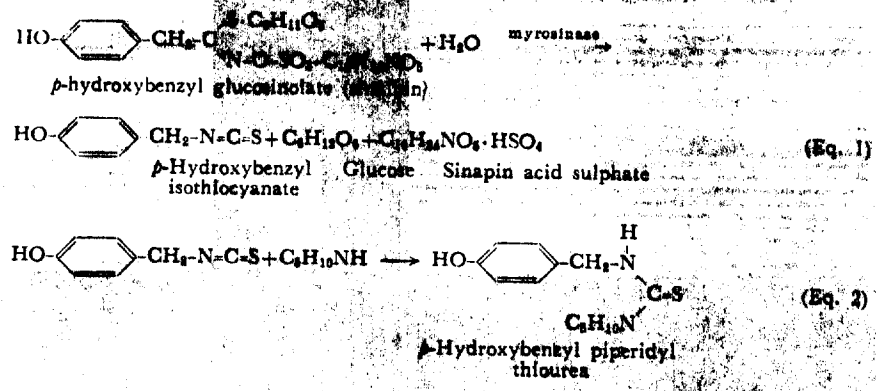

Piperidine solution. An approximately 0.05 N solution was prepared by transferring about 0.9 g of piperidine (Fluka) into a 200 ml. Volumetric flask and making up the volume to the mark using A. R. acetone. The purity of this was determined using the above standard sulphuric acid solution.

Mixed indicator. This was prepared by dissolving 0.02 g of methyl red and 0.1 g of bromo cresol green in 100 ml of alcohol solution.

White mustard seed. A Swedish variety of white mustard kindly supplied by Dr. E. Josefsson, Swedish Seed Association, Svalof, Sweden and a variety purchased from the local market were finely ground and used.

Procedure. A weighed sample of white mustard powder was transferred into a 100 ml Erlenmeyer flask. To this about 30 ml of hot water (65°C) were added and the temperature of the solution was maintained at about 65°C for 1 hr using a water-bath. The solution was cooled to room temperature and filtered through a cotton plug. To the filtrate 20 ml of piperidine solution were added and kept aside for 45 min for completion of the reaction. The mixture was filtered through a filter paper and the filtrate containing the excess unreacted piperidine was determined by titrating against the standard sulphuric acid solution using the mixed indicator till the appearance of a wine red colour. A blank using the same quantity of white mustard and without the addition of piperidine solution was carried out simultaneously and the titre value found was deducted from the above. From the volume of piperidine reagent consumed, the p-hydroxybenzyl isothiocyanate was computed using the following equation 1 ml of 1 N piperidine = 0.165 g of p-hydroxybenzyl isothiocyanate. The results of analysis are given in Table I. In this table are also included the results obtained by the iodometric method[8] which has been used as a method of comparison.

TABLE I. ANALYSIS OF WHITE MUSTARD

Variety	Weight of mustard taken in g	Weight of p-hydroxybenzyl isothiocyanate found in g	
		By the piperidine method	By the iodometric method
Local (Indian)	2.0	0.0200	0.0241
		0.0208	0.0243
		0.0215	0.0238
	3.0	0.0310	0.0361
		0.0318	0.0359
		0.0328	0.0365
	4.0	0.0442	0.0480
		0.0446	0.0485
		0.0458	0.0487
Swedish	2.0	[illegible]	0.0680
		[illegible]	[illegible]
		0.0659	0.0688
	2.5	0.0818	0.0856
		0.0827	0.0862
	3.0	0.0971	0.1015
		0.0985	0.1023

DISCUSSION

A study of the results given in Table I shows that the piperidine method compares

well the iodometric method. The advantage of the present method over all the existing methods is that the method is quick and specific for the *p*-hydroxybenzyl isothiocyanate. Ammonia and aliphatic amines react with isothiocyanate very slowly whereas a cyclic base like piperidine reacts quickly and quantitatively. In addition the piperidine solution has a good stability.

Acknowledgement. The authors wish to thank Dr. H. A. B. Parpia, Director of the Institute, for his keen interest in the work.

243. Biological Activity of Isothiocyanates

The isothiocyanates display a range of pharmacological characteristics which are still being exploited in today's therapy. Thus, allyl-isothiocyanate is used in the form of a mustard poultice as a remedy for rheumatism; radish and watercress are used as bile stimulators.

The studies by Schmidt and Marquardt (80) as well as those of Kosser (89) provide evidence for the bacteriostatic and antimycotic activity of isothiocyanates; thus, certain isothiocyanates can be used with good results against ground parasites (e.g. fungi.).

Many animal feeds contain glucoside oils of mustard; when these are split by enzymatic action, isothiocyanates are produced which can be poisonous to animals. Findings on the constituent contents of such feeds are given be Kjaer et al (36).

However, the most important role played by the isothiocyanates is in human nutrition. Many spices and varieties of vegetables derive their characteristic oder and taste from the isothiocyanates and their breakdown products. These have been demonstrated to produce certain physiological effects as well.

As we mentioned earlier, among the intermediate products arising from certain isothiocyanates are cyclic compounds which exhibit strong anti-thyroid action. Thus, rhodanide (thiocyanide), which according to Virtanen (82,83) arises from the hydrolysis by p-HBI of two hypothetical isothiocyanates, produces a disturbance in the formation of the thyroid hormones by inhibiting the iodation processes. ($SCN^{\ominus}$ - has the same ion radius as $I^{\ominus}$ ions (72,91,92). Rhodanides (thiocyanides) also act to depress blood pressure (72).)

The possibility whether milk from cows fed with plants of the Brassica variety can, because of the thiocyanide content of the plants, work goitrogenically on man has been denied by the studies of Virtanen et al (82,83).

As earlier mentioned, the work cited above (Virtanen 82,83) occupied itself with the study of the production and stability of p-HBI in table mustard. These studies found no p-HBI in table mustard allowed to stand for a one to two-week period. The breakdown products apparently contributed scarcely anything to the taste. The products formed by the action of p-HBI, p-hydoxybenzylalcohol and thiocyanide (from 1 g white seed mustard 7-8 mg SCN are produced) to be sure have anti-thyroid effects (72,91,92); however, in the normal consumption of table mustard, no harmful amounts are ingested.

The use of white seed mustard (Sinapis alba L.) in table mustard causes no lasting piquancy in the mustard since the p-HBI cannot be stabilized because of its labile character. Technically therefore, the white seed mustard serves in table mustard only as a thickening agent.

The presence of Sinapis alba L. in table mustard can be conclusively demonstrated by the thin-film-chromatographic evidence of p-hydroxybenzylalcohol (X), and quantitative analysis for thiocyanides permits us to calculate the original amount of Sinapis alba L. in the table mustard.

Prom. Nr. 3537

Über p-Hydroxybenzyl-isothiocyanat, das Senföl aus Samen von Sinapis alba L.

Von der
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HOCHSCHULE IN ZÜRICH

zur Erlangung
der Würde eines Doktors der technischen Wissenschaften
genehmigte

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Meinen verehrten Lehrern,

Herrn Prof. Dr. Hans Deuel,

dem am 17. Januar 1962 verstorbenen Vorstand des Agrikulturchemischen Instituts der Eidgenössischen Technischen Hochschule, Zürich, und

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unter deren Leitung die vorliegende Arbeit ausgeführt wurde, möchte ich für das mir und meiner Arbeit stets entgegengebrachte Wohlwollen und Interesse danken.

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1. EINLEITUNG

Das Samenmehl des weissen Senfs (Sinapis alba L.) bildet neben dem des schwarzen Senfs (Brassica nigra K.) und dem des Sarepta-Senfs (Brassica juncea Cz & C) einen der wichtigsten Bestandteile des Tafelsenfs.

Die Schärfe des Tafelsenfs rührt von den Isothiocyanaten oder Senfölen her, die in den Pflanzen als Senfölglucoside vorliegen und während der Senfbereitung von der in den gleichen Pflanzen vorhandenen Myrosinase enzymatisch freigesetzt werden.

Das Samenmehl von Sinapis alba L. wird hauptsächlich deshalb zur Senfherstellung herangezogen, weil dessen nichtflüchtiges Isothiocyanat, neben dem flüchtigen Allylisothiocyanat von Brassica nigra K., dem Tafelsenf einen nachhaltend brennenden Geschmack verleiht und damit den Würzeffekt erhöht.

In der Technologie der Senfherstellung bedient man sich auch heute noch meistens traditioneller Rezepte, ohne dass deren Grundlage je qualitativ und quantitativ untersucht wurde.

Das Senfölglucosid des Samens von Sinapis alba L. wurde zwar schon von Robiquet und Boutron (1) 1831 kristallin erhalten, das daraus enzymatisch gebildete Isothiocyanat konnte jedoch noch nie in analytisch reiner Form isoliert werden. Es wurde von Salkowski (2), Schneider (3), Kjaer und Rubinstein (4) anhand der Reaktionen des zugehörigen Senfölglucosids und mit Hilfe von Thiobarnstoffderivaten des Isothiocyanats eindeutig als p-Hydroxybenzyl-isothiocyanat (im folgenden als p-HBI abgekürzt) erkannt. Die Identifizierung konnte jedoch nie durch Synthese des reinen p-HBI bestätigt noch konnten seine Eigenschaften exakt untersucht werden. Es ist lediglich klar geworden, dass das p-HBI einen äusserst labilen Charakter besitzen muss, der durch seine leichte Oxydier- und Polymerisierbarkeit erklärt wurde. Die Zersetzungsprodukte sind aber nicht untersucht worden.

Die für die Senfbereitung wichtigen Fragen der Entstehungsweise und Stabilität des p-HBI blieben ebenfalls bis anhin ungeklärt.

Der Zweck der vorliegenden Arbeit bestand deshalb darin, das p-HBI synthetisch zu gewinnen, aus fermentiertem Samen von Sinapis alba L. (alle folgenden Angaben über Samen oder Samenmehl beziehen sich auf Sinapis alba L.) zu isolieren, seine Eigenschaften zu untersuchen und die Wirkung der wichtigsten Inhaltsstoffe des Samens auf die Entstehung und Stabilität von p-HBI zu studieren.

Die Identität des synthetischen p-HBI mit dem aus dem enzymatisch abgebauten Samenmehl isolierten sollte den endgültigen Beweis der Struktur der Muttersubstanz dieses Isothiocyanats liefern.

2. THEORETISCHER TEIL

21. Synthese von p-Hydroxybenzyl-isothiocyanat

211. Literatur der Isothiocyanatsynthesen

Für die Darstellung von Isothiocyanaten existieren zahlreiche Methoden mit teilweise ausgezeichneten Ausbeuten (5, 6).

Aus den Alkylhalogeniden und Metallrhodaniden erhält man durch thermische Umlagerung der intermediär gebildeten Alkylrhodanide glatt die Alkylisothiocyanate.

$$R - X \xrightarrow{SCN^{\ominus}} [R - SCN] \xrightarrow[150^{0}]{Dest.} R - NCS$$

Nach einer amerikanischen Patentschrift (7) kann das Allylisothiocyanat, das Senföl des Samens von Brassica nigra K., sogar durch Erhitzen von Allylchlorid, Natriumcyanid und Schwefel in Methanol direkt hergestellt werden.

$$CH_2 = CHCH_2 - Cl + NaCN + S \rightarrow [CH_2 = CHCH_2 - SCN] \rightarrow CH_2 = CHCH_2 - NCS$$

Für die Synthesen von Arylisothiocyanaten muss man in den meisten Fällen von den entsprechenden Aminen ausgehen.

Mit Thiophosgen werden sowohl die alipathischen wie auch die aromatischen primären Amine nach Rathke (8), Dyson und George (9, 10, 11) seit langem mit Erfolg direkt zu Isothiocyanaten umgesetzt.

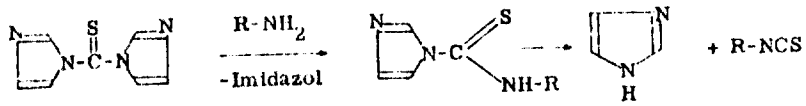
$$R - NH_2 + Cl_2CS \xrightarrow{HCO_3^{\ominus}} [R - NH - CS - Cl] + HCl$$

$$\downarrow$$

$$R - NH - CS - NH\text{-}R \xleftarrow{R\text{-}NH_2} R - N = C = S + HCl$$

Da die Ausbeute öfters durch die Bildung von symetrischen Thioharnstoffen aus den gebildeten Isothiocyanaten mit den freien Aminen vermindert wird, geht man am besten von den Aminsalzen aus. Die Umsetzungen werden immer im wässrigen alkalischen Milieu durchgeführt, wenn das Isothiocyanat nicht wasser- und alkali-empfindlich ist, gibt die Thiophosgenmethode eine gute Ausbeute.

Vor kurzem wurde eine interessante Isothiocyanatsynthese von Staab und Walther (12) beschrieben, die aus primären aliphatischen und aromatischen Aminen mit NN' - Thiocarbonyl-di-imidazol in äquimolarer Mischung Imidazol und Isothiocyanat erhielten.



Das Isothiocyanat konnte durch Destillation oder durch Extraktion mit Benzin oder Tetrachlorkohlenstoff, in denen Imidazol unlöslich ist, abgetrennt werden. Die Reaktion wurde immer in vollkommen wasserfreiem Chloroform durchgeführt.

Die primären Amine werden mit Schwefelkohlenstoff und Alkalihydroxyd oder Ammoniak öfters zuerst in die Salze der entsprechenden Dithiocarbaminsäuren übergeführt, welche nach verschiedenen Methoden in Isothiocyanate gespalten werden können.

Einfache Arylisothiocyanate werden aus Dithiocarbaminaten nach Hofmann (13) durch Erhitzen mit Metallsalzen erhalten (14). Nach einer ebenfalls von Hofmann (15) stammenden Modifikation können die dithiocarbaminsauren Salze mit Jod zersetzt werden. Dithiocarbaminat ergibt aber auch durch Oxydation mit Jod Thiuramdisulfid, das nach Braun und Deutsch (16) mit Natriumäthylat- und weiterer Jodbehandlung unter vorübergehender Bildung eines unbeständigen zyklischen Sulfids ein Gemisch von Schwefel und Isothiocyanaten liefert. Schmidt et al. (17,18) oxydierten die Dithiocarbaminaten mit Natriumhypochlorit oder Natriumchlorit zu Isothiocyanaten.

Für die Herstellung aromatischer Isithiocyanate bewährte sich hauptsächlich die Methode von Slotta und Dressler (19), nach der Phosgen mit Dithiocarbaminaten in wasserfreiem Benzol ohne störende Nebenreaktionen Isothiocyanate gibt.

$$R\text{-}NH\text{-}CSS^{\ominus}\ NH_4^{\oplus} + Cl_2CO \rightarrow R\text{-}N{=}C{=}S + HCl + NH_4Cl + COS$$

Die Reaktion verläuft schon bei 0 Grad fast quantitativ. Das Ammoniumchlorid wird abgenutscht, Chlorwasserstoff und Kohlenoxysulfid entweichen beim Einengen der Benzollösung, worauf das Isothiocyanat durch fraktionierte Destillation oder Kristallisation rein erhalten wird. Diese Synthese ist besonders für die Darstellung von phenolischen Isothiocyanaten geeignet, da Slotta und Dressler (19) das p-Hydroxyphenyl-isothiocyanat nur mit dieser Methode rein erhalten konnten. Dyson und George (9) bekamen mit der Thiophosgenmethode nur schmutzige ölige Umsetzungsprodukte, während sämtliche anderen Isothiocyanatsynthesen versagten.

Dithiocarbaminaten geben nach Andreasch (20), Kaluza (21) und Nägele (22) mit Chlorameisensäureester Isothiocyanate in guter Ausbeute. Obwohl die Reaktion gerade in der aromatischen Reihe entdeckt wurde (23), ist sie dort

meistens nicht brauchbar, da sie zum grössten Teil symetrische Thioharnstoffe liefert. Von Moore (24) sowie van der Kerk, Pluygers und de Vries (25) wurden verschiedene Modifikationen der Andreasch-Kaluza-Methode beschrieben, nach denen man auch aromatische Isothiocyanate erhalten sollte.

Bei aromatischen Aminen, deren basischer Charakter zu schwach ist, bereitet die Herstellung von Dithiocarbaminaten Schwierigkeiten, da das Gleichgewicht in Richtung der symetrischen Diarylthioharnstoffe verschoben ist. Aromatische Amine ergeben mit Schwefelkohlenstoff allein leicht Diarylthioharnstoffe, die nach der alten Methode von Hofmann (26) oder nach Bly, Perkins und Lewis (27) mit heissen Mineralsäuren in aromatische Isothiocyanate und in Aminsalze überführt werden können. Ebenso kann man die Monoarylthioharnstoffe durch Erhitzen in einem geeigneten Lösungsmittel in Isothiocyanate und Ammoniak aufspalten (28).

212. Frühere Syntheseversuche von p-Hydroxybenzyl-isothiocyanat

Obwohl, wie erwähnt, viele Möglichkeiten zur Darstellung von Isothiocyanaten bestehen, führen bei phenolischen Isothiocyanaten die meisten nicht zum Erfolg. Die bisherigen Darstellungsversuche des p-HBI zeigen dies ebenfalls.

Salkowski (2) war 1889 der erste, der versuchte, das Senföl durch Zersetzung des entsprechenden Dithiocarbaminats (VII) mit Quecksilberchlorid in reiner Form zu erhalten. Er setzte deshalb p-Hydroxybenzylamin (IV) in Alkohol mit Schwefelkohlenstoff um. Das in geringer Menge entstandene Dithiocarbaminat (VII) erhitzte er in wässriger oder alkoholischer Quecksilberchloridlösung, um das p-HBI zu erhalten. Mit Aether extrahierte er aus der wässrigen Lösung eine ölige Substanz, welche sehr scharf schmeckte. Da er das Rohöl durch Wasserdampfdestillation nicht reinigen konnte, verzichtete er auf die Reindarstellung des Senföls und gab für die Substanz weder Analyse noch sonstige Eigenschaften bekannt.

In neuerer Zeit versuchte auch André (29) erfolglos p-HBI herzustellen und vermutete, dass das Senföl äusserst temperaturempfindlich und an der Luft oxydierbar sei. Auch den p-Hydroxybenzylthioharnstoff konnte er aus dem Senföl nicht erhalten.

Kjaer und Rubinstein (4) führten zahlreiche Vorversuche aus, um die Möglichkeit der Reindarstellung des Senföls zu studieren. Die Hofmann'schen Synthesen (15), die modifizierte Andreasch-Kaluza-Synthese (20, 21, 22) und verschiedene Varianten der Dysonsmethode (10, 11) lieferten alle unverarbeitbare gummiartige Produkte polymerer Natur, aus denen sie mit Aminen keine Thioharnstoffderivate herstellen konnten.

Lediglich aus p-Hydroxybenzylamin (IV) und Thiophosgen in einem alkalischen Aether-Wasser-Gemisch konnten sie etwas p-HBI in der Aetherphase darstellen. Ohne dieses zu isolieren, identifizierten sie das Senföl als N-p-Hydroxybenzyl-N'-phenylthioharnstoff-Derivat (IX), das sie einerseits aus der Aetherphase mit Anilin und andererseits aus p-Hydroxybenzylamin (IV) mit Phenylisothiocyanat erhielten.

Nachdem schon Will und Laubenheimer (30) zeigten, dass p-HBI in stark alkalischem Milieu viel Rhodanid bildet, musste sicherlich bei den Versuchen von Kjaer und Rubinstein (4) in der Aetherphase nur noch ganz wenig Senföl neben seinen Zersetzungsprodukten vorliegen, wodurch die Isolierung von reinem Senföl sehr erschwert würde. Auch Kjaer und Rubinstein (4) machten die ausserordentliche Labilität für die bisherigen Misserfolge in der Isolierung des reinen Senföls verantwortlich.

In Anbetracht der Reaktionsträgheit der Isothiocyanate, ist unserer Auffassung nach nicht zu befürchten, dass eine Addition der Phenolgruppe an die Isothiocyanatgruppe bei Zimmertemperatur eintritt, diese kommt nur nach langem Kochen des Reaktionsgemisches zustande (31). Das reine p-Hydroxyphenyl-isothiocyanat destilliert bei 165-170°/10 Torr ohne Zersetzung, ein weiterer Hinweis wie wenig man mit intermolekularen Kondensationen bei phenolischen Isothiocyanaten rechnen muss.

Es erscheint dagegen viel wahrscheinlicher, dass die Wasser- und vor allen Dingen die Alkaliempfindlichkeit des p-HBI für das Scheitern der bisherigen Syntheseversuche verantwortlich ist.

Aus diesen Ueberlegungen erschienen die Methoden von Slotta und Dressler (19) und von Staab und Walther (12) am aussichtsreichsten, denn hier erfolgt die Bildung der aromatischen Senföle in vollkommen inerten Lösungsmitteln unter Ausschluss von Feuchtigkeit und Luft.

213. Darstellung von p-Hydroxybenzyl-isothiocyanat

Um die Methode von Slotta und Dressler (19) zu prüfen, wurden Anilin, Benzylamin, p-Anisidin und p-Aminophenol über ihre Ammonium-dithiocarbaminate mit Phosgen zu Phenyl-, Benzyl-, p-Methoxyphenyl- und p-Hydroxyphenyl-isothiocyanaten umgesetzt. Diese einfachen Isothiocyanate sollten später auch für die Darstellung von Thioharnstoffderivaten und für dünnschichtchromatographische Untersuchungen dienen.

Anilin, Benzylamin und p-Anisidin ergaben die Ammonium-dithiocarbaminate glatt sowohl mit wässrigem Ammoniak als auch in wasserfreiem Toluol mit trockenem Ammoniak. Hingegen konnte Ammonium-p-hydroxyphenyl-dithiocarbaminat nur mit

einem Zehntel der von Slotta und Dressler (19) angegebenen Menge konzentriertem Ammoniak in kristalliner Form erhalten werden.

Die Reinheit der Ammonium-dithiocarbaminate wurde durch eine Ammoniakbestimmung ermittelt. Die durchwegs 98 - 99 % reinen trockenen Ammoniumsalze lieferten mit Phosgen vorschriftsmässig die Isothiocyanate.

Das p-Methoxyphenyl-isothiocyanat wurde nach der Destillation als eine weisse kristallinische Masse erhalten, Smp. 20 - 21° C.

Das p-Hydroxybenzyl-isothiocyanat erstarrte nach der Destillation ebenfalls zu einer kristallinen Masse und konnte aus Petroläther umkristallisiert werden. Die Kristalle waren im Gegensatz zu den Angaben von Slotta und Dressler (19) vollkommen farblose Nadeln, Smp. 49 - 50° C.

Bei einem zweiten Ansatz wurde p-Hydroxyphenyl-isothiocyanat direkt aus der eingeengten Benzollösung, ohne vorherige Destillation, durch Auflösen und Umkristallisieren in Petroläther erhalten.

Um die Eignung der Isothiocyanatsynthese von Staab und Walther (12) für phenolische Isothiocyanate zu untersuchen, wurde das p-Aminophenol mit NN' - Thiocarbonyl-di-imidazol (V) umgesetzt. V kann nach Staab und Walther (12) auch mit Phenolen reagieren, deshalb findet man neben dem Isothiocyanat auch Nebenprodukte, wie das Dünnschichtchromatogramm des Rohproduktes zeigte. Das p-Hydroxyphenyl-isothiocyanat konnte von den Nebenprodukten nur schwer abgetrennt werden. Aus dem Petroläther erhielt man schliesslich ein farbloses kristallines Produkt, Smp. 47 - 48° C, Ausbeute 51 %.

Das UV-Spektrum (Fig. 1) von p-Hydroxyphenyl-isothiocyanat wurde in n-Heptan aufgenommen und zeigt charakteristische Maxima bei 228 mμ (log ε 4,395), 274 mμ (log ε 4,164) und 283 mμ (log ε 4,155).

Das IR-Spektrum (Fig. 2) von p-Hydroxyphenyl-isothiocyanat als Kaliumbromidpressling ergibt die intensive für Isothiocyanate charakteristische Bande bei 2120 cm^{-1} (32) und eine intensive Bande bei 1505 cm^{-1}, welche nach Pestemer und Lauerer (33) durch Addition von Lewis-Basen bzw. -Säuren an Isothiocyanate herrühren soll.

Im Gegensatz zu den Angaben von Slotta und Dressler (19) ist p-Hydroxyphenyl-isothiocyanat in Aether sehr gut, in Alkohol und Benzol weniger gut löslich.

Aus dem p-Hydroxyphenyl-isothiocyanat wurde mit Ammoniak der p-Hydroxyphenyl-thioharnstoff dargestellt, Ausbeute 78 %, Smp. 216° C. Das IR-Spektrum des Thioharnstoffderivates (Fig. 3) wurde als Kaliumbromidpressling aufgenommen und zeigt, dass die intensive Bande bei 2120 cm^{-1} nicht mehr vorhanden ist.

Die erfolgreiche Herstellung des p-Hydroxyphenyl-isothiocyanats nach beiden Methoden, berechtigten zur Hoffnung, dass auf einem der beiden Wege auch das p-Hydroxybenzylamin (IV) in p-HBI übergeführt werden kann. Da p-Hydroxybenzylamin (IV) im Handel nicht erhältlich ist, musste von p-Hydroxybenzaldehyd (I) ausgegangen werden. (Reaktionsschema Seite 16)

p-Hydroxybenzaldehyd (I) wurde mit Hydroxylamin-hydrochlorid in p-Hydroxybenzaldoxim (II) überführt. Für die katalytische Hydrierung des Oxims (II) nach Ott und Zimmermann (34) stand eine Vorschrift von Kjaer und Rubinstein (4) zur Verfügung. Während diese bei der Hydrierung mit Adam's Katalysator 74 % Ausbeute erhielten, konnten, wie Tabelle I zeigt, durch 5 %-ige Pd-Kohle schnellere und ausgiebigere Hydrierungen erzielt werden.

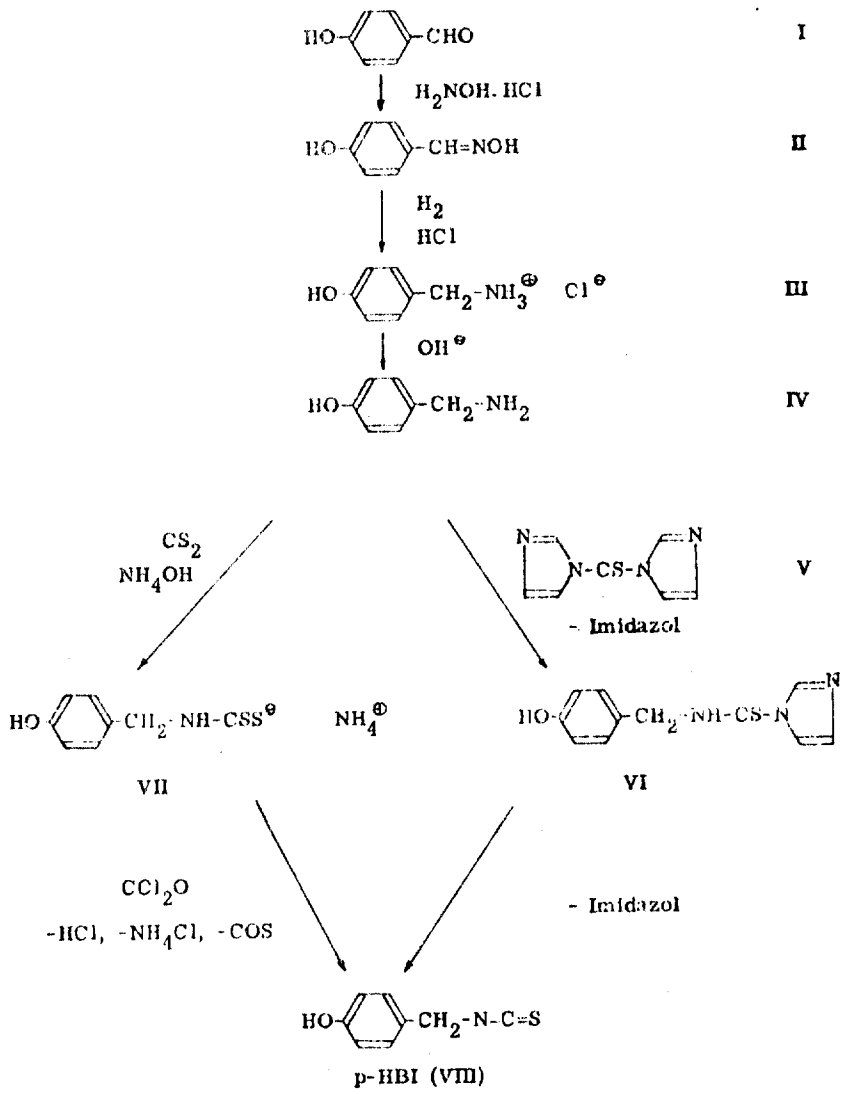
Tabelle I: Hydrierung von p-Hydroxybenzyldoxim (II)

Oxim (II)	Katalysator		H_2-Aufnahme		Hydrochlorid (III)	
Menge [g]	Typ	Menge [mg]	Menge [L]	Zeit [Min.]	Menge [g]	Ausbeute [%]
7,5	PdO (Fluka)	300	2,85	180	6,95	79,5
7,5	PdO (Fluka)	300	2,75	180	6,37	73,0
7,5	Pd-Kohle 10 %	750	2,94	82	7,51	86,0
7,5	Pd-Kohle 5 %	750	2,85	101	8,00	92,0
15,0	Pd-Kohle 5 %	750	5,90	196	12,56	72,0

Aus dem p-Hydroxybenzylamin (IV) versuchten wir das p-HBI einerseits via Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) und andererseits durch Reaktion mit NN'-Thiocarbonyl-di-imidazol (V) darzustellen.

p-Hydroxybenzylamin (IV) löst sich nicht in Benzol oder Toluol. Deshalb war man gezwungen, das Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) entweder in methanolisch-wässriger Ammoniaklösung oder in wasserfreier Methanol-Aether-Lösung mit trockenem Ammoniak herzustellen. Beide Möglichkeiten wurden untersucht. Aus der Methanol-Wasser-Lösung schied sich VII durch Zugabe von Aether in 71 - 73 %-iger Ausbeute aus. Dagegen musste die wasserfreie Methanol-Aether-Lösung zuerst eingedampft werden, um das reine Ammoniumsalz (VII) aus dem Rohprodukt durch Umkristallisieren aus Aethanol-Aether mit 44 %-iger Ausbeute erhalten zu können.

Die Synthese von p-HBI gestaltete sich gemäss nachfolgendem Reaktionsschema.



Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) wurde zuerst wie beim Ammonium-p-hydroxyphenyl-dithiocarbaminat, bei 50° C mit Phosgen umgesetzt. Nachdem der benzolunlösliche Teil abgenutscht, die Benzollösung schonend eingedampft und das p-HBI mit warmem Petroläther aufgelöst wurde, kristallisierte das p-HBI aus der eingeengten Petrolätherlösung in langen farblosen Nadeln aus. Die Kristalle schmolzen bei 36 - 38° C unscharf. Die Ausbeute war mit 10,4 % sehr gering, bezogen auf das Ammoniumsalz (VII) (7,0 % bezogen auf p-Hydroxybenzylamin (IV)).

Die Umsetzung bei Zimmertemperatur (20° C) ergab fast dieselbe Ausbeute, hingegen verlief sie bei 0° C unter Stickstoffatmosphäre und längerer Reaktionszeit etwas günstiger, Ausbeute 14,2 %, bezogen auf VII respektive 10,3 % bezogen auf IV. Die niedrigen Ausbeuten stammen offenbar nicht von Einflüssen der Reaktionstemperatur oder der Oxydation des p-HBI.

Alle weiteren Versuche zur Erhöhung der Ausbeute verliefen ergebnislos.

Es fiel auf, dass bei allen Umsetzungen der benzolunlösliche Teil erheblich mehr betrug als man aus den theoretischen Mengen des gebildeten Ammoniumchlorids erwartet hätte. Aus einer Umsetzung von 3 g (VII) wurden 0,23 g (10,6 %) p-HBI und 2,6 g benzolunlösliche Substanz erhalten. Letztere wurde mit Benzol gewaschen, 20 Stunden getrocknet und nachher quantitativ untersucht. Nur 0,986 g waren wasserlöslich, während 1,614 g einer wasserunlöslichen, weissen amorphen Substanz mit einem Schmelzpunkt von 80 - 90° C erhalten wurden. Der wasserlösliche Teil bestand aus 0,74 g Ammoniumchlorid, und enthielt kein Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) mehr. Theoretisch sollten aus 3 g (VII) 1,56 g flüchtige oder benzollösliche Substanz entstehen (0,5 g Chlorwasserstoff, 0,83 g Kohlenoxysulfid und 0,23 g p-HBI). Demnach sollte 1,44 g benzolunlösliche Substanz zurückbleiben, wovon 0,74 g (Ammoniumchlorid) wasserlöslich sein sollte. Benzol- und wasserunlöslich wären 0,7 g, gefunden wurden aber 1,614 g. Nach dieser theoretischen Berechnung kann man aussagen, dass die Reaktion von Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) mit Phosgen offenbar hauptsächlich durch Einbau von Phosgen unter Bildung eines Polymeren, Chlorwasserstoff, Ammoniumchlorid und nur ganz wenig p-HBI und Kohlenoxysulfid verläuft.

Die Umsetzung von p-Hydroxybenzylamin (IV) mit NN'-Thiocarbonyl-di-imidazol (V) liefert zwar p-HBI, wie dünnschichtchromatographisch nachgewiesen werden kann, doch konnte das Isothiocyanat aus den öligen Nebenprodukten nicht rein isoliert werden.

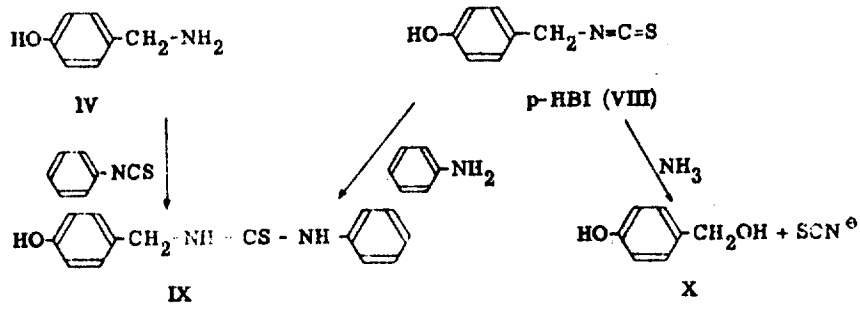
Die Umsetzung von VII mit Phosgen bei 0° C war daher die einzige Methode, welche die Herstellung einer grösseren Menge von reinem, kristallinem p-HBI gestattete.

Das zweimal aus Petroläther umkristallisierte p-HBI schmolz scharf bei 42° C und zersetzte sich oberhalb von 50° C.

Die Elementaranalyse des kristallinen p-HBI ergab Werte, die etwas ausserhalb der erlaubten Fehlergrenze für C_8H_7ONS lagen. Wahrscheinlich ist die leichte Zersetzlichkeit des p-HBI bereits durch die Luftfeuchtigkeit dafür verantwortlich.

Das kristalline p-HBI war dünnschichtchromatographisch vollkommen rein.

Das p-HBI konnte mit Anilin in N-p-hydroxybenzyl-N'-phenyl-thioharnstoff (IX) übergeführt werden, welcher andererseits aus dem p-Hydroxybenzylamin (IV) mit Phenylisothiocyanat erhalten wurde, wie das folgende Reaktionsschema zeigt.



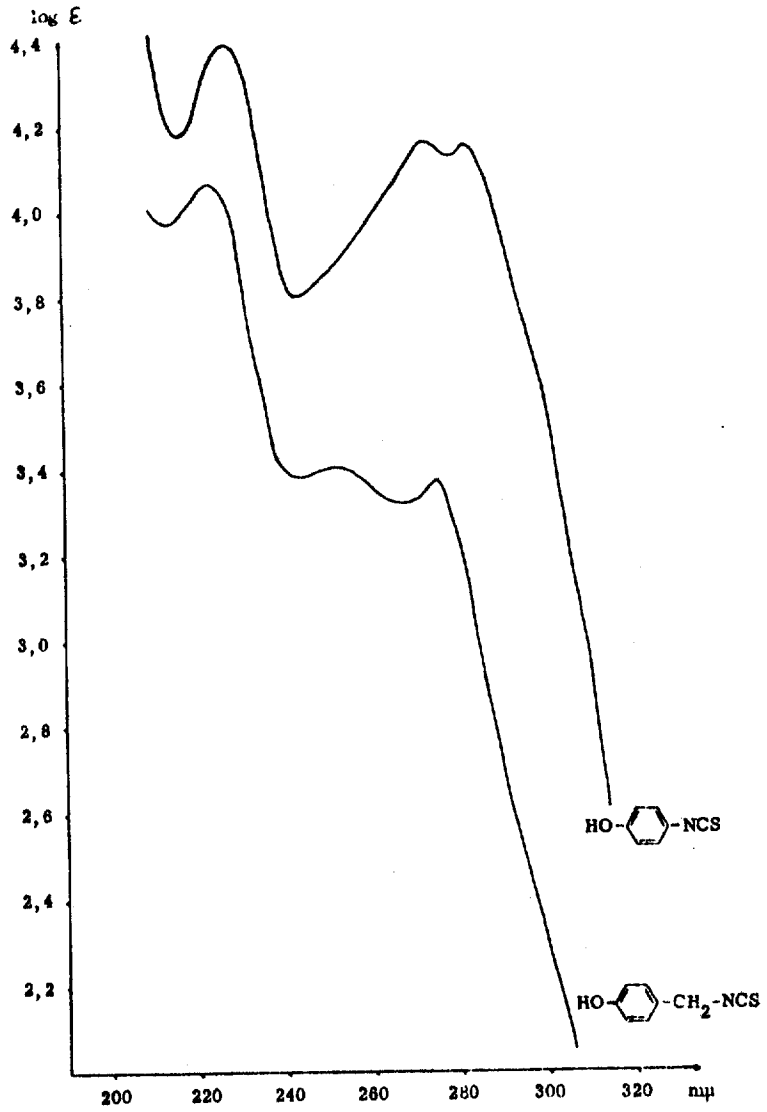
Die beiden Derivate hatten die gleichen Schmelzpunkte (3,4), ihre Mischung zeigte keine Depression. Das IR-Spektrum dieses Thioharnstoffderivates (Fig. 5) ist vollkommen identisch mit demjenigen von Kjaer und Rubinstein (4).

p-HBI konnte mit Ammoniak sowohl in wässrigem wie in vollkommen wasserfreiem Medium nicht in den Monothioharnstoff übergeführt werden, in beiden Fällen erfolgte quantitative Spaltung in p-Hydroxybenzylalkohol und Rhodanid.

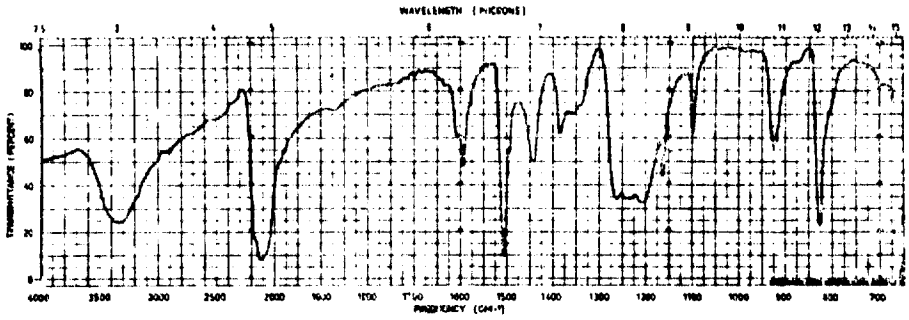
Das UV-Spektrum von p-HBI (Fig. 1) wurde in n-Heptan aufgenommen, λ max. bei 224 mμ (log. ε 4,07) und 275 mμ (log. ε 3,38).

Das IR-Spektrum wurde von reinem, geschmolzenem p-HBI aufgenommen (Fig. 4), da Druck, Temperatur und eine geringe Hydrolyse im Natriumchloridprisma die Kristalle zum Schmelzen brachten. Innerhalb der für das Isothiocyanat charakteristischen Bandbreite trat eine Bande mit 2 Maxima bei 2160 cm^{-1} resp. 2080 cm^{-1} auf. Das Maximum bei 1345 cm^{-1} dürfte von der Methylengruppe herrühren, während die intensive Bande bei 1515 cm^{-1} wiederum durch Anlagerung an die Isothiocyanatgruppe zustande kommen könnte (33). Diese Bande fehlt sowohl bei Phenyl- wie bei Benzylisothiocyanat, so dass man bei phenolischen Isothiocyanate intermolekulare Additionen annehmen muss.

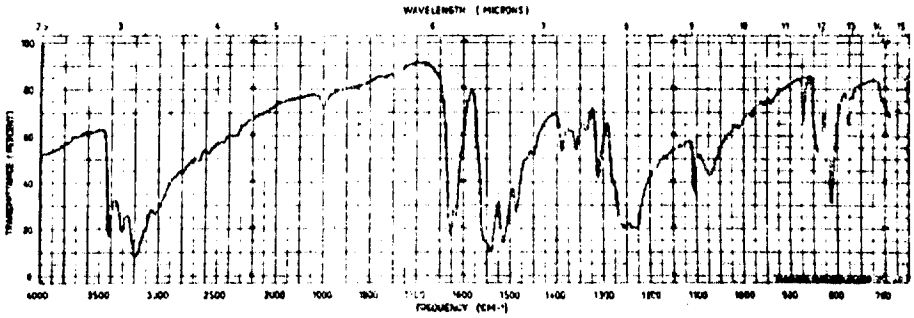
Die UV-Absorptionsspektren wurden mit einem Carl-Zeiss-Spektralphotometer PMQ II gemessen. Die IR-Absorptionsspektren wurden mit einem Perkin-Elmer-Spektrographen, Typ 21, aufgenommen.



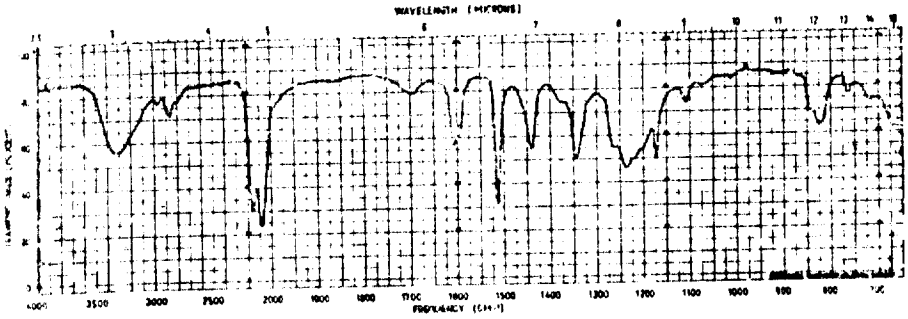
Figur 1 UV-Absorptionsspektren von p-Hydroxyphenyl-isothiocyanat und von p-HBI in n-Heptan



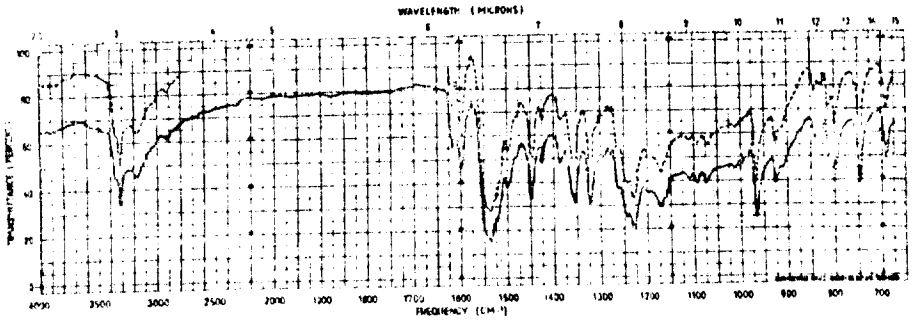
Figur 2 IR-Absorptionsspektrum von p-Hydroxyphenyl-isothiocyanat (KBr)



Figur 3 IR-Absorptionsspektrum von N-p-Hydroxyphenyl-thioharnstoff (KBr)



Figur 4 IR-Absorptionsspektrum von p-HBI (Reine Substanz)



Figur 5 IR-Absorptionsspektrum von N-p-hydroxybenzyl-N'-phenyl-thioharnstoff (IX) (KBr)

——— aus synth. p-HBI ------- aus nat. p-HBI

22. Dünnschichtchromatographie von aromatischen Isothiocyanaten und ihren Zersetzungsprodukten

Zur Trennung leichtflüchtiger Isothiocyanate bedient man sich der Gaschromatographie (35), für die Papierchromatographie müssen sie entweder in Thioharnstoffe oder in Thiosemicarbazide übergeführt werden (36).

In der Literatur der Isothiocyanate (5, 6) und der Dünnschichtchromatographie (37) findet man keine Angaben über dünnschichtchromatographische Trennmethoden der Isothiocyanate. Gmelin und Virtanen (38) verwendeten die Dünnschichtchromatographie bei der Strukturaufklärung von Glucobrassicin mit Erfolg. Allerdings diente sie ihnen zur Identifizierung der Indolderivate, die als Nebenprodukte beim enzymatischen und chemischen Abbau von Glucobrassicin (XIX) auftraten.

Mit den nachfolgenden Untersuchungen versuchten wir eine dünnschichtchromatographische Trennmethode mit Hilfe der erhaltenen Isothiocyanate und ihrer Zersetzungsprodukte auszuarbeiten.

Für die Auswahl der Dünnschichtmittel wurden die Isothiocyanate auf Zellulose, Polyamid (Woelm), Kieselgur G und Kieselgel G mit zwei Standard-Laufmitteln

Benzol/Methanol/Essigsäure (48:8:4)
n-Heptan/90 % HCOOH/Butanol (20:20:15)

ohne Rücksicht auf die Wirkung der Laufmittel auf die Isothiocyanate chromatographiert. Auf den Kieselgel G-Platten wanderten die Isothiocyanate und ihre Zersetzungsprodukte ohne Schwanzbildung als kompakte Flecken, die anderen Adsorbentien bewährten sich nicht.

Neben den bekannten Isothiocyanat-Reagenzien wurden auch diejenigen, die wegen ihrer Aggressivität nur bei der Dünnschichtchromatographie verwendet werden, untersucht.

Sprühreagenzien:	Farbe der Flecken:
Diazotierte Sulfanilsäure	hellgelb
konz. Schwefelsäure/Anisaldehyd	braun-schwarz
Ferrinitrat	hellbraun
Silbernitrat	braun
2, 6-Dichlor-chinon-chlorimid (2, 6-DCC)	rotbraun, blau
Jod in Chloroform	rotbraun

Von den angeführten Sprühreagenzien hat sich 2,6-DCC am besten bewährt. Es gab mit Phenyl- und Benzyl-isothiocyanat rotbraune, mit p-Methoxyphenyl-isothiocyanat blassrote und mit p-Hydroxyphenyl- und p-Hydroxybenzyl-isothiocyanat eine intensiv blaue Farbreaktion.

Weil die blaue Farbreaktion bei p-HBI mit 2,6-DCC auf die Phenolgruppe zurückzuführen ist, war zu erwarten, dass man mit diesem Sprühmittel auch die Zersetzungsprodukte dieses Isothiocyanats, falls sie auch Phenolgruppen enthalten, nachweisen kann.

Jod in Chloroform hat sich noch als einigermassen brauchbar gezeigt, aber die Flecken verblassten nach dem Besprühen viel zu schnell.

Um ein Laufmittelsystem zu finden, mit welchem die Substanzen ohne Zersetzung getrennt und ungefähr bis zur Mitte der Laufstrecke getragen werden, mussten ausgedehnte Versuchsreihen durchgeführt werden. Die Laufmittel und die zugehörigen R_f-Werte der einzelnen Isothiocyanate sind in Tabelle II zusammengestellt.

Tabelle II R_f-Werte der Isothiocyanate in verschiedenen Laufmitteln

Laufmittel	R_f-Werte von Isothiocyanaten (R-N=C=S)				
	Phenyl-	Benzyl-	p-Methoxy-phenyl-	p-Hydroxy-phenyl-	p-Hydroxy-benzyl-
Benzol/Methanol/Essigsäure (45:8:4)	0,78	0,78	0,78	0,58	0,61
	0,77	0,77	0,74	0,54	0,54
Benzol/Dioxan/Essigsäure (90:25:4)	0,71	0,71	0,72	0,57	0,60
	0,75	0,75	0,71	0,58	0,62
n-Heptan/Ameisensäure/Butanol (20:20:15)	0,55	0,39	0,33	0,06	0,03
n-Heptan/Benzol/Wasser (2:9:9)	0,68	0,59	0,54	0,09	--
	0,65	0,58	0,57	0,09	--
n-Heptan/Benzol/Tetrahydrofuran (2:2:1)	--	--	0,58	0,39	0,35
n-Heptan/Benzol/Dioxan (2:3:4)	--	--	0,71	0,55	0,55
n-Heptan/Benzol/Dioxan (2:3:2)	0,68	0,68	0,71	0,50	0,53
n-Heptan/Benzol/Dioxan (4:3:1)	--	--	0,58	0,29	0,31
Dioxan	0,58	0,58	0,60	0,58	0,61
Chloroform	--	--	--	--	0,20
Petroläther	--	--	--	--	0,00

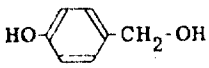
Mit Dioxan erhält man für alle Isothiocanate gleiche R_f-Werte. Die Zugabe von n-Heptan und Benzol bewirkt eine deutliche Trennung zwischen den phenolischen und den anderen Isothiocyanaten. Die flüchtigen Phenyl- und Benzol-isothiocyanate eignen sich nicht für Dünnschichtchromatographie.

Mit Benzol/Methanol/Essigsäure Laufmittel traten immer mehrere Flecken auf, wahrscheinlich tritt Zersetzung der Isothiocyanate in diesem Laufmittel ein.

Es wurde mehrheitlich mit folgender dünnschichtchromatographischer Methodik gearbeitet:

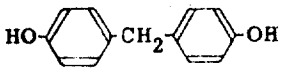
Dünnschichtmittel:	Kieselgel G
Laufmittelsystem:	n-Heptan/Benzol/Dioxan (2:3:2)
Sprühreagens:	2,6-DCC

Mit dieser Methode wurden auch zwei vermutliche Zersetzungsprodukte des p-HBI untersucht und die durchschnittlichen R_f-Werte aus mehreren Versuchen ermittelt und zusammengestellt.



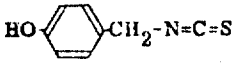
p-Hydroxybenzyl-alkohol (X)

R_f-Werte: 0,23



44'-Dihydroxy-diphenyl-methan (XI)

0,37



p-HBI (VIII)

0,50

23. Isolierung von p-Hydroxybenzyl-isothiocyanat aus Samen von Sinapis alba L.

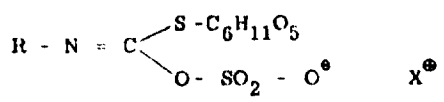
231. Senfölglucoside und ihre enzymatische Spaltung

Senfsamen dürften der Menschheit schon sehr früh als Gewürzmittel oder Heilmittel bekannt gewesen sein. Wie Zinner (39) berichtet, wurden Senfsamen als Bestandteile von Arzneistoffen schon bei Theophrastus und Dioskorides aufgeführt. Mit Sicherheit weiss man nur, dass Trallianus schon um 600 n. Chr. den schwarzen Senf als Arzneimittel verwendete. Ein Leydener Arzt, Boerhave, erkannte um 1730 das wirksame Prinzip dieses Samens, ein stechend riechendes ätherisches Oel, welches aus dem wässrigen Samenteig mit Wasserdampf destillierte. 1868 gelang es Hofmann (13) eine Reihe ähnlicher ätherischer Oele zu synthetisieren und als Isothiocyansäure-ester zu identifizieren. Er nannte diese neue Stoffgruppe deshalb "Senföle".

Bevor aber Hofmann (13) die Struktur der Isothiocyanate sichergestellt hatte, isolierte Bussy (40) bereits 1863 aus dem schwarzen Senf (Brassica nigra L.) einen kristallinen Körper, das Sinigrin, für den Will und Körner (41) eine Summenformel $C_{10}H_{18}NO_{10}S_2K$ aufstellen konnten.

Die Myrosinase, ein Enzym, welches von Bussy (40), Boutron und Fremy (42) im Senfsamen entdeckt wurde, spaltete das Sinigrin zu Allylisothiocyanat, D-Glucose und Kaliumhydrogensulfat.

In mühevoller Arbeit klärte Gadamer (43,44) die Art der Verknüpfung dieser enzymatischen Abbauprodukte auf und konnte wahrscheinlich machen, dass die "Precursors" aller natürlichen Senföle die gleiche Grundstruktur (XII) besitzen. Je nach dem Substituent R- am Stickstoffatom entstehen verschiedene Senföle.

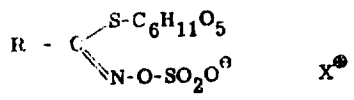


XII

Da die Myrosinase-Spaltung von Senfölglucosiden immer nur D-Glucose ergab, konnte der sichere Beweis der Thioglucosidstruktur erst 1914 von Schneider und Wrede (45) durch Bildung von 1-Thio-D-Glucose mit Kaliummethoxylat erbracht werden. 1930 bewiesen Schneider, Fischer und Specht (46) auch, dass die vom enzymatischen Abbau der Senfölglucoside entstehende D-Glucose, welche bereits 1905 von Ter Meulen (47) als solche erkannt wurde, aus einer β-glucosidischen Bindung stammt.

Die Strukturform von Gadamer (XII) vermochte die öfters beobachtete (48,49,50) Nitrilbildung aus Senfölglucosiden bei verschiedenen Reaktionen nicht zu erklären. Nitrile können nämlich nur gebildet werden, wenn in den Senfölglucosiden eine durchgehende Kohlenstoffkette vorhanden wäre.

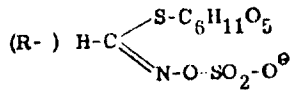
Ettlinger und Lundeen (51) erhielten durch schonenden hydrierenden Abbau aus Sinigrin n-Butylamin, aus Sinalbin Tyramin. Damit wurde die durchgehende Kohlenstoffkette in den Senfölglucosiden sicher bewiesen. Bei der sauren Hydrolyse wurde das Stickstoffatom der Senfölglucoside quantitativ als Hydroxylamin-Salz gefunden. Somit konnten sie die endgültige Struktur der Senfölglucoside (XIII) aufstellen. Schliesslich wurde von Ettlinger und Lundeen (52) 1957 durch die Synthese von Glucotropeolin ($R = C_6H_5-CH_2$) die revidierte Strukturformel von Senfölglucosiden sichergestellt.



XIII

Die Senfölglucoside sind demnach am Stickstoff mit Schwefelsäure acylierte und am Schwefel glucosidierte Thiolhydroximsäuren, die je nach Löslichkeit ihrer Salze als K-, Na-, $(CH_3)_4N$-, Sinapin-Salze etc. isoliert werden.

Als freie Säuren sind sie unbeständig. In den Pflanzen kommen sie wahrscheinlich in gelöster Form als Anionen vor. Mit den Trivialnamen Sinigrin resp. Sinalbin werden kristalline Salze bezeichnet. Die meisten Senfölglucoside, die zum Teil noch gar nicht isoliert wurden, erhalten nach Gadamer (48) und Kjaer (53) das Praefix "Gluco" und eine Ableitung der lateinischen Namen der Pflanze, in der das betreffende Senfölglucosid erstmals nachgewiesen wurde (z. B. Glucotropaeolin aus Tropaeolum, Anion: Glucotropaeolat; Anion von Sinalbin: Glucosinalbat). Diese Nomenklatur ist in Anbetracht der zunehmenden Zahl der entdeckten Senfölglucoside nicht einheitlich durchführbar (z. B. Neoglucobrassicin (54)). Deshalb erscheint ein Vorschlag von Ettlinger und Dateo (55) besonders begrüssenswert. Sie nennen das folgende Anion Glucosinolat (XIV).



XIV

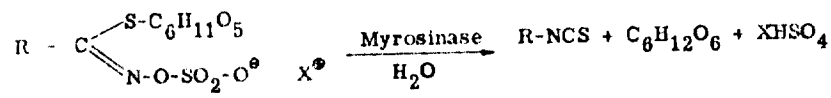
Mit dem Namen des Substituenten R bilden sie den Namen des Senfölglucosidanions. Diese Nomenklatur ist chemisch einwandfrei und ordnet ein Senfölglucosid, das meistens in mehreren Pflanzen vorkommt, nicht einer bestimmten Pflanze zu.

Besonders in den letzten zehn Jahren isolierten und identifizierten verschiedene Forscher, vorallem Schultz, Ettlinger, Virtanen, Kjaer (53 Sammelreferat, vgl. dort auch die ältere Literatur) und ihre Mitarbeiter zahlreiche Senfölglucoside, deren ständig wachsende Zahl heute bereits ca. 45 erreicht hat, nur ein Viertel davon wurden kristallin erhalten.

Unter den etwa viertausend senfölglucosidführenden Pflanzen, die wenigen Familien (Cruciferae, Capparidaceae, Resedaceae, Moringaceae, Tropeolaceae, Caricaceae, Euphorbiaceae, Limnanthaceae, Phytolaceae, Plantaginaceae, Salvadoraceae) angehören, befinden sich viele für die menschliche Ernährung wichtige Gemüsesorten, Gewürzstoffe und für die Tierernährung wichtige Futtermittel (Kohlarten, Raps, Rettich, Kresse, Senfsamen, Meerrettich etc.). Viele Geruchs- und Geschmacksstoffe unserer Gemüse und Gewürzsorten bestehen aus Isothiocyanaten und ihren Zersetzungsprodukten.

Die enzymatische Spaltung der Senfölglucoside und Bildung der Senföle ist sehr kompliziert.

Die folgende Bruttogleichung zeigt Anfangs- und Endprodukte, die durch Umlagerungen über zahlreiche Zwischenstufen gebildet werden.



Euler und Erikson (56) nahmen als erste an, dass die Spaltung von Senfölglucosiden durch das Zusammenwirken von mehreren Enzymen erfolgt. Neuberg und Wagner (57) sowie Neuberg und Schönebeck (58) gelang es durch fraktionierte Fällungen der Myrosinase eine Thioglucosidase und eine spezielle Sulfatase für die Spaltung der Senfölglucoside wahrscheinlich zu machen. Diese Befunde wurden von Sandberg und Holly (59) bestätigt. Hingegen deuten die neueren Untersuchungen von Nagashima und Uchiyama (60) darauf hin, dass die Myrosinase nur aus einer speziellen Thioglucosidase besteht. Nach Abspaltung der D-Glucose würde darnach die Spaltung der Aether-Schwefelsäure-Bindung spontan durch intermolekulare Umlagerung stattfinden. Dieser Reaktionsmechanismus stützt sich auf ähnliche Beobachtungen von Hurd (61) bei Umlagerungen von Hydroximsäuren. Die beiden japanischen Forscher stellten auch fest, dass SH-Inhibitoren hemmend, Ascorbinsäure dagegen stark aktivierend auf die Spaltung wirken. Zwei verschiedene Enzyme hingegen sollten auf die Inhibitoren und Aktivatoren verschieden reagieren.

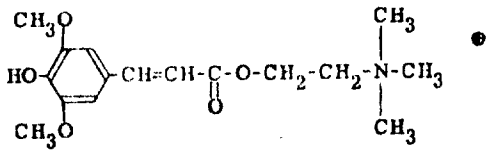
Im Gegensatz zu den ausführlichen Arbeiten von Nagashima und Uchiyama (60) berichteten Gaines und Göring (62) von einer Trennung der Myrosinase an einer DEAE-Cellulose in eine Thioglucosidase und eine Sulfatase ähnlich den früheren Befunden.

Ettlinger et al. (63) vertreten die Meinung, dass im Samen von Sinapis alba L. mindestens zwei Enzyme, eine auf L-Ascorbinsäure indifferente, klassische Myrosinase und eine Thioglucosidase, für die L-Ascorbinsäure als Coenzym dient, vorhanden sind. Die Spaltung der Senfölglucoside erfolgt nach diesen Autoren durch die Thioglucosidasewirkung und anschliessende Lossen'sche Umlagerung.

232. Strukturaufklärung des Senfölglucosids aus den Samen von Sinapis alba L.

1831 erhielten Robiquet und Boutron (1) aus dem Samen eine kristalline Substanz für die Will und Laubenheimer (30) 1879 die Summenformel $C_{30}H_{44}N_2O_{16}S_2$ aufstellten. Diese Summenformel wurde von Gadamer (44) zu $C_{30}H_{42}N_2O_{15}S_2 \cdot 5H_2O$ modifiziert.

Die Myrosinase-Spaltung lieferte ein brennend schmeckendes Senföl, D-Glucose und ein Bisulfat mit folgender Summenformel: $(C_{16}H_{24}NO_5)^{\oplus}\ HSO_4^{\ominus}$. Das organische Kation wurde später von Gadamer (44) als Cholinester der 3,5-Dimethoxy-4-hydroxy-zimtsäure (Sinapinsäure) identifiziert und Sinapin (XV) genannt.



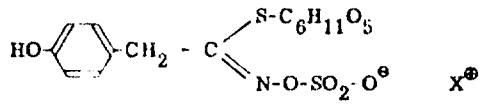
XV

Das kristalline Senfölglucosid aus Sinapis alba L. erhielt den Namen Sinalbin (XVII). Will und Laubenheimer (30) zerlegten das Silbersalz von XVII mit H_2S und erhielten ein Nitril, welches durch Säurehydrolyse eine Hydroxyphenylessigsäure ergab, bei der sie die Hydroxygruppe in ortho-Stellung vermuteten. Salkowski (2) stellte inzwischen die p-Hydroxyphenylessigsäure dar und erkannte die Identität dieser mit derjenigen von Will und Laubenheimer (30). So konnte er das Senföl von XVII als p-HBI postulieren, ohne dass er diese Annahme durch Synthese bestätigen konnte, da er dabei nur eine ölige Substanz erhielt.

Schneider (3) berichtete in einem Sammelreferat über Lauch- und Senföle kurz über einige charakteristische Derivate des Senföls von Sinalbin (XVII), den N-p-hydroxybenzyl-N'-phenylthioharnstoff (IX) und das Phenyl-p-hydroxysemicarbazid, die er in unveröffentlicht gebliebenen Versuchen erhalten hat.

Kjaer und Rubinstein (4) stellten in neuerer Zeit das p-HBI in ätherischer Lösung dar und erhielten den obigen Thioharnstoff (IX) durch Zugabe von Anilin. Dasselbe Derivat (IX) konnten sie aus dem Aetherextrakt des Samens mit Anilin isolieren.

Nachdem Ettlinger und Lundeen (51) durch schonenden hydrierenden Abbau von Sinalbin (XVII) Tyramin erhielten, war die folgende Strukturformel des Senfölglucosidanions (XVI) gesichert.



XVI XVII, $X^{\oplus}$ = Sinapin

Bis jetzt wurde XVI in kristalliner Form nur als Tetramethylammonium- und Sinapinsalz (Sinalbin XVII) isoliert.

Das Senfölglucosidanion XVI wäre nach Kjaer (53) als Glucosinalbat, nach Ettlinger und Dateo (55) als p-Hydroxybenzylglucosinolat zu bezeichnen.

Es muss darauf hingewiesen werden, dass das p-Hydroxybenzylglucosinolat-Anion (XVI) in der Pflanze wahrscheinlich nicht als Sinapinsalz vorliegt, da noch andere Kationen, besonders Kalium-Ionen als Gegenionen ebenfalls denkbar sind. Kalium-p-hydroxybenzylglucosinolat konnte bis jetzt aber aus dem Samen nicht kristallin erhalten werden.

Sowohl Sinapin (XV) wie das p-Hydroxybenzylglucosinolat-Anion (XVI) kommen in mehreren Pflanzen vor, von denen besonders die Samen von Sinapis arvensis (64) nach Messungen von Ettlinger et al. (65) beinahe so viel enthalten, wie die Samen von kultiviertem Sinapis alba L.

Vor kurzem wurde von Kjaer et al. (66) auch das m-Hydroxybenzylglucosinolat (Glucolepigramin) in grasblättriger Kresse (Lepidium graminifolium L.) entdeckt. Weiter wurde auch das p-Methoxybenzylglucosinolat (Glucoaubrietin) in Aubrietia deltoidea DC (67) sowie das m-Methoxybenzylglucosinolat (Glucolimnathin) in Limnanthes dauglasii R. Br. (68) aufgefunden.

233. Entfettung und Entglucosidierung von Sinapis alba L. Samen

Die Samen sind hellgelb mit einem Durchmesser von etwa 1,8 - 2,5 mm. Sie sind von einer harten Samenschale umhüllt, welche in ihren Epidermiszellen beträchtliche Mengen Pflanzenschleimstoffe enthält, deren Eigenschaften und Zusammensetzung in zwei älteren Arbeiten von Bailey und Norris (69) und Bailey (70) beschrieben werden. Zur Samenmehlherstellung werden die Samen in den meisten Fällen von ihren Schalen befreit, durch Auspressen teilweise entfettet und schliesslich gemahlen.

Das Untersuchungsmaterial der vorliegenden Arbeit war ein aus geschälten Samen stammendes fetthaltiges Mehl, dessen Wassergehalt 3,4 % betrug.

Da der Fettgehalt des Samens sich bei der Isolierung von Myrosinase, p-Hydroxybenzylglucosinolat (XVI) und p-HBI sehr störend auswirkt, musste das Samenmehl entfettet werden und zwar so, dass sowohl das Senfölglucosid wie die Myrosinase bei dieser Behandlung intakt blieben.

Schwimmer (71) entfettete das Samenmehl zur Myrosinaseherstellung bei 0° C durch zehnmaliges Ausschütteln mit Aceton. Weil für die nachfolgenden Untersuchungen grössere Mengen entfettetes Samenmehl benötigt wurden, musste eine rationelle, kontinuierliche Kaltextraktionsmethode ausgearbeitet werden. Um das Senfölglucosid aus dem entfetteten Samenmehl zu isolieren, musste ferner ein Lösungsmittel gefunden werden, welches es möglichst vollständig und rein aus dem Samen herauslöst. Die Einflüsse verschiedener organischer Lösungsmittel auf das Samenmehl wurden parallel in einem gewöhnlichen Soxhlet-Extraktor (Warmextraktion) und in einem speziellen Kaltextraktionsapparat (Fig. 11) untersucht. Es wurde auch versucht, das mit Petroläther entfettete Samenmehl anschliessend mit Methanol zu extrahieren. In den Extrakten wurde qualitativ festgestellt, welche Stoffe vom betreffenden Lösungsmittel herausgelöst wurden. Die getrockneten extrahierten Samenmehle wurden andererseits qualitativ auf Myrosinaseaktivität geprüft. Die Resultate der Vorversuche von Warm- und Kalt-Extraktion sind in Tabelle III aufgeführt.

Tabelle III Extraktionsversuche von Sinapis alba L. Samenmehl mit verschiedenen Lösungsmitteln bei verschiedenen Temperaturen

Lösungsmittel	Warm-Extraktion			Kalt-Extraktion			Qualitative Zusammensetzung der Extrakte
	Extr. Temp. [°C]	Extrakt-Ausbeute [%]	Myr. Akt.	Extr. Temp. [°C]	Extrakt-Ausbeute [%]	Myr. Akt.	
Petroläther	30-40	35	+	12	37	+++	Fette und Farbstoffe
Aceton	55	38	-	19	37	++	Fette und Farbstoffe
Methanol	55-60	20	-	20	21	++	Fette, Farbstoffe, Zucker, Aminosäuren, Sinapin, p-Hydroxybenzylglucosinolat
Petroläther 3 Std.	---	---	---	14	34	+++	Fette und Farbstoffe
gefolgt von Methanol 10 Std.	---	---	---	18	22 total: 56	++	Farbstoffe, Zucker, Aminosäuren, Sinapin, p-Hydroxybenzylglucosinolat

Myr. Akt. = Myrosinase-Aktivität

Wie aus Tabelle III ersichtlich, lösen Petroläther und Aceton ausser den gesamten Pflanzenfetten der Samenmehle nur noch Farbstoffe, während Methanol allein nur einen Teil der Pflanzenfette und das gesamte Sinapin und p-Hydroxybenzylglucosinolat mit Begleitstoffen herauslöst.

Nach der Kaltextraktion war die Myrosinase-Aktivität noch erhalten, während die höhere Temperatur bei der gewöhnlichen Soxhlet-Extraktion die Myrosinase inaktivierte. Diese Vorversuche zeigten, dass es am zweckmässigsten ist, das Samenmehl zuerst nach dem Kaltextraktionsverfahren mit Petroläther restlos zu entfetten und anschliessend das Senfölglucosid mit Methanol zu extrahieren. Nach diesem

Verfahren wurden grössere Mengen Samenmehl entfettet, aus denen später Myrosinase und p-HBI isoliert wurden. Ein Teil dieses Mehls wurde mit Methanol entglucosidiert und aus der Methanollösung verschiedene Salze des p-Hydroxybenzylglucosinolats (XVI) und des Sinapins gewonnen. Die Ergebnisse der Extraktionen sind in Tabelle IV aufgeführt.

Tabelle IV Entfettung und Entglucosidierung von Sinapis alba L. Samenmehl nach dem Kaltextraktions-Verfahren

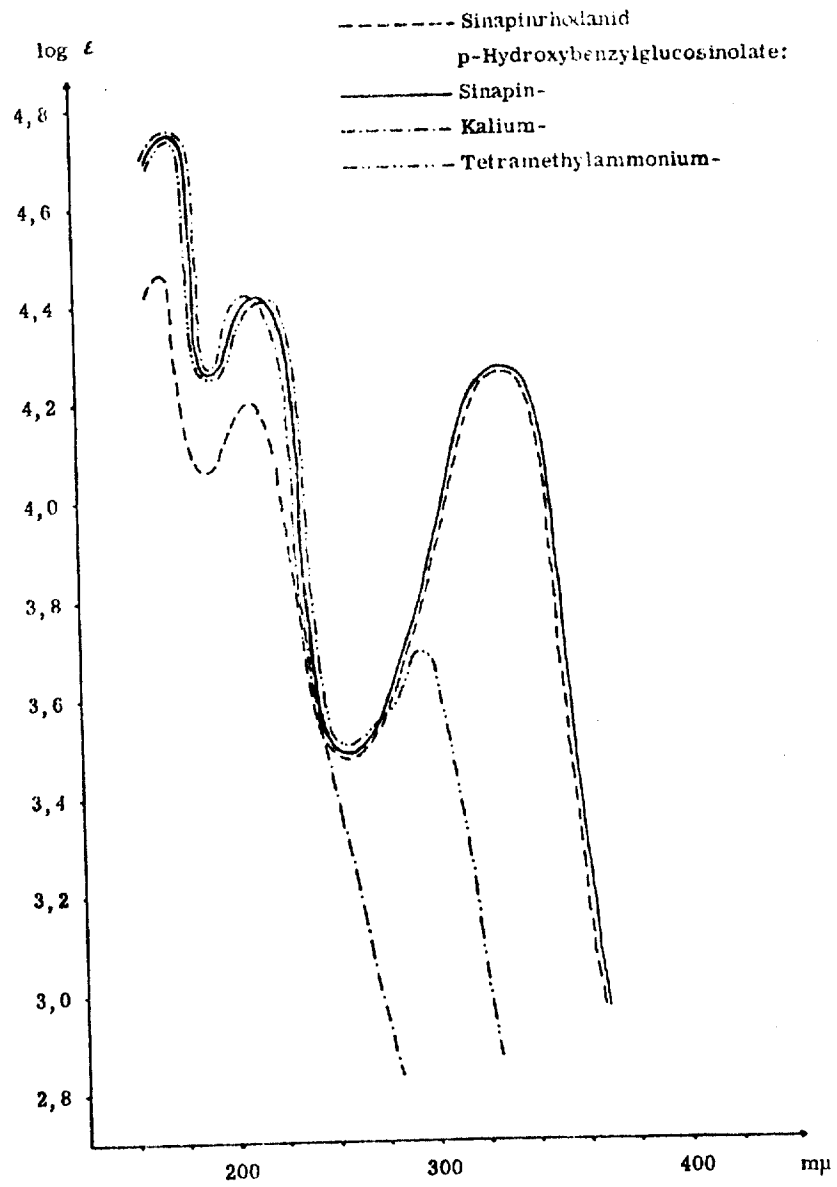
Lösungsmittel	Samenmehl [g]	Extrakt [g]	Extrakt [%]	Myrosinase-Aktivität
Petroläther	356	128	36,0	aktiv
	374	135	36,0	aktiv
	385	141	36,5	aktiv
Petroläther Methanol	394	145	36,8	aktiv
		64,7	16,4	
			total: 53,2	

Aus sechs Kaltextraktionen mit Petroläther oder Aceton ergab sich ein durchschnittlicher Fettgehalt von 36,55 %. Der Gesamtextrakt erhalten mit Petroläther und Methanol, 53,2 % bzw. 56,6 %, dürfte den gesamten in organischen Lösungsmitteln löslichen Anteil des Samenmehls darstellen.

Aus dem senfölglucosidhaltigen methanolischen Extrakt des entfetteten Samenmehls (145 g) konnten mit Hilfe eines Anionenaustauschers das Kalium-p-hydroxybenzylglucosinolat und das Sinapin-rhodanid isoliert werden.

Die UV-Spektren der verschiedenen Salze des p-Hydroxybenzylglucosinolats (XVI) und des Sinapins sind in Fig. 6 dargestellt. Das UV-Spektrum von Kalium-p-hydroxybenzylglucosinolat zeigt, dass es noch nicht ganz rein vorliegt.

Das entfettete und entglucosidierte Samenmehl stellte ein aktives trockenes Myrosinase-Rohpräparat dar.



Figur 6 UV-Absorptionsspektren von Sinapin- und p-Hydroxybenzylglucosinolatsalzen in destilliertem Wasser

234. Senfölglucosidbestimmung des Samens von Sinapis alba L.

Um die erzielte Ausbeute von p-Hydroxybenzylglucosinolat und p-HBI aus dem Samen berechnen zu können, musste der Senfölglucosidgehalt des Samenmehls ermittelt werden.

Die Bestimmungen des ursprünglichen Senfölglucosidgehaltes in Pflanzenmaterialien stützen sich ausnahmslos auf die Analyse eines der drei primären Abbauprodukte der Senfölglucoside.

Die enzymatisch freigesetzten Isothiocyanate können, wenn sie wasserdampfflüchtig sind und bei der Abtrennung aus dem Reaktionsgemisch nicht zersetzt werden, argentometrisch oder jodometrisch bestimmt werden. Für das nicht flüchtige, leichtzersetzliche p-HBI ist diese Methode nicht anwendbar.

Ettlinger et al. (65) entwickelten speziell für die p-Hydroxybenzylglucosinolatbestimmung eine Analysemethode, in der die starke Alkali-Labilität des enzymatisch freigesetzten p-HBI ausgenützt wird.

Eine bestimmte Menge Samenmehl wird mit Wasser in Gegenwart von Aether enzymatisch abgebaut. Die p-HBI-haltige Aetherphase wird abgetrennt und mit Natronlauge ausgeschüttelt. In der alkalischen Lösung zersetzt sich das p-HBI quantitativ in Rhodanid, das nachher abgetrennt und nach Barker (72) kolorimetrisch bestimmt wird. Aus den gemessenen Rhodanid-Werten kann das enzymatisch freigesetzte p-HBI und daraus der ursprüngliche Senfölglucosidgehalt berechnet werden.

Nach der Senfölglucosidbestimmung von Schultz und Gmelin (73) wird die abgespaltene Glucose mit Hilfe des Anthronreagens kolorimetrisch bestimmt. Leider versagt diese Methode gerade bei dem p-Hydroxybenzylglucosinolat, da die Phenol-Gruppe die Analyse stört.

Bauer und Holle (74) hydrolisieren den senfölglucosidhaltigen Extrakt mit Salzsäure und bestimmen das abgespaltene Sulfat gravimetrisch als Bariumsulfat.

Während des enzymatischen Abbaus von Senfölglucosiden in ungepufferter wässriger Lösung sinkt das pH sofort rapid durch das gebildete saure Hydrogensulfat und die Reaktion bleibt bei pH 2-3 endgültig stehen. Wird das optimale pH von 6,2 durch automatische Titration des freigesetzten Hydrogensulfats konstant gehalten, dann verläuft der enzymatische Abbau quantitativ und die verbrauchte Menge Lauge kann zur Senfölglucosidbestimmung verwendet werden. Durch den enzymatischen Abbau von Lösungen, die bestimmte Mengen kristalline Senfölglucoside enthielten, wurde die Genauigkeit dieser Titrationsmethode ermittelt, wobei folgende Werte in Prozent der eingewogenen Menge Senfölglucosid gefunden wurden:

Kalium-allylglucosinolat (Sinigrin)	Sinapin-p-hydroxybenzylglucosinolat (Sinalbin)	Kalium-p-hydroxybenzylglucosinolat	Tetramethylammonium-p-hydroxybenzylglucosinolat
gefunden:			
99 %	102 %	98 %	106 %

Bei den p-Hydroxybenzylglucosinolaten wurden durchschnittlich 2 % zuviel Senfölglucosid erhalten.

Die Anwendung dieser Methode der quantitativen Senfölglucosidbestimmung im Pflanzenmaterial erfordert ein aktives Myrosinasepräparat, sowie die vollständige Extraktion des Senfölglucosids und die Abtrennung sämtlicher Begleitstoffe, welche die Titration stören könnten.

p-Hydroxybenzylglucosinolat ist das einzige Senfölglucosid im Samenmehl wie durch Dünnschichtchromatographie gezeigt werden konnte. Zu dessen Bestimmung durch Titration wurden 5 g Samenmehl zuerst entfettet, anschliessend entglucosidiert und das erhaltene Glucosinolat in 100 ml Wasser aufgelöst und davon 20 ml analysiert. Aus der Reaktionsgleichung des enzymatischen Abbaus von Senfölglucosiden ist ersichtlich, dass 1 ml 0,01-N Natronlauge 10^{-5} Mol p-HBI oder Senfölglucosid entspricht. Bei den gewählten Analysebedingungen wird der theoretische Prozentgehalt an p-HBI oder Senfölglucosid mit dem Molekulargewicht MG gleich Anzahl ml 0,01-N Natronlauge x 10^{-3} MG.

In vier Parallelversuchen mit Doppelbestimmungen wurden durchschnittlich 12,54 ml 0,01-N Natronlauge verbraucht, die daraus berechneten Senfölglucosidgehalte des Samenmehls sind in Tabelle V aufgeführt. Zur Bestätigung der erhaltenen Werte wurde der Senfölglucosidgehalt des Samenmehls auch nach der Methode von Ettlinger (65) bestimmt. Da in der Literatur über den Einfluss der enzymatischen Abbauzeit des p-Hydroxybenzylglucosinolats (XVI) sowie der Verseifungszeit des p-HBI auf die Resultate nichts bekannt war, wurde derselbe in einer Versuchsreihe untersucht. Die Ergebnisse sind zusammen mit den Werten der Titrationsmethode in Tabelle V zusammengestellt. Es ergibt sich, dass der enzymatische Abbau von p-Hydroxybenzylglucosinolat (XVI) bei der Ettlinger'schen Methode innerhalb einer Stunde praktisch vollständig ist. Die Analysenwerte ändern sich nach weiteren Abbauzeiten nicht wesentlich, so dass für Reihenuntersuchungen eine einstündige Hydrolysedauer genügend ist. Die Verlängerung der Verseifungszeit von p-HBI, besonders die Erwärmung der Natronlauge-Auszüge bewirkt eine leichte Erhöhung der Resultate bei der Ettlinger'schen Analyse. Dünnschichtchromatographisch konnte tatsächlich nach 15-minütiger Verseifung noch p-HBI nachgewiesen werden, sodass die Resultate nach der warmen Verseifung eher dem effektiven Senfölglucosidgehalt entsprechen.

235. Anwendung der Dünnschichtchromatographie bei der Identifizierung und Isolierung des Senföls von Sinapis alba L.

Entfettetes Samenmehl wurde bei Raumtemperatur in mehreren Ansätzen mit Wasser versetzt, und das Senfölglucosid eine Stunde enzymatisch abgebaut. Der wässrige Brei wurde entweder mit Aether, Petroläther oder Chloroform gut verrührt. Durch Zentrifugieren wurden die Extrakte von der wässrigen Phase getrennt und mit Natriumsulfat getrocknet. Je 10 - 50 µl der Extrakte und der wässrigen Phase wurden auf eine Kieselgel-G Platte neben dem synthetischen p-HBI mit n-Heptan/Benzol/Dioxan (2:3:2) chromatographiert. Von sechs Dünnschichtchromatogrammen wurden je zwei mit 2, 6-DCC, Schwefelsäure-Anisaldehyd und mit Chloroform-Jod-Lösung entwickelt, um mit Sicherheit aller organischen Substanzen nachweisen zu können. Auf den Chromatogrammen erschienen aber mit allen Sprühmitteln immer nur die gleichen Flecken, deren durchschnittliche R_f-Werte nachfolgend zusammengestellt sind:

Extrakte				p-HBI
Wasser	Aether	Petroläther	Chloroform	
	0,88			
	0,71			
0,50	0,50	0,51	0,50	0,50
0,23	0,23			

Diese R_f-Werte beweisen, dass das Senföl des Samens mit dem synthetischen p-HBI identisch ist. Die Resultate deuten auch darauf hin, dass neben p-HBI kein weiteres Senföl gebildet wird und somit ausser p-Hydroxybenzylglucosinolat (XVI) auch kein weiteres Senfölglucosid im Samen vorhanden ist.

Petroläther und Chloroform lösten keine weiteren Stoffe ausser p-HBI aus der wässrigen Samenpaste. Die beiden Lösungsmittel sind deshalb für die Extraktion von p-HBI sehr gut geeignet.

236. Isolierung von p-Hydroxybenzyl-isothiocyanat aus wässrigem und lyophilisiertem Samenmehl von Sinapis alba L.

In einer deutschen Patentschrift von Scheib (75) werden mehrere Verfahren zur Gewinnung von reinem p-HBI beschrieben. Das entfettete Samenmehl wird dabei in Wasser 24 - 48 Stunden fermentiert, anschliessend abgepresst und der Rückstand mit Aether ausgezogen. Nach Abdampfen des Lösungsmittels hinterbleibt ein gelbes Oel, welches reines p-HBI darstellen sollte. Aether löst aber, wie dünnschichtchromatographisch gezeigt wurde, mehrere Substanzen des Samenmehls heraus, so dass auf diese Art kein reines Produkt erhältlich ist. Ausserdem werden während 1 - 2 Tagen in wässriger Lösung bereits 65 - 75 % des gebildeten p-HBI hydrolisiert. Die Isolierung von p-HBI nach dieser Methode, auch wenn sie etwas modifiziert wird (3), liefert stets ein gelbes Oel.

Selbst die Aetherextraktion der enzymatischen Abbauprodukte von kristallinem Sinalbin (XVII) ergab nur braune ölige Substanzen.

Nachdem die Dünnschichtchromatographie die Brauchbarkeit von Petroläther und Chloroform als Extraktionsmittel zeigte, wurden aus entfettetem und eine Stunde enzymatisch abgebautem Samenmehl, Petroläther und Chloroform-Extrakte hergestellt. Die Auszüge wurden in der Kälte bis zur Trübung eingeengt. Die Farbe der Extrakte wurde dabei gelblich, bei -10° C schied sich nur ein bräunlich-gelber öliger Niederschlag von unreinem p-HBI aus. Wahrscheinlich wurden durch Petroläther und Chloroform aus dem Samen noch Reste von Fett, sowie Farbstoffen und anderen Substanzen zusammen mit dem p-HBI herausgelöst, die das Auskristallisieren von p-HBI verhinderten. Deshalb wurde versucht, durch präparative Säulenchromatographie die Begleitstoffe vom p-HBI abzutrennen. Als Kolonnenmaterial diente Silikagel, als Elutionsmittel n-Heptan/Benzol/Dioxan.

Nachdem feststand, dass auf Kieselgel-G Platten das p-HBI mit Petroläther nicht wandert, wurde der Petrolätherextrakt direkt auf die Kolonne gegeben. Der Chloroformextrakt musste dagegen zuerst auf einige ml eingeengt werden, da Chloroform das p-HBI eluiert. Die farblosen Fraktionen, die p-HBI enthielten, werden vereinigt und schonend eingeengt. Aus warmem Petroläther (40° C) erhielt man in beiden Fällen bei -10° C das p-HBI in schönen weissen Nadeln, Smp.: 39 - 40° C, nach 2-maligem Umkristallisieren aus Petroläther 41 - 42° C. Ausbeute: aus 100 g Samenmehl beim Petrolätherextrakt 370 mg (11,2 %), beim Chloroformextrakt 545 mg (16,5 %, berechnet auf dem theoretischen p-HBI-Gehalt des Samenmehls).

Die UV- und IR-Spektren des natürlichen p-HBI, sowie Schmelzpunkt und IR-Spektrum der N-p-hydroxybenzyl-N'-phenylthiobarnstoffe (IX) aus natürlichem und synthetischem p-HBI waren identisch.

Eine Vereinfachung der Isolierung von p-HBI unter Umgehung der Chromatographie könnte durch Lyophilisierung des enzymatisch abgebauten Samenmehls und anschliessender Petrolätherextraktion erreicht werden. In Vorversuchen wurde festgestellt, dass das p-HBI während der Lyophilisierung nicht sublimiert und praktisch nicht hydrolysiert wird (vgl. Kapitel 342.). Bei der Petrolätherextraktion des p-HBI aus der lyophilisierten Samenpaste musste aber festgestellt werden, dass zum Herauslösen der gesamten p-HBI-Menge mindestens 25 Stunden erforderlich sind. Die gelblichen Extrakte wurden zur Entfärbung mit etwas Kieselgel geschüttelt. Nach dem Einengen der Lösung kristallisierte sich reines p-HBI aus, jedoch mit geringerer Ausbeute als im obigen Versuch.

Es wurde beobachtet, dass eine kurze Kaltextraktion von lyophilisiertem Samenmehl mit Petroläther, Chloroform oder Aether nur ganz geringe Menge p-HBI herauslöst, obwohl dieselben Lösungsmittel die gesamte Menge p-HBI aus wässrigem Samenmehl in der Kälte herauslösen.

Die Lyophilisierung des Samenmehls brachte daher für die Isolierung des p-HBI keine Vereinfachung, der Arbeitsaufwand scheint im Gegenteil bedeutend grösser zu sein als bei der Isolierung aus der wässrigen Samenmehlsuspension. Das lyophilisierte Samenmehl könnte hingegen als Gewürzmittel eine gewisse Bedeutung erlangen, stellt es doch ein p-HBI-haltiges, sehr scharf schmeckendes streubares Pulver dar, welches selbst nach monatelanger Lagerung seine Schärfe nicht verliert.

24. Chemische Eigenschaften von p-Hydroxybenzyl-isothiocyanat

241. Reaktionen von synthetischen und natürlichen Isothiocyanaten

Die Isothiocyanate gehen die gleichen Reaktionen ein, wie die Isocyanate, nur mit bedeutend geringerer Reaktionsgeschwindigkeit, deshalb spielen sie in der Kunststoffherstellung keine nennenswerte Rolle.

Die wichtigsten Reaktionstypen der Isothiocyanate sind Additions- und Ringschlussreaktionen.

Addiert werden z. B. Ammoniak, Amin, Hydrazine, Hydroxylamin, Alkohole, Phenole, Merkaptane, Carbonsäuren, Thiocarbonsäuren, Verbindungen mit aktiven Methylgruppen, Sulfonamide und Natriumhydrogensulfit.

Die Isothiocyanate haben eine ausgeprägte Tendenz zu Ringschlussreaktionen. In der Literatur existieren zahlreiche Methoden für die Darstellung fünf- oder sechsgliedriger heterocyclischer Verbindungen mit Hilfe von Isothiocyanaten. Einige werden auch grosstechnisch ausgeführt (76, 77).

Greer (78), Kjaer (79) und Schultz (80, 81) konnten nachweisen, dass aus bestimmten Senfölglucosiden durch Myrosinasespaltung intermediär Isothiocyanate entstehen, die durch sofortigen Ringschluss Thiooxazolidon-Derivate bilden.

Gmelin und Virtanen (38, 54) fanden beim enzymatischen Abbau von Glucobrassicin (XIX) und Neoglucobrassicin sowohl bei pH 7 wie bei pH 3,5 quantitative Mengen Rhodanid neben anderen Sekundärprodukten. Dieser für Isothiocyanate neuartige Reaktionstyp wird von Virtanen (82) damit erklärt, dass die Spaltung der hypothetischen Isothiocyanate aus den beiden Senfölglucosiden durch die Bildung resonanzstabilisierter Carboniumionen begünstigt wird. Die Isothiocyanate der beiden Senfölglucoside konnten gar nicht isoliert werden, da ihre Zersetzung so schnell erfolgt.

Da das p-HBI gegenüber Alkali und Wasser ebenfalls sehr labil ist und die Möglichkeit einer resonanzstabilisierten Zwischenstufe bei der Zersetzung von p-HBI ebenfalls denkbar ist, lag die Vermutung nahe, dass im Falle des p-HBI eine Parallele mit den hypothetischen Isothiocyanaten von Virtanen (82) vorliegen muss.

Dünnschichtchromatographisch wurde tatsächlich nachgewiesen, dass sich das p-HBI sowohl bei pH 4,8-5 als auch bei pH 10-12 in p-Hydroxybenzylalkohol (X) und Rhodanid zersetzt (Reaktionsgleichung siehe Seite 47). In alkalischen Lösungen von p-HBI konnte neben p-Hydroxybenzylalkohol (X) noch das 4,4'-Dihydroxydiphenylmethan (XI) aber kein p-HBI mehr nachgewiesen werden. Die erwähnten Zersetzungsprodukte wurden neben zwei nicht weiter untersuchten Substanzen mit R_f-Werten von 0,15 und 0,09 auch in alten Präparaten von kristallinem p-HBI nachgewiesen. Natronlauge spaltet hingegen sowohl aus p-Hydroxyphenyl- wie aus Benzyl-isothiocyanat kein Rhodanid ab.

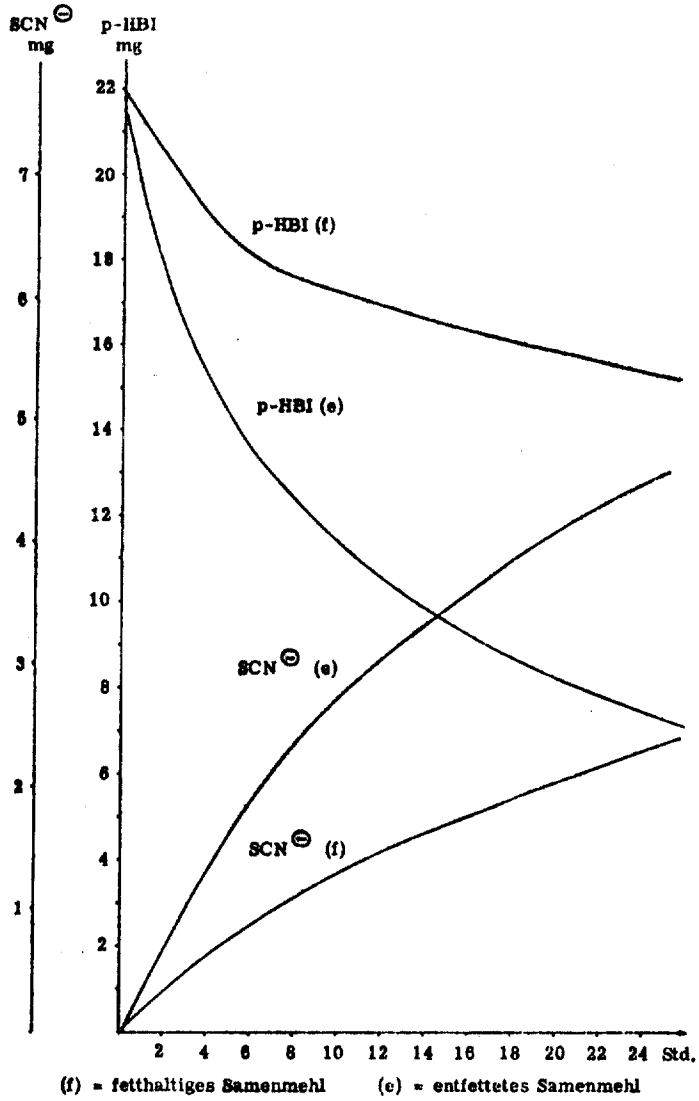
Andererseits zeigten die umfangreichen Untersuchungen von Zahradnik et al. (83, 84), dass die meisten Isothiocyanate mit OH- stets unter Bildung von Monothiocarbamaten reagieren und kein Rhodanid abspalten.

242. Zersetzung von p-Hydroxybenzyl-isothiocyanat in wässrigem und lyophilisiertem Samenmehl von Sinapis alba L.

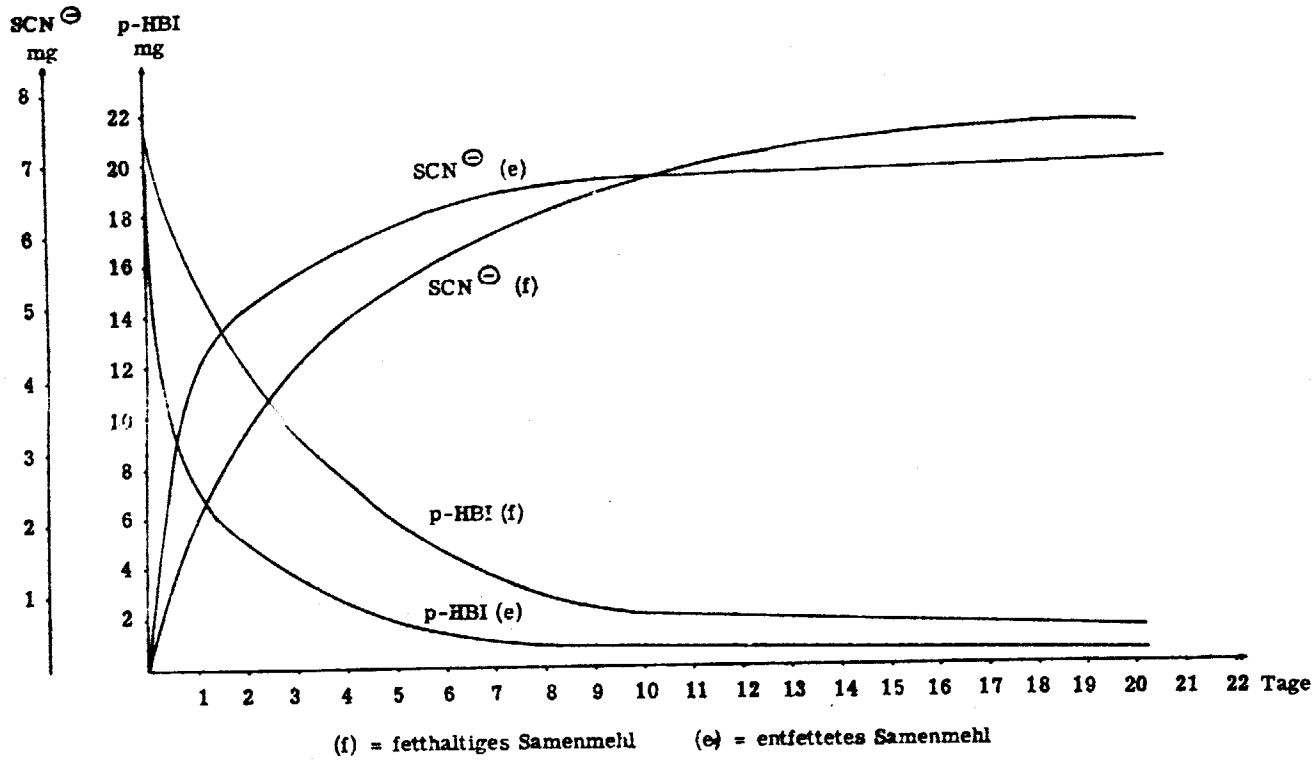
Die Verfolgung der Hydrolyse von p-HBI wurde sowohl in fetthaltigen wie in entfetteten Samenmehl-Wassergemischen ermittelt, da für ihr Ausmass der Fettgehalt des Samenmehls eine sehr wichtige Rolle zu spielen scheint. Um die Hydrolyse in beiden Fällen miteinander vergleichen zu können, wurden sämtliche Ergebnisse der Untersuchungen auf intaktes Samenmehl bezogen. Die Abnahme der p-HBI-Konzentration und die durch die Hydrolyse gebildeten Rhodanidmengen wurden nach verschiedenen Zeiten gemessen und in Fig. 7 und 8 graphisch dargestellt. Die prozentuale Hydrolyse wurde auf zwei Arten berechnet; einerseits aus der Differenz der ursprünglichen und der zur Zeit t bestimmten p-HBI-Konzentration im Samenmehl-Wassergemisch und andererseits aus der zur Zeit t gefundenen Rhodanidmenge, die der Menge hydrolisiertem p-HBI entspricht. Die Resultate sind in Fig. 9 und 10 dargestellt.

Die Fig. 7 - 10 zeigen deutlich, dass die Hydrolyse des p-HBI im entfetteten Samenmehl ungefähr doppelt so schnell vor sich geht wie im fetthaltigen Samenmehl. Das gebildete p-HBI löst sich offensichtlich zum grössten Teil im Fett und wird dadurch vor der Hydrolyse geschützt. Selbst im fetthaltigen Samenmehl-Wassergemisch findet man aber nach 10 Tagen praktisch kein p-HBI mehr sondern nur noch p-Hydroxybenzylalkohol (X), sowie dessen Kondensationsprodukte und Rhodanid.

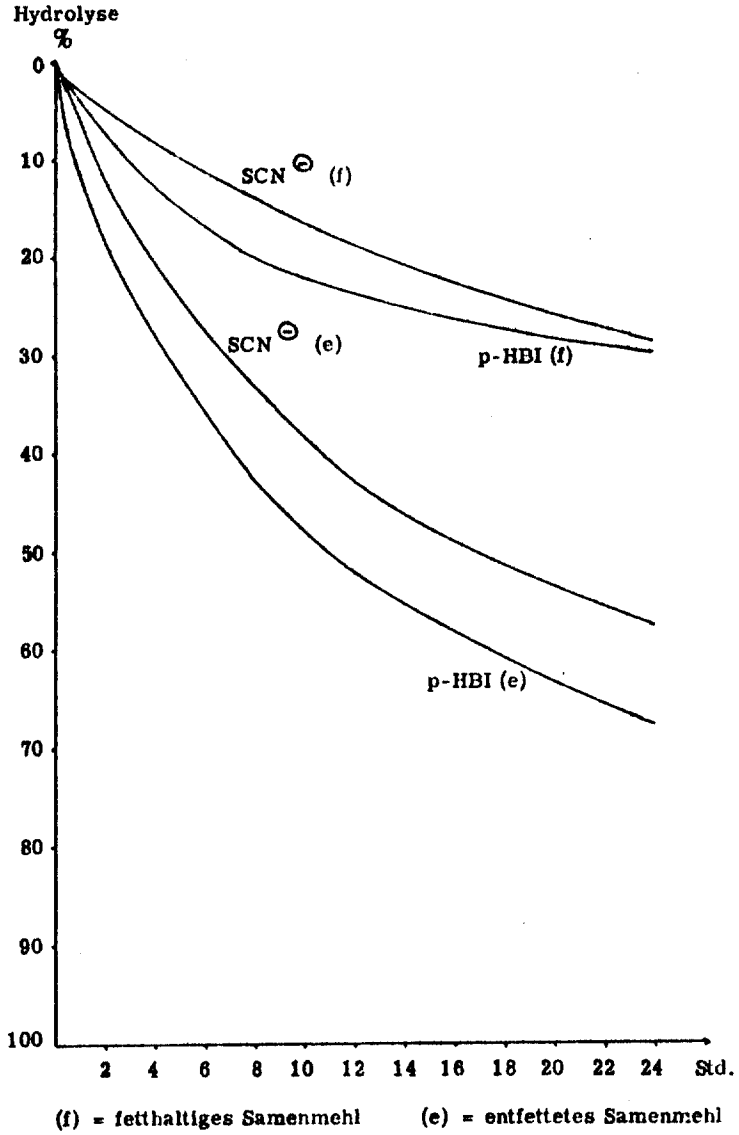
Um die Zersetzung des p-HBI während der Lyophilisation der fetthaltigen und entfetteten Samenmehle und während der Aufbewahrung der lyophilisierten Samenmehle zu ermitteln, wurden ebenfalls die zur Zeit t vorhandenen p-HBI-Konzentrationen und die durch Hydrolyse gebildeten Rhodanidmengen gemessen und daraus die prozentuale Hydrolyse berechnet. Die Resultate sind in Tabelle VI zusammengestellt. Die Untersuchungen über die Lyophilisierung des Samenmehls zeigen auch, dass die Zersetzung des p-HBI nur davon abhängig ist, wie lange dieses mit dem Wasser in Kontakt ist. Tabelle VI zeigt auch, dass die auf die beiden Arten berechneten Hydrolysewerte von p-HBI weitgehend übereinstimmen.



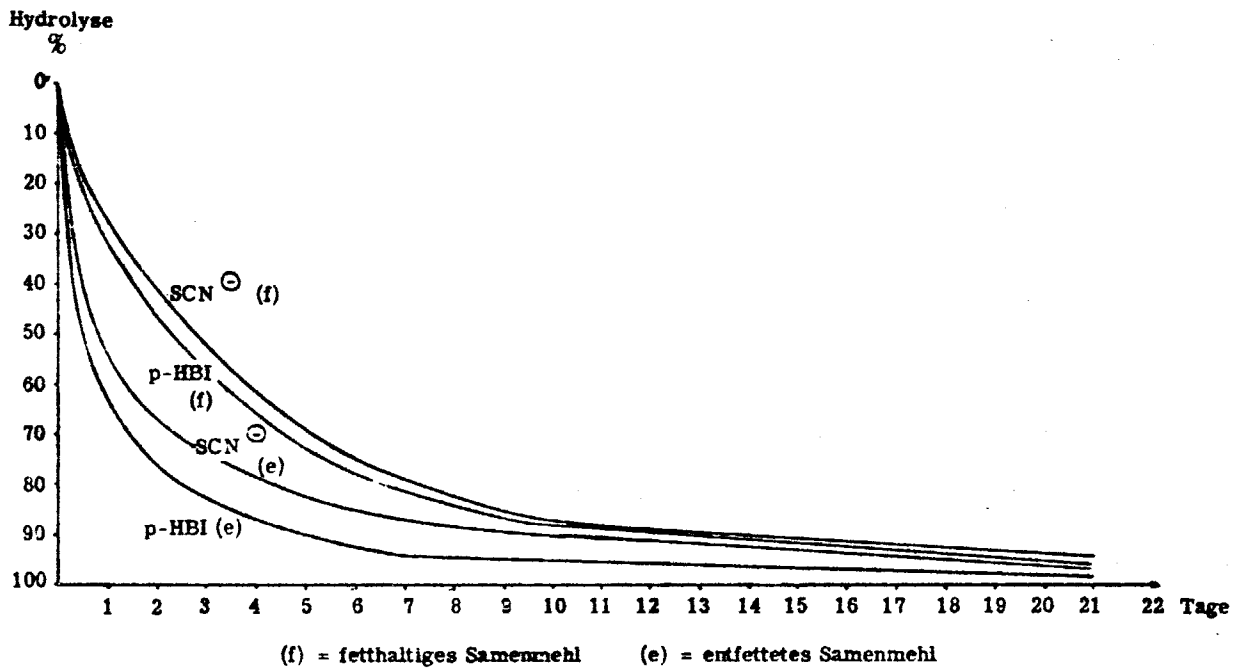
Figur 7 Aenderung des p-HBI- und $SCN^{\ominus}$ - Gehaltes in wässrigen Suspensionen von fetthaltigem (1 g) und entfettetem (0,6345 g) Samenmehl als Funktion der Zeit (0 - 24 Std.)



Figur 8 Aenderung des p-HBI- und $SCN^{\ominus}$ - Gehaltes in wässrigen Suspensionen von fetthaltigem (1 g) und entfettetem (0,6345 g) Samenmehl als Funktion der Zeit (0 - 22 Tage)



Figur 9 Prozentuale Hydrolyse des p-HBI von fetthaltigem und entfettetem Samenmehl in Wasser als Funktion der Zeit (0 - 24 Std.)



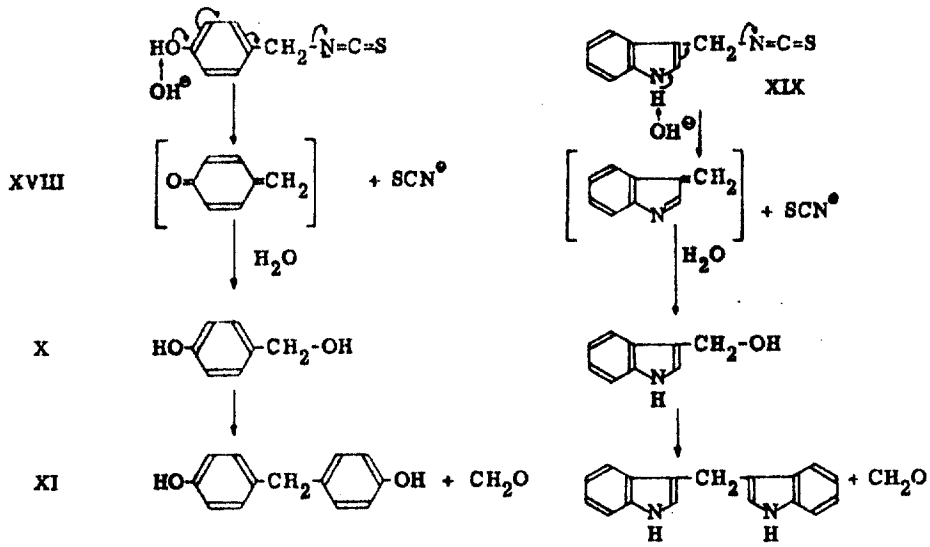
Figur 10 Prozentuale Hydrolyse des p-HBI von fetthaltigem und entfettetem Samenmehl in Wasser als Funktion der Zeit (0 - 22 Tage)

Tabelle VI Hydrolyse von p-HBI in fetthaltigem und entfettetem hyophilisiertem Samenmehl

	p-HBI gef. [mg/g]		p-HBI hydr. [mg/g]		Hydrolyse [%]		$SCN^{\ominus}$ gef. aq. Extr. [mg/g]		p-HBI hydr. [mg/g]		Hydrolyse [%]		Total gef. p-HBI in % d. Th.	
	f	e	f	e	f	e	f	e	f	e	f	e	f	e
Unbehandeltes Samenmehl	22,0	21,4	0	0	0	0	0,60	0,33	1,71	0,95	0	0	100	100
Vor der Lyophilisierung	21,2	19,0	0,8	2,4	3,6	11,2	0,21	0,63	0,60	1,8	2,7	8,4	99,2	98
Nach der Lyophilisierung	17,9	17,4	4,1	4,0	18,6	18,7	1,24	1,27	3,55	3,62	16,1	16,9	97,5	98,4
Nach 1 monat. Aufbewahrung	14,2	12,2	7,8	9,2	35,4	43,0	2,52	2,87	7,18	8,2	32,6	38,5	97,0	95,5
Nach 3 monat. Aufbewahrung	10,0	6,9	12,0	14,5	54,6	67,7	3,90	4,92	11,10	14,0	50,5	65,5	96,0	97,6

hydr. = hydrolisiert
f = fetthaltiges Samenmehl
e = entfettetes Samenmehl

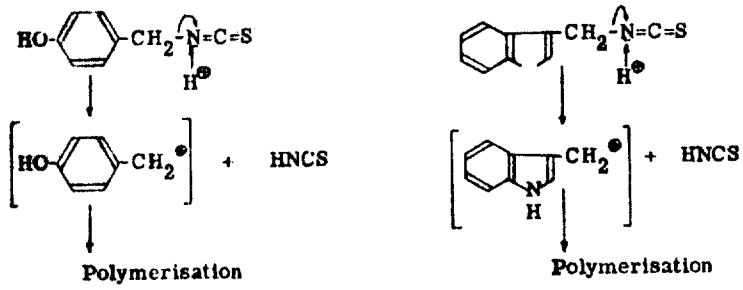
Die Untersuchung der Zersetzung des p-HBI zeigt, dass sowohl p-HBI wie die beiden theoretischen Isothiocyanate von Virtanen (82) sich in ihren Reaktionen mit $OH^{\ominus}$ und $H^{\oplus}$ - Ionen abnormal verhalten. Die Zersetzung von p-HBI und des theoretischen 3-Indolylmethyl-isothiocyanat verläuft im basischen Milieu wahrscheinlich wie folgt:



Die Zersetzung des p-HBI verläuft mit aller Wahrscheinlichkeit über das Chinonmethid (XVIII) als Zwischenstufe. Diese Verbindung ist äusserst reaktionsfähig und konnte noch nie isoliert werden. (Ueber Chinonmethide und ihre Polymerisation vgl. z. B. 85, 86, 87). Chinonmethide addieren leicht Phenole, Zucker, Wasser etc.

Von der Analogie der Beobachtungen (38, 85) ausgehend müsste unter den Zersetzungsprodukten von p-HBI im alkalischen Milieu auch Formaldehyd vorhanden sein.

In stark saurem Milieu dürfte das Proton am Stickstoffatom angreifen und durch Abspaltung des Rhodanids ein resonanzstabilisiertes Carboniumion bilden, welches in diesem pH-Bereich zu Polymeren führen müsste:



Nach diesen Beobachtungen kann man die Isothiocyanate in zwei Gruppen einteilen, nämlich in solche, die mit $OH^{\ominus}$ - Ionen Monothiocarbamate bilden und in solche, die Rhodanid-Ionen abspalten.

243. Biologische Wirkung von Isothiocyanaten

Die Isothiocyanate zeigen eine Reihe von pharmakologischen Eigenschaften, die heute noch ausgenützt werden. So wird Allyl-isothiocyanat in Form von Senfpackungen als Antirheumaticum, Rettich und Brunnenkresse als galletreibende Mittel verwendet.

Die Arbeiten von Schmidt und Marquardt (88) sowie von Foter (89) wiesen auf die bakteriostatische und antimykotische Wirkung von Isothiocyanaten hin. Neuere Untersuchungen (90) zeigten, dass gewisse Isothiocyanate erfolgreich gegen Schädlinge im Boden angewendet werden können.

Viele Futtermittel enthalten Senfölglucoside, deren enzymatische Spaltung Isothiocyanate liefert, die für Tiere giftig sein können. Die Kenntnisse der Inhaltsstoffe solcher Futtermittel werden von Kjaer et al. (36) ständig erweitert.

Die wichtigste Rolle spielen die Isothiocyanate aber in der menschlichen Ernährung. Viele Gewürze und Gemüsesorten erhalten ihren charakteristischen Geruch und Geschmack von Isothiocyanaten und ihren Zersetzungsprodukten, die letzteren können zudem auch bestimmte physiologische Wirkungen aufweisen.

Wie bereits erwähnt, entstehen aus gewissen intermediär gebildeten Isothiocyanaten cyclische Verbindungen, die stark antithyreotische Wirkungen aufweisen. Auch das Rhodanid, das durch Hydrolyse der beiden hypothetischen Isothiocyanate von Virtanen (82, 93) und wie gefunden von p-HBI entsteht, bewirkt durch Hemmung des Jodierungsprozesses eine Störung der Bildung des Schilddrüsenhormons [$SCN^{\ominus}$ - Ionen haben den gleichen Ionenradius wie $J^{\ominus}$ - Ionen (72, 91, 92), Rhodanide wirken auch blutdrucksenkend (72)].

Die Frage, ob die Milch von einseitig mit Pflanzen von Brassica-Arten gefütterten Kühen, wegen ihres Rhodanidgehaltes auf die Menschen kropferregend wirken kann, wurde von Virtanen et al. (82, 93) nach eingehenden Untersuchungen verneint.

Wie einleitend erwähnt, befasste sich die vorliegende Arbeit mit der Untersuchung der Entstehung und Stabilität von p-HBI im Tafelsenf. Diese ergab zusammenfassend, dass im ein- bis zweiwöchigen gelagerten Tafelsenf kein p-HBI mehr vorhanden ist und die Zersetzungsprodukte kaum zum Geschmack desselben beitragen. Die Zersetzungsprodukte des p-HBI, p-Hydroxybenzylalkohol und Rhodanid (aus 1 g Samenmehl entsteht 7-8 mg $SCN^{\ominus}$) haben zwar antithyreotische Wirkungen (72, 91, 92), jedoch werden bei normalem Verbrauch von Tafelsenf keine schädlichen Mengen eingenommen.

Die Verwendung von Samenmehl (Sinapis alba L.) zur Tafelsenfherstellung bewirkt keine anhaltende Schärfe des Senfs, da das p-HBI wegen seines labilen Charakters nicht stabilisiert werden kann. Dieses Samenmehl dient deshalb im Tafelsenf technologisch nur als Verdickungsmittel.

Das Vorhandensein von Sinapis alba L. Samenmehl im Tafelsenf kann durch dünnschichtchromatographischen Nachweis von p-Hydroxybenzylalkohol (X) bestimmt werden und die quantitative Rhodanidanalyse erlaubt, die ursprüngliche Menge Samenmehl im Tafelsenf zu berechnen.

3. EXPERIMENTELLER TEIL

31. Synthese von p-Hydroxybenzyl-isothiocyanat

311. Darstellung einfacher Arylisothiocyanate

Im folgenden wird die Herstellung einiger aromatischer Isothiocyanate nach den Vorschriften von Slotta und Dressler (19) beschrieben. Die Ammonium-aryldithiocarbaminate wurden jeweils durch folgende Ammoniakbestimmung analysiert:

Eine abgewogene Menge Ammoniumsalz wurde in eine Gaswaschflasche gebracht und daraus mit 20 - 30 ml 2 %-iger Natronlauge Ammoniak freigesetzt, welches mit getrockneter Luft (konz. Schwefelsäure) in 100 ml 0,05 N Salzsäure übergetrieben wurde. Die Apparatur bestand aus drei miteinander verbundenen Gaswaschflaschen, durch die während der 10-stündigen Analyse mittels einer Wasserstrahlpumpe Luft gesaugt wurde. Die Methode wurde zuerst mit genau eingewogenem Ammoniumchlorid geeicht, wobei man 98,5 % der eingewogenen Menge wiederfand.

Für die Umsetzung der Ammonium-aryldithiocarbaminate mit Phosgen, benützte man einen 100 ml Dreihalskolben, welcher mit einem KPG-Rührer, einem Tropftrichter und einem Rückflusskühler mit Gasableitungsrohr versehen war. Die Umsetzungen sind in Tabelle VII zusammengestellt, die Ausbeuten sind alle auf die Amine bezogen.

Tabelle VII Synthese von Arylisothiocyanaten

R -	Amine $R\text{-}NH_2$	Ammonium-dithiocarbaminate $R\text{-}NH\text{-}CSS^{\ominus}\ NH_4^{\oplus}$				Isothiocyanate R-NCS		
	Ausg.-Menge [g]	Ausbeute [g]	Ausbeute [%]	Smp. [°C]	$NH_4^{\oplus}$-Gehalt [%]	Ausbeute [g]	Ausbeute [%]	Sdp. [°C]
Phenyl-	9,3	16,5	89	95	98	10,2	75	221
Benzyl-	10,7	14,1	71	118	97	8,9	60	225
p-Methoxy-phenyl-	12,3	17,1	80	88	99	11,6	70	Smp. 21

312. p-Hydroxyphenyl-isothiocyanat

16,2 g sublimiertes Aminophenol (150 mMol), Smp.: 183 - 184° C, wurden mit 22 ml Methanol schwach erwärmt und mit 18 ml konz. Ammoniak (spez. Gew. 0,91) versetzt (1/10 der in der ursprünglichen Vorschrift angegebenen Menge Ammoniak). Zum Reaktionsgemisch wurden unter ständigem Rühren 12,6 g Schwefelkohlenstoff zugegeben. Das p-Aminophenol ging in Lösung und nach weiteren 10 - 20 minütigem Rühren schieden sich beim Abkühlen gelbliche Kristalle aus. Man erhielt 25,7 g (85,4 %) Ammonium-p-hydroxyphenyldithiocarbaminat, Smp. 102° C, Ammoniumgehalt 98 % der Theorie.

10,1 g Ammoniumsalz (50 mMol) wurden in 10 ml Benzol suspendiert, langsam auf 50° C erwärmt und unter kräftigem Rühren tropfenweise mit 27,5 ml Benzol-Phosgen-Lösung versetzt. Während der halbstündigen Umsetzung wurde die Farbe des Reaktionsgemisches gelb. Das überschüssige Phosgen wurde durch die Apparatur gesaugt. Das Ammoniumchlorid wurde abgenutscht, mit Benzol gewaschen und die Benzollösung fraktioniert destilliert. Das reine Isothiocyanat destillierte zwischen 165° und 170° C bei 10 mmHg, Ausbeute 4,8 g (55 %). Das p-Hydroxyphenyl-isothiocyanat erstarrte bei Raumtemperatur zu einer weissen, fettigen kristallinen Masse, Smp. 40 - 42° C, nach dem Umkristallisieren aus heissem Petroläther weisse Nadeln mit Smp. 49 - 50° C (Lit. (19) 43 - 44° C).

Bei einer zweiten Umsetzung von 10,1 g Ammoniumsalz mit Phosgen wurde die Benzollösung zur Trockene eingedampft und das Isothiocyanat in heissem Petroläther gelöst und kristallisiert, Ausbeute 4,1 g (46,4 %), Smp. 49° C.

p-Hydroxyphenyl-isothiocyanat ist in Aether sehr gut, in Alkohol und Benzol weniger gut löslich. Es besitzt einen sehr scharfen Geschmack und erzeugt auf der Haut sogar bei grosser Verdünnung langsam heilende Blasen.

Das p-Hydroxyphenyl-isothiocyanat wurde auch nach der Staab'schen Methode (12) wie folgt dargestellt:

Zunächst wurden 6 g gelbes kristallines N N'-Thiocarbonyl-di-imidazol (V) aus 12,3 g (180 mMol) Imidazol und 5,2 g (45 mMol) Thiophosgen hergestellt, Ausbeute 75 %, Smp. des Rohproduktes 96° C.

3 g (17 mMol) NN'-Thiocarbonyl-di-imidazol (V) (Rohprodukt) wurden in 50 ml äthanolfreiem Chloroform gelöst und diese Lösung zu einer Aufschlämmung von 1,83 g (17 mMol) sublimiertem p-Aminophenol in 30 ml Chloroform getropft. Das Gemisch wurde bei 0° C und unter Stickstoff zwei Stunden gerührt. Das Amin löste sich und bildete ein grünliches Oel, welches abgetrennt wurde. Aus dem Oel (1,7 g) konnte durch Tetrachlorkohlenstoff- und Petrolätherextraktion 1,3 g (51 %) kristallines p-Hydroxyphenyl-isothiocyanat mit Smp. 47 - 48° C gewonnen werden.

N-p-Hydroxyphenylthioharnstoff wurde aus je 300 mg p-Hydroxyphenyl-isothiocyanat (2 mMol) auf zwei verschiedene Arten hergestellt. Das Isothiocyanat wurde einerseits in 20 ml absolutem Benzol mit trockenem Ammoniak, andererseits in 0,3 ml absolutem Aethanol und mit 0,6 ml konzentriertem Ammoniak umgesetzt. Es resultierten weisse, schuppenartige Kristalle. Man erhielt 260 mg (78 %) nach der ersten und 250 mg (75 %) nach der zweiten Methode, Smp. 215 - 216° C.

313. p-Hydroxybenzaldoxim (II)

Das p-Hydroxybenzaldoxim wurde nach einer Vorschrift von Kjaer und Rubinstein (4) bereitet. Zu einer Suspension von 95 g p-Hydroxybenzaldehyd (770 mMol) und 81,5 g Hydroxylamin-hydrochlorid in 500 ml Wasser wurde eine Lösung von 40,5 g Natriumhydroxyd in 320 ml Wasser gegeben. Das Reaktionsgemisch wurde mit soviel Alkohol versetzt, bis eine klare Lösung entstand. Die Lösung wurde unter Rückfluss während einer Stunde zum Sieden erhitzt. Das Aethanol wurde im Vakuum abgedampft und die wässrige Phase 1 mal mit 200 ml und 3 mal mit 100 ml Aether extrahiert. Der Aetherextrakt wurde mit Natriumsulfat getrocknet und der Aether eingedampft. Das rohe p-Hydroxybenzaldoxim wurde aus 6000 ml Benzol umkristallisiert, wobei man 68,8 g (64,5 %) kristallines Produkt mit Smp. 109 - 112° C erhielt. Aus der Mutterlauge wurden weitere 8,3 g Substanz gewonnen.

314. p-Hydroxybenzylamin (IV)

Das Oxim (II) wurde nach folgender Modifikation der Vorschrift von Ott und Zimmermann (34) katalytisch hydriert. Zu einer Lösung von 7,5 g bzw. 15,0 (II) in 375 ml 96 %-igem Alkohol wurden 10 ml konz. Salzsäure und verschiedene Mengen PdO, Pd-Kohle- 5 und 10 % gegeben. Das Reaktionsgemisch wurde bei Raumtemperatur unter kleinem Wasserstoffüberdruck geschüttelt. Nachdem keine weitere Wasserstoffaufnahme mehr eintrat, wurde durch Celit filtriert und das Filtrat auf ein kleines Volumen im Vakuum eingeengt. Nach Zugabe von Aether schied sich das kristalline p-Hydroxybenzylamin-hydrochlorid (III) aus.

Die Resultate der verschiedenen Hydrierungen sind in Tabelle I zusammengestellt (siehe Seite 16).

Die p-Hydroxybenzylamin-hydrochlorid-Kristalle (III) waren bei der PdO-Hydrierung farblos, während sie bei der Pd-Kohle-Hydrierung rötlich gefärbt waren, Smp. 186 - 188° C. Die in der Literatur angegebene Ausbeute betrug 74 % und der Smp. 190 - 191° C. 15,5 g III wurden in etwas Methanol gelöst und mit der berechneten Menge konz. Ammoniaklösung (20,4 ml, 23 %-ig) in das Amin überführt. Man erhielt 10,8 g (90 %) farbloses, kristallines Amin (IV), Smp. 99 - 102° C.

315. Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII)

In zwei Ansätzen wurden je 2,5 g (20 mMol) pulverisiertes p-Hydroxybenzylamin (IV) mit 3 ml Methanol vermischt und mit 2,4 ml konz. Ammoniak leicht erwärmt. Unter kräftigem Rühren wurden portionenweise 1,68 g Schwefelkohlenstoff zugegeben. Nach 1/4 Stunde Rühren ging alles Amin in Lösung, nach einer weiteren Viertelstunde Rühren wurde die Lösung mit Aether versetzt. Ein Teil des gebildeten Ammoniumsalzes (VII) fiel darauf sofort aus, der Rest kristallisierte bei -5° C über Nacht aus. Nachdem die Kristalle mit kaltem Aether gewaschen wurden, erhielt man 3,14 g (71,5 %) und 3,2 g (73 %) schwach gelbliches VII, Smp. 117 - 120° C (Zersetzung), Ammoniumgehalt von VII 98 % der Theorie.

1,25 g Amin (IV) (10 mMol) wurden in 10 ml Methanol und 30 ml Aether aufgelöst. Unter starkem Rühren sättigte man die Lösung mit trockenem Ammoniak und tropfte 0,84 g Schwefelkohlenstoff, gelöst in 10 ml Aether, zu. Nach einstündiger Reaktionszeit entstand eine gelbe Lösung die zur Trockene eingedampft wurde. Man erhielt 1,96 g (90 %) einer gelben, kristallinen Masse. Dieses Rohprodukt enthielt 84,5 % ammoniumhaltiges Salz, es wurde aus Aethanol-Aether umkristallisiert, wobei 0,92 g weisses, kristallines VII mit Smp. 116 - 118° C ausfiel, Ammoniumgehalt 97 % der Theorie.

316. p-Hydroxybenzyl-isothiocyanat

6,54 g (30 mMol) Ammonium-p-hydroxybenzyl-dithiocarbaminat (VII) wurden in 30 ml Benzol aufgeschlämmt und auf 40 - 50° C erwärmt. Unter ständigem Rühren wurden 15 ml Phosgen-Benzol-Lösung (3 g Phosgen) während 15 Minuten portionenweise zugetropft. Das Reaktionsgemisch wurde weitere 15 Minuten gerührt und abgekühlt. Zur Entfernung von überschüssigem Phosgen wurde während 30 Minuten Stickstoff durch den Apparat geleitet. Der benzolunlösliche Teil wurde abgenutscht, mit Benzol gewaschen und getrocknet; er wog 5,83 g. Das Filtrat wurde am Dünnschichtverdampfer zur Trockene eingedampft und das p-HBI mit warmem Petrolaether (30 - 40° C) herausgelöst. Die Petrolätherlösung wurde bis zur Trübung eingeengt und schied bei -10° C p-HBI in Form von farblosen Nadeln aus. Der Petroläther wurde unter Ausschluss von Luftfeuchtigkeit abgesaugt. Die getrockneten Kristalle wogen 520 mg (10,4 % bez. auf das Ammoniumsalz) und schmolzen unscharf zwischen 36 - 38° C.

2,2 g (10 mMol) Ammoniumsalz (VII) wurden bei 20° C mit Phosgen unter den obigen Bedingungen umgesetzt. Nach dem Trocknen erhielt man 180 mg kristallines p-HBI (10,7 % bez. auf Ammoniumsalz), Smp. 36 - 38° C.

0,97 g VII wurden in 30 ml Benzol aufgeschlämmt und unter kräftigem Rühren bei 0° C während 15 Minuten mit 0,6 g Phosgen (gelöst in Benzol) versetzt. Die Reaktion wurde eine Stunde unter Ausschluss von Feuchtigkeit unter Stickstoff ausgeführt. Nach Entfernung des Phosgens mit Stickstoff wurde das Reaktionsgemisch filtriert und das schwach gelbliche Filtrat sofort auf Isothiocyanat-Gehalt analysiert, es enthielt 75 mg-HBI. Der Niederschlag wurde noch einmal mit Phosgen umgesetzt, da vermutet wurde, dass die vorher angewandte Reaktionszeit zu kurz war. Nach einer Reaktionszeit von 2,5 Stunden wurden im Filtrat weitere 62,2 mg p-HBI gefunden. Die Benzollösungen wurden vereinigt und wie oben aufgearbeitet. Aus Petroläther wurden 105 mg (14,2 %) reines p-HBI mit Smp. 38° C erhalten.

2,1 g (17 mMol) p-Hydroxybenzylamin (IV) wurden nach Staab und Walther (12) mit 3 g (17 mMol) NN'-Thiocarbonyl-di-imidazol (V) unter den gleichen Bedingungen wie beim p-Hydroxyphenyl-isothiocyanat beschrieben, umgesetzt. Man versuchte aus den öligen Endprodukten das p-HBI mit Petroläther zu extrahieren, konnte jedoch kein kristallines p-HBI erhalten. Ein Dünnschichtchromatogramm zeigte aber eindeutig, dass sich während der Umsetzung p-HBI gebildet hat.

Eine p-HBI-haltige Benzollösung, erhalten durch Umsetzung von 5 g (25 mMol) Salz (VII) mit Phosgen, wurde im Dünnschichtverdampfer auf einige ml eingeengt und im Kugelrohr unter Hochvakuum destilliert. Die anfänglich bräunliche Flüssigkeit begann sich bei 110° C zu zersetzen unter Bildung weisser Dämpfe. Die Flüssigkeit erstarrte zu einer gelblich-bräunlichen Masse, die nicht mehr destillierbar war (erhitzt bis 250° C). In der Kugel sammelte sich ein gelber harzartiger Stoff, der in Aether unlöslich, in Alkohol löslich war, er wurde nicht weiter untersucht.

Der benzolunlösliche Teil aus einer Umsetzung von 3 g Ammoniumsalz (VII) mit der aequivalenten Menge Phosgen wurde nach 1/2 Stunden Reaktionszeit quantitativ abgenutscht, mit Benzol gewaschen und 20 Stunden im Vakuum getrocknet. Das gelblich-weisse Pulver (2,6 g) wurde mit 100 ml heissem Wasser ausgezogen und nochmals abgenutscht. Zurück blieben 1,614 g einer farblosen, wasser- und benzolunlöslichen Substanz mit Smp. 80 - 90° C.

Das aus den verschiedenen Ansätzen erhaltene p-HBI wurde vereinigt und zweimal aus heissem Petroläther umkristallisiert, wobei farblose Nadeln erhalten wurden, die bei 41 - 42° C scharf schmolzen. Die Kristalle wurden 24 Stunden im Hochvakuum getrocknet und analysiert.

		C %	H %	N %	S %
Mikroanalyse	Gef.:	57,27	4,55	8,18	18,80
C_8H_7ONS	Ber.:	58,18	4,27	8,48	19,41

N-p-hydroxybenzyl-N'-phenylthioharnstoff (IX) wurde einerseits aus p-Hydroxybenzylamin (IV) andererseits aus p-HBI dargestellt.

0,53 g (4 mMol) Amin (IV) wurden in 40 ml Aethanol aufgelöst und mit 0,64 g Phenyl-isothiocyanat unter Rückfluss eine halbe Stunde gekocht. Nach Kühlen und Zugabe von Wasser schied sich das weisse kristalline IX aus, das abgenutscht und dreimal aus wässrigem Alkohol umkristallisiert wurde, Smp. 166° C, Lit. (4) 167 - 168° C.

260 mg (1,6 mMol) synthetisches p-HBI wurden in 3 ml Aether gelöst und mit einer Lösung von 150 mg Anilin in 1 ml Aether tropfenweise versetzt. Es wurde 24 Stunden bei Zimmertemperatur geschüttelt, der Aether abgedampft und der Thioharnstoff (IX) zweimal aus verdünntem Aethanol umkristallisiert, Smp. 166 - 167° C.

Umsetzung von p-HBI mit Ammoniak:

248 mg (1,5 mMol) p-HBI wurden in Petroläther gelöst und mit trockenem Ammoniak umgesetzt. Es resultierte ein voluminöser weisser Niederschlag, welcher abgenutscht und mit Petroläther und Benzol gewaschen wurde. Das Produkt (110 mg) wurde aus Alkohol-Wasser zweimal umkristallisiert. Man erhielt 75 mg farblose Kristalle, Smp. 119° C.

Da die Substanz keinen Stickstoff erhielt, konnte es sich nicht um den zunächst vermuteten N-p-hydroxybenzylthioharnstoff handeln. Die Dünnschichtchromatographie ergab, dass die Substanz mit p-Hydroxybenzylalkohol (X) identisch war. Der Mischschmelzpunkt mit reinem X ergab keine Depression. Die erhaltene Menge von 75 mg entspricht einer Ausbeute von 40,3 % p-Hydroxybenzylalkohol (X).

32. Dünnschichtchromatographie von aromatischen Isothiocyanaten und ihren Zersetzungsprodukten

Bei allen Versuchen wurde stets in den mit Laufmitteldämpfen gesättigten Trennkammern aufsteigend chromatographiert. Bei der Auswahl geeigneter Dünnschicht-, Sprüh- und Laufmittel wurden die in der Literatur beschriebenen (37) Bedingungen und Konzentrationen angewandt. Ausser in den Vorversuchen wurde später immer Kieselgel G (Camag, Muttenz/Schweiz) als Dünnschichtmittel verwendet, welches etwa 0,5 mm dick auf die Platten aufgetragen wurde. Die beschichteten Platten wurden 30 Min. bei 105° C getrocknet und bis zum Gebrauch im Exsikkator über Silikagel aufbewahrt.

Die Substanzen wurden mittels Mikropipetten in Mengen zwischen 10 - 100 µl aufgetragen. Die Laufstrecke betrug durchschnittlich 15 cm. Nach Markierung der Grenzlinie wurden die Chromatogramme zuerst 5 Min. bei 105° C getrocknet, anschliessen mit 1 %-iger alkoholischer Lösung von 2,6-Dichlor-chinon-chlorimid (Merk), nachher mit 2 N-Natriumcarbonatlösung besprüht.

Die R_f-Werte der einzelnen Substanzen wurden sofort nach der Entwicklung der Chromatogramme berechnet. Da die R_f-Werte bei der Dünnschichtchromatographie nicht genau reproduzierbar sind, wurden die einzelnen Flecken stets mit Hilfe von Vergleichssubstanzen identifiziert.

Vergleichssubstanzen und ihre R_f-Werte:

p-Hydroxybenzylalkohol (X) (Fluka AG.) Umkrist. aus Alkohol-Wasser, Smp. 118 - 120° C, DC-rein. R_f-Werte: 0,22 - 0,26; Durchschnitt: 0,23.

4,4'-Dihydroxydiphenylmethan (XI) wurde nach Staedel (94) und Egli (95) hergestellt, farblose Blättchen, Smp. 155° C, DC rein. R_f-Werte: 0,33 - 0,40; Durchschnitt 0,37.

p-HBI synthetisches und isoliertes aus dem Samen, DC rein. R_f-Werte: 0,47 - 0,57; Durchschnitt: 0,50.

33. Isolierung von p-Hydroxybenzyl-isothiocyanat aus Samen von Sinapis alba L.

331. Samenmaterial

Alle Untersuchungen wurden mit einem handelsüblichen fetthaltigen Samenmehl von weissem Senf (Sinapis alba L.) der Firma J. J. Colman Ltd., Norwich, England, durchgeführt.

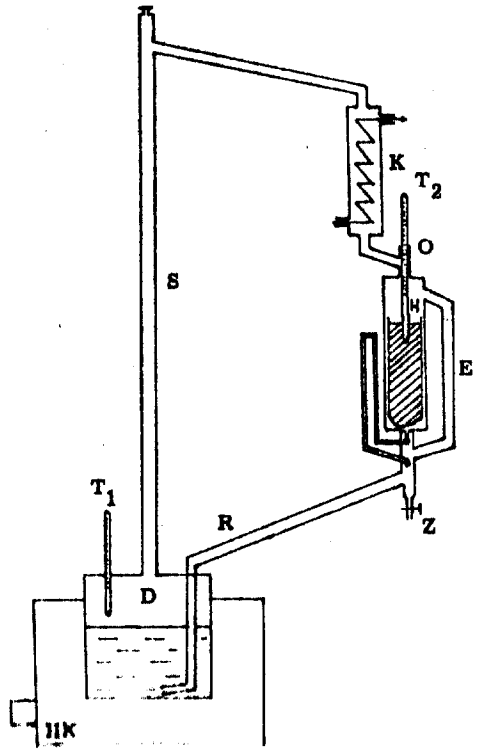
332. Entfettung und Entglucosidierung von Sinapis alba L. Samenmehl

Zur Warmextraktion wurde ein gewöhnlicher Soxhlet-Extraktor verwendet, in dem die Extraktion jeweils beim Siedepunkt des betreffenden Lösungsmittels vor sich ging.

Beim Kaltextraktionsverfahren konnte immer zwischen 10 - 15° C extrahiert werden, da die Lösungsmitteldämpfe keine Erwärmung des Extraktionsgutes bewirken konnten. Der Kaltextraktionsapparat (Fig. 11) bestand aus einem mit Lösungs-

mittel gefüllten Destillationskolben (D), von dem das Lösungsmittel durch Destillation über ein senkrechtes 1,5 m langes Steigrohr (S) in einen wirksamen Kühler (K) gelang. Aus dem Kühler tropfte das auf 10 - 15° C gekühlte Lösungsmittel direkt auf das in einem gewöhnlichen Soxhlet-Extraktor (E) befindliche zu extrahierende Samenmehl. Sobald das Lösungsmittel die Abflusshöhe erreichte, floss es durch ein mit Zweiweghahn (Z) versehenes Rohr (R) wieder in den Destillationskolben (D) zurück. Da dieses Rohr in den unteren Teil des Destillationskolbens (D) führte, konnten die Lösungsmitteldämpfe nur den vorher beschriebenen Weg passieren. Der ganze Apparat wurde ausser einer kleinen Oeffnung (O) beim Uebergangsstück zum Soxhlet-Apparat (E) durch Schliffe hermetisch abgeschlossen, es ergab sich somit eine kontinuierliche Kaltextraktion. Beim Zweiweghahn (Z) konnte jeweils eine kleine Probe entnommen werden um das Fortschreiten der Extraktion zu kontrollieren. Die Temperaturen wurden sowohl im Extraktionsgut (T_2) wie im Destillationskolben (D), (T_1) kontrolliert, wobei die Temperatur bei T_1 jeweils etwa dem Siedepunkt des betreffenden Lösungsmittels entsprach. Sowohl beim Warm- wie beim Kaltextraktionsverfahren dauerte die Extraktion drei Stunden. Es wurden jeweils 10 g Samenmehl und 1000 ml Lösungsmittel verwendet. Das extrahierte Mehl wurde in der Hülse (H) über Nacht am Wasserstrahlvakuum im Exsikkator getrocknet und gewogen. Die im Destillationskolben (D) zurückgebliebenen Extrakte wurden qualitativ untersucht, um die von den betreffenden Lösungsmitteln gelösten Substanzen zu bestimmen. Die getrockneten Senfsamenmehle wurden auch qualitativ auf Myrosinase-Aktivität geprüft. Die Resultate der Vorversuche sind in Tabelle III zusammengestellt (siehe Seite 31).

Die Verwendung eines grösseren Soxhlet-Extraktors erlaubte die Extraktion von 350 - 400 g Samenmehl. Dieses Samenmehl wurde zuerst mit 2000 ml Petroläther (Sdp. 30 - 50° C) während acht Stunden bei 13 - 14° C entfettet. Anschliessend wurde das Mehl samt Hülse im Wasserstrahlvakuum getrocknet und gewogen, um den extrahierten Teil, der vorwiegend aus Pflanzenfett bestand, quantitativ zu ermitteln. Das Samenmehl wurde dann mit 2000 ml Methanol während acht Stunden bei 16 - 17° C entglucosidiert, wieder getrocknet und gewogen. Die Resultate der Extraktion sind in Tabelle IV zusammengestellt (siehe Seite 32).



Figur 11 Modifizierte Soxhlet-Apparatur für Kaltextraktion

Abkürzungen:

HK	=	Heizkalotte
D	=	Destillationskolben
S	=	Steigrohr (1,5 m)
K	=	Kühler
O	=	Oeffnung
E	=	Soxhlet-Extraktor
H	=	Hülse
Z	=	Zweiweghahn
R	=	Rohr
T_1, T_2	=	Temperaturmessstellen

333. Darstellung von Myrosinase-Trockenpräparaten

64,4 g getrocknetes, entfettetes Senfsamenmehl, entsprechend 100 g unbehandeltem Mehl, wurden mit je 300 ml destilliertem Wasser bei Zimmertemperatur zweimal eine halbe Stunde extrahiert. Die wässrigen Auszüge wurden filtriert und zentrifugiert. Aus den klaren Extrakten wurde die Myrosinase durch Acetonfällung (30 - 70 %) gewonnen. Das ausgefallene Enzym wurde abzentrifugiert, in 200 ml destilliertem Wasser aufgelöst, 48 Stunden dialysiert, erneut zentrifugiert und die klare Lösung anschliessend lyophilisiert, Ausbeute: 1,5 g weisses, flockiges Enzympräparat.

Der gleiche Versuch, durchgeführt bei 0 - 4° C ergab 1,3 g Enzympräparat.

In einem dritten Versuch wurde Myrosinase ebenfalls bei 0 - 4° C durch Acetonfällung zwischen 30 - 40 % gewonnen. Das lyophilisierte Enzympräparat (545,3 mg) erwies sich bedeutend aktiver als die beiden ersten. Die Aktivität der verschiedenen Myrosinase-Präparate wurde nach Schwimmer (71) durch die Abnahme der optischen Dichte einer Kalium-allylglucosinolat-(Sinigrin)-Lösung (1,9 µMol/ml) als Funktion der Zeit gemessen. Aus dem Verlauf des enzymatischen Abbaus des vorgelegten Senfölglucosids wurde die Aktivität der Myrosinase qualitativ ermittelt.

334. Isolierung von Senfölglucosiden

Sinapin-p-hydroxybenzylglucosinolat (Sinalbin) (XVII) wurde nach einer Vorschrift von Schneider (3) dargestellt.

Aus 200 g entfettetem Samenmehl wurden nach Umkristallisation aus Alkonol-Wasser 4,4 g Sinapin-p-hydroxybenzylglucosinolat · $5H_2O$ als schwach gelbliche Nadeln erhalten. Ausbeute: 13,5 % bezogen auf den Senfölglucosidgehalt des Samenmehls, Smp. 84° C. Nach dem Trocknen beim Hochvakuum während vier Tagen verloren die Kristalle 4 Moleküle H_2O und schmolzen bei 120° C.

Kalium-p-hydroxybenzylglucosinolat

2000 ml methanolische Senfölglucosidlösung, erhalten aus 145 g entfettetem Samenmehl (Kapitel 332.), wurden zur Trockene eingedampft und der Rückstand in 1500 ml destilliertem Wasser aufgenommen. Die wässrige Lösung wurde auf eine Amberlite IR - 4B Kolonne (1,5 x 35 cm) ($Cl^{\ominus}$) gegeben. Die Durchlaufgeschwindigkeit des Senfölglucosid-Extraktes betrug 200 ml pro Stunde. Die glucosidfreie Lösung wurde in 100 ml-Portionen aufgefangen und im UV auf eventuell durchgehendes p-Hydroxybenzylglucosinolat (XVI) geprüft. Das Perkolat war klar, schwach gelblich,

reagierte neutral und enthielt kein Senfölglucosid mehr, sondern nur Sinapinchlorid. Die Kolonne wurde mit 400 ml destilliertem Wasser gewaschen.

Die Ablösung des Glucosids erfolgte mit 0,5 N Kaliumhydroxyd bis in dem Eluat die erste alkalische Reaktion wahrnehmbar wurde. Das Eluat wurde dann bei 30° C am Dünnschichtverdampfer zur Trockene eingedampft. Der bräunliche, gummiartige Rückstand wurde dreimal mit je 50 ml heissem Pyridin extrahiert. Der Pyridin-Extrakt wurde am Vakuum eingedampft und ergab 10 g rohes Kalium-p-hydroxybenzylglucosinolat. Das Rohprodukt wurde in absolutem Methanol aufgelöst und mit Aktivkohle entfärbt. Aus heissem 90 %-igen Alkohol erhielt man das Endprodukt als weisse, amorphe Substanz, Smp. 148° C unscharf, Ausbeute 4,5 g (33,8 % bez. auf den Senfölglucosidgehalt des Samenmehls).

Kalium-allylglucosinolat (Sinigrin) wurde nach Stoll und Sebeck (96) aus 3 kg Meerrettichwurzeln isoliert. Man erhielt 12,9 g rohes Sinigrin, welches aus wässrigem Alkohol 8,6 g farbloses, kristallines Sinigrin ergab, Smp. 128° C, der Mischschmelzpunkt mit reinem Kalium-allylglucosinolat (Fluka AG.) ergab keine Depression.

Kalium-allylglucosinolat wurde auch aus entfettetem Brassica nigra Samenmehl durch Methanol-Extraktion und Ionenaustauschchromatographie wie bei Kalium-p-hydroxybenzylglucosinolat beschrieben, erhalten, Smp. 128° C.

Tetramethylammonium-p-hydroxybenzylglucosinolat wurde von der California Corp. for Biochem. Res., Los Angeles (USA), bezogen, Smp. 191 - 192° C (zers.), analytisch rein.

Sinapinrhodanid wurde aus der eingeengten Sinapinchloridlösung (Durchlauf der Amberlite IR-4B Kolonne beim Austausch von Sinapin durch Kalium) durch Kaliumrhodanidzusatz gewonnen, Smp. 178° C.

335. Senfölglucosidbestimmung mittels Titration

3351. Analyse von reinen senfölglucosidhaltigen Lösungen

Aus $3,8 \times 10^{-3}$ N (3,8 µMol/ml) wässriger Kalium-allylglucosinolat-Lösung sowie aus 10^{-3} N (1,0 µMol/ml) wässrigen Sinapin-, Kalium- und Tetramethylammonium-p-hydroxybenzylglucosinolat-Lösungen wurden jeweils 10 ml mit ebenfalls 10 ml wässriger Myrosinaselösung (0,5 mg/ml) unter Rühren versetzt. Der sofort beginnende pH-Abfall wurde durch ständige Zugabe von 0,01-N Natronlauge kompensiert, der pH-Wert während des enzymatischen Abbaus konstant auf 6 - 7 gehalten und die verbrauchte Menge Lauge mittels einer Mikrobürette gemessen. Die Titration wurde solange unter Stickstoff durchgeführt, bis der pH-Wert ohne Zugabe von Natronlauge konstant blieb. Von jedem Senfölglucosid wurden zwei Parallelanalysen gemacht.

Durchschnittlicher Laugeverbrauch:

Kalium-allylglucosinolat 3,76 ml, Salze von p-Hydroxybenzylglucosinolat: Sinapin 1,02 ml, Kalium 0,98 ml und Tetramethylammonium 1,06 ml.

3352. p-Hydroxybenzylglucosinolatbestimmung des Samens von Sinapis alba L.

5 g gemahlener Samen wurde im Soxhlet drei Stunden mit 500 ml Petroläther entfettet (Fettbestimmung). Die Extraktionshülse wurde mit dem entfetteten Samen einige Minuten im Vakuum getrocknet und im gleichen Apparat mit 200 ml Methanol drei Stunden weiter extrahiert. Der Methanolextrakt wurde dann im Dünnschichtverdampfer zur Trockene eingedampft und der Rückstand in Wasser gelöst, die Lösung mit Kieselgur filtriert und auf 100 ml aufgefüllt. 20 ml dieser Lösung wurden mit etwa 5 mg aktiver trockener Myrosinase versetzt und der pH-Wert der Lösung während des enzymatischen Abbaus mit 0,01 N Natronlauge unter ständigem Rühren bei pH 6 - 7 konstant gehalten.

Die vier Parallelversuche mit je zwei Titrationen ergaben folgenden durchschnittlichen Verbrauch von 0,01 N Natronlauge in ml: 12,55 12,40 12,75 12,46. Die Resultate der Bestimmungen sind in Tabelle V zusammengestellt (siehe Seite 36).

336. p-Hydroxybenzylglucosinolatbestimmung des Samens von Sinapis alba L. nach Ettlinger (65)

1 g Senfsamenmehl wurde mit 5 ml Wasser und 100 ml peroxydfreiem Aether drei Stunden bei Zimmertemperatur geschüttelt. Der Aether wurde dekantiert und der Rückstand mit etwas Aether gewaschen. Die vereinigten Aetherauszüge wurden 2 mal mit 10 ml Wasser gewaschen. Der Aether wurde dann mit 25 ml und 2 mal mit 10 ml 0,1 N Natronlauge ausgeschüttelt. Die vereinigte, alkalischen Auszüge wurden 15 Minuten bei Zimmertemperatur stehengelassen und anschliessend vorsichtig mit 0,5 N Salpetersäure neutralisiert. Die neutralisierte Lösung wurde 3 mal mit 50 ml Aether gewaschen, und die wässrige Lösung auf dem Dampfbad erwärmt, um den Aether zu entfernen. Schliesslich wurde die Lösung abgekühlt, filtriert und auf genau 100 ml aufgefüllt. Der Rhodanidgehalt der Lösung wurde kolorimetrisch nach Barker (72) bestimmt:

Reagens:

50 g Ferrinitrat wurden in 500 ml Wasser aufgelöst und nach Zugabe von 25 ml konz. Salpetersäure auf 1 Liter verdünnt.

Messung:

1 ml Lösung wurde mit 2 ml Reagens vermischt und mit destilliertem Wasser auf 10 ml verdünnt. Die optische Dichte wurde bei 470 mμ gegen einen Blindwert (8 ml destilliertes Wasser und 2 ml Reagens) gemessen.

Auswertung:

Die optische Dichte der unbekannten Rhodanidlösungen wurde mit der optischen Dichte von bekannten Verdünnungen einer durch Vollhard-Titration gegen Silbernitrat standardisierter Kaliumrhodanidlösung verglichen (Eichkurve). Jedes mg Rhodanid entspricht 2,85 mg p-HBI.

Es wurden vier Analysen durchgeführt und von jeder Analyse vier Rhodanidbestimmungen ausgeführt, von denen das arithmetische Mittel genommen wurde.

In weiteren Untersuchungen nach der gleichen Methode wurde die enzymatische Abbauzeit auf 1, 2 und 17 Stunden verlängert, sowie die Verseifungszeit variiert (60 Minuten ohne, und 30 Minuten mit kurzer Erwärmung auf 50° C). Die Resultate sind in Tabelle V (Seite 36) zusammengestellt.

337. Isolierung von p-Hydroxybenzyl-isothiocyanat aus wässrigem und lyophilisiertem Samenmehl von Sinapis alba L.

Je 100 g entfettetes Samenmehl wurden bei 25° C mit je 30 ml Wasser zu einem Brei gut verrührt und eine Stunde fermentiert. Zur Extraktion des gebildeten p-HBI wurden in einem Fall 1200 ml Petroläther, im anderen 700 ml Chloroform zugesetzt und mechanisch zu homogenen Emulsionen vermischt. Die Emulsionen wurden zentrifugiert, die Petroläther- bzw. die Chloroformlösungen vorsichtig herauspipetiert, mit Natriumsulfat getrocknet und filtriert.

Eine Rückstandbestimmung von 25 ml getrocknetem Extrakt ergab, dass von Petroläther insgesamt 1,21 g Substanz und von Chloroform 3,38 g gelöst wurde.

30 g Silikagel (Merk, 0,05-0,2 mm) wurden in Petroläther aufgeschlämmt und in ein Chromatographierohr von 22 mm Durchmesser eingefüllt. Der Petrolätherextrakt wurde tropfenweise auf die Kolonne gegeben, und mit 500 ml Petroläther nachgewaschen. Man eluierte das p-HBI mit n/Heptan/Benzol/Dioxan (2:3:2). Fraktionen von 2 ml wurden mit einem automatischen Fraktionensammler aufgefangen. Bis zur Fraktion No. 30 waren die Fraktionen nacher farblos. Von den farblosen Fraktionen wurden je 10 μl auf einen Filterpapierstreifen aufgetragen und mit 2,6-DCC besprüht. Die Fraktionen, die stark blaue Flecken zeigten, wurden vereinigt und das Lösungsmittel am Dünnschichtverdampfer abdestilliert. Der Rückstand wurde dann mit 3 mal 300 ml Petroläther bei 40° C extrahiert und die vereinigten

Lösungen bis zur Trübung eingeengt. Bei -10° C schieden sich innerhalb von 24 Stunden weisse Nadeln mit dem Smp. von 39 - 40° C aus. Ausbeute: 370 mg (11,2 %), berechnet auf den theoretischen p-HBI-Gehalt des Samenmehls.

Der Chloroformextrakt wurde zunächst auf 5 ml eingeengt und erst dann auf die Säule aufgetragen. Das p-HBI wurde gleich wie oben eluiert. Die qualitativen Resultate der Fraktionierung zeigten, dass die Fraktionen No. 15 - 35 die Begleitstoffe, No. 35 - 50 den grössten Teil des p-HBI enthielten. Bis zur Fraktion No. 90 konnten noch kleinere Mengen p-HBI nachgewiesen werden. Die Fraktionen No. 35 - 50 wurden vereinigt und wie oben verarbeitet. Man erhielt ebenfalls weisse Nadeln, Smp. 38 - 39° C, Ausbeute: 545 mg (16,5 %), berechnet auf den theoretischen p-HBI-Gehalt des Samenmehls.

100 g entfettetes Samenmehl wurden mit 400 ml Wasser von 0° C im "Turmix"-Apparat gemischt, wobei die Temperatur trotz kräftigem Durchmischen nicht über 30° C stieg. Nach einstündigem Abbau bei Zimmertemperatur wurde das wässrige Samenmehl während 24 Stunden lyophilisiert. Das vollkommen trockene Produkt wurde pulverisiert und unter Stickstoff wasserdicht aufbewahrt.

16 g lyophilisiertes Samenmehl wurden in einem Soxhlet-Apparat mit Petroläther bei 40° C so lange extrahiert, bis kein p-HBI mehr herausgelöst wurde (25 Stunden). Die hellgelbe Lösung wurde eingeengt, mit etwas Kieselgel G entfärbt und filtriert. Bei -10° C kristallisierte p-HBI in farblosen Nadeln, Smp. 39 - 40° C, Ausbeute: 35 mg (6,7 % bez. auf den theoretischen p-HBI-Gehalt des Samenmehls).

34. Zersetzung von p-Hydroxybenzyl-isothiocyanat

341. Zersetzung von p-Hydroxybenzyl-isothiocyanat in wässrigem Samenmehl von Sinapis alba L.

In Reagensgläsern von 8 ml Inhalt wurden 1 g fetthaltiges oder 0,6345 g entfettetes Samenmehl mit 5 ml destilliertem Wasser verrührt und die verschlossenen Röhrchen unter gelegentlichem Schütteln verschieden lang bei Zimmertemperatur stehen gelassen. Den Inhalt goss man sorgfältig in einen Erlenmeyerkolben (500 ml), wusch das Röhrchen mit etwas Aether aus und extrahierte das p-HBI mit 100 ml peroxydfreiem Aether durch einstündiges Schütteln. Die Aetherschicht wurde in einem Scheidetrichter dekantiert und das wässrige Samenmehl noch zweimal mit 40 ml Aether gewaschen. Der Aetherextrakt wurde zweimal mit 10 ml destilliertem Wasser gewaschen und sein p-HBI-Gehalt nach der modifizierten Ettlinger'schen Methode (Kap. 336.) bestimmt.

Die wässrige Samenmehlpaste, welche im Erlenmeyerkolben zurückblieb, wurde mit 40 ml destilliertem Wasser durchgeschüttelt, auf einen Faltenfilter gespült und in einem 500 ml Rundkolben filtriert. Der Erlenmeyerkolben wurde noch mit weiteren 20 ml Wasser ausgewaschen. Das Filtrat und die Waschflüssigkeit des Aetherextraktes wurden auf das Samenmehl im Faltenfilter gegeben und so die Filtrate vereinigt. Das wässrige Filtrat wurde am Dünnschichtverdampfer vom Aether befreit, auf 100 ml aufgefüllt und der Rhodanidgehalt kolorimetrisch (Kap. 336.) bestimmt.

Um den Anfangsgehalt von p-HBI und Rhodanid im fetthaltigen und entfetteten Samenmehl zu ermitteln, wurden 1,0 g bzw. 0,6345 g Samenmehl mit 5 ml Wasser und 100 ml peroxydfreiem Aether drei Stunden geschüttelt und wie oben auf den p-HBI- und Rhodanidgehalt analysiert. Die so gefundenen Rhodanidwerte wurden den während des Abbaus erhaltenen Rhodanidmengen abgezogen, um das zusätzlich nur durch die Hydrolyse des p-HBI entstandene Rhodanid zu erhalten.

Es wurden jedesmal zwei Parallelunterschungen und zu jeder Analyse vier Rhodanidbestimmungen durchgeführt. Die Resultate sind in Fig. 7 - 10 graphisch dargestellt (Seite 42 - 45).

342. Zersetzung von p-Hydroxybenzyl-isothiocyanat in lyophilisiertem Samenmehl von Sinapis alba L.

Je 5 g fetthaltiges und entfettetes Samenmehl wurden mit je 20 ml Wasser gut vermischt, eine Stunde bei Zimmertemperatur stehen gelassen, eingefroren und lyophilisiert. Nach der Lyophilisation konnte im Wasserabscheider weder p-HBI noch Rhodanid nachgewiesen werden.

Der p-HBI- und Rhodanidgehalt des Samenmehls vor der Lyophilisation wurde wie in Kapitel 341. beschrieben, nach einstündiger Hydrolyse ermittelt.

Der p-HBI- und Rhodanidgehalt des lyophilisierten Samenmehls wurde sofort nach der Lyophilisation sowie nach einem und nach drei Monaten folgendermassen bestimmt:

1 g fetthaltiges, bzw. 0,6345 g entfettetes lyophiliertes Samenmehl wurden mit 5 ml Wasser und 100 ml peroxydfreiem Aether zwei Stunden geschüttelt. In der Aetherphase wurde die p-HBI-Konzentration und in der wässrigen Phase die Rhodanidkonzentration nach Kapitel 341. bestimmt.

Die Resultate sind in Tabelle VI zusammengestellt (Seite 46).

Den Herren dipl. Ing.-Agronomen K. Herrmann und G. Hrazdina sowie den Laboranten R. Wehrli und A. Walter † danke ich für ihre wertvolle Mitarbeit.

4. ZUSAMMENFASSUNG

1. p-Hydroxybenzyl-isothiocyanat (p-HBI), das scharfe Prinzip des weissen Senfsamen (Sinapis alba L.) wurde erstmals in Form langer, weisser Nadeln (Smp. 42° C) sowohl durch Synthese aus p-Hydroxybenzylamin (Ausbeute 10,3 %) als auch durch Extraktion und anschliessende präparative Säulenchromatographie aus fermentierten Senfsamen gewonnen.

2. Das p-HBI ist eine sehr instabile Verbindung, es hydrolisiert in jedem pH-Bereich primär zu p-Hydroxybenzylalkohol und Rhodanid. Der Reaktionsmechanismus der Hydrolyse ist analog demjenigen der hypothetischen Isothiocyanate, die intermediär aus Glucobrassicin resp. Neoglucobrassicin entstehen.

3. Die Hydrolyse des p-HBI wurde in wässrigen, fetthaltigen und entfetteten Samenmehl-Suspensionen quantitativ verfolgt. Beim fetthaltigen Samenmehl betrug die Hydrolyse nach 24 Stunden 30 %, beim entfetteten Samen dagegen 65 %. Nach 10 Tagen betrug die Hydrolyse in beiden Fällen über 90 % und nach 20 Tagen enthielten die beiden Suspensionen praktisch kein p-HBI mehr.

4. Das untersuchte Samenmehl enthielt neben 36,5 % Fett 5,2 - 5,7 % Senfölglucosid (p-Hydroxybenzylglucosinolat), daraus bilden sich bei der enzymatischen Spaltung theoretisch 2 - 2,2 % p-HBI.

5. Der Senfölglucosid-Gehalt wurde durch Titration des enzymatisch freigesetzten Hydrogensulfats sowie durch Bestimmung des gebildeten p-HBI ermittelt.

6. Das Samenmehl wurde nach einem Kaltextraktionsverfahren entfettet und entglucosidiert. Das auf diese Weise extrahierte Samenmehl besass noch die volle Myrosinase-Aktivität. Aus dem entfetteten Samenmehl wurden hochaktive Myrosinase-Trockenpräparate sowie Sinapin- und Kalium-p-hydroxybenzylglucosinolat dargestellt. Fermentation und anschliessende Lyophilisierung ergab ein sehr scharfes, trockenes Gewürzpulver in dem das p-HBI monatelange stabil blieb.

7. Die physiologische Wirkung der Zersetzungsprodukte des p-HBI wurde diskutiert. Die Verwendung des Samenmehls von Sinapis alba L. zur Tafelsenfherstellung sollte wegen der leichten Zersetzlichkeit des p-HBI und der Rhodanidbildung neu überprüft werden.

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LEBENSLAUF

Am 8. März 1935 wurde ich in Budapest, Ungarn, geboren. Hier besuchte ich die Primarschule und das Gymnasium, welches ich mit der Maturitätsprüfung im Frühling 1953 abschloss.

Nach einjähriger Tätigkeit im Staatlichen Forschungsinstitut für das Leder-, Schuh- und Pelzgewerbe erhielt ich das Diplom als Laborant und begann im Herbst 1954 mit dem Studium an der Agrarwissenschaftlichen Universität in Budapest.

Im Herbst 1955 setzte ich mein Studium an der Hochschule für Garten- und Weinbau, Budapest, fort und zu Beginn des fünften Semesters im November 1956 flüchtete ich in die Schweiz, wo ich unmittelbar das Studium an der Abteilung für Chemie der Eidgenössischen Technischen Hochschule, Zürich, begann.

Im Frühling 1961 erwarb ich das Diplom eines Ingenieur-Chemikers und arbeitete seither als wissenschaftlicher Mitarbeiter am Agrikulturchemischen Institut der Eidgenössischen Technischen Hochschule, wo ich die vorliegende Promotionsarbeit ausführte und im Frühling 1964 abschloss.

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THE BLOOD CYANATES IN THE TREATMENT OF HYPERTENSION

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A few years ago I gave a number of patients with hypertension potassium or sodium thiocyanate with results that were generally unsatisfactory. Some showed extreme weakness, nausea and dizziness, while an occasional one seemed to be considerably improved as far as the symptoms and blood pressure level were concerned. In general, it seemed that older patients or those who had a blood pressure elevation over a long period of time seemed to tolerate cyanate therapy less well than the younger group. Careful observation indicated that individual dosage was necessarily dependent on the individual response and the toxicity. An attempt has been made, therefore, to gage the dosage by a study of the cyanate clearance from the body through the urine and a correlation by the blood cyanate level and the blood pressure. The following material is being presented as a preliminary report on such observations extending over a period of four years.

LITERATURE

The pharmacology of the cyanates is very little understood. Claude Bernard[1] made the first observations, which were reported in 1857, and he regarded them as a muscle poison which abolished muscular

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activity. Pauli[2] and LeRoy[3] independently noted decreases in blood pressure while studying the cyanates. Nichols[4] reviewed the pharmacologic and therapeutic properties of thiocyanates in 1925. Schreiber[5] showed that it took from two to three weeks for cyanates to return to normal levels in the saliva and in the blood after the administration was discontinued. He indicated that toxic manifestations in the normal individual appeared when the blood cyanates reached 40 or 50 mg. More recently Healy[6] has shown that the cut surface of the adrenal body was strongly positive for the thiocyanates in the cortical portion when potassium thiocyanate was administered to rabbits, which suggested the possibility of the accumulation of the drug in the adrenal. Smith and Rudolf[7] administered sodium thiocyanate to normal individuals. The cyanate was stopped when the blood pressure fell below 100, and none complained of symptoms. From one to eight weeks' time was required for the blood pressure to return to the previous normal level.

A review of the clinical literature brings a great divergence of opinion on the value of the administration of cyanates in hypertension. Goldring and Chasis[8] noted a constant relation between the persistence of the hypotensive effect and the amount of thiocyanate administered. They cautioned that the dose should not exceed 5 grains (0.3 Gm.) daily and that the drug should be discontinued at the first indication of nausea, fatigue or vomiting or with the first distinct fall in blood pressure. Contrary to opinions generally expressed, these authors feel that thiocyanate carefully administered is just as effective and no more apt to produce toxicity in the patient with glomerulonephritis than in hypertension. Their reports on fatal cases, however, show no antemortem blood studies for cyanates. Representative toxic manifestations have been reported by Palmer and Sprague[9] and others.[10] Some[11] are strenuously opposed to the administration of cyanates. Others[12] report no toxic effects in patients closely observed. A review of the individual protocols of dosages reported by the various authors indicates that patients given larger doses more regularly suffered toxic manifestations, while those who gave small doses reported little or no toxicity and often no decrease in blood pressure.

MATERIAL AND METHOD

This report covers observations on forty-five patients with systolic blood pressures well over 200, who have been personally studied in the renal clinic and in private practice during a period of from one to four years. The technic consisted in selecting patients who had been seen regularly and whose blood pressures were followed through periods of from one to four years on various forms of therapy. This should familiarize one with individual variations, influence of seasonal changes and the like, or factors that are so important in evaluating any therapy in this group, as emphasized by Ayman,[13] Davis[14] and others. The patients have been repeatedly studied from the cardiorenal-vascular standpoint, but the details of this work are too extensive to be included here.

It was thought that the factor of individual variations in toxicity might be avoided or controlled by following the blood cyanate level. If so, it was thought that a dose of cyanate might be attained which would reduce blood pressure without causing toxic symptoms.

A modification of Schreiber's[5] technic for the determination of thiocyanates in the blood was developed, which has been simple, once the following standards were made up:

THE ESTIMATION OF THIOCYANATES IN THE BLOOD

Solutions.—1. Ten per cent trichloroacetic acid solution.

2. Ferric nitrate reagent. Dissolve 50 Gm. of crystallized ferric nitrate in 500 cc. of distilled water. Add 25 cc. of concentrated nitric acid and make up to 1 liter with distilled water.

3. Thiocyanate standards: Stock solution. Dissolve about 1 Gm. of potassium thiocyanate in 800 cc. of distilled water. Titrate a 20 cc. portion of a standard silver nitrate solution (made by dissolving exactly 2.9195 Gm. of silver nitrate in 1 liter of distilled water) acidified with 5 cc. of concentrated nitric acid, with the potassium thiocyanate solution, using ferric ammonium sulfate as an indicator. Calculate the amount of water which it will be necessary to add to the potassium thiocyanate solution to make 20 cc. equivalent to 20 cc. of silver nitrate solution. Add the calculated amount of water, mix thoroughly and check the solution by another titration to make sure the potassium thiocyanate solution is exactly equivalent to the silver nitrate solution.

Standard solutions. Make three dilutions of the stock solution to give the following three standards: (1) 100 cc. of stock diluted to 1 liter with water gives a standard which contains 0.5 mg. of the thiocyanate ion in 5 cc. of solution. (2) 70 cc. of stock diluted to 1 liter with water gives a standard which contains 0.35 mg. of the thiocyanate ion in 5 cc. of solution. (3) 40 cc. of stock diluted in 1 liter with water gives a standard which contains 0.2 mg. of the thiocyanate ion in 5 cc. of solution.

Method.—Transfer 5 cc. of the 10 per cent trichloroacetic acid solution to a test tube. Add 5 cc. of serum or plasma. Stopper and shake well. Allow to stand from ten to fifteen minutes. Filter through a small filter paper. The filtrate should be perfectly clear. If it is not, filter again through the same filter paper. Measure 5 cc. of the filtrate into a clean, dry test tube. Add 1 cc. of the ferric nitrate reagent: Mix and read in a colorimeter with the standard solution set at 20 mm., choosing that standard which most nearly matches the unknown. The standards are made as follows: Transfer 5 cc. of each of the three standard solutions to three test tubes. Add 5 cc. of trichloroacetic acid solution and 2 cc. of the ferric nitrate reagent to each. Mix.

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Calculation.—With the standard solution set at 20 mm. for the colorimetric comparison the calculation may be simplified to the three following forms, depending on the strength of the standard.

1. Using the 0.5 mg. standard, 200/reading-mg. of the thiocyanate ion in 100 cc. of serum.
2. Using the 0.35 mg. standard, 140/reading-mg. of the thiocyanate ion in 100 cc. of serum.
3. Using the 0.2 mg. standard, 80/reading-mg. of the thiocyanate ion in 100 cc. of serum.

Before the patient was started on cyanate, all therapy was discontinued and control observations for cyanates in the blood and urine were made. The patients were then given 0.3 Gm. of potassium or sodium thiocyanate daily. They were seen twice a week for the first two weeks and once a week thereafter until an equilibrium between the dosage of cyanate and blood pressure was established. Blood cyanate determinations were made at each visit. A number of patients were hospitalized and then given doses of from 0.3 to 1 Gm. daily for a number of days, until sharp falls in blood pressure or toxicity were noted. Daily cyanate determinations were made on the blood and urine of this group. The urine clearance of cyanates varied greatly, and that feature will be discussed at a later date as a factor of individual tolerance. In the main, no clear-cut information has been gained from the urine clearance alone, but there was a fairly good correlation between the blood cyanates, the toxicity and the reduction of the blood pressure.

RESULTS AND COMMENT

Whenever the cyanates in the blood were raised above 5 or 10 mg., a fall in the systolic and diastolic blood pressures occurred in thirty-five of the forty-five patients studied. Slight toxic manifestations, namely, weakness, ease of fatigue and dizziness, were noted in many of these patients but were not especially disturbing until the blood cyanates were raised above 10

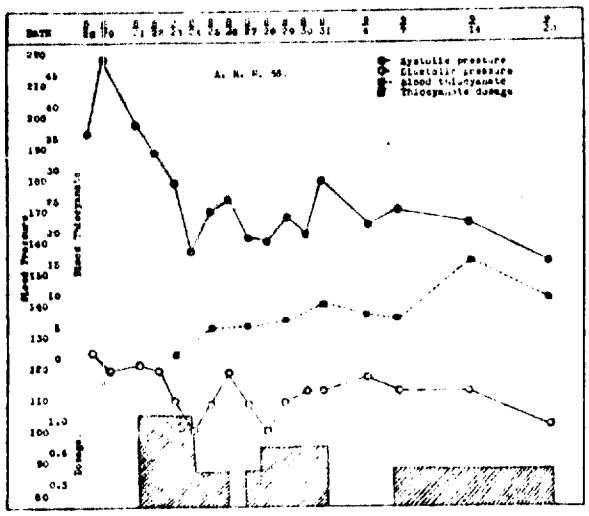

Chart 1.—Clinical course in case 1.

or 15 mg. Toxicity increased rapidly above the blood level of 20 mg., but serious manifestations were not noted until levels from 35 to 50 mg. were reached. From the standpoint of the relief of symptoms and the drop in blood pressure, it seemed that a blood cyanate level from 6 to 10 mg. was ordinarily required. The dosage was found to be individual in each case. For example, one patient required a dosage of only 60 mg., while another required 720 mg. a day to maintain a blood cyanate level of 10 mg. As the cyanate clearance through the kidney improved, the dosage had to be gradually increased during the weeks that followed, if the blood pressure and the blood cyanate levels were to be maintained.

It will be impossible to go into the many details incident to the observations and care of this group of

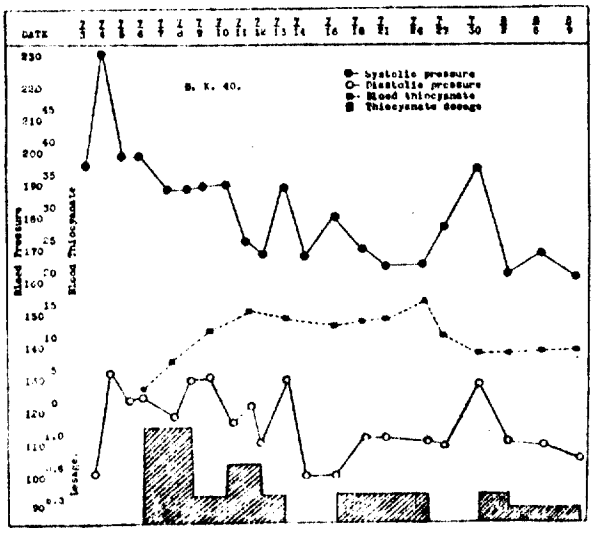

Chart 2.—Clinical course in case 2.

patients, but charts 1 and 2 will indicate typical experiences in general.

Early in the study of two of these patients, severe weakness, vascular collapse and cerebral thrombosis with ultimate recovery was experienced. Both patients had had severe hypertension of long standing and their pressures ranged from 250 to 275 most of the time. The neurologic evidence of thrombosis occurred twelve and thirty-six hours after the vascular accidents were noted. In both of these patients the blood cyanates were found to have risen sharply to levels of 33 and 45 mg. respectively on doses considerably under that recommended in the literature. Without the blood cyanate observations, no doubt such responses would have been regarded as merely intolerance to the drug rather than to an actual overdosage. Such experiences also cause me to suspect that deaths may have occurred in the past, in the course of cyanate therapy, which may have been attributed to the vascular accidents common to the patient with vascular disease (charts 3 and 4). Two other patients were carried to 35 mg. per hundred cubic centimeters without collapse. One complained of much fatigue with only slight reduction of the blood pressure, while the other one complained of great fatigue and somnolence associated with a sharp fall in the blood pressure (chart 5). The return of the blood pressure to the previous high levels lagged behind the reduction of the blood cyanates. This experiment was repeated several times, and the blood pressure was now maintained at from 150 to 180 mm. by a dosage of potassium thiocyanate, which maintains a blood cyanate of 10 mg.

Of the forty-five patients studied with the blood cyanate level controlled, no two have been found that were comparable. Thirty-five of the forty-five have responded with respect to symptoms and blood pressure levels in essentially the following way: A dosage of 0.3 Gm. a day usually was associated with a decrease in nervousness, diminution of headaches and often a beginning fall of blood pressure in from five to seven

days. At this time the blood cyanates were generally found to be between 5 and 7 mg. per hundred cubic centimeters. The patients then frequently complained of fatigue. Insomnia often changed to somnolence and the blood pressure generally fell from 30 to 50 mm. in the first ten or fifteen days. At that time the blood cyanates were commonly found to be between 8 and 10 mg. per hundred cubic centimeters. To prevent elevations over 10 mg. the dosage was now decreased to 0.3 Gm. three or four times a week. If the blood cyanates were then found to be 10 mg. or over, the administration was discontinued, because, in the instances in which the blood cyanate level rose above 15 mg., increasing symptoms of toxicity were noted. A peculiar aching of the legs and body disturbed an occasional long standing case. Quite a number of patients commented on their increased urinary output. Some of this group had congestive heart failure so that such a diuresis was associated with a return of compensation and loss of edema. A reduction in the size of the heart of four patients was noted. Such responses indicate the importance of the reduction of the load on the cardiac mechanism. One young patient with severe hypertension now under control has noted a great increase in seminal fluid formation, which has persisted for several months. In some patients treated over a long period a severe anemia has developed.

As reported by Borg,[15] this study has not revealed any difference between the sodium and the potassium salt. The toxic manifestations, hypotensive effect and blood levels for these two salts have been essentially the same. No skin manifestations have been noted to date. Three patients have shown a peculiar myxedematous swelling of the tissues of the face, orbital areas and cervical regions. One occurred in a woman after one year and the other occurred in a woman after fifteen months of the administration of cyanate. In the latter

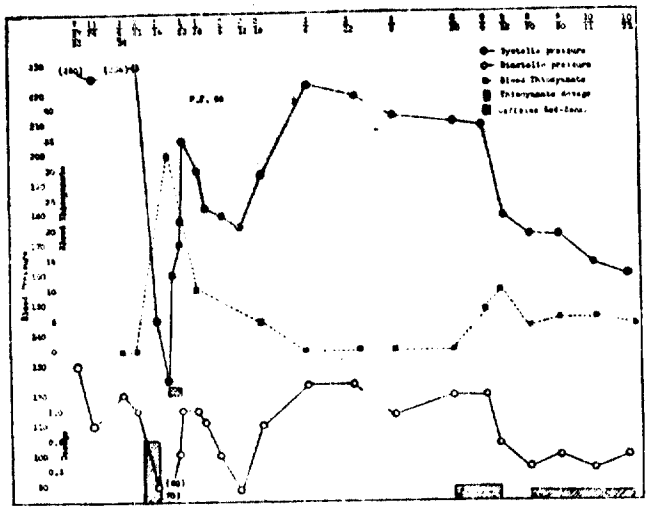

Chart 3.—Clinical course in case 3.

a large thyroid, hoarseness, swollen face and heavy jowls developed. The basal metabolic rates were only slightly reduced (—18, —9). In one man a diffusely enlarged thyroid gland developed after ten months' administration of potassium thiocyanates. His basal metabolism had fallen from +19 to —9. The enlarged thyroids returned to normal size on the administration of desiccated thyroid. Such observations have questionable relation to the cyanate therapy, but they cause one to be alert for other evidences of possible endocrine effect. One cannot help recalling the diffuse thyroid enlargement noted in rabbits after the feeding of cabbage, which has been considered to be possibly of cyanate origin.

Ten of the forty-five patients showed little or no response to cyanate therapy. Two of this group

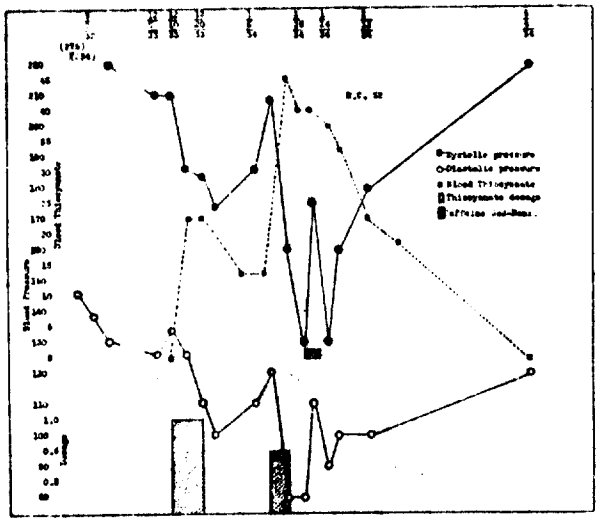

Chart 4.—Clinical course in case 4.

required larger doses (0.6-1 Gm. daily) to maintain the blood cyanates over 8 mg. No relief of blood pressure or its attendant symptoms could be otherwise obtained. Symptoms of toxicity, especially fatigue, often were as annoying as those of the hypertension. Although occasionally one of these patients felt better on such doses, it has been difficult to keep the pressure below 200 mm. Attempts to effect a further reduction were attended by toxic manifestations, and the cessation of cyanate therapy was soon followed by a return of the blood pressure to its former levels of from 230 to 280, with all the old symptoms. Three of this group showed no immediate response, but, on a dosage sufficient to maintain a blood cyanate level of from 9 to 15 mg. for from three to four months, a cessation of the fluctuations to high levels was noted. In these patients the systolic pressure appeared to stabilize at the lower level; namely, about 200 mm. for a time and then a gradual reduction of both systolic and diastolic levels occurred, so that these three patients are now maintained around 150 or 170 mm. The remaining five patients showed no response other than toxic manifestations of a severe degree. Although the reason for the patients to fail to respond to cyanate therapy is not clear, it was evident that the most resistant cases presented well advanced arteriosclerosis. Some older patients with severe hypertension of several years' standing were "cyanate sensitive" and have been almost as easily stabilized as any of the younger nonsclerotic group.

In general, if the patient is found to be able to tolerate the cyanates it seems much more satisfactory to effect a gradual reduction of the blood pressure so that he may become adjusted to the change. After the blood pressure has been maintained at a lower level for from one to three months, a great improvement of the patients' symptoms is generally noted. The first period of weakness passes and a feeling of well being and a return of energy follow. Although the complications are many and varied, the benefits derived in those

15. Borg, J. F.: Experiences in the Use of Sulphocyanates, Minnesota Med. 13:293 (May) 1930.

responding favorably, as manifested by a decrease in blood pressure with the relief of subjective symptoms and congestive heart failure together with the improvement of urea and uric acid clearance, the reduction of total serum proteins and phenols in the blood so frequently noted would indicate that the cyanates are worthy of further study.

SUMMARY

Forty-five patients with hypertension have been given sodium or potassium thiocyanate and the concentration of the cyanates in their blood has been followed. The reduction of blood pressure and the relief of symptoms obtained in thirty-five of the forty-five roughly corresponded to the level of the cyanates in the blood. The optimum therapeutic level would seem to range between 8 and 12 mg. per hundred cubic centimeters and significant toxicity begins to appear at from 15 to 30 mg. The individual tolerance varies greatly, the different levels being obtained with widely varying doses. The cyanates may reach hazardous concentrations very quickly in some individuals, so that the administration of the thiocyanates is believed to be dangerous unless controlled by close observation and blood cyanate determinations.

REPORT OF CASES

Case 1.—A. H. M., a man, aged 56, an executive under observation for three years, complained of nervousness, heart consciousness, tremor and occipital headaches. Blood pressure

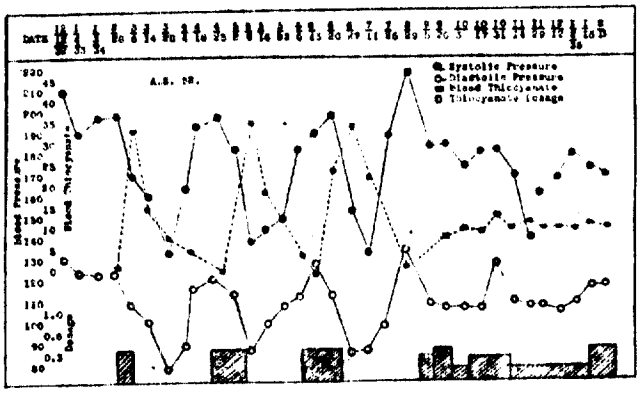

Chart 5.—Clinical course in case 5.

fluctuations were noted between 190 and 230 systolic and 120 and 130 diastolic, the average being 200/120. A dosage of 1 Gm. of potassium thiocyanate for four days was followed by a drop in both systolic and diastolic pressure. A fluctuation of readings was noted for a few days, followed by a leveling of the pressure at about 165/110 on 0.6 Gm. (chart 1). During the past two years his blood pressure has been maintained between 155 and 170 systolic and 90 and 100 diastolic on a dosage which maintains the blood cyanates at about 10 mg. A complete relief of symptoms was noted after the first three months of cyanate therapy. His maintenance dose is between 2 and 3 Gm. a week. Two attempts at stopping the drug were associated with a return of blood pressure elevation and symptoms after about four weeks.

Case 2.—B. K., a housewife, aged 40, complained of severe pounding occipitofrontal headaches, dizziness, ringing in the ears, nervousness, insomnia, emotional instability, heart consciousness and weight loss. A known hypertension for five years and personal observation for one year revealed a blood pressure of 195-230 systolic and 100-130 diastolic, the average being 215/120. A dosage of 1 Gm. of potassium thiocyanate for two days was reduced to 0.3 Gm., and in sixteen days the fluctuant period had passed and the patient's blood pressure was rather constant at 165 systolic and 110 diastolic (chart 2). She was most grateful, because she was now sleeping very well and was entirely free from headaches. The blood cyanates increased to 15 mg. by the nineteenth day after the cyanate therapy was started and she started to complain of fatigue and somnolence. The cyanates were discontinued for one week and a return of pressure began, which was again reduced with readministration of the drug. The maintenance dose was found to be 0.2 Gm. of potassium thiocyanate for the next two months and during the next eight months was 0.3 Gm. daily. The blood cyanate level noted at the optimum blood pressure readings was between 8 and 10 mg.

Case 3.—F. P., a man, aged 68, a retired broker, admitted to the hospital Aug. 27, 1933, had had a severe hypertension for a known duration of five years, with moderately severe congestive failure. A diuretic regimen of low sodium diet, ammonium nitrate, digitalis and mercurials brought him to a fair circulatory balance, but as soon as he was allowed up or out of the hospital the congestive failure returned. The blood pressure ranged between 238 and 250 systolic after six months of care. He was markedly sclerotic but it was decided to try cyanate therapy. He was given 0.6 Gm. of potassium thiocyanate and on the fourth day he became pale, very weak and confused, and the blood pressure fell to 124/70 within a few hours. A cerebral thrombosis with a right hemiplegia, loss of speech and difficulty in swallowing came twelve hours later. The blood cyanates were found to be 33 mg. Caffeine with sodium benzoate in 5 grain (0.3 Gm.) doses every two hours seemed to revive the vascular tone (chart 3, 1/16/34). The cerebral lesion gradually cleared and the blood cyanates returned to normal in fifty days. The blood pressure returned gradually to somewhat over 200 and the heart failure reappeared. A cautious resumption of a dosage of 0.2 Gm. of potassium thiocyanate again caused a sharp fall of the blood pressure and later the maintenance dose was found to be 0.1 Gm. Renal clearance has improved so that one year later he requires 0.5 Gm. potassium thiocyanate daily to maintain a blood cyanate of 8 to 10 mg. He seems to be in splendid health without diet, rest or any other form of therapy. The blood pressure averages 160/100.

Case 4.—R. C., an executive, aged 52, who had had a severe hypertension of seven years' duration, had had a cerebral hemorrhage with slight residuals five years before this study was made. Personal observation of two years had shown blood pressure fluctuations of 240-300 systolic and 140-170 diastolic, and a moderately severe congestive heart failure was present much of the time despite energetic therapy. Sedatives and venesections reduced the average pressure to 210/130 on three periods of hospitalization. Within two or three weeks after the patient resumed activity the blood pressure would be found at the previous high levels. He was again hospitalized and, after his blood pressure seemed stabilized, ten doses of 1 Gm. of potassium thiocyanate administered on consecutive days were associated with a significant drop of the pressure (185/110). The medication was stopped and the blood pressure soon began to return to its former level. After discharge from the hospital he was instructed to take 0.6 Gm. of potassium thiocyanate daily and to return biweekly for observation. The patient drank the medication directly from the bottle without measuring the dosage and he was found at home in vascular collapse (chart 4, 1/16/34) with a blood pressure of 128/80. Large doses of caffeine with sodium benzoate seemed to revive him greatly and the blood pressure rose much after the manner noted following caffeine administration in quinidine intoxication. His blood cyanates were found to be 45 mg. and it required nearly four months for them to return to normal. During the first half of this recovery period the patient was disoriented, confused and extremely weak. There was a marked defect of speech. The return of the blood pressure with resistant heart failure caused a cautious resumption of the thiocyanates. At first 0.3 Gm. a week was sufficient to maintain a 10 mg. blood cyanate level and an associated blood pressure of 180-200 systolic and 90-110 diastolic. The renal cyanate clearance has improved in the past year so that it is necessary to give 3 Gm. a week to maintain the blood cyanate level of 10 mg. During this period the heart failure has not returned and the pressure remains slightly under 200/110, which he seems able to sustain without untoward effects.

Case 5.—A. S., a woman, aged 52, unemployed, had had a known hypertension of sixteen years' duration. She had been

under my personal observation for two years prior to this study. Her symptoms were headache, insomnia, emotional instability, nocturia and chronic congestive heart failure. Her record is striking in that she was "cyanate sensitive," and the blood cyanate and the blood pressure curves are quite reciprocal. At first a dosage of 0.3 Gm. would raise the blood cyanates to 35 mg. and the blood pressure would drop sharply. This fall continued for about three weeks after the drug had been discontinued (chart 5). Extreme fatigue was associated with elevations of blood cyanate over 15 mg. Renal clearance of cyanates gradually improved so that as time went on the dosage period required to raise the blood cyanates to the previous level of 35 mg. had to be increased. Chart 5 shows this relation clearly, and it also shows that the patient had now reached a continuous dosage of 0.3 Gm. of potassium thiocyanate daily in order to maintain a blood cyanate of 10 mg. This level of blood cyanates has continued to be associated with a blood pressure of 170 systolic and 110 diastolic most of the time. It is noteworthy that the patient has been without any dietary program or cardiac therapy for one year and feels quite well. She sleeps well and suffers no more headaches, and the emotional state is normal.

303 East Superior Street.

Food Res. 5:147-152. 1940.

BACTERICIDAL PROPERTIES OF ALLYL ISOTHIOCYANATE AND RELATED OILS

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Walton, Herbold, and Lindegren (1936) and Lovell (1937) showed that the vapors from crushed garlic and onions contained bactericidal substances. Foter and Golick (1938) reported the same property for the vapors from crushed horse-radish. Vollrath, Walton, and Lindegren (1937) and Ingersoll, Vollrath, Scott, and Lindegren (1938) attributed the bactericidal effects of the vapors from crushed garlic and onions to acrolein and crotonaldehyde.

In an attempt to attribute the inhibitory properties of the vapors from crushed horse-radish to some definite compound or compounds, a study was made of the literature relative to its composition. Chemical analyses of crushed horse-radish reported by Gildemeister and Hoffmann (1916) and by Heiduschka and Zwergal (1931) show the main volatile constituent to be mustard oil, allyl isothiocyanate ($CH_2{=}CHCH_2NCS$), 20 to 25 per cent phenyl ethyl isothiocyanate ($C_6H_5{-}CH_2CH_2NCS$), and a trace of phenyl propyl isothiocyanate ($C_6H_5{-}[CH_2]_3NCS$). This report is concerned with a study of the bactericidal effects of allyl isothiocyanate and two other oils similar in structure, namely, methyl isothiocyanate (CH_3NCS) and ethyl isothiocyanate (CH_3CH_2NCS). An attempt is being made at the present time to synthesize the other isothiocyanates reported in crushed horse-radish.

EXPERIMENTAL PROCEDURE

The bactericidal properties of the oils were studied by three different methods, using 10 test organisms.

Method 1: Agar plates were poured using approximately 15 ml. of a suitable medium and the agar was allowed to solidify. The plates were inverted and sterile filter paper placed in the tops. Various amounts of the oils were placed on the filter paper, the plates were sealed, and the agar was exposed to the vapors from the oils at 37°C.(98.6°F.) for varying lengths of time (Table 1). After the exposure periods the covers of the Petri plates were removed and replaced by sterile tops and immediately streaked, using a standard four-mm. loopful of a 24- to 48-hour broth culture of the test organism as the inoculum. The plates were then resealed and incubated

TABLE 1

Growth Score of Organisms on Agar Plates After Exposure to Oil Vapors

Organism	Oil	Allyl isothiocyanate						Methyl isothiocyanate						Ethyl isothiocyanate					
		0	8[2]	16	32	60	120	0	8	16	32	60	120	0	8	16	32	60	120
	ml.																		
Serratia marcescens	.1	4	0[3]	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	0	0	0	0	0	4	0	0	0	0	0	4	4	3.5	3	3	2.5
	.001	4	4	3	3	3	2	4	4	4	3	2	1	4	4	4	4	4	4
	.0001	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4
Bacillus subtilis	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	0	0	0	0	0	4	0	0	0	0	0	4	4	3	3	3	2
	.001	4	4	4	3	2.5	2	4	4	3	2.5	2	1	4	4	4	3.5	3	3
	.0001	4	4	4	4	3	3	4	4	4	4	4	4	4	4	4	4	3.5	3
Escherichia coli	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	2	2	1	1	0	4	0	0	0	0	0	4	4	4	4	4	4
	.001	4	4	4	3	3	2	4	4	4	4	4	4	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Bacillus mycoides	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	2	0	0	0	0	4	0	0	0	0	0	4	4	2	2	2	2
	.001	4	4	4	4	3.5	3	4	3	2.5	1	1	0	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
MRX-1[1]	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	0	0	0	0	0	4	0	0	0	0	0	4	2	2	2	2	2
	.001	4	4	3	3	2	1	4	4	4	4	3.5	3	4	3.5	3	2.5	2	2
	.0001	4	4	4	4	4	4	4	4	4	4	4	3	4	4	4	4	4	4
Eberthella typhosa	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	0	0	0	0	0	4	0	0	0	0	0	4	4	4	4	4	4
	.001	4	4	4	4	4	4	4	4	3.5	2.5	3	1.5	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Staphylococcus aureus	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	4	4	4	4	4	4	0	0	0	0	0	4	4	4	4	4	4
	.001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mycobacterium tuberculosis var. *hominis*	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	4	4	3	3	3	4	0	0	0	0	0	4	4	4	4	4	4
	.001	4	4	4	4	4	3	4	4	4	4	4	4	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mycobacterium tuberculosis var. *bovis*	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	3	3	3	0	0	4	0	0	0	0	0	4	4	4	4	4	4
	.001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
Mycobacterium phlei	.1	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
	.01	4	3	2	2	1	1	4	0	0	0	0	0	4	4	3.5	3	3	1
	.001	4	4	4	3	3	3	4	4	4	4	4	4	4	4	4	4	4	4
	.0001	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4

[1] Isolated from cases of rat pneumonia. Probably identical with *Bacillus bronchisepticus*. [2] The number at the top of each column indicates the time of exposure in minutes. [3] Each number represents an average of two to three experiments.

at 37°C. for at least a week. Controls were made in all experiments.

A comparative scale was used in estimating the amount of growth after the incubation period by which 4 indicated full growth as in the controls, 3 indicated from three-fourths to full growth, 2 indicated one-half to three-quarters of full growth, and 1 indicated any growth to one-half of full growth.

Method 2: Dilutions of the oils, from 1:100 to 1:1,000,000, were prepared in a suitable agar medium. Plates were poured and streaked, using again a standard four-mm. loopful of a 24- to 48-hour broth culture of the test organism. The plates were sealed and incubated at 37°C. for one week, after which they were scored for growth as described above.

Method 3: In order to determine the approximate times required for killing and the effect of the presence of organic matter on bactericidal efficiency of the oils, two series each of aqueous suspensions of the oils were prepared in dilutions from 1:000 to 1:1,000,000. One series contained five per cent by volume of sterile cow serum. The aqueous suspensions were brought to a temperature of 37°C., after which each tube containing 10 ml. was inoculated with .1 ml. of a 24- to 48-hour broth culture of the test organism. The cotton plugs were then replaced with sterile rubber stoppers to prevent loss of the oils by vaporization and the tubes were incubated at 37°C. At various time intervals (.5, 1, 2, 4, 6, 8, 12, 24, 48, and 72 hours) standard four-mm. loop transfers were made from the dilutions to suitable sterile broth in tubes. These in turn were reincubated for at least a week before the results were recorded. Inoculated sterile distilled water controls with and without cow serum were made.

Attempts were made to prepare emulsions of the oils rather than suspensions, using various emulsifying agents with little success. Most of the emulsifying agents were themselves bactericidal for the test organisms. In all the methods where the growth of the test organism in broth did not yield uniform suspensions of cells for inoculations, the broth culture was transferred to a sterile glass-stoppered bottle containing glass beads, shaken vigorously, and filtered through sterile cotton.

RESULTS

The bactericidal effects of vapors from the volatile oils tested (Table 1) show that complete or marked inhibition of growth resulted in most cases when agar plates were exposed to the vapors from .1 ml. or .01 ml. of the oil for as short a time as eight minutes. Vapors from allyl isothiocyanate, the main volatile constituent found in crushed horse-radish, inhibit the growth of microörganisms in a manner similar to the vapors from crushed horse-radish itself. The vapors from

methyl isothiocyanate appear to be the most effective, those from ethyl isothiocyanate the least effective.

The bactericidal efficiency of the oils was more evident when exact dilutions were prepared in agar (Table 2). Complete or marked inhibition of growth of the test organisms resulted in most cases from dilutions as high as 1:100,000 and 1:1,000,000. Ethyl isothiocyanate was again the least effective of the oils.

Three test organisms were employed in the study of the oils by Method 3, *Escherichia coli*, *Bacillus subtilis*, and *Mycobacterium tuberculosis* var. *hominis*. In practically all cases the results confirm those obtained by Method 2. *Escherichia coli* was the most resistant test organism requiring a 24- to 48-hour exposure to a 1:10,000 dilution of the oils to obtain complete killing. Growth of the human strain of *Mycobacterium tuberculosis* was completely inhibited by a 48- to 72-hour exposure to dilutions of 1:100,000 or 1:1,000,000. *Bacillus subtilis* was the least resistant requiring only an eight- to 12-hour exposure to a 1:1,000,000 dilution of allyl and methyl isothiocyanate to obtain complete inhibition of growth. In all cases exposure to the 1:100 dilution of the oils for the shortest time inter-

TABLE 2

Bactericidal Effects of Oils Diluted in Agar

Organism	Allyl isothiocyanate						Methyl isothiocyanate						Ethyl isothiocyanate					
	Control	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	Control	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000	Control	1:100	1:1,000	1:10,000	1:100,000	1:1,000,000
Serratia marcescens	4	0[1]	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
Bacillus subtilis	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	1
Escherichia coli	4	0	0	1	1	1	4	0	0	0	0	1	4	0	0	2	4	4
Bacillus mycoides	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
MRX-1	4	0	0	0	3	4	4	0	0	0	0	0	4	0	0	0	4	4
Eberthella typhosa	4	0	0	0	0	0	4	0	0	0	0	0	4	0	0	0	0	0
Staphylococcus aureus	4	0	0	1	2	3	4	0	0	1	1	2	4	0	2	3	4	4
Mycobacterium tuberculosis var. *hominis*	4	0	0	0	0	2	4	0	0	0	0	0	4	0	0	0	0	1
Mycobacterium tuberculosis var. *bovis*	4	0	0	0	1	2	4	0	0	0	0	0	4	0	0	0	0	1
Mycobacterium phlei	4	0	0	0	0	1	4	0	0	0	0	0	4	0	0	0	0	0

[1] Each number represents an average of three to four experiments.

val, one-half hour, resulted in complete destruction of the cells. The presence of the five-per cent sterile cow serum slightly inhibited the bactericidal efficiency of the oils.

DISCUSSION

The apparent discrepancy in efficiency of the oils on the test organisms when agar plates are exposed to their vapors and when various dilutions of the oils are prepared in agar or aqueous suspensions may best be explained by the fact that when agar plates are exposed to the vapors, the oil is at first concentrated at the surface. During incubation the oils diffuse through the agar and concentration at the surface becomes too low to inhibit growth.

The test organisms employed differ in their resistance to the oils. *Escherichia coli* and *Staphylococcus aureus* appear to be the most resistant; while *Serratia marcescens*, *Bacillus subtilis*, and *Bacillus mycoides* are the least, with the other test organisms between these.

CONCLUSIONS

1. Allyl isothiocyanate, the main volatile constituent found in crushed horse-radish, and the related compounds, methyl and ethyl isothiocyanate, exhibit bactericidal effects on a variety of organisms when tested by several methods. The organisms employed differ in their resistance to these compounds.

2. The bactericidal efficiency of the oils may be more accurately determined by dilution in agar or by the preparation of aqueous suspensions than by exposure of agar plates to the vapors from similar dilutions.

3. The presence of five per cent by volume of sterile cow serum slightly interferes with the bactericidal efficiency of the oils.

4. Allyl isothiocyanate is a pungent volatile oil which irritates the eyes and burns the skin. Its presence in crushed horse-radish, and identification by several workers, probably explains the characteristic effects obtained when handling this substance and also the inhibitory properties of its vapors on microörganisms.

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The Metabolism of the Thiocyanate Ion

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The thiocyanate ion is a biogenic compound which is found in the body, although in very slight amounts. It is excreted and found in the blood in large amounts in poisoning by cyanide and hydrocyanic acid. Thiosulphate, pure sulphur, glutathione and cystine reduce the toxicity of cyanide in man and in animals and by the participation of these substances, cyanide is converted into the far less toxic thiocyanate. The cyanide ion must also be regarded as a biogenic substance, since it is found in the body even under normal conditions. Thiocyanate is formed from it in vivo and according to some of the latest results (Boxer and Rickards 1952, Goldstein and Rieders 1953), thiocyanate is broken down again into cyanide, so that, as a result of this interconversion, some of the pharmacological properties of thiocyanate are attributed to cyanide. The metabolism of thiocyanate should be regarded as being in very close functional connection with cyanide.

The thiocyanate ion has mild goitrogenic properties. Findings have been submitted demonstrating that in patients with hyperplasia of the thyroid gland, the level of the thiocyanate ion is increased (Šilink and Maršíková 1951). In view of the fact that the thiocyanate ion is found in many forms of food and that its seasonal variations resemble the variations of the ascorbic acid saturation curve, Šilink (1953) assumed that the thiocyanates originate exclusively in food and that they are indicators of an intake of more potent goitrogenic substances, or that they develop from these. The thiocyanate ion, however, is also increased in febrile diseases (Mayer 1904), in cancer (Saxl 1913) and its excretion does not fall to zero even in protracted fasting (Dezani 1918, Christomanos 1936). On the basis of a comparison of the conditions under which an increase in the level of thiocyanate in the blood or urine occurred, older authors also came to the conclusion that the metabolism of thiocyanate is bound to conditions of nitrogen metabolism.

It still remains to be determined how much thiocyanate is of exogenous and how much of endogenous origin. Older communications do not provide any definite standpoint and deviate substantially from one another according to the experimental conditions used. The investigation of this relationship is rendered difficult by the interconversion of cyanide and thiocyanate and this is probably the main cause of the lack of agreement hitherto. We have made an attempt to contribute to the solution of this question indirectly by a study of the metabolism of the thiocyanate ion in the following ways a): By conducting research for thiocyanogenic substances among structurally related compounds and other theoretically possible compounds, which could form a chain of precursors up to the development of the thiocyanate ion,

b) By research on the relationship of the level of thiocyanate in connection with the influence of the thyroid hormone on the endogenous metabolism of the organism,

c) By research on precursors of thiocyanate in food, with particular reference to cabbage as a widely consumed food with goitrogenic action.

Thiocyanogenic Substances in Food

In addition to a small amount of already formed thiocyanate, some foods contain substances from which thiocyanate develops in the organism. These are the cyanide ion already mentioned, which is also found only in small amounts, and substances form which cyanide is produced by the action of hydrolytic enzymes. As far as I am aware, no investigation has been made of whether thiocyanate is formed, for example, from amygdalin, although it is almost certain that this is the case. In our department an investigation was made of the formation of thiocyanate from the kernels of "cyanogenic" plants, such as apricots, plums and peaches and it was found that after the administration of these the excretion of thiocyanate shows a sharp increase.

Further, substances exist which do not split off cyanide on hydrolysis but are nevertheless a source of increased thiocyanate in the urine and in the blood. These are the nitriles of aliphatic and arylaliphatic acids (Lang 1894), contained in some cruciferous plants. To this family belong the mustard oils (isothiocyanates), the structure of which very closely resembles that of thiocyanate. A study was therefore made of the thiocyanogenicity of the isothiocyanates and an investigation made for a natural precursor of the thiocyanate ion in cabbage, the most widely consumed of the Brassica genus.

Investigation for Metabolic Precursors

The mechanism by which nitriles are converted into thiocyanate has not yet been explained, but conversion depends on thyroid secretion (Baumann, Sprinson and Metzger 1933). Further precursors have also been sought, which, structurally or functionally, could be linked up with the nitriles.

The increase in the level of thiocyanate in some pathological conditions which are characterised by changes in nitrogen metabolism, led to a search for the precursors of thiocyanate among the amino acids, the purines and some biogenic amines. No final conclusions have so far been drawn from the results, which are contradictory (Dezani 1917, 1918, Willanen 1906). Of other, non-biogenic substances which have been investigated as precursors of thiocyanate, mention may be made of the barbiturates; a higher level of thiocyanate in the blood has also been found after carbon monoxide and sulphur dioxide poisoning. Thiourea, however, does not cause an increase in the level of thiocyanate either in the urine or in the blood.

A search was made for substances which could lie between the nitriles and the amino acids or purines, which might be amines or amides; according to a working hypothesis, the chain of the development of thiocyanate from amino acids or purines could take place theoretically in the following manner:

$$\text{'SCN'} \leftarrow \text{HCN} \leftarrow \text{nitriles} \left\langle \begin{array}{l} \text{amides} \leftarrow ? \leftarrow \text{purines} \\ \text{amines} \leftarrow \text{amino acids} \rightleftarrows \text{proteins} \end{array} \right.$$

The formation of SCN' via HCN could, according to this hypothetical process, take place by simple reactions, by the removal of water, hydrogen etc. In this way, the [illegible] of compounds of the appropriate type was investigated.

Amino Acids as Thiocyanogenic Substances

It has been thought, already according to the results of older work, that the amino acids, as components of proteins, are a source of the thiocyanate ion, and their connection with the catabolism of nitrogen has also been pointed out. The thiocyanogenicity of amino acids has been investigated, but the results are contradictory. The demonstration of their thiocyanogenicity would show whether there is a disturbance in protein metabolism in conditions where the level of thiocyanate is increased. It would also be useful to investigate the thiocyanogenicity of amino acids in view of the fact that they are nitrogenous substances, which when broken down by oxidation, in vitro, under certain circumstances split off hydrocyanic acid as a subsidiary product of oxidation and could therefore form thiocyanate in other ways than that suggested above.

The Influence of the Thyroid Hormone on the Formation of Thiocyanate.

The results of the work of Baumann et al. (1933), Stöa (1952) and also of Šilink and Maršíková (1951) show a connection between the formation of thiocyanate and conditions in the thyroid gland. Since the state of the thyroid gland is manifested by changed conditions in the secretion of the thyroid hormone, which can influence the metabolism of thiocyanate, an investigation was made of the influence of this hormone on the production of thiocyanate. This investigation at the same time shows a relation to increased total metabolism in the organism and provides evidence of the variability of the endogenous component of thiocyanate metabolism. It was assumed that it would be possible to increase this component by thyroxine or thyroglobulin and demonstrate that thiocyanate is a catabolite of oxidation processes.

Methods

Rats were selected as the experimental animals. White rats of both sexes of the Zverex breed were used, with an average weight of about 100 g. Rats of the same sex were kept in pairs in metabolic cages with a wire floor and their combined urine was caught in a conical utensil lined with umaplex dissolved in chloroform and evaporated to form a surface not detergent for the urine. The values of the daily excreted amount of thiocyanate, therefore, correspond to that of two rats, but have been calculated for one rat. The urine then ran into a calibrated container through a glass funnel containing only a single layer of cellulose paper, so as to filter off the food remainders which fell through the netting. Control experiments showed that the values of thiocyanate were virtually unaffected by the extraction of these remnants from the urine as it flowed through. There should not, however, be many remnants, so as not to cause any considerable retention of the urine. The volume of the urine was measured and diuresis was observed daily.

Since all the urine of the experimental animals cannot be collected quantitatively, the values must be evaluated relatively and compared with those preceding the administration of the test substances. Losses are caused by small amounts remaining on the wire floor of the cage, the walls of the utensil and the filter with the food which has fallen through. In order to ascertain the character of the relationship between the amount of urine lost and the amount obtained, a correction curve was constructed in the following way: various amounts of urine were allowed to drip, in small quantities, into a cage and the quantity of the urine which dripped through was compared with the quantity of the original amount. The loss is linear and therefore the values of rat urine obtained were not corrected in any way. The urine was not rinsed out of the cage because of decreasing the concentration, which in most cases would have prevented the carrying out of the estimation.

The rats were kept in the metabolic cages for eight days before commencing the administration of the substances under investigation, so as to ascertain the normal level of excretion and to ensure stabilisation of the values. The temperature of the room was maintained at 19—22° C. Lighting conditions corresponded approximately to room conditions.

The rats were fed on a Larsen diet without lucerne, in daily amounts of 10 g with water ad lib. Several batches of the Zverex Larsen diet were used and the basic level of thiocyanate depended to some extent on the quantity of the green batch. Larger groups were therefore used, which had been

kept in a cool, dry, place, with a previously determined low content of Aldridge-positive substances in the standard extract.

The substances under investigation were administered in amounts marked in the individual experiments and were mixed with the food, unless stated otherwise. They were preparations of usual purity (a) degree of purity unspecified, b) purum, c) p. a.) and were not further purified; only a test was made for the presence of Aldridge-positive substances. The substances administered did not contain impurities in amounts which could cause a change in the excretion of thiocyanate. They were mixed into the food shortly before it was given to the rats. Fresh watery solutions of the appropriate substances were prepared for parenteral administration. It was assessed that 70 - 90% of the food was consumed, the remainder was spoilt by excrements and trodden on.

In experiments for determining the level of thiocyanate in the blood, the rats were kept in normal cages, in two groups. In the experimental group each rat was given one tablet of Thyreoglobulin forte Spofa daily in its food. The Thyreoglobulin forte was administered for 10 days and the animals were then exsanguinated and the thiocyanates in the blood determined.

The thiocyanates were determined in the urine and in the serum by a modification of the method of Aldridge (1944, 1945).

For the determination of thiocyanate in the urine, 4.5 ml. of a 5% solution of trichloracetic acid was added to 0.5 ml. urine and the mixture centrifuged: 2 ml. of the supernatant fluid was then taken and 0.2 ml. bromine water saturated at 20° C, 0.2 ml. 2% solution of sodium arsenite, 3.8 ml. pyridine reagent and 0.2 ml 2% solution of benzidine hydrochloride were added successively. The pyridine reagent was prepared by mixing 250 ml. of pure pyridine with 10 ml. conc. hydrochloric acid p. a. and made up to 1,000 ml. with distilled water. The mixture was allowed to stand for 30 minutes and submitted to colorimetric determination through a green filter. The values were calculated against the standard after deducting the blind values, (without the addition of benzidine).

The same method was used for carrying out tests for Aldridge-positive substances—impurities (thiocyanates and cyanides)—in an extract of the Larsen diet and in the substances being investigated. In this case, 1% solutions or, in the case of insoluble substances, extracts in the proportion of 1 : 100 were prepared. Because of the small amount, the test was not carried out in the case of 3-indoylacetonitrile.

For the determination of thiocyanates in the serum, 0.5 ml. serum was used, to which 2 ml. 5% trichloracetic had been added. After centrifuging, 1.5 ml. of the supernatant fluid was taken and the same amount of bromine water and arsenite added as in the urine determination, together with 5 ml. pyridine mixture (4.5 ml. pyridine reagent plus 0.5 ml. benzidine solution).

When determining thiocyanates in the urine, it was not possible to use methods involving ferric salts (red coloration) because of the disturbing effect of urine chromogens. Precedence was also given to the method of Aldridge because of its greater sensitivity.

The whole method was verified by the administration of 1 mg. potassium thiocyanate and 2 mg acetonitrile and 2 mg. benzyl cyanide as known thiocyanogenic substances. The characteristic increase in the excretion curves bears out the data on the thiocyanogenicity of these substances, described in the literature.

The excretion of thiocyanates was observed for eight days before administering the substances and the averages, when given in the tables, were calculated from four daily values prior to the experiments. The values measured are expressed in mcg. for 24 hours; an evaluation in units of concentration was not made because of the dependence on the amount of urine, which was often considerably affected by the properties of the experimental substance administered. The substances were administered in the afternoon at about 3 pm. the urine being collected in the morning at about 9 a. m. The values on the first day after administering the substances in all experiments therefore theoretically contained only $^3/_4$ urine following administration of the substance, while $^1/_4$ is still normal urine. In view of the time required by the rats for consuming the food, this $^3/_4$ value is also in fact reduced. For this reason the increase in the level of thiocyanate on the first day after administration is less than it would be if the rats consumed the food at the moment when the calibrated containers for the urine were changed.

Results

Allylisothiocyanate and phenylisothiocyanate were administered to rats in the manner described without any discernible influence on the curve of excretion of thiocyanate in the urine in 24 hours. This negative result in the demonstration of the thiocyanogenicity of nutrient isothiocyanates led to an investigation of the thiocyanogenicity of natural nitriles. Not long ago Henbest et al. (1953) isolated a new growth factor in cabbage, which they identified as 3-indoylacetonitrile. It was possible that this substance might have thiocyanogenic properties and this we were

Table 1.

Excretion of Thiocyanate following Daily Administration of 2 mg. Indoylacetonitrile and of 2 mg. Benzyl Cyanide in Rats

Substance	No. of rats	Sex	(g.) Weight	mcg. SCN' in 24 hrs. per rat				
				Ø 4 days before	1st day	2nd day	3rd day	4th day
3-indoyl-acetonitrile	2×2	♀	83 92 111 116	58.3 ±22.3	131.3 ±19.7	196.0 ±21.3	367.3 ±40.1	285.7 ±36.9
	1×2	♂	98 108					
Benzyl Cyanide	2×2	♀	75 95 124 130	45.9 ±14.2	141.9 ±31.1	374.0 ±18.0	341.1 ±41.0	325.0 ±50.4
	2×2	♂	80 91 99 104					

able to confirm. Thanks to the kindness of Dr Henbest, we obtained a sample of 3-indoylacetonitrile, which we submitted to investigation by the method described above.

The excretion of thiocyanates following the administration of 3-indoylacetonitrile is shown in tab. 1, which also gives the course of the excretion of thiocyanate after the administration of benzyl cyanide as a control substance.

By analogy, indoylacetonitrile should also have a thiocyanogenic action in man. In the amounts in which it occurs in cabbage, according to the preliminary data of Henbest, i. e. about 2 mg./kg., however, its thiocyanogenic action in man after administration in a single dose is not significant. These preliminary results in man have not yet been thoroughly verified because of insufficient quantity of the substance and of non-smoker volunteers.

Table 2.

The Influence of Fruit Kernels on the Excretion of Thiocyanate in the Urine of Rats

Type of kernel	No. of rats	g. kernel per day/rat	mcg SCN' in 24 hrs. per rat			
			Ø 4 days before	1st day	2nd day	3rd day
Peach	2	0,55	50.6 ± 9.8	51.3	204.5	301.6
Plum	2	0,25	41.2 ± 7.0	35.9	113.8	224.0
Apricot	2	[illegible]	47.8 [illegible]	[illegible]	[illegible]	100.1

For the verification of the thiocyanogenicity of cyanogenic fruits, the crushed kernels of plums, apricots and peaches were administerd in daily amounts of 250 mg. per rat. Increases in values were found as shown in tab. II. It can be seen that peach kernels contain most thiocyanogenic substances and that plums are also a rich source. These substances are of nutritive importance in the consumption of fruit, particularly stewed fruit.

In investigating metabolic precursors, acetamide was tested. Doses increasing from 10—30 mg. daily led to an irregular increase in the value of thiocyanate in the urine. The administration of fixed doses of 20 mg. of acetamide brought about an increase in values that was only temporary and even when the administration of acetamide was continued, the values returned to normal. In a single experiment in which acetamide was administered in a watery solution par enteris, a smaller effect was found, but of the same character. In connection with the behaviour of acetamide, several acetyl derivatives were investigated in detail. Acetyl urea administered in

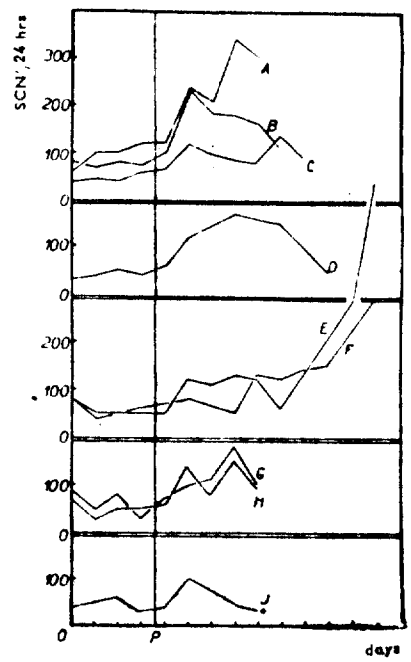


Fig. 1. Thiocyanate excretion curves in the urine of rats after administration of nitrogenous substances. Curve A: Acetamide, increasing daily doses of 10, 10, 20 and 30 mg. successively, further 30 mg. daily. Curve B: Acetamide, constant daily dose of 20 mg. Curve C: Acetamide administered par enteris in a watery solution in daily doses of 10, 10, 30, 30 and 50 mg. successively, further 50 mg. daily. Curve D: Acetylglycine, daily doses of 100, 200, 300, 500 mg. successively, further 500 mg. daily. Curve E: Urea, daily doses of 100, 200, 300, 500 successively, further 500 mg. daily. Curve F: Alloxan, constant daily dose of 100 mg. Curve G: Diethylamine, daily doses of 10, 20, 30 mg. successively, further 30 mg. daily. Curve H: Methylamine, daily doses of 10, 20, 30 mg. successively, futher 30 mg. daily. Curve J: Ammonium acetate par enteris in watery solution, daily doses of 10 mg. The commencement of administration is denoted by the straight line at P. The substances were administered in the amounts given, per rat; the individual curves give the values of the combined urine of two rats calculated for one rat.

daily doses of up to 500 mg. per rat, did not prove to be thiocyanogenic and had no influence on the course of the excretion curve. Acetylglycine was administered in doses of up to 500 mg. daily and acted on the curve in the same way as acetamide, i. e. a temporary increase occurred with a return of the values to the original amounts. As compared with the values obtained with amino acids (v. below), the curve has a protracted character. Benzoylglycine (hippuric acid) does not influence the excretion curve.

Urea (the diamide of carbonic acid) did not produce discernible changes in an experiment lasting the usual time. On prolonged administration, however, a very marked increase in the amount of excreted thiocyanate occurred, seven to ten days after commencing the administration of urea. Because of its close relationship to urea (carbamide) and as an inhibitor of enzymatic processes, a test was made with [illegible]. The administration of this substance, however, produced no changes.

Guanidine also had no effect on the course of the curves. Alloxan, however, showed thiocyanogenic properties, which were discernible after prolonged administration, as in the case of urea.

In some experiments the administration of the nucleic acid, Lachema, showed an increase in the excretion of thiocyanate after a long period, while in other experiments with the same preparation, thiocyanogenic properties were not found. The cause of this conflicting result was not found.

In this connection the amines were also tested as possible sources of endogenous thiocyanate. Theoretically these could give rise to nitriles by oxidative dehydrogenation. The thiocyanogenicity of methylamine and diethylamine was tested and it was found that both these substances influence the course of the excretion curve in the same way as acetamide, by a temporary maximum course. On increasing the dose the maximum is repeated.

Since many substances influence the excretion of thiocyanate by a temporary increase followed by a return to the original values, an attempt was made to ascertain whether these substances are not changed in the alimentary tract. They are nitrogenous substances, the common denominator of which could be ammonium. Certain ammonia salts (acetate, chloride, carbonate) were therefore administered par enteris

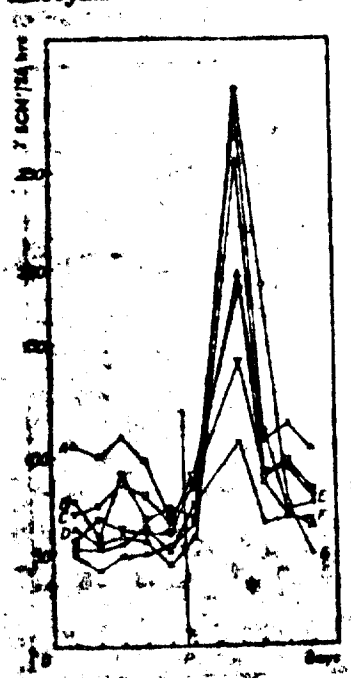


Fig. 2. The influence of the administration of amino acids on the excretion of thiocyanate in the urine of rats. — Curve A: aspartic acid, B: arginine, C: glycine, D: glutamic acid, E: methionine, F: cystine, G: alanine. — The amino acids were administered in daily amounts of 100 mg. per rat, from the point denoted on the ordinate by the perpendicular at P. The values of the combined urine of two rats are calculated for one rat.

in a watery solution. With these substances also the thiocyanate excretion curve was influenced temporarily, as is seen in the ammonium acetate curve.

The changes in the excretion of thiocyanate following the administration of acetamide, acetyl glycine, urea, alloxan, methylamine, diethylamine and ammonium acetate are shown in the form of a graph in fig. 1.

The influence of the administration of amino acids on diuresis was shown clearly only in the case of methionine, less clearly with histidine. With the other amino acids the amount of urine was within the limits of normal variation.

The curves of the excretion of the thiocyanate ion, on the other hand, all show a course characterised by a maximum after which the values again fall to those of normal excretion (fig. 2). The differences in the height of the maximum and the gradient of its course are determined partly by the properties of the amino acids under investigation and partly by the physiological state of the animal. This fact is mentioned so that the increases in the curves shall be taken only as a relative criterion of the degree of interference of the substance investigated into thiocyanate metabolism and so that the curves should be evaluated [illegible]

values before administration of the substances and less with regard to the mutual relationship of the individual amino acids.

The course of the excretion of thiocyanate with the various substances is given in fig. 2. From a number of experiments with individual amino acids (2—5), the curves of one experiment (2 rats) are given. The course of the maximum was marked in all experiments. The height of the maximum is dependent on the height of the original excretion, as may be seen from the course of a number of experiments with the same amino acid.

The maximum is most marked with arginine, alanine and glutamic acid; because of dependence on the experimental conditions, this particular property of the amino acids named would require more extensive material.

Table 3.

Levels of SCN′ in the Serum of Rats

Rat No.	Weight (g.)		mcg % SCN′ in serum after		$\bar{x} \pm \sigma$
	Before exper.	After expor.	Thyro-globulin	Control	
1	134	121	588		
2	122	109	552		
3	112	102	414		514.8
4	104	92	558		± 65.7
5	98	87	462		
6	119	127		426	
7	117	128		420	
8	108	122		462	421.2
9	96	119		378	± 26.7
10	92	112		420	

With the significance test of A. Aspin (Biometrika 36 : 290, 1949) a significant difference was found for the 5% level of probability.

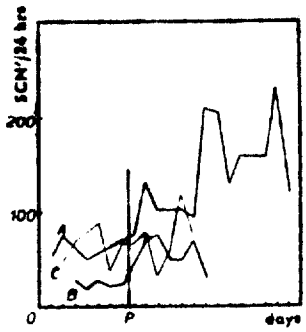
Thyreoglobulin forte Spofa, administered to rats in daily doses one one tablet per rat for 10 days, produced a clear picture of hyperthyroidism. As compared with the controls, the experimental animals were much more restless and more easily tired, and had an increased appetite. They lost weight. The basal metabolism was not determined because of the technical difficulties. The level of thiocyanates in the serum, following exsanguination, are included in tab. III, which shows an increase in values as compared with the controls.

On the administration of thyroxine and its analogues, an increase in diuresis occurs. In the case of tetrabromothyronine this does not occur until after the administration of the high dose of 50 mg. per rat per day. Following the administration of 1 mg. thyroxine there is a temporary increase in values with a maximum, which is repeated on increasing the dose. The values then fall, but still remain higher than the original values. On prolonged administration they slowly increase. Following the administration of thyroglobulin the character of the curve is similar, but is somewhat more protracted. Diiodotyrosine, administered in doses of 10 mg. per rat per day brings about a small, but discernible increase in the amount of thiocyanate excreted, which is [illegible] by a maximum. It is not clearly shown whether the values return to their original level or whether they remain somewhat raised.

Tetrabromothyronine, administered in doses of 20, 20, 30, 50 and a further 50 mg. per rat per day did not produce any noticeable changes.

The long-term observation of the excretion of thiocyanate with the continuous administration of thyroxine or thyroglobulin showed that after the maximum had been reached, the values remained raised and continued to rise (fig. 3).

A pilot experiment was also carried out to investigate whether this effect could be suppressed by a goitrogenic substance. Methylthiouracil was used and was administered for three days in daily amounts of 50 mg. Since methylthiouracil reacts with the Aldridge reagent, the curves for this period are recorded in dashed lines



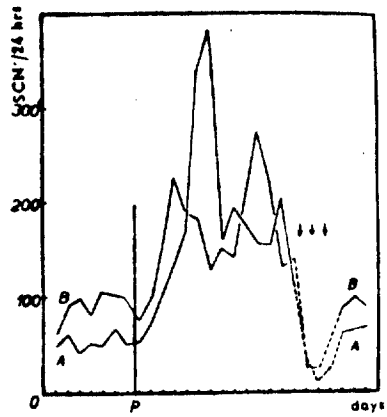


Fig. 3. Course of the excretion of thiocyanate after the administration of thyroglobulin and analogues. Curve A: Thyreoglobulin forte Spofa (0.5 mg. bound iodine), one tablet per rat daily. Curve B: Diiodotyrosine in daily doses of 10, 10, 10, 10, 20 mg. successively, further 20 mg. per rat daily. Curve C: Tetrabromothyronine, in daily doses of 20, 20, 30, 50 mg. successively, further 50 mg. per rat daily. The substances were administered from the point denoted on the ordinate by the perpendicular at P; the values of the combined urine of two rats are calculated for one rat.

Fig 4. The influence of the administration of thyroxine and thyroglobulin on the amount of thiocyanate excreted in the urine of rats and the effect of methylthiouracil (MTU), Curve A: Thyreoglobulin forte Spofa (0.5 mg. bound iodine), two tablets per rat daily. Curve B: Thyroxine, one tablet per rat daily. The substances were administered from the moment P to the moment when the administration of methylthiouracil was commenced. The administration of methylthiouracil for three days denoted by arrows. The values are shown by an interrupted line because of influencing of the Aldridge reaction (v. text). The values of the combined urine of two rats are calculated for one rat.

(v. fig. 4). After the excretion of methylthiouracil, the level of thiocyanate remains at the original value. It has not yet been ascertained what course the excretion of thiocyanate takes when thyroxine is discontinued, without the addition of a goitrogenic substance.

The values of the permanent increase are dependent on the amount of thyroxine or thyroglobulin administered. The larger the dose, the greater the increase in the amount of thiocyanate excreted.

Discussion

In the presence of iodine deficiency, thiocyanate [illegible] includes the residue of iodine from the cells of the thyroid gland and [illegible] the condition of iodine deficiency worse. The goitrogenic [illegible] can be explained

by this mechanism, but it is also possible that the increased level of cyanides resulting from the shift in the thiocyanate-cyanide balance leads to a greater suppression of oxidation processes, since the cyanides are powerful inhibitors of oxidation enzymes. In this case there would be an antagonistic relationship between thyroxine and the cyanides, in which thiocyanate would act as a link neutralising the toxicity of the cyanides. Such a mechanism naturally places great emphasis on the endogenous metabolism of thiocyanate. The raised level of thiocyanate in thyropathic conditions would then be due to a metabolic disturbance related to the changed conditions in the thyroid gland. In placing greater stress on exogenous thiocyanate in thyropathic conditions it would, on the contrary, be necessary to assume that the goitrogenic action is due to increased intake. Some data of the present work show that the endogenous metabolism of thiocyanate has its own particular importance and that its existence in relation to thyropathic conditions must not be neglected.

As the source of exogenous thiocyanate, the precursors of thiocyanate from food—nitriles, cyanides, cyanogenic substances, etc.—must also be taken into account. The nitriles are considered to be a degradation product of the isothiocyanates, which are likewise contained in plant food in the group of Cruciferae. The genus Brassica, belonging to this group, contains cabbage, Savoy cabbage, kohlrabi and cauliflower, that is to say foods to which goitrogenic action has been attributed and which appear frequently at our table. The thiocyanogenicity of these plants has already been described (Dezani 1918). The source of the thiocyanates in cabbage, for example, was not known. Thiocyanogenic properties of the nitriles have been found in synthetic derivatives and in derivatives found in plants of little or no nutritive significance for man. The existence of such nitriles in cabbage leaves had not been described up to their isolation by Henbest (1953). The 3-indoylacetonitrile isolated by Henbest was found by us to be a substance with thiocyanogenic properties. This is the case of a natural thiocyanogenic substance from food which is consumed in considerable quantities. Although the results of investigations on its thiocyanogenicity in man are not yet complete, this finding supplements the data on nutritive thiocyanogenic substances.

The isothiocyanates were not found to be thiocyanogenic substances. They influence the course of the Aldridge reaction and their presence might therefore be a source of negative results. They are, however, rapidly metabolised and do not reach the urine. A qualitative determination of their presence in the urine of rats was made in specimens taken at random after the administration of isothiocyanates but their presence was never demonstrated. On the basis of this the isothiocyanates would not appear to be nutritive thiocyanogenic substances which might be responsible for the increased secretion and accumulation of thiocyanide in the blood following the intake of food from the genus Brassica. There still remains a possibility, however, that the isothiocyanates are present in the urine in the form of an unknown, bound derivative, which would also inhibit the Aldridge reaction. This possibility could naturally not be investigated within the scope of the present work.

In a study of the substances which might act as precursors of the thiocyanates in endogenous metabolism—i. e. the amines, amides etc.— the increase in values to a new, higher level is not shown on the thiocyanate excretion curves as being dependent on the dose administered, as is the case with typical thyiocyanates like the nitriles. A temporary maximum of the values occurs, after which the curve again falls to the original levels. This course is also found, to a greater or lesser degree, in all the substances investigated. An analysis of these curves in relation to diuresis shows [illegible] no more than a cut of thiocyanates from the tissues by an increased

volume of urine. Diuresis is a factor only with some substances and has no bearing on the course of maximum excretion. Where the excretion curve has a maximum, i. e. following the administration of acetamide, acetylglycine, methylamine, diethylamine and all the amino acids and ammonium salts investigated, these substances might be regarded as being thiocyanogenic only if the repeated administration of the given substance led to inhibition of the system which forms the thiocyanate from them. This is, however, very improbable. A more probable explanation is that on the administration of these substances already formed thiocyanate or cyanide, possibly bound to protein, is released. This explanation is also supported by the ability of thiocyanate to become bound to protein and also by the relationship to the height of the maximum level of thiocyanate excreted before the administration of the amino acid or substance with a similar action. On the basis of this interpretation the amino acids cannot be considered to be thiocyanogenic substances, the same applying to the amines, amides, etc. This excludes the possibility that the metabolism of the thiocyanates takes place according to the scheme described above, via amines and nitriles from amino acids or proteins.

In discussing the behaviour of the amino acids, mention should be made of the behaviour of cystine. Cystine is considered to be the substance, the sulphur of which is the source of the sulphur of the thiocyanate ion. In our experiments, however, it was not shown as having a thiocyanogenic action. Since excess cystine, however, becomes a source of thiocyanate in cyanide poisoning, it can be assumed that the limiting factor of the formation of thiocyanate under normal conditions is the formation of cyanide in vivo. Under the conditions of cyanide poisoning, on the contrary, the limiting factor is cystine sulphur, since on the administration of cystine or glutathione the toxicity of cyanide decreases and the formation of thiocyanate is increased.

Urea and alloxan, which bring about an increase in excretion without a maximum, are likewise not thiocyanogenic substances of the type of the nitriles. Their action does not begin sufficiently promptly. These substances are either metabolised in another manner and only their metabolites become a source of thiocyanate, or their presence produces a diseased state of the organism, which is manifested in the increased excretion of thiocyanate from quite different sources. This is perhaps the case with nucleic acid in experiments in which, after the administration of this substance, increased excretion occurred.

As regards the question of the thiocyanogenicity of proteins on the one hand and of derivatives of the purines or pyrimidines on the other, these results tend to indicate the development of thiocyanate from the nucleic acids rather than from proteins. These two groups of substances are considered in the literature as possible sources of thiocyanate, on the basis of studies on the content of thiocyanate in the blood and of its excretion under various physiological conditions. The thiocyanogenicity of proteins appears to be less probable since the amino acids administered did not show thiocyanogenic properties. On the other hand, thiocyanogenicity—even though of a special type—in urea and alloxan, as substances structurally close to the purines and pyrimidines, tends to lend support to the view that thiocyanate develops from nucleic acid. The metabolism of the nucleic acids, however, has its own peculiar characteristics, in that administered purines are not used as such for building up nucleic acids, but are first of all degraded. Because of the present difficulty of estimating these processes, the question of the thiocyanogenicity of the nucleic acids is a complicated one, even though on the basis of our results it seems the more feasible.

A chain of reactions which could take place with a certain degree of probability

[illegible] living organism, from the purines via alloxan to urea and cyanide or thio[illegible] does not exist. Cyanide develops from the substances mentioned above, [illegible]ever, as a subsidiary oxidation product in some oxidation processes in vitro. [illegible] logical to assume that the metabolism of thiocyanate is linked up with the [illegible] break-down of some nitrogen components. Such a type of oxidation, [illegible] cannot be carried out by any known oxidation-reduction system, but the [illegible]tion of thyroxine is evidence that the process of the formation of thio[illegible] is of an oxidative character.

In hyperthyroidism produced experimentally in rats by the administration of thyroglobulin, an increase occurs in the level of thiocyanate in the serum. Increased [illegible] in the urine likewise occurs, and in long-term experiments also, in which the amount of thiocyanate excreted daily remains permanently raised. The increase in the values of excreted thiocyanate depends more or less on the physiological effectiveness of the substances administered, in the series: thyroxine, thyroglobulin, diiodotyrosine and tetrabromothyronine. All these substances have a calorigenic action, which is in agreement with the series mentioned, i. e. they increase the basal metabolism. In the amounts used, diiodotyrosine had only a small effect and the action of tetrabromothyronine was not discernible. Thyroxine and thyroglobulin interfere in a marked manner in the regulation of the level of the thiocyanate ion.

These results appear to contradict the findings of Stöa (1952), who found that under the influence of thyroxine, acetonitrile is converted into thiocyanide to a far lesser degree than without thyroxine. He investigated the mechanism of the Reid-Hunt reaction, with which, under the influence of thyroxine, reduced sensitivity to lethal doses of acetonitrile occurs in mice, and found that thyroxine decreases the amount of thiocyanate in the blood after the injection of acetonitrile. In the case of thiocyanate being of completely exogenous origin (from nitriles contained in the food), thyroxine ought also to decrease the excretion of thiocyanate in the urine and its level in the blood. Our results, using large doses of thyroxine or thyroglobulin, showed the exact opposite. A constant increase in the amount of excreted thiocyanate occurred and higher values were also found in the blood, which shows that the amount of nutritive thiocyanates in the food used was minimal. Stöa did not make a more detailed study of the system which brings about these processes, but he assumed speculatively that the thyroxine inhibits the oxidative breaking-down of acetonitrile into cyanide. That would not correspond to the known properties of thyroxine, which is to accelerate oxidation processes. Our results would be more in keeping with this property, since we are of the opinion that the formation of cyanide as the precursor of thiocyanate is of the character of an oxidation. We are as yet [illegible] to give an explanation of this difference, but it is possible that a number of [illegible] participate in the metabolism of thiocyanate with the influence of thyroxine [illegible] in various ways.

[illegible] of tissue oxidation processes by goitrogenic substances antagonises [illegible] of thyroxine. Methylthiouracil administered experimentally for three [illegible] level of thiocyanate, which had been raised by thyroxine, back to [illegible] In [illegible] of the fact that methylthiouracil decreases values in the [illegible] remains at its original level after the methyl[illegible] it was not established in what way the [illegible] This contributes to the conception that [illegible] thyroxine. These results [illegible] substances [illegible] the endogenous

[illegible] of the thiocyanate ion is not a negligible factor in the regulation of the [illegible] in the blood in the [illegible] of [illegible].

Summary

[illegible] was made of the metabolism of the thiocyanate ion with reference to the [illegible] the level of this ion is raised in that part of the population suffering from [illegible] the influence of nutritive precursors, particular attention was [illegible] a widely consumed form of food with thiocyanogenic and goitro- [illegible] and it was demonstrated that the indoylacetonitrile contained in [illegible] is one of the nutritive thiocyanogenic substances. The mustard oils were not [illegible] to be thiocyanogenic.

[illegible] previous studies on the conditions of excretion and of the level of [illegible] in the blood, it is thought that thiocyanide develops when amino acids, [illegible] or nucleic acids are broken down. In studying the precursors which might [illegible] in the endogenous metabolism of thiocyanate, special thiocyanogenic [illegible] were found in urea and allozan; this points to the possibility of a con- [illegible] between the metabolism of thiocyanate and the metabolism of the purines [illegible] from nucleic acid. Thiocyanogenic properties were not found in the [illegible] acids.

[illegible] amino acids and some nitrogenous substances, e. g. amines, amides and [illegible] exert a temporary influence on the excretion of thiocyanate, [illegible] freeing of adsorbed thiocyanate, probably, from proteins.

[illegible] of thiocyanate in vivo, the limiting factor under normal condi- [illegible] cyanide, as follows from the behaviour of cystine under these [illegible]

[illegible] of the opinion that the formation of thiocyanate is an oxidation [illegible] is accelerated by the hormone of the thyroid gland; the responsible [illegible] of the living organism is not known. The dependence of [illegible] on thyroxine or thyroglobulin is also proof of the [illegible] of thiocyanate.

[illegible] to Dr. H. E. Henbest for his kindness in supplying a specimen [illegible] for technical collaboration.

[illegible]

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СООБЩЕНИЕ К МЕТАБОЛИЗМУ РОДАНИСТОГО ЙОНА

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Резюме

Изучался метаболизм роданистого йона с особым вниманием к факту, что уровень этого йона является повышенным у популяционных групп страдающих зобом. При определении действия принимаемых в пище прекурсоров, внимание обращалось на капусту, как на пищевой продукт массового потребления с роданогенным и струмигенным влиянием и было доказано, что индолилацетонитрил, содержащийся в капусте, является одним из принимаемых в пище роданогенов. Горчичные масла в качестве роданогенных веществ не проявлялись.

На основании предшествующего изучения условий выделения и уровня роданита в крови имелось подозрение, что при распаде аминокислот или соотв. протеинов и нуклеиновых кислот образуется роданит. При изучении прекурсоров, которые бы могли, возможно, появляться в эндогенном метаболизме роданита, были обнаружены особые роданогенные свойства цистеина и аспарагина, что указывает на возможность существования связи между метаболизмом роданита и метаболизмом пуринов и пиримидинов из нуклеиновых кислот. Напротив, роданогенные свойства аминокислот обнаружены не были.

Аминокислоты и некоторые простые вещества, напр. амины, амиды и аминокислоты влияют на выделение роданита. Промывание желудочного, что свидетельствует об освобождении роданита, с абсорбцией, например, бальмами.

При образовании роданита in vivo лимитирующим фактором является при нормальных условиях содержание цианида, как это матеиает на поведения цистина при этих условиях.

Автор предполагает, что образование роданита является описательным процессом, так как оно увеличивается гормоном щитовидной железы, однако соответствующая описательная система [illegible] сотается неизвестной. Зависимость образования роданита от гормонов или гормонобразующих тканей является доказательством, свидетельствующим в пользу [illegible] метаболизма роданита.

Kjaer-'56

Fortschritte der Chemie Organischer Naturstoffe 18: 123-176. 1956.

Naturally Derived *iso*Thiocyanates (Mustard Oils) and Their Parent Glucosides.

By ANDERS KJÆR, Copenhagen.

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I. Introduction.

The *iso*thiocyanate producing glucosides constitute a well-defined and unique class of natural products which occur in a large variety of higher plants, belonging to a relatively small number of botanical families. They are characterized by the ability to undergo enzymic hydrolysis to *iso*thiocyanates (mustard oils), hydrogen sulphate and *D*-glucose. Invariably, the latter has been encountered as the sugar moiety of the more than thirty individual compounds so far recorded, justifying the designation of the latter as glucosides.

On account of the conspicuously pungent properties of many mustard oils this group of glucosides has attracted scientific interest for hundreds of years. The time period prior to about 1850, with its fragmentary concepts of chemical structure, was followed by a period of considerable progress, culminating around 1900 with the proposal of a general chemical structure of these glucosides. After almost five decades of little activity, this province of natural product chemistry has enjoyed a considerable revival since the end of the Second World War. Recently, the subject covered in this essay has been reviewed by various authors (*10, 37, 46, 57, 58, 60, 62, 157*). However, a more comprehensive treatise, including data for the individual representatives of this class of compounds, may be of help in future studies.

Particular emphasis will be placed on recent developments, whereas only scant attention will be given to the analytical aspects of the subject which have recently been surveyed elsewhere (*145*). Possible trends for future activities will be indicated.

II. Historical Development.

References to higher plants as sources of volatile, pungent principles date back to antiquity. Mustard, and allied species, have been employed as condiments and remedies for centuries, and numerous speculations concerning the active compounds appeared in the literature, long before modern organic chemistry was founded a hundred years ago.

A valuable key to the oldest literature is the monograph by GILDEMEISTER and HOFFMANN (*36*).

About 1830, various investigators demonstrated that the production of volatile mustard oil from seed material required the presence of water. A decade later, the enzymic character of the reaction was envisaged by BUSSY (*8*) who isolated the parent substrate from black mustard seeds (*Brassica nigra* KOCH) in form of a crystalline potassium salt of an acid, termed "acide myronique" (myron = balsam), which has subsequently become known as *sinigrin*. An auxiliary compound, known today as an enzyme (myrosinase), was termed "myrosyne" (syn = with). As early as in 1831, ROBIQUET and BOUTRON (*113*) isolated from the seeds of white mustard (*Sinapis alba* L.) a crystalline, sulphur-containing constituent which later was named *sinalbin* and recognized as a mustard-oil producing glucoside. Only a single addition was made to the list of crystalline glucosides prior to the present post-war period, viz. *glucocheirolin*, a compound isolated by SCHNEIDER and SCHÜTZ (*128*) in 1913 from wall-flower seeds. Today, the number of crystalline glucosides of this type exceeds ten, in addition to half as many which have been characterized as crystalline acetates. The existence of a considerable number of further glucosides is indicated by current knowledge of their enzymic fission products. The latter comprise a variety of *iso*thiocyanates, XNCS, which, for the sake of the present discussion, will be divided into groups according to their structural types. Up to 1952, when interest in this field was revived, eight mustard oils of established structure had been recorded. Today, the number runs close to thirty, and several further additions may be expected in the near future.

A reliable survey covering the literature to about 1930 was presented by SCHNEIDER (*124*).

Clearly, this progress is a result of the development of modern analytical tools. The discovery within a few years of about four times as many glucosides and mustard oils as known earlier illustrates the immense influence of new methods such as paper chromatography on the study of natural products.

The traditional interest in the *iso*thiocyanate glucosides, virtually limited to their applications as condiments and remedies in folk medicine, has recently been deepened by several biological effects of the glucosides or rather their enzymic fission products.

III. Parent Glucosides.

1. General Properties.

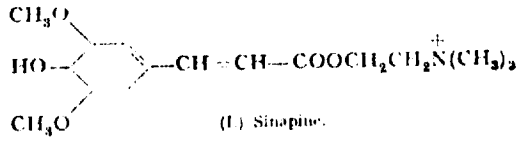
All glucosides discussed in this Chapter are of the same general chemical character and hence possess similar properties. The nine *iso*-

References, pp. 169—170.

thiocyanate-producing glucosides thus far described in the crystalline state are listed in *Table 1*, p. 157, which also includes references to the original botanical sources of the individual compounds.

It will be noted that, apart from the long-established trivial names sinigrin and sinalbin and the lately introduced designation progoitrin (*42*)*, the glucosides have been consistently named by adding the prefix "gluco" to an appropriate part of the Latin name of the botanical species in which the compound was first recognized. This arbitrary nomenclature often leads to rather unwieldy designations, which might conceivably be rationalized by an alternative nomenclature based on a generic trivial name for the molecular entity common to all glucosides, preceded by the systematic chemical name of the side-chain of the individual compound.

All glucosides of this class contain a sulphuric acid residue and are accordingly isolated and handled as salts; they contain mostly potassium as the cation, because of the abundant presence of this element in plant tissues. Sinalbin, the classical glucoside of white mustard, is unique in the sense that it contains sinapine (I), a rather widely distributed quaternary base, as its cationic moiety (cf. *124*).



(I.) Sinapine.

The excellent crystallization properties of a few glucosides such as sinigrin, sinalbin, glucocapparin and glucoiberin, are in marked contrast to the generally experienced difficulties in inducing the other purified mustard oil glucosides to crystallize. This explains, why only nine out of more than thirty glucosides have hitherto been obtained as crystals. Recently, ETTLINGER and LUNDEEN (*23, 24*) have shown that certain tetramethylammonium salts possess good crystallization properties.

In a number of instances, acetylation of amorphous *iso*thiocyanate glucosides was advantageously employed for characterization purposes. The well described crystalline glucoside acetates are listed in *Table 2*, p. 158. Usually, four acetyl groups are introduced, all located in the glucose moiety. In one instance, viz. that of glucorapiferin (= progoitrin), a fifth acetyl enters a secondary hydroxyl group in the side-chain (*141*), whereas glucoconringiin, possessing a tertiary hydroxyl function in the aglucone, is not acetylated in this moiety under the conditions employed (*82*).

* Independently, the name "glukorapiferin", constructed in accord with the general practice within this field, was proposed for the same glucoside by other authors (*138*) but, unfortunately, it seems to have received less recognition than the rather unorthodox designation "progoitrin".

In addition to the acetyl derivatives listed in Table 2, several non-homogeneous preparations of other glucoside acetates have been reported (*151*). The acetylated products of glucoberteroin (*151*) and glucomatronalin (*151*) have been omitted from Table 2 because the infrared spectra (*142*), which display only minor differences in such glucoside acetates, are the sole documentation available for these derivatives. As seen from Tables 1 and 2 (pp. 157, 158), most glucosides and their acetates crystallize from water or aqueous alcohols as monohydrates.

In accordance with their structure, the crystalline glucosides are colourless and water-soluble. In aqueous solution they all exhibit *levo*-rotation. Apart from glucoiberin which possesses an asymmetric sulphoxide-group in its side-chain, all known glucosides display rotations of roughly the same magnitude suggesting β-glucoside character*.

2. Distribution in Plant Tissues.

The distribution of the various *iso*thiocyanate glucosides within the vegetable kingdom will be discussed in Chapter VI, p. 154. It should be emphasized that the occurrence of more than one glucoside in a given botanical species is the rule rather than the exception. As many as eight individual glucosides have been clearly distinguished in a single seed specimen. Within the glucoside-containing species the parent compounds often seem to be distributed over the entire plant. According to GUIGNARD (*44*), the glucosides are diffusively present in parenchymal tissues, especially in the bark. In seeds, the embryos constitute the site of accumulation. Only few systematic studies have been undertaken to establish the variation of the glucoside content as a function of the stage of growth, or of environmental factors such as climate, soil composition, etc. It is interesting in this connexion that STAHLMANN et al. (*144*) found 2-phenylethyl *iso*thiocyanate, the aglucone of gluconasturtiin, to be the predominant mustard oil enzymically liberated from roots of black mustard (*Brassica nigra* KOCH); the seeds represent the classical source of sinigrin which yields allyl *iso*thiocyanate upon enzymic fission. Further, DELAVEAU (*16*) has studied the total and relative amounts of the individual glucosides in *Alliaria officinalis* during its growth cycle and noticed considerable variation. Numerous (mostly unpublished) observations from the author's laboratory clearly indicate significant quantitative, and frequently also qualitative, changes in the glucoside pattern of a given species, from the roots to green organs and to seeds (cf. Table 5, p. 161). Likewise, considerable variations were observed occasionally when the glucoside contents in botanically

* The rotatory dispersion curves through the wavelength region 295–600 mμ of a number of representative glucosides from the author's laboratory have kindly been determined by Dr. W. KUYNE, Postgraduate Medical School, University of London. They all exhibit negative plain curves through the entire range, i.e. curves devoid of anomalous dispersion (no Cotton effect).

References, pp. 169–176.

identical seed specimens of different provenance were compared. Hence, it is imperative, here as in most provinces of natural product chemistry, to define the botanical source very carefully.

Most glucoside studies have been conducted with seeds which are usually rich in the desired compounds. Seeds are often more easily available than the fresh plants and the isolation procedures are simpler. Moreover, seasonal independence is another convenience when working with seeds.

From the above it would appear that much remains to be learned about the location of mustard oil glucosides in various tissues. Clarification of such problems may prove most helpful in attempts to elucidate the biogenesis and metabolic pathway of this class of natural products.

3. Detection, Isolation, Separation and Determination.

a. Paper Chromatography.

Until a few years ago, all references to the occurrence of *iso*thiocyanate glucosides in higher plants were based on the detection of mustard oils, liberated upon enzymic hydrolysis, whereas no analytical procedure existed for the detection of the glucosides themselves. As in most other areas of biochemistry, paper chromatography has been put to good service in the class under discussion.

SCHULTZ et al. (*38, 131, 133, 139*) developed a method for paper chromatography of the genuine glucosides, applicable to crude extracts of plant material, with the use of various solvent systems (*n*-butanol : acetic acid : water, collodine : water etc.) and with ammoniacal silver nitrate as a spray reagent. This method, combined with chromatography of certain derivatives of the corresponding *iso*thiocyanates (p. 139), has afforded the experimental basis for practically all the progress in this field during the last decade; it has helped to establish the glucoside patterns of numerous plant species (*38, 131, 133, 139, 151*) and yielded many new glucosides. The ease of performance and the minimum requirement of material render paper chromatography well suited also for chemotaxonomic studies (*87*).

In view of the large number of well-known, genuine *iso*thiocyanate glucosides, paper chromatography alone does not provide sufficient evidence for the identification of an individual spot but it provides valuable assistance in this task. Evidently, a need does exist for developing of still better differential-diagnostic assays. At present, caution is recommended when assigning definite glucosides to a given plant solely on paperchromatographic evidence; several erroneous statements have appeared in the literature on this account.

The paperchromatographic technique has proved to be very useful also in purifying extracts as well as in synthetic studies.

b. Isolation and Separation Methods.

The procedure of extraction and purification of *iso*thiocyanate glucosides involves disintegration of plant tissues in such a way that enzymic hydrolysis be prevented or minimized, extraction of the glucosides with water or aqueous alcohols, removal of impurities, crystallization and purification.

The classical isolation of sinigrin from black mustard seed (*124*) was improved by STOLL and SEEBECK (*146*), who employed fresh horse-radish as a source. Three kg. of fresh roots, containing 60–70% water, afforded 10.6 g. of analytically pure sinigrin. Well-crystallized glucoiberin was isolated from seeds of *Iberis amara* L. by a very simple procedure (*136*). In most instances, however, particularly when the plant extracts contained a mixture of glucosides, the traditional procedures were unsuccessful.

Fortunately, modern methods of *ion-exchange* have made the old-known glucosides, as well as a series of new compounds, more easily accessible in crystalline or highly purified form (*Table 1*, p. 157). SCHUITZ et al. (*38, 137*) studied the applicability of various anion exchange resins (Lewatit M1, Amberlite IR-400 and Amberlite IR-4 B) in the isolation of mustard oil glucosides and found that the latter, due to their character of substituted sulphates, can be retained quantitatively on the resins and thus be freed from impurities, such as sinapine, and other cationic or neutral contaminants (carbohydrates, etc.). Subsequent elution with KOH or sulphate solutions afforded a highly purified glucoside which in several cases crystallized. A very useful modification, introduced by the same authors (*38, 135, 136, 138, 140, 141, 151*) utilizes the ion-exchange properties of acid-washed ("anionotropic") alumina. This exchanger has the additional ability of retaining coloured and other impurities. In a few instances, fractional elution of glucoside-loaded alumina columns has allowed the resolution of a glucosidic mixture into its components (*151*), although this selectivity seems to be rather limited. Much experience in the author's laboratory has confirmed the broad applicability of anionotropic alumina in the purification and isolation of rapeseed oil glucosides; we consider this as the tool of choice. Clearly, ion-exchange resins are well suited also for introducing other cations, such as tetramethylammonium (*27*) or rubidium (*61*), into the glucosides.

Lead acetate, a commonly used reagent in the purification of glucosides (*149*) and other plant products also removes some impurities from crude extracts of mustard oil glucosides prior to ion exchange (*138, 151*), although prolonged contact with lead-containing reagents may lead to losses of thioglucosides as was demonstrated long ago (*128*). In the author's laboratory, lead acetate precipitations have been used extensively, with favourable results (*67, 75, 80, 83, 90*).

Other procedures include electrophoresis (*130*) and partition chromatography on cellulose powder (*38, 132*). Neither of these proved entirely satisfactory, though the latter technique, when applied to acetylated glucosides, has afforded purified (*138*) or homogeneous (*140*) preparations. Isolated examples of the use of countercurrent distribution (*67*) and adsorption chromatography in 80% ethanol (*12*) for the separation of chemically similar glucosides suggest broader applicability.

References, pp. 169–176.

To summarize, the method of isolation and purification should be selected in each case with due regard to the nature and amount of contaminants. Mostly, the available methods will prove satisfactory for the preparation of glucoside fractions free of extraneous matters. A great need exists, however, for efficient preparative procedures which would resolve complex mustard oil glucoside mixtures.

c. *Quantitative Determination.*

Only a few systematic studies have hitherto been undertaken to determine the quantities of glucosides present in plant material. Customarily, the contents have been evaluated indirectly, by estimating the enzymically produced *iso*thiocyanates; for this purpose several methods exist [cf. (*145*)]. Repeated observations indicate, however, that the enzymic fission rarely affords quantitative yields of mustard oils. Hence, an analytical procedure, based on cleavage of the glucosides in strong sulphuric acid and followed by a colourimetric glucose determination, using the anthrone reagent (*38*, *134*), was helpful. A feature that detracts from the usefulness of this method is the necessity of removing, prior to analysis, all disturbing impurities, such as free sugars, glycosides, etc., by paper chromatography or ion exchange.

On the basis of an extensive series of paper chromatograms of *iso*-thiocyanate glucosides from higher plants, and also from literature references concerning the quantities of mustard oils liberated by enzymic hydrolysis, it can now be claimed that the total and relative glucoside contents are subject to considerable variation. The amounts range from traces to several per cent of the dry weight. As in many phytochemical comparisons, doubt may arise as to whether or not a trace glucoside should be counted as a characteristic constituent of a plant. This, of course, is entirely dependent on the sensitivity of the analytical method. Furthermore, the glucoside pattern of even a single organ of a given species may show considerable variation depending on environmental factors. Pertinent quantitative studies have received much less attention so far than did qualitative aspects.

4. Chemical Structure.

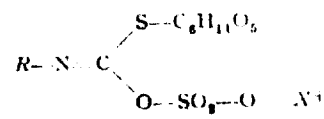
a. *Earlier Formulation.*

Studies of the chemistry of sinigrin and sinalbin, the classical mustard oil glucosides, commenced several years before A. W. HOFMANN (*49*) established in 1868 the true structure of the *iso*thiocyanates and their isomeric relationship to the thiocyanates. Of particular importance in this connexion is a contribution by WILL and KÖRNER (*155*) (1863). They established the correct elementary composition of sinigrin and

demonstrated the formation of free sulphur and allyl cyanide, in addition to allyl *iso*thiocyanate, glucose and sulphate, upon enzymic decomposition of sinigrin. Furthermore, they studied the cleavage of sinigrin with silver nitrate, resulting in precipitation of a glucose-free silver salt which on decomposition with hydrogen sulphide afforded elementary sulphur and allyl cyanide. The latter was established also as a minor constituent of allyl mustard oil of natural origin. WILL and KÖRNER concluded that sinigrin, in addition to glucose and sulphate, contained the elements of sulphur and allyl cyanide, arranged in such a manner as to allow for the simultaneous formation of allyl *iso*thiocyanate, allyl nitrile and sulphur, upon enzymic hydrolysis or chemical cleavage.

HOFMANN (*51*) was the first to report on the presence of 0.32–0.56% of carbon disulphide in naturally derived, as well as synthetic allyl *iso*thiocyanate. The origin of this contamination has been discussed by CHALLENGER (*10*).

On this background it may surprise that GADAMER (*30*) in 1897 ventured to propose for sinigrin and sinalbin the structures (II) which remained virtually unchallenged until a few years ago and still appear in most elementary textbooks.



(II.)

(IIa.) $R = CH_2 = CH - CH_2$, X = K. Sinigrin.
(IIb.) $R = (p)HOC_6H_4CH_2$, X = Sinapine. Sinalbin (GADAMER).

Structure (II) makes it difficult to explain the formation of nitriles. On the other hand, it satisfactorily accounts for the enzymic hydrolysis to *iso*thiocyanates, $R-N=C=S$, glucose and sulphate. Additional evidence given by GADAMER for structure (II) included the reaction of sinigrin with silver nitrate (*30*). When one equivalent of the latter was used, glucose was detached from the glucoside, and a silver mercaptide appeared, suggesting the original location of the sugar moiety in a thioglucosidic linkage. In case of an additional equivalent of silver nitrate, potassium was exchanged by silver to give a crystalline compound, $(C_3H_5NCS)(Ag)(SO_4Ag)$. Information on the molecular site of the sulphate-grouping was sought in the behaviour of sinigrin towards barium hydroxide. Whereas the glucoside was unaffected by boiling barium chloride, the hydroxide caused instantaneous precipitation of barium sulphate, indicating the presence in sinigrin of sulphuric acid in an ester linkage.

Analogous reasoning led GADAMER to propose the structure (IIb) for sinalbin, the parent glucoside of white mustard (*Sinapis alba* L.) (*30*), which furnishes 4-hydroxybenzyl *iso*thiocyanate upon enzymic hydrolysis,

References, pp. 169–176.

as rendered likely by SALKOWSKI (*114*) and confirmed in our laboratory (*94*). An unusual feature of the sinalbin structure is its content of the base sinapine (I, p. 125), the choline ester of sinapic acid, which GADAMER has identified as 3,5-dimethoxy-4-hydroxycinnamic acid (*31*).

A welcome corroboration of the presence of a thioglycoside linkage in sinigrin originated from studies by SCHNEIDER and WREDE (*129*) who showed that treatment of the glucoside with potassium methoxide yielded 1-thio-*D*-glucose, isolated as the silver salt. This observation was later extended by the same research group, with the result that sinigrin, and other analogous glucosides as well, are 1-β-*D*-thio-glucosides (*125*).

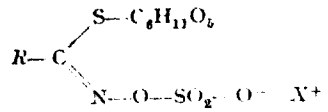
The same authors (*129*) demonstrated the formation also of "mero-sinigrin", for which a cyclic structure was proposed, during treatment of sinigrin with potassium methoxide. The strongly *dextro*rotatory compound yielded a triacetate and was obviously formed by elimination of one molecule of sulphuric acid from sinigrin.

In spite of the early recognized nitrile formation already mentioned, and several observations of the production of varying amounts of organic cyanides during both enzymic hydrolysis and chemical fission of other glucosides (cf. *10*), the GADAMER structure was not seriously questioned until a few years ago. This appears even more astonishing in view of the formation of other recognized by-products during the enzymic cleavage, such as free sulphur and carbon disulphide, the appearance of which is not easily reconcilable with GADAMER's formulation. It is only fair to point out that these difficulties were not ignored by GADAMER but he assumed that the positive evidence mentioned was sufficiently convincing to justify his proposed structure.

A thorough, critical discussion of these developments has been presented by CHALLENGER (*10*) in a recent monograph, that also contains speculations on the formation of the various by-products in the light of present-day knowledge. Especially, the many instances of concomitant nitrile formation during enzymic hydrolysis of thioglucosides are reviewed.

b. Revised Structures.

In 1956, ETTLINGER and LUNDEEN (*23*) published an important communication in which structure (III) was convincingly established as a correct expression for sinigrin and sinalbin.



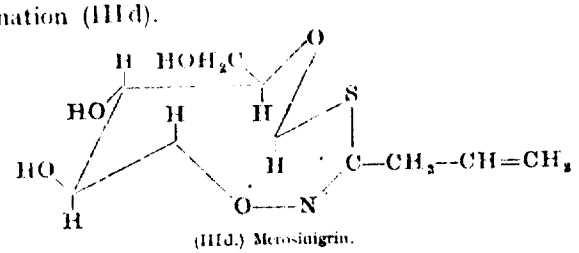
(III)

(IIIa.) $R = CH_2{=}CHCH_2$, $X^+ = K^+$ Sinigrin.
(IIIb.) $R = (p)HOC_6H_4CH_2$, X^+ Sinapine Sinalbin.
(IIIc.) $R = (p)HOC_6H_4CH_2$, $X^+ = (CH_3)_4N^+$.

The revised structure differs significantly from (II) by having the side-chain attached to carbon, rather than nitrogen, which is accommodated in an oxime-like arrangement. Accordingly, the glucosides may be interpreted as substituted thioimino acid esters or, rather, *iso*thiohydroxamic acids.

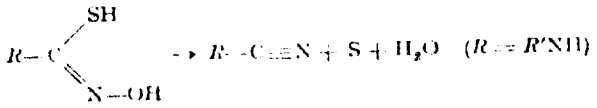
Conclusive support for the revised structure was provided by the following reactions: (i) Hydrogenolysis of sinigrin and the tetramethylammonium salt (IIIc) with Raney nickel furnished, respectively, *n*-butylamine and tyramine; (ii) acid hydrolysis of the same glucosides afforded, respectively, vinylacetic and *p*-hydroxyphenylacetic acid; (iii) the acid fission was accompanied in both instances by formation of hydroxylamine in 50–90% yields. None of these results are compatible with the GADAMER structure. In addition, the American authors presented evidence for sinigrin being a *β*-1-thio-*D*-glucopyranoside by desulphurizing its tetraacetate to 1,5-anhydro-*D*-glucitol tetraacetate. The only structural detail which still remains to be settled is the configuration around the C=N-double bond. Indirect evidence, quoted below, appears to support the *anti*-configuration (III) of the side-chain *R* and the OSO_2O^--grouping (*23*).

In the light of current knowledge, merosinigrin, the transformation product of sinigrin mentioned, almost certainly possesses the structure and conformation (IIId).



(IIId.) Merosinigrin.

Structure (III) makes the frequently observed nitrile and sulphur formation more understandable. As pointed out by ETTLINGER and LUNDEEN (*23*), thiohydroxamic acids have formerly been demonstrated (*9, 150*) to undergo facile decomposition to nitriles and elementary sulphur. Thus, *iso*thiocyanates ($R'NCS$), mainly of the aromatic type, add hydroxylamine to give compounds of the type $R'NHCSNHOH$ which readily decompose in the following manner:



Similar cleavage of the silver mercaptides, or intermediates in the enzymic hydrolysis, proceeding concurrently with the *iso*thiocyanate

References, pp. 169—176.

formation, may be responsible for the observed formation of these products.

The new formulation (III) implies that an intramolecular rearrangement takes place both during enzymic hydrolysis of glucosides and, occasionally, by nucleophilic displacement of the metal from the silver mercaptides. The analogy of this reaction with the well-known Lossen rearrangement of hydroxamic acids has been pointed out by the American authors (*23*).

This mechanism lends support to the suggested configuration around the C=N-bond, in view of the generally recognized *anti* configuration of the migrating group in such rearrangements. Moreover, there is some evidence available (*24, 67, 86*), though not as yet conclusive, to indicate that the rearrangement proceeds with retention of the configuration of the migrating group as expected for a reaction of this kind. The hydrolysis of the glucoside is probably unique in the sense that it involves an enzyme-initiated, intramolecular, nucleophilic displacement; hence, it is of considerable interest. A more detailed study of the factors influencing the relative amounts of *iso*thiocyanates and nitriles produced in the enzymic reaction would be desirable.

In this connexion, the recent demonstration by GMELIN and VIRTANEN (*40*) of an enzyme occurring in the seeds and fresh plants of *Thlaspi arvense* L. and *Lepidium ruderale* L. deserves attention: it cleaves the glucosides sinigrin and glucotropaeolin present to allyl and benzyl thiocyanate, respectively. Apparently, no concomitant production of *iso*thiocyanates takes place in these plants, whereas ordinary garden cress (*Lepidium sativum* L.) gives rise to a mixture of benzyl thiocyanate and benzyl *iso*thiocyanate when reacting with the plant's own enzyme system. Other species have been listed by the same authors (*39*) as additional sources of thiocyanates. Possibly, an unknown factor governing the course of enzymic attack is operative. Indeed, it was questioned by SCHMIDT (*122*) many years ago whether allyl *iso*thiocyanate was the primary reaction product of enzymic sinigrin hydrolysis, considering the facile isomerization of allyl thiocyanate to the corresponding *iso*thiocyanate. Enzymic cleavage experiments conducted at 0° by the same author afforded, however, only traces of the rhodanide and did not support the assumption that the latter is the first reaction product.

In conclusion, at the present time three pathways seem to exist for the enzymic attack on this class of glucosides: (i) A course involving intramolecular rearrangement to *iso*thiocyanates, (ii) an alternative route, predominant in certain plants, that leads to thiocyanates, obviously by rearrangement; and (iii) the formation of nitriles and elementary sulphur with no change in the carbon skeleton.

Differences in the enzymic systems as well as in the initial sites of attack are likely to be responsible for this multiplicity of the observed

end products. It would be important to clarify the detailed mechanism of this remarkable enzyme reaction.

Shortly after the publication of structure (III, p. 131), SCHULTZ and WAGNER (*142*) expressed doubt as to its correctness, because they failed to detect the expected C=N stretching mode in the infrared spectra of various glucoside acetates, and were unable to hydrogenate the same group catalytically by means of Raney nickel. Neither of these arguments seems, however, to affect the validity of (III). There are numerous cases on record (*26*) of substituted oximes, whose C=N stretching bands are very weak or absent. In fact, a complete set of infrared spectra of the crystalline tetraacetates listed in Table 2 (p. 158), recorded in the author's laboratory (*64*), invariably display a weak, but consistent band at ~ 1640 cm.$^{-1}$, the expected position for the C=N stretching mode. The failure to saturate the C=N linkage under the conditions employed can hardly surprise considering the sluggish reaction of a concurrently studied, synthetic model glucoside acetate and the insufficient analytical assays employed.

There are good reasons to believe that all glucosides encountered thus far in nature possess the same general structure as that of sinigrin, sinalbin and glucotropaeolin, with the individual features residing solely in the side-chains. Thus, various other glucosides (progoitrin (*23*), glucomalcolmiin (*90*), glucohirsutin (*66*), glucoerypestrin (*78*), glucocamelinin (*83*), glucoalyssin (*75*), glucocapparin (*74*), glucocinringiin (*41*), etc.) which have been subjected to degradation, all afforded at least two of the fission products: glucose, sulphate and hydroxylamine, the typical fragments of structure (III).

c. Synthesis.

Shortly after the announcement of the glucoside structure (III), ETTLINGER and LUNDEN (*24*) reported on the first successful synthesis of an *iso*thiocyanate glucoside, viz. that of glucotropaeolin. This noteworthy achievement provides an important argument for the correctness of (III).

The synthesis (*Chart 1*) proceeded from magnesium dithiophenylacetate (IV), obtained from benzylmagnesium chloride and carbon disulphide, which upon treatment with hydroxylamine yielded phenylaceto-thiohydroxamic acid (V). Interaction of the latter with aceto-bromoglucose afforded S-*β*-*D*-1-(tetraacetyl-glucopyranosyl)-phenylaceto-thiohydroximic acid (VI), which could be converted into the tetraacetyl-glucotropaeolate ion upon treatment with sulphur trioxide in pyridine. The ion was isolated as the potassium salt, which proved identical with a salt of natural origin (Table 2, p. 158), and as the tetramethylammonium salt (VII) which was then deacetylated to give glucotropaeolin as a salt

References, pp. 169–170.

of the same base (VIII); the latter was indistinguishable from a sample prepared by ion-exchange of the potassium salt that originated from natural sources.

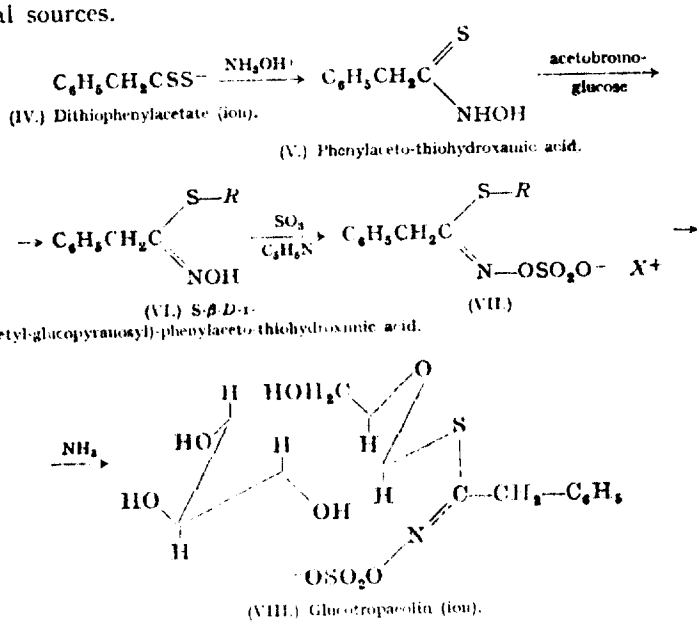


Chart 1. Synthesis of Glucotropaeolin.
R = Tetraacetyl β-D-1-glucopyranosyl, X^+ = $(CH_3)_4N$.

The glucotropaeolate ion (VIII) is formulated above in its supposedly favoured conformation, with all substituents of the pyranose ring located in equatorial positions.

5. Individual Glucosides.

In addition to the natural glucosides which have been obtained in crystalline form, either as the genuine compounds (Table 1, p. 157) or as acetates (Table 2, p. 158), a considerable number exists, whose structures can be inferred from the chemical nature of the derived mustard oils. Most of the parent compounds have been assigned names in accordance with the general usage in this field. The complete series of glucosides for which the derived *iso*thiocyanates have so far been chemically established appears in *Table 3*, p. 159. For the sake of completeness, the glucosides listed in Tables 1 and 2 are retabulated in Table 3, together with their aglucones. A number of glucosides with unknown or only partially clarified side-chains, such as glucocaulorapin (*138*, *151*) and gluconatronalin (*142*, *151*), have not been included in the Table.

A more detailed discussion of the individual *iso*thiocyanates (cf. Table 3) will be presented on p. 141. It has become an increasingly common practice to denote the more complex *iso*thiocyanates by trivial names derived from the corresponding glucoside designations by omitting the prefix gluco- (e. g. arabin, iberin, berteroin, etc.).

The natural sources for the individual glucosides (Table 3) will be treated on p. 154. As an outcome of paperchromatographic scanning of an extensive collection of botanical taxa for *iso*thiocyanate glucosides in the author's laboratory and elsewhere, it can be predicted that several additions to those listed in Table 3 will be forthcoming.

IV. Enzymic Hydrolysis.

1. Distribution of Myrosinase.

The recognition of *iso*thiocyanate production as a result of enzymic hydrolysis (*8*), paired with the unique chemical structure of the substrates and the multiplicity of the reaction products, has stimulated early interest in myrosin, particularly its anatomical localisation and its distribution in the vegetable kingdom. The practical interest, associated with the manufacture of table mustard, should not be overlooked in this connexion.

In contrast to the glucosides, which are distributed diffusely throughout the parenchymal tissues, the enzyme is accumulated in particular cells (idioblasts), as was first demonstrated for Crucifers by GUIGNARD (*44*). The same author later showed a similar occurrence of myrosin in species of the families *Capparidaceae*, *Resedaceae*, *Tropaeolaceae* and *Limnanthaceae* (*45*). It appears that myrosin-containing cells are present in all tissues of *iso*thiocyanate glucoside-producing plants and can be histochemically distinguished by staining with Millon's reagent, iodine, or orcinol in hydrochloric acid (*143*). PECHE (*106*) employed a sinigrin solution, saturated with barium chloride, to locate myrosin whereby a precipitate of barium sulphate formed inside the enzyme-containing cells. In accordance with the distribution of substrate and enzyme within the plant, no hydrolytic fission takes place until the tissues are disintegrated and the reactants brought into contact.

In an early, important contribution by LEPAGE (*99*) it was demonstrated that a large variety of Crucifer seeds contained myrosin. Since then, numerous references have appeared in the literature to myrosin-containing species, and a list of these has been published by SCHMALFUSS and MÜLLER (*117*). There are good reasons for believing that the presence of myrosin in plant species is usually connected with the glucoside content. However, the same enzyme system seems to be operative in the hydrolysis of all glucosides of this type.

References, pp. 169–176.

2. Properties of Myrosinase.

A more detailed study of myrosin was initiated in 1926 by NEUBERG and WAGNER (*104*) as part of a broader investigation of sulphatases. They adopted the term "myrosinase" instead of "myrosin", coined in analogy with "sulphatase". Directions were also presented for the preparation, from white mustard seeds, of a cell-free, active enzyme solution, which has been employed in most subsequent work. An alternative procedure for the preparation of myrosinase, in form of a dry powder, was described by BRAECKE (*7*).

Surprisingly enough, few systematic studies have as yet been reported on the purification of myrosinase; and most authors have been concerned with rate studies of the enzymic fission. NEUBERG and WAGNER (*104*) clearly demonstrated that myrosinase is distinctly different from all known plant and animal sulphatases and possesses a high degree of specificity that is limited to the natural glucosides discussed. The existence in myrosinase of two entities, one liberating glucose in a fast reaction and another acting as a slow sulphatase, was suggested by VON EULER and ERIKSON (*25*). This interpretation was strengthened by SANDBERG and HOLLY (*115*), yet with the modification that both components seemed to start their action simultaneously. Whereas a quantitative yield of sulphuric acid was obtained, the yields of glucose never exceeded 60% of the theoretically possible value. STAHMANN et al. (*144*) have demonstrated that myrosinase at equilibrium had produced about 80% of the possible amount of allyl *iso*thiocyanate.

In contrast to these investigations, NAGASHIMA and UCHIYAMA (*103a*) have recently provided good experimental evidence to support the conception of myrosinase as a single enzyme that hydrolyses the thioglucosidic linkage. The subsequent intramolecular rearrangement is visualized as a spontaneous, non-enzymic reaction. The same authors have observed inhibition of myrosinase by SH-inhibitors and a remarkable activation by ascorbic acid.

Several investigators have tested the stability of myrosinase by varying pH and temperature [cf. e. g. (*153, 103a*)]. It appears that myrosinase has a pH optimum at 6.5–7.5 and a temperature optimum between 30° and 40°.

It is obvious that detailed studies of the enzyme and its action, in the light of our recent knowledge concerning the chemical constitution of the substrates, are greatly needed. The diversity of the products suggests that enzymic and non-enzymic conversions may proceed concomitantly and at various rates, depending on the conditions. Moreover, the participation of unknown co-factors or inhibitors in the

enzymic hydrolysis is suggested by the recent finding of thiocyanates as ultimate reaction products in certain plants (*39, 40*).

It is noteworthy that REESE et al. (*112*), as a result of screening 300 microorganisms, have recently reported the discovery of three fungi of the *Aspergillus versicolor* group that produce a specific thioglucosidase, for which the term *sinigrinase* was introduced (not to be confused with the same name used earlier for myrosinase). In one of these fungi, an *Aspergillus sydowi* strain, it was shown that sinigrinase represents a constitutive enzyme. Though distinctly different from myrosinase, the fungus enzyme acts on sinigrin to yield the same products as myrosinase. Sinigrinase effects virtually complete glucose formation from sinigrin but attacks phenyl-*β*-*D*-thioglucoside as little as does myrosinase. The relative activities of the two enzymes on various *iso*thiocyanate glucosides are different and so are the respective stabilities and inhibition patterns.

It is of interest in this connexion that GOODMAN et al. (*41a*) have recently demonstrated the ability of myrosinase to hydrolyze a series of synthetic *β*-*D*-thioglucopyranosides which contain thiol-substituted purines, pyrimidines, pyridazines or aromatic rings as aglycones. In this respect, myrosinase resembles a remarkable new enzyme detected by the same authors as a constituent of mammalian tissues of every living species investigated. The natural substrates for the latter enzyme are still obscure.

Evidently, several unanswered problems would be clarified by a more detailed knowledge of myrosinase and its mode of action.

V. Naturally Derived *iso*Thiocyanates.

1. General Properties.

We have referred repeatedly to *iso*thiocyanates which result from enzymic hydrolysis of naturally occurring glucosides. The formulae of the thirty known mustard oils of natural derivation with clarified structures are listed in *Table 3*, p. 159. In the present Chapter the individual *iso*thiocyanates will be discussed.

The ability to liberate pungent principles upon disintegration is a conspicuous property of some plants which have been used extensively as potherbs, condiments or remedies. Evidently, the production of *iso*thiocyanate will often be the first indication of the presence of the corresponding glucoside. Accordingly, much pertinent information has originated from studies of enzymically produced mustard oils.

Notwithstanding the early recognition of such non-volatile *iso*thiocyanates as cheirolin, erysolin and *p*-hydroxybenzyl mustard oil, there

References, pp. 169—175.

has been a general tendency to consider the natural *iso*thiocyanates as "essential oils", i. e. steam-volatile compounds. This should be discontinued, however, because about half of all known natural mustard oils are not steam-distillable (cf. Table 3). Most of them are liquids, and it is a common practice to characterize them by their thiourea derivatives obtained by means of ammonia or amines.

The spectroscopic properties of *iso*thiocyanates, both in the ultraviolet and infrared regions, have recently been thoroughly studied (*147*).

When applied to the tongue, all mustard oils cause a sharp and burning sensation. Their odours, though mostly pungent, display characteristic individual differences which often are helpful in the detection and classification of mustard oils. Certain *iso*thiocyanates, that undergo rapid intramolecular cyclization, give rise to a transient biting taste, followed by a sensation of bitterness. Like most synthetic mustard oils, those of natural origin show vesicant and frequently also lachrymatory properties.

2. Detection, Isolation, Separation, and Determination.

a. Chromatographic Methods.

Besides taste and smell, various chemical assays have been employed to detect *iso*thiocyanates in plant materials (*145*). While most earlier procedures were limited to the detection of steam-volatile mustard oils, paper chromatography has provided a more general and efficient analytical tool. It is also useful for the tentative identification of individual *iso*thiocyanates. In the author's laboratory, a method was developed several years ago for the separation of thioureas by chromatography on paper (*93*). Transformation of natural *iso*thiocyanates into thiourea derivatives, followed by paper chromatography (*72*), has been found very useful in the detection, isolation and identification of many new natural mustard oils. Originally, the chromatographic method had been developed for steam-volatile mustard oils (*72*), but it has since been extended to the study of non-volatile representatives.

Water-saturated chloroform has proved to be particularly useful for paper chromatography of thioureas (*93*), but other solvent systems also have been employed, such as ethyl acetate : water and 2-butanone : water (*56*), pyridine : amyl alcohol : water and heptane : 95% formic acid : *n*-butanol (*73, 85*); *n*-butanol : ethanol : water (*74, 75*), formamide : chloroform (*65*), and aromatic hydrocarbons : water, eventually with varying additions of ethanol, acetone or ethyl acetate (*131*). Grote's reagent, a modified nitroprusside reagent, has been extensively used for spraying, whereby the thioureas appear as blue spots (*93*). Ammoniacal silver solutions are also useful (*12, 131*) for locating thiourea spots on paper. When not too volatile, the *iso*thiocyanates themselves can be chromatographed, with silver

nitrate as a suitable spray reagent (*76, 109*). A Roumanian research group has reported on paper chromatography of various aromatic *iso*thiocyanates, after conversion of the latter into thiosemicarbazides by means of 2,4-dinitrophenylhydrazine (*27*). Alternatively, the same authors chromatographed simple thioureas in form of their yellow bismuth acetate complexes (*28*).

The powerful new tool of gas chromatography is a promising supplement to the paperchromatographic technique for the separation and identification of mustard oils (*88*).

b. Isolation.

The procedures selected for the isolation of *iso*thiocyanates from enzymic hydrolysates depend, of course, on the chemical character. Provided the mustard oils are sufficiently stable and volatile, steam-distillation still affords a convenient method. Furthermore, steam-volatility of one or more components in a complex mixture of *iso*thiocyanates may help in identifying individual constituents. From the aqueous distillates the substance can be isolated either by extraction, or by conversion directly into a thiourea derivative (references in Table 3, p. 159). When extraction is required, certain solvents, such as ether, do not interfere with the enzymic reaction and can be employed for continuous removal of the mustard oils during hydrolysis (*94*); other solvents, e. g. chloroform, may damage the enzyme system.

c. Separation.

As mentioned, most glucoside-containing plant materials afford a mixture of *iso*thiocyanates upon enzymic hydrolysis. This raises the important problem of separating the individual constituents on a preparative scale in order to secure sufficient material for structural studies. Occasionally, the material available will allow fractional distillation in vacuo of the mustard oil mixture (*20, 89, 116*), but oftentimes thiourea derivatives will have to be prepared. Distribution between partly miscible solvents has been successfully applied to *iso*thiocyanates (*119, 120*) and thioureas (*67, 71, 85, 103*). A similar method, followed by column chromatography on alumina, has proved useful also for the separation of free mustard oils (*109*). In many instances, however, the solubility properties of the individual thiourea derivatives are sufficiently different to permit fractional crystallization (e. g. (*90, 119*)).

No systematic studies have yet been undertaken to develop general methods for the separation on a preparative scale of *iso*thiocyanates or their derivatives. All workers active in the field would welcome progress along these lines.

References, pp. 169–176.

d. Quantitative Determination.

A detailed discussion of the quantitative analysis of mustard oils falls outside the scope of the present paper. Special interest is attached to this problem in pharmacy and food industry, and an extensive pertinent literature exists (*3, 145*). Evidently, the methods must be based on a preceding liberation of the *iso*thiocyanates from the glucosides and hence depend on the character and amount of accompanying by-products or contaminants. The frequent occurrence of *iso*thiocyanate mixtures in plants has often been disregarded.

Within the last decade, spectrophotometric assays have been increasingly used. In the author's laboratory, a method, taking advantage of the intense band shown by thioureas at $\sim 240\ m\mu$, has given very satisfactory results (*72*).

The determination in plant extracts and milk samples of that particular group of mustard oils which undergo cyclization to biologically potent 2-oxazolidinethiones represents a problem of particular interest. The methods used for this purpose are based on the spectrophotometric evaluation of the heterocyclic ring (*4, 97, 154*).

3. Chemical Structure.

The following discussion includes a brief outline of the evidence on which the chemical structures are based, and also some references to the most important botanical sources. A more complete survey of the plants investigated appears in *Table 5*, p. 161. Only mustard oils of well-authenticated chemical structures, as listed in Table 3, will be considered.

a. Saturated Alkyl iso*Thiocyanates.*

Methyl *iso*thiocyanate. This simple mustard oil, CH_3NCS, has not been definitely identified in any species of the *Cruciferae* but appears to be widely distributed in form of the glucoside *glucocapparin* throughout the *Capparidaceae* (*74, 78*). For the purpose of identification it was transformed into 1-methylthiourea (*84*).

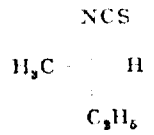
Ethyl *iso*thiocyanate. Seeds of the North-American crucifer *Lepidium Menziesii* DC. represent, thus far, the sole recorded source of this simple compound derived from the hypothetical glucoside "glucolepidiin". Conversion into 1-ethylthiourea has proved its structure (*56*).

***iso*Propyl *iso*thiocyanate.** The first recorded source of this mustard oil was the Indian plant *Putranjiva Roxburghii* WALL. (*110*) – a remarkable finding since this compound is the sole well-authenticated mustard oil found in *Euphorbiaceae*. The same *iso*thiocyanate, deriving from the

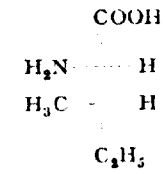
parent glucoside *glucoputranjivin*, was isolated from seeds of the crucifer *Lunaria biennis* MNCH. (*69*). It is rather widely distributed in *Cruciferae*, occurring e. g. in species of *Cochlearia*, *Lunaria* and *Sisymbrium*. Furthermore, the species *Tropaeolum peregrinum* (*Tropaeolaceae*) afforded *iso*propyl mustard oil upon enzymic hydrolysis. The structural evidence is based on the conversion into 1-*iso*propylthiourea (*69*).

(+)-2-Butyl *iso*thiocyanate (IX) represents one of the "classical" mustard oils, recognized by HOFMANN (*50*) in 1870, as part of distillates of *Cochlearia officinalis* L.; it was further studied by GADAMER (*33*). More recent investigations have established its occurrence in form of the glucoside *glucocochlearin*, in species of the cruciferous genera, *Cardamine*, *Cochlearia*, *Draba*, *Lunaria*, *Sisymbrium*, etc. (cf. Table 5, p. 161). Other families, such as *Euphorbiaceae* *Phytolaccaceae* and *Tropaeolaceae*, also include species which afford, on enzymic fission, (+)-2-butyl *iso*thiocyanate. The latter is frequently accompanied by varying amounts of *iso*propyl *iso*thiocyanate, possibly indicating a common biogenetic pathway of the two compounds.

By chemical correlation, the *dextro*rotatory, natural 2-butyl *iso*-thiocyanate has been shown to possess the absolute configuration (IX) (*86*) which is identical with that prevailing around the β-carbon atom of natural *iso*leucine (X).



(IX.) (+)-2 Butyl isothiocyanate.



(X.) *L-Iso*leucine.

Methyl 4-*iso*thiocyanatobutyrate (Erypestrin). The detection in the seeds of *Erysimum rupestre* DC. (and other species of the same genus) of an alkali-labile glucoside, *glucoerypestrin*, was followed by a study of the corresponding mustard oil, *erypestrin*, in the author's laboratory (*78*). On treatment with ammonia, aniline and 1-naphthylamine, the *iso*thiocyanate afforded crystalline thioureas that were indistinguishable from those prepared from synthetic methyl 4-*iso*thiocyanatobutyrate, $CH_3OOCCH_2CH_2CH_2NCS$. No other sources than *Erysimum* spp. have been reported thus far for glucoerypestrin which seems to be invariably accompanied by glucocheirolin.

b. Unsaturated Alkyl iso*Thiocyanates.*

Allyl *iso*thiocyanate. This is the volatile mustard oil par excellence, $CH_2=CHCH_2NCS$, formed by enzymic fission of sinigrin. Seeds of black

References, pp. 169–179.

mustard (*Brassica nigra* KOCH) serve as the traditional source for this *iso*thiocyanate but other materials, such as horse-radish root (*146*), may serve equally well.

Numerous references exist to the production of allyl mustard oil upon disintegration of various botanical species, but the older literature should be accepted with reservation because of meagre documentation in many instances. A list of well-established and of doubtful sinigrin sources is available (*58*). Attention should be drawn to the recently observed enzymic production in *Thlaspi arvense* L. seeds of allyl thiocyanate rather than the mustard oil (*40*).

3-Butenyl *iso*thiocyanate. A rather contradictory literature exists on the occurrence in rape seeds (*Brassica napus* L.) of a glucoside, *gluconapin*, that affords on enzymic cleavage an unsaturated C_5-*iso*-thiocyanate. The identity of the latter as 3-butenyl *iso*thiocyanate, $CH_2{=}CHCH_2CH_2NCS$, was established by synthesis independently by ETTLINGER and HOPKINS (*20*), and KJÆR et al. (*71*). Some additional sources exist for this mustard oil, e. g. *Alyssum*, *Brassica*, *Cardamine* and *Isatis* spp. (cf. Table 5, p. 161).

4-Pentenyl *iso*thiocyanate. This higher homologue, $CH_2{=}CHCH_2CH_2CH_2NCS$, has been identified as a minor constituent of the volatile mustard oil fraction of rape seed, originating, by enzymic hydrolysis, from the glucoside, *glucobrassicanapin* (*89*). The structure is based on infrared evidence and on the desulphuration of the 1-naphthylthiourea derivative (XI) to the urea derivative (XII), which upon catalytic hydrogenation afforded the saturated urea (XIII), identical with a sample synthesized from *n*-pentylamine and 1-naphthyl *iso*-cyanate.

$$CH_2{=}CH{-}CH_2{-}CH_2{-}CH_2{-}NHCSNH{-}C_{10}H_7$$

(XI) $\downarrow AgNO_3$

$$CH_2{=}CH{-}CH_2{-}CH_2{-}CH_2{-}NHCONH{-}C_{10}H_7$$

(XII) $\downarrow H_2/Pt$

$$CH_3{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}NHCONH{-}C_{10}H_7$$

(XIII.) Pentyl-(1-naphthyl)-urea.

$\uparrow$

$$CH_3{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}NH_2 + C_{10}H_7NCO$$

Synthesis of 4-pentenyl *iso*thiocyanate confirmed the identity of the mustard oil mentioned (*89*). In addition to *Brassica* spp., the 4-pentenyl compound seems to occur in members of *Alyssum* and possibly other Crucifers as well.

c. ω-Methylthioalkyl iso*Thiocyanates and Related Sulphoxides and Sulphones.*

Under this heading an interesting group of natural products will be discussed, having in common the skeleton, $CH_3S(CH_2)_nNCS$, in which a double bond may be present in the alkyl chain and the sulphide atom may carry oxygen. Mustard oils of this type occur abundantly in Crucifers, and there is much interest in the problem of their biogenesis. The discovery within the last decade of ten new individual *iso*thiocyanates of this general structure was preceded by the structural elucidation of cheirolin, $CH_3SO_2(CH_2)_3NCS$ (*123*), and erysolin, $CH_3SO_2(CH_2)_4NCS$ (*126*), by SCHNEIDER et al. fifty years ago.

3-Methylthiopropyl *iso*thiocyanate (Ibervirin). Seeds of the Crucifer *Iberis sempervirens* L. were shown in this laboratory to afford two steam-volatile *iso*thiocyanates subsequent to enzymic hydrolysis. The corresponding thiourea mixture was subjected to countercurrent distribution, resulting in the separation of two pure fractions (*85*). One of these proved to be 1-(3-methylthiopropyl)-thiourea upon comparison with a synthetic specimen, whereas the other, minor constituent was the homologous 1-(4-methylthiobutyl)-thiourea. This result indicates the enzymic production of 3-methylthiopropyl *iso*thiocyanate, $CH_3SCH_2CH_2CH_2NCS$, *(ibervirin)* from a parent glucoside, named *glucoibervirin*. The botanical distribution of the latter is still unknown but appears to be rather limited. Glucoibervirin is present in green parts of *Cheiranthus cheiri* L. (wallflower) and in *Iberis amara* L. roots (unpublished).

3-Methylsulphinylpropyl *iso*thiocyanate (Iberin). The foregoing mustard oil is closely related to *iberin*, derivable from the glucoside *glucoiberin*, which was isolated by SCHULTZ and GMELIN (*136*) in crystalline form from the seeds of *Iberis amara* L. They presented evidence, later confirmed in the writer's laboratory (*75*), for the corresponding, optically active *iso*thiocyanate to possess the structure $CH_3SO(CH_2)_3NCS$. The sulphoxide group is very probably formed by in vivo oxidation of glucoibervirin. Among possible botanical sources of glucoiberin, the green parts of certain cabbage species deserve special interest. Thus, PROCHÁZKA et al. (*109*) found iberin in the press juice of Brussels sprouts, for example.

3-Methylsulphonylpropyl *iso*thiocyanate (Cheirolin). A still higher oxidation level than in iberin is present in the well-known mustard oil of wallflower seeds (*Cheiranthus cheiri* L.), for which SCHNEIDER (*123*) proved the structure $CH_3SO_2CH_2CH_2CH_2NCS$, derivable from the glucoside *glucocheirolin*. The latter has been encountered in several species of the cruciferous genera *Cheiranthus*, *Erysimum* and *Malcolmia*. It is most likely related biogenetically to the structurally similar mustard

References, pp. 169—176.

oils ibervirin and iberin (see above). Recently, BACHELARD et al. (*40*) have isolated cheirolin from enzymically hydrolyzed extracts of fruits (1.2 g. per kg. dry weight) and fresh leaves (0.4 g. per kg. wet weight) of the common weed, *Rapistrum rugosum* (L.) ALL. A convenient synthesis of cheirolin has been developed in the author's laboratory (*94*).

4-Methylthiobutyl *iso*thiocyanate (Erucin). This sulphide mustard oil, $CH_3SCH_2CH_2CH_2CH_2NCS$, was first found in enzymically hydrolyzed glucoside extracts of *Eruca sativa* MILL., and its structure was established by comparison with synthetic preparations (*73*). The parent glucoside, ***glucoerucin***, seems to be rather widespread throughout the Crucifer family. Thus, erucin was isolated by DELAVEAU (*17*) from *Diplotaxis tenuifolia* (L.) DC. roots and, as pointed out above, in this laboratory from *Iberis sempervirens* L. (*85*). Furthermore, there is strong paper-chromatographic evidence for the presence of glucoerucin in certain species of the genera *Brassica*, *Cheiranthus*, *Farsetia*, *Hesperis*, *Iberis*, *Matthiola* and *Vesicaria* (*73*).

4-Methylsulphinylbutyl *iso*thiocyanate (Sulphoraphane). It was found only recently that this next higher homologue, $CH_3SO(CH_2)_4NCS$, of iberin, synthesized by SCHMID and KARRER (*121*) more than ten years ago, is a natural product. In the author's laboratory a rather widespread occurrence of sulphoraphane has been noted (*66*), e. g. in species of *Brassica*, *Eruca* and *Iberis*. PROCHÁZKA (*108*) was able to isolate partially racemized sulphoraphane from the leaves of *Lepidium draba* L. According to the same author, sulphoraphane, together with iberin, is also present in the fresh juice of certain cabbage varieties (*109*). No name has yet been suggested for the parent glucoside, but *glucoraphanin* would be a logical term.

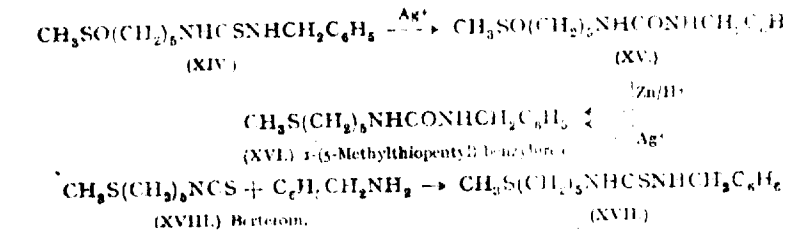
4-Methylsulphinyl-3-butenyl *iso*thiocyanate (Sulphoraphene). SCHMID and KARRER (*119*) concluded in 1948 that radish seeds (*Raphanus sativus* L.) produce, upon enzymic hydrolysis, the strongly *levo*rotatory mustard oil, sulphoraphene, $CH_3SOCH{=}CHCH_2CH_2NCS$, accompanied by the corresponding nitrile (*120*). It seems, that sulphoraphene is the first natural product whose optical activity is due solely to the presence of asymmetric sulphur. GMELIN (*38*) has introduced the designation *glucoraphenin* for the parent glucoside, which probably is also a constituent of *Matthiola bicornis* DC. seeds (*138*). The recent finding by PROCHÁZKA (*107*) of sulphoraphene in the press juice of *Plantago major* L. is remarkable, since it is the first recorded case of the presence of a mustard oil in *Plantaginacea*.

4-Methylsulphonylbutyl *iso*thiocyanate (Erysolin). This higher homologue, $CH_3SO_2(CH_2)_4NCS$, of cheirolin was reported by SCHNEIDER

and KAUFMANN (*126*) as a constituent of enzymically hydrolyzed seed extracts of *Erysimum Perofskianum* FISCH. et MAY. Although this mustard oil, conceivably arising from the glucoside *glucoerysolin*, fits well into the present series, it has not been possible in the author's laboratory or elsewhere to prove its presence in the seeds mentioned or in any other species investigated. Hence, its occurrence may be dependent on special conditions of growth. A practical synthesis of erysolin was reported by KJÆR and CONTI (*70*).

5-Methylthiopentyl *iso*thiocyanate (Berteroin). It was shown in the author's laboratory, that seeds of the cruciferous weed *Berteroa incana* (L.) DC. furnish enzymically a volatile mustard oil, *berteroin*, whose 5-methylthiopentyl *iso*thiocyanate structure, $CH_3S(CH_2)_5NCS$, was established by comparison with suitable thiourea derivatives and by synthesis (*91*). The progenitor, *glucoberteroin*, was shown to be present also in seeds of *Lunaria rediviva* L., as well as in several *Alyssum* species. Usually, the glucoside is accompanied by varying amounts of the corresponding sulphoxide (see below).

5-Methylsulphinylpentyl *iso*thiocyanate (Alyssin). This sulphoxide mustard oil was first isolated in the author's laboratory from seeds of *Alyssum argenteum* VITM., following enzymic hydrolysis. Its structure, $CH_3SO(CH_2)_5NCS$, was established by transformation of the corresponding benzylthiourea derivative (XIV) into the benzylurea compound (XV). The optical activity of the latter disappeared on reduction with zinc and acid which yielded the sulphide-urea (XVI); this indicates the original sulphoxide-grouping as the sole centre of asymmetry in alyssin. Compound (XVI) was proved to be 1-(5-methylthiopentyl) benzylurea by comparison with an authentic sample, produced by desulphurization of the corresponding thiourea (XVII); the latter had been prepared from berteroin (XVIII) and benzylamine (*75*).



The progenitor, *glucoalyssin*, was encountered in several *Alyssum* and *Berteroa* spp. (*75*) and may well be related biogenetically to glucoberteroin. SCHULTZ and WAGNER (*140*) isolated glucoalyssin, in form

References, pp. 169–170.

of the crystalline tetraacetate, from seeds of an *Alyssum* species and proposed (though on meagre evidence) the above alyssin structure.

8-Methylsulphinyloctyl *iso*thiocyanate (Hirsutin). The class of naturally derived sulphoxide *iso*thiocyanates was extended when it was observed in this laboratory that enzymic hydrolysis of seed extracts of the crucifer *Arabis hirsuta* (L.) SCOP. afforded *hirsutin*, for which the structure $CH_3SO(CH_2)_8NCS$ was established upon degradation of the corresponding phenylthiourea derivative to 1-(8-methylthiooctyl)-3-phenylurea (*66*) by a procedure similar to that outlined for alyssin. The glucoside, *glucohirsutin*, was not further characterized and its natural distribution is still unknown.

9-Methylsulphinylnonyl *iso*thiocyanate (Arabin). This next-higher homologue of hirsutin was encountered as the enzymic hydrolysis product of *glucoarabin*, present in seeds of the common crucifer *Arabis alpina* L. The arabin structure, $CH_3SO(CH_2)_9NCS$, was suggested on the basis of customary degradation methods (*77*), and was later unequivocally proved by partial synthesis (*65*). Although presumably present in several *Arabis* species, the exact distribution of glucoarabin remains to be clarified.

10-Methylsulphinyldecyl *iso*thiocyanate (Camelinin). This highest known member of the sulphoxide mustard oil series appears in enzymically cleaved seed extracts from *Camelina sativa* (L.) CRANTZ that contain *glucocamelinin*. Its structure, $CH_3SO(CH_2)_{10}NCS$, was established in the author's laboratory (*83*) by a conventional scheme of degradation. The parent glucoside is present also in other *Camelina* species (*83*), but otherwise its occurrence in the vegetable kingdom is unknown.

Stereochemistry.

The homologous series of optically active *ω*-methylsulphinylalkyl *iso*thiocyanates of natural extraction, $CH_3SO(CH_2)_nNCS$ (n = 3, 4, 5, 8, 9 and 10), discussed above, raises the question as to the relative and absolute steric configurations. Convincing evidence is on hand that all members of this series possess the same steric configuration around the asymmetric sulphoxide grouping. Thus, an extensive series of analogous derivatives, listed in Table 4, p. 160, shows comparable rotations, with regard to sign as well as magnitude. Furthermore, rotatory dispersion studies of a selected number of these derivatives over a rather broad wavelength region (*96*) lend even stronger support to our assumption.

Absolute configurations, however, have not yet been established. The problem requires the stereochemical correlation of any representative of the mustard oil series with (+)-S-methyl-*L*-cysteine S-oxide, the

sole sulphoxide of secured absolute configuration. The latter was established by HINE and ROGERS (*48*) by X-ray analysis.

d. Aromatic iso*Thiocyanates.*

Benzyl *iso*thiocyanate. This structurally simple, aromatic mustard oil, $C_6H_5CH_2NCS$, was first recognized by GADAMER in 1899 as an enzymic hydrolysis product of *glucotropaeolin*, present in seeds and fresh parts of *Tropaeolum majus* L. (*34*) and in *Lepidium sativum* L. seeds (*35*). Since then, several other sources of benzyl mustard oil have been found, e. g. *Coronopus didymus* (L.) SM. (*101*) and *Lepidium* spp. Even more interesting is the frequent appearance of benzyl *iso*thiocyanate in species belonging to other families than *Cruciferae*. Thus, it has been encountered in *Caricaceae*, *Moringaceae*, *Salvadoraceae*, *Tropaeolaceae* and, possibly, *Phytolaccaceae* (cf. *Table 6*, p. 167). There is, furthermore, some support for its occurrence in *Jatropha multifida* L. *(Euphorbiaceae)* (*29*).

It is noteworthy that benzyl cyanide has been observed repeatedly as a product formed during fission of the parent glucoside (*6*, *10*). Accordingly, SCHULTZ and GMELIN (*135*) suggested a possible relationship between glucotropaeolin and an unknown growth factor of *Lepidium sativum* L. which may plausibly be phenylacetic acid, formed by hydrolysis of benzyl cyanide. An alternative enzymic cleavage of glucotropaeolin in the seeds of *L. ruderale* L. and *L. sativum* L., viz. to benzyl thiocyanate, was recently reported (*40*). Further breakdown of the latter may be responsible for the benzyl mercaptan production observed by FORSS (*28a*) in macerates of the crucifer *Coronopus didymus*. Under different circumstances the same plant afforded mixtures of benzyl cyanide and benzyl *iso*thiocyanate (*28a*, *101*).

***p*-Hydroxybenzyl *iso*thiocyanate.** The non-volatile mustard oil formed during enzymic hydrolysis of the "classical" glucoside *sinalbin* was studied by SALKOWSKI (*114*) who suggested its structure, $(p)HOC_6H_4CH_2NCS$. His conclusion was confirmed in the author's laboratory by comparing the phenylthiourea derivative with an authentic sample of 1-(4-hydroxybenzyl)-3-phenylthiourea. The labile character of the initially formed mustard oil calls for special precautions during isolation (*94*). Thus, WILL and LAUBENHEIMER (*156*) have demonstrated the facile formation of thiocyanate upon treatment of the mustard oil with hot ammonia or alkali. Though abundantly present in white mustard seeds (*Sinapis alba* L.), sinalbin does not appear to be a glucoside of very wide distribution. There is good evidence for the presence of the glucosidic anion of sinalbin also in seeds of *Sinapis arvensis* L., *Lepidium*

References, pp. 169—176.

campestre (L.) R. Br. and in some species of the genera *Aubrietia*, *Brassica* and *Bunias*.

p-Methoxybenzyl *iso*thiocyanate (Aubrietin). In view of the common occurrence of O-methylated phenolic compounds in plants, it was not unexpected to find *p*-methoxybenzyl mustard oil, $CH_3OC_6H_4CH_2NCS$, as an enzymic fission product of *glucoaubrietin*, in fresh parts and seeds of various *Aubrietia* spp. *(Cruciferae)*. Its structure was secured by comparison of thiourea derivatives with authentic samples (*81*). Thus far, *Aubrietia* spp. represent the sole botanical source of aubrietin, easily recognizable by its taste which, besides being pungent, is reminiscent of anis.

m-Methoxybenzyl *iso*thiocyanate (Limnanthin). More surprising was the finding by Ettlinger and Lundeen (*22*) of *m*-methoxybenzyl mustard oil, $CH_3OC_6H_4CH_2NCS$, as an enzymic product of an unnamed glucoside in the seeds of the North-American plant *Limnanthes douglasii* R. Br., belonging to the small family of *Limnanthaceae*. No other sources have yet been recorded. The structural proof was provided by synthesis. As pointed out by the American authors, the limnanthin structure deserves special interest considering the rare occurrence of *m*-disubstituted aromatic compounds in nature.

2-Phenylethyl *iso*thiocyanate. This next higher homologue, $C_6H_5CH_2CH_2NCS$, of benzyl mustard oil, liberated from *gluconasturtiin*, was first encountered by Gadamer (*35*) in fresh parts of the Crucifers *Nasturtium officinale* R. Br. and *Barbarea praecox* R. Br., but has since been recognized as a mustard oil of extensive distribution. In fact, there is strong evidence that it is the predominant volatile *iso*thiocyanate in root tissues of several *Brassica* species, such as turnip, black and white mustard, as well as cabbage (*144*). Horse-radish also has a high content of this aromatic *iso*thiocyanate (*144*). André and Delaveau (*2*) found it to be a minor constituent of the volatile mustard oil fraction of rape seed cake. Outside the family *Cruciferae*, 2-phenylethyl *iso*thiocyanate has been observed in root tissues of various *Resedaceae* (*5*).

3-Benzoyloxypropyl *iso*thiocyanate (Malcolmiin). A rather unexpected aromatic mustard oil was discovered in the author's laboratory when studying seed extracts of the Crucifer *Malcolmia maritima* (L.) R. Br. Upon enzymic cleavage of *glucomalcolmiin*, a mustard oil with absorption characteristics indicative of aromatic character was obtained. Its thiourea derivative, $C_{11}H_{14}O_2N_2S$, yielded (in alkali) benzoic acid in accordance with the infrared evidence of an ester linkage. The alcoholic entity of the latter was established as 3-hydroxypropyl *iso*thiocyanate, $HOCH_2CH_2CH_2NCS$, by its spontaneous cyclization to a heterocyclic

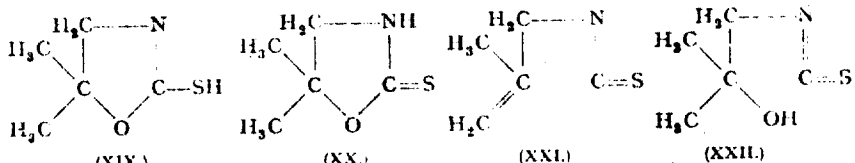
compound, which was identified with synthetic tetrahydro-1,3-oxazine-2-thione (*76*). Subsequently, the malcolmiin structure was confirmed by synthesis (*90*).

Since the genus *Malcolmia* is taxonomically closely related to *Cheiranthus*, the traditional botanical source of cheirolin, it is not surprising to find the latter accompanying malcolmiin in seed hydrolysates of *M. maritima* (*76*, *90*). The further distribution of glucomalcolmiin is unknown.

e. Hydroxy-substituted iso*Thiocyanates.*

There exist some natural *iso*thiocyanate glucosides whose aliphatic side-chains are substituted by a β-hydroxy group. The corresponding *iso*thiocyanates undergo a presumably spontaneous, intramolecular cyclization to 2-oxazolidinethiones, which are the recognizable end products of the enzymic reaction. This conclusion has been reached as a result of recent studies of several representatives of this type (see below).

2-Hydroxy*iso*butyl *iso*thiocyanate. More than twenty years ago, HOPKINS (*52*) isolated from enzymically cleaved seed extracts of the weed *Conringia orientalis* L. (DUMORT) [more correctly designated as *C. orientalis* (L.) ANDRZ. and synonymous with *Erysimum orientale* MILL.] a compound to which the structure (XIX) was attributed; it was corrected by ETTLINGER (*18*) to the isomeric form (XX) on the basis of infrared evidence. HOPKINS suggested as the likely pathway in the formation of (XIX) the initial formation of β-methallyl *iso*thiocyanate (XXI), followed by hydration of the double bond and cyclization. That this reaction sequence cannot be responsible for the production of (XX) became apparent, however, when a synthetic specimen of (XXI), studied in the author's laboratory, displayed no tendency to cyclize (*95*).



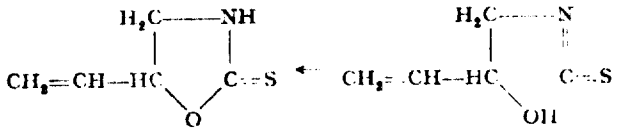
(XIX) (XX) (XXI) β-Methallyl isothiocyanate. (XXII) 2-Hydroxy*iso*butyl *iso*thiocyanate.

More recently, the parent glucoside, *glucoconringiin*, was isolated as a crystalline acetate from seeds of *C. orientalis* by KJÆR et al. (*82*), who demonstrated spectroscopically that 2-hydroxy*iso*butyl *iso*thiocyanate (XXII) is the initial product of the enzymic hydrolysis and subsequently cyclizes to give the HOPKINS compound (XX). Similar conclusions were reached by SCHULTZ and WAGNER (*141*). Likewise, the presence of

References, pp. 169–176.

glucoconringiin in various species of the genus *Cochlearia* was proved, and 5,5-dimethyl-2-oxazolidinethione was isolated from the seeds of *C. officinalis* L. (*82*); its further distribution is unknown.

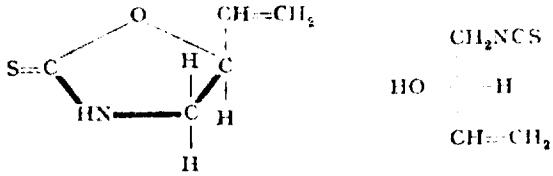
2-Hydroxy-3-butenyl *iso*thiocyanate. In 1949, ASTWOOD et al. (*4*) isolated from yellow turnip and various *Brassica* seeds an antithyroid factor, which was identified as (−)-5-vinyl-2-oxazolidinethione (XXIII), a structure later confirmed synthetically by ETTLINGER (*19*). The same investigators pointed to an *iso*thiocyanate glucoside as a plausible progenitor of the heterocyclic compound (*43*)—a suggestion confirmed by studies in the author's laboratory (*82*) and elsewhere (*141*). These indicated one of the stereoisomeric 2-hydroxy-3-butenyl *iso*thiocyanates (XXIV) to be the first reaction product.



(XXIII.) Goitrin. (XXIV.) 2-Hydroxy-3-butenyl isothiocyanate.

GREER (*42*) succeeded in isolating from rutabaga seeds, the genuine glucoside, *progoitrin*, which is identical with *glucorapiferin*, obtained as the crystalline pentaacetate from seeds of a rape variety [SCHULTZ and WAGNER (*141*)].

In this laboratory, the absolute configuration of goitrin (XXIII), and hence of the parent mustard oil (XXIV) as well as the side-chain of progoitrin, was recently established by chemical correlation with configurationally known compounds (*68*); see the formulas (XXV) and (XXVI).

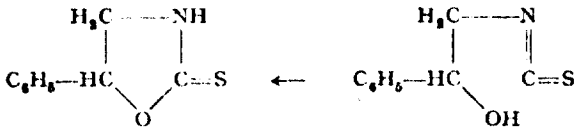


(XXV.) Goitrin. (XXVI.) 2-Hydroxy-3-butenyl isothiocyanate.

Progoitrin occurs largely in *Brassica* spp., predominantly in seed materials, including rapeseed oil meal (2–4 mg. goitrin per g. of meal) (*111*). This occurrence is interesting, because progoitrin is a potential precursor of goitrin. In contrast to seeds, fresh cabbage contains only very small amounts of goitrin (*1*).

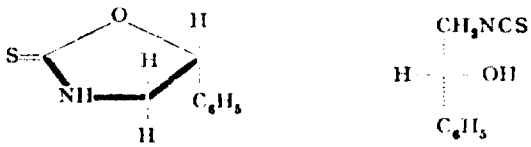
2-Hydroxy-2-phenylethyl *iso*thiocyanate. On evidence, similar in nature to that presented for the last two hydroxy-substituted mustard

oils, it was established in this laboratory that seed material of various species of the cruciferous genus *Barbarea* afforded on enzymic hydrolysis a hydroxy-substituted *iso*thiocyanate, that underwent spontaneous cyclization to a 2-oxazolidinethione. Seed extracts from *B. vulgaris* R. BR., containing the parent glucoside *glucobarbarin* in addition to gluconasturtiin, were enzymically cleaved to a mixture of 2-phenylethyl mustard oil, originating from the latter glucoside, and (—)-5-phenyl-2-oxazolidinethione (barbarin) (XXVII), the cyclization product of primarily formed 2-hydroxy-2-phenylethyl mustard oil (XXVIII) (*79*).



(XXVII.) Barbarin. (XXVIII.) 2-Hydroxy-2-phenylethyl isothiocyanate.

A stereospecific synthesis of both enantiomers of (XXVII), starting from configurationally known compounds, has established the absolute configurations of barbarin (XXIX) and its mustard oil precursor (XXX) (*79*, *80*).



(XXIX.) Barbarin. (XXX.) 2-Hydroxy-2-phenylethyl isothiocyanate.

In addition to *Barbarea* species, leaves and inflorescenses of *Reseda luteola* L. (dyer's weed, weld) yielded barbarin on enzymic treatment. Seeds of the same plant, as well as other *Reseda* species, are additional sources of barbarin (*80*).

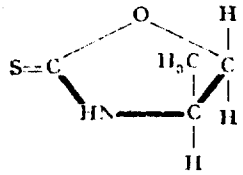
2-Hydroxy-*iso*propyl *iso*thiocyanate. A β-hydroxy-substituted, branched, aliphatic mustard oil represents the newest addition to the *iso*thiocyanates that undergo cyclization. The glucoside *glucosisymbrin* was reported from this laboratory as a constituent of seeds of the Crucifer *Sisymbrium austriacum* JACQ. It was characterized by its

(XXXI.) Sisymbrin. (XXXII.) 2-Hydroxy-isopropyl isothiocyanate.

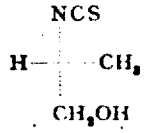
References, pp. 169—176.

ability to undergo enzymic decomposition to *dextro*rotatory 4-methyl-2-oxazolidinethione (sisymbrin) (XXXI), produced by cyclization of the initially liberated 2-hydroxy-*iso*propyl *iso*thiocyanate (XXXII) (*67*).

A stereospecific synthesis starting from alanine of known configuration has given full information on the absolute configuration of the ring compound. Accordingly, the configurations (XXXIII) and (XXXIV) were established for the naturally occurring sisymbrin and its parent mustard oil (*67*).



(XXXIII.) Sisymbrin.



(XXXIV.) 2-Hydroxy *iso*propyl *iso*thiocyanate.

In the same paper (*67*), the possible biogenetic relationship of (XXXIV) and the non-hydroxylated *iso*propyl mustard oil, formerly encountered in plants, was discussed as well as its formal similarity with β-hydroxy-*iso*butyric acid of the sesquiterpene lactone arctiopicrin, as well as with (—)-β-amino-*iso*butyric acid, isolated from bulbs of *Iris tingitana*.

f. iso*Thiocyanates of Doubtful Structure.*

In addition to the naturally derived mustard oils of established structure, a number of other *iso*thiocyanates have been reported without sufficient documentation to permit conclusive formulations. The existence of still others is questionable, because their isolation could not be repeated.

The first category includes the compound, $CH_3CH_2CH_2CH_2SCH{=}CHCH_2CH_2NCS$, which HEIDUSCHKA and ZWERGAL (*47*) claimed to be present in fresh radish root. The same authors postulated the presence in horse-radish of 3-phenylpropyl *iso*thiocyanate.

Quite incompatible with the physical and chemical properties, ZWERGAL (*150*) formulated a component from kohlrabi seeds as 4,4-dimethyl-5-vinyl-2-oxazolidinethione, a structure which was later revised by SCHULTZ and WAGNER (*141*) to $CH_3SOCH{=}CHCH_2CH_2CH_2NCS$, apparently again with no other evidence than its resemblance to the corresponding nitrile, $CH_3SOCH{=}CHCH_2CH_2CH_2CN$; the latter represents ZWERGAL's original proposal (*158*). The parent glucoside, isolated as a non-homogeneous tetraacetate from kohlrabi seeds, was named glucocaulorapin (*141, 151*).

From the seeds of *Hesperis matronalis* L., WAGNER (*151*) isolated glucomatronalin as a crystalline hexa- or heptaacetate, suggesting the presence of two or three acetylable groupings in the otherwise unknown, derivable mustard oil.

An interesting observation deserving repetition was made by PUNTAMBEKAR (*110*) who claimed, in addition to *iso*propyl and *sec*-butyl *iso*thiocyanates, the isolation of phenyl mustard oil (as its thiourea derivative) from seed kernels of the Indian *Euphorbia* species *Putranjiva Roxburghii* WALL.

As already pointed out the presence of numerous unidentified glucoside spots in paper chromatograms obtained from a large collection of plant species indicates that additional mustard oils will be forthcoming in the future.

VI. Botanical Distribution of *iso*Thiocyanate Glucosides.

It is remarkable that practically all investigated plants of the nearly cosmopolitan family *Cruciferae* contain one or more *iso*thiocyanate-producing glucosides. Although only a minor fraction of a total of about 1500 species, that belong to this family, has as yet been subjected to analysis, it appears likely that most Crucifers, in some or all parts of the plant, contain such glucosides. In *Table* 5 (p. 161) an attempt has been made to present a rather complete survey of all those Crucifer species in which either *iso*thiocyanates or parent glucosides have been found up to the end of 1959. The Table does not include numerous other plants, studied in the author's laboratory, whose analyses are unpublished. In general, Table 5 contains the chemical identity of the *iso*thiocyanates or their glucosides. In some cases, however, and particularly when the evidence was exclusively of paperchromatographic nature, it was preferable to list the reported number of constituents rather than chemical structures, even when these were suggested in the original paper.

On the whole, the number of observed individual *iso*thiocyanate glucosides is now so large that chemical identifications based solely on paper chromatography are more than questionable. Furthermore, the number of the observed glucosides may vary considerably, depending on the sensitivity of the analytical method and the concentration of the extracts. It was noted repeatedly in the author's laboratory that plant materials, generally regarded as containing only one or two glucosides, on closer examination displayed a notable number of additional trace glucosides.

Where provided in the original text, or where no doubt seems to exist, botanical authors' names appear in Table 5. It should be stressed at this point that botanical designations should be as unambiguous as the proof of the chemical structure, when new chemical compounds are related to botanical sources. To illustrate: the labelling of more than half of 300 seed specimens, collected by us from botanical gardens and other sources all over the world, proved to be incorrect on closer inspection.

Although preponderant in *Cruciferae*, *iso*thiocyanate glucosides are by no means confined to this family. It may not surprise that species of other families, such as *Capparidaceae*, *Moringaceae* and *Resedaceae*, which belong to the same order *(Rhoeadales)* as *Cruciferae*, may occasionally contain the same glucosides. Quite unexpected, however, was the isolated appearance of *iso*thiocyanate glucosides in some species

References, pp. 169–170.

of plant families which are taxonomically remote from *Rhoeadales*, such as *Caricaceae*, *Euphorbiaceae*, *Limnanthaceae*, *Phytolaccaceae*, *Plantaginaceae*, *Resedaceae* and *Tropaeolaceae*. *Table 6* (p. 167) presents a survey of reported glucoside-containing species which do not belong to *Cruciferae*.

Attempts to correlate chemical structure of plant constituents with the botanical character have become more frequent during recent years, and often rewarding. Such chemotaxonomic approaches have also been attempted (*87*) in the glucoside group discussed and will probably be extended. It should not be forgotten, however, that the structure of a selected type of compounds is only one of numerous factors that contribute to biological individualism. Nevertheless, further comparative studies of the distribution of mustard oil glucosides, when conducted with due criticism, may provide new and interesting results concerning the chemistry of natural products as well as botanical taxonomy.

VII. Biological Properties of *iso*Thiocyanates and Their Parent Glucosides.

In connexion with the taxonomic and phylogenetic implications of comparative studies of *iso*thiocyanates and glucosidic progenitors in individual botanical species, biogenesis is a challenging problem. No systematic studies have as yet been undertaken to elucidate the anabolic or catabolic pathway of these compounds in plants. The conspicuous structural regularities, however, make such investigations most desirable. Thus, the formal similarity of several side-chains of the glucosides with those of some amino acids occurring in proteins is striking and would suggest a possible common pathway in biogenesis (*23*, *59*). Other products, however, belong to a type not ordinarily encountered in nature, such as the long-chain methylthioalkyl derivatives. Another interesting feature is the recognition of the glucosides as hydroxylamine derivatives (*23*). Bound hydroxylamine groups have been extensively discussed in the literature in connexion with the nitrogen metabolism of higher plants. The frequent appearance of homologous mustard oils in extracts is another notable characteristic of potential interest for biogenetic considerations. As a whole, the detailed knowledge of an extensive series of formally simple glucosides with well-established structures calls for thorough biogenetical investigations.

From time to time, several biological actions have been ascribed to mustard oils or their simple conversion products, but a detailed discussion of this subject falls outside the scope of the present survey. It suffices to draw attention to the long recognized antibacterial and fungiostatic property of most mustard oils, on which an extensive literature exists.

Recently, McKay et al. (*102*) published the results of bacteriostatic assays of a large series of *iso*thiocyanates.

Much interest is being devoted to some *iso*thiocyanates originating from weeds, as factors responsible for a "scorched" or "burnt" flavour of cream and butter. McDowall et al. (*101*), however, were unable to confirm such suggestions in case of the land-cress taint in New Zealand dairy products (cf. *28a*).

The goitrogenic effect of several oxazolidinethiones, particularly those of plant origin, has led to a variety of biological studies to assess the possible importance of such compounds in animal feeding and in endemic goiter. In connexion with such problems, Bachelard et al. (*4a*) have recently isolated cheirolin from ripe fruits and fresh leaves of *Rapistrum rugosum* (L.) All., a widely distributed weed in Australian pastures, and showed that cheirolin is goitrogenic in rats. Rumen liquor effected in vitro conversion of cheirolin into the less goitrogenic 1,3-bis-(3-methylsulphonylpropyl)-2-thiourea. Moreover, certain toxic effects observed occasionally in animals after feeding materials that contained excessive amounts of *iso*thiocyanates, have called for much attention.

An interesting biological effect has been attributed to the genuine glucosides rather than the free mustard oils, viz. that of stimulating the feeding of certain insects. According to Thorsteinson (*148*), two oligophagous insects [*Plutella maculipennis* (Curt.) and *Pieris brassicae* (L.)] could be induced to feed on leaves which they ordinarily refuse, by painting the leaves with sinigrin or sinalbin solutions, but not with allyl *iso*thiocyanate. The importance of such observations is obvious, considering the immense, world-wide damages caused by host-specific insects to crops of important *Cruciferae*, such as cabbage, turnip and rape. These, and many other examples may characterize the *iso*thiocyanate glucosides and their enzymic fission products as natural products of considerable and increasing interest to a diverse group of modern scientists.

References, pp. 169—176.

VIII. Tables.

Table 1. Crystalline *iso*Thiocyanate Glucosides, Known at the End of 1959.

Glucoside	Cation	Seed source	M. p.	Rotation in water			References
				$[\alpha]_D$	Temp. (°)	c	
Glucocapparin	K	*Cleome spinosa* JACQ.	208–210°	— 28.6°	25	2.2	(*74*)
Sinigrin[a]	K	*Brassica nigra* KOCH	127°	— 17.6°	27		(*124*)
Glucoiberin[a]	K	*Iberis amara* L.	142–144° (dec.)	— 55.3°	20	4.9	(*136*)
Glucocheirolin[a]	K	*Cheiranthus cheiri* L.	158–160° [c]	— 21.6°	27	4.9	(*128*)
Sinalbin[b]	Sinapine	*Sinapis alba* L.	83–84°	— 8.4°			(*124*)
Glucotropaeolin	$(CH_3)_4N^+$	*Tropaeolum majus* L.	188–189°	— 16.7°	28		(*24*)
Gluconasturtiin[d]	K	*Nasturtium officinale* R. BR.	163°	— 21°			(*11*)
Glucoconringiin	K	*Conringia orientalis* (L.) ANDRZ.	168° (dec.)	— 10.9°	21	3.7	(*41*)
Progoitrin	Na	*Brassica napa* L.	145°	— 22.3°			(*42*)

[a] Monohydrate.

[b] Pentahydrate. An anhydrous preparation was reported (*124*) to have the m. p. 138.5–140°. The anhydrous tetramethylammonium salt of the sinalbin anion (tetramethylammonium glucosinalbate), m. p. 191–192° (dec.); $[\alpha]_D^{35}$ — 19° (H_2O), was described by ETTLINGER and LUNDEEN (*23*).

[c] A sample prepared in this laboratory decomposed over a much broader range (*61*).

[d] Non-analyzed preparation of unstated purity.

Table 2. Crystalline *iso*Thiocyanate Glucoside Tetraacetates, Known at the End of 1959.

Parent glucoside	M. p.[a]	Rotation in water			References
		$[\alpha]_D$	Temp. (°)	c	
Glucocapparin[b]	209–210°	−31.0°	25	1.8	(*74*)
Glucoputranjivin	180°	−15.4°	23	1.2	(*61*)
Glucocochlearin	193–194°				(*138*)
Sinigrin	192–193°[c]	−18.6°	24	3.6	(*82*)
Glucoiberin[d]	145–147°	−16.6°	23	2.0	(*61*)
Glucocheirolin	193–194°	−18.0°	24	1.0	(*61*)
Glucoraphenin[e]	155–156°	−23.5°	27	1.5	(*61*)
Glucoalyssin	158°	−10.9°	20	1.7	(*140*)
Glucoerypestrin[f]	188–190°	−18.6°	23	1.7	(*78*)
Glucotropaeolin[g]	186–187°	−20.0°	25	1.0	(*63*)
Glucoconringiin[h]	152°	−5.3°	25	5.3	(*82*)
Glucorapiferin[i] (= Progoitrin)	178–180°	−9.5°	25	2.6	(*42, 138, 141*)

[a] All glucoside acetates decompose on heating; the decomposition temperatures are much dependent on the rate of heating.

[b] If not otherwise stated the glucoside acetates are crystalline potassium salts.

[c] SCHULTZ and WAGNER (*138*) reported the m. p. 195–196° (dec.); no rotation data were given.

[d] Monohydrate. A preparation, with no reference to its water content, was reported by WAGNER (*138, 151*); m. p. 148–149°, $[\alpha]_D^{20}$ −12.2° (*c* 1.4, H_2O).

[e] Monohydrate. The name glucoraphenin was introduced by GMELIN (*38*) for the parent (hypothetical) glucoside. WAGNER (*151*) reported the m. p. 157–159°, but no rotation data, for a tetraacetate sample containing less than 1 H_2O.

[f] Monohydrate.

[g] Monohydrate. Other reported data: m. p. 197–198° (*151*) and 187–189° (dec.) (*138*); no rotations were given. The anhydrous tetramethylammonium salt was described by ETTLINGER and LUNDEEN (*24*), m. p. 182–183° (dec.), $[\alpha]_D^{28}$ −18.9° (H_2O).

[h] SCHULTZ and WAGNER (*141*) reported m. p. 160° (dec.), $[\alpha]_D^{23}$ −2° (*c* 2.3, H_2O).

[i] Pentaacetate.

References, pp. 169–176.

Table 3. *iso*Thiocyanate Glucosides with Established Side-chains, Known at the End of 1959.

No.	Parent glucoside	*R* of the derived isothiocyanate, *R*—NCS	References
1	Glucocapparin[a, b]	CH_3[j]	(74, 84)
2	Glucolepidiin[d]	CH_3CH_2[j]	(56)
3	Glucoputranjivin[b]	$CH_3CH(CH_3)$[j]	(69, 110)
4	Glucocochlearin[b]	(+)-$CH_3CH_2\overset{*}{C}H(CH_3)$[j]	(33, 50, 86)
5	Sinigrin[a, b]	$CH_2{=}CHCH_2$[j]	(124, 155)
6	Gluconapin	$CH_2{=}CHCH_2CH_2$[j]	(20, 71)
7	Glucobrassicanapin	$CH_2{=}CHCH_2CH_2CH_2$[j]	(89)
8	Glucoibervirin	$CH_3SCH_2CH_2CH_2$[j]	(85)
9	Glucoiberin[a, b]	$CH_3SOCH_2CH_2CH_2$	(75, 136)
10	Glucocheirolin	$CH_3SO_2CH_2CH_2CH_2$	(123)
11	Glucoerucin	$CH_3SCH_2CH_2CH_2CH_2$[j]	(73)
12	(Glucoraphanin)[d]	$CH_3SOCH_2CH_2CH_2CH_2$	(108)
13	(Glucoraphenin)[b]	$CH_3SOCH{=}CHCH_2CH_2$	(119)
14	Glucoerysolin[e]	$CH_3SO_2CH_2CH_2CH_2CH_2$	(126)
15	Glucoberteroin	$CH_3SCH_2CH_2CH_2CH_2CH_2$[j]	(91)
16	Glucoalyssin[b]	$CH_3SOCH_2CH_2CH_2CH_2CH_2$	(75, 140)
17	Glucohirsutin	$CH_3SO(CH_2)_8$	(66)
18	Glucoarabin	$CH_3SO(CH_2)_9$	(77)
19	Glucocamelinin	$CH_3SO(CH_2)_{10}$	(83)
20	Glucotropaeolin[a, b]	$C_6H_5CH_2$[j]	(34, 35)
21	Sinalbin[a]	(*p*)$HOC_6H_4CH_2$	(94, 114)
22	Glucoaubrietin	(*p*)$CH_3OC_6H_4CH_2$[j]	(81)
23	(Glucolimnanthin)[d, f]	(*m*)$CH_3OC_6H_4CH_2$	(22)
24	Glucomasturtiin[a]	$C_6H_5CH_2CH_2$[j]	(32, 35)
25	Glucoerypestrin[b]	$CH_3OOCCH_2CH_2CH_2$[k]	(78)
26	Glucomalcolmiin	$C_6H_5COOCH_2CH_2CH_2$[k]	(76, 90)
27	Progoitrin[a] (Glucorapiferin[c])	$CH_2{=}CH\overset{*}{C}HOHCH_2$[j]	(42, 82, 141)
28	Glucocoumingiin[a, b]	$(CH_3)_2C(OH)CH_2$[g]	(41, 82, 141)
29	Glucobarbarin	$C_6H_5\overset{*}{C}HOHCH_2$[h]	(79, 80)
30	Glucosisymbrin	$HOCH_2\overset{*}{C}H(CH_3)$[i]	(67)

[a] Known in crystalline form, cf. Table 1, p. 157.

[b] Known as crystalline tetraacetate, cf. Table 2, p. 158.

[c] Known as crystalline pentaacetate, cf. Table 2.

[d] This name was not proposed in the original paper.

[e] The occurrence of this glucoside could not be verified in the author's laboratory.

[f] Cyclizes to (−)-5-vinyl-2-oxazolidinethione.

[g] Cyclizes to 5,5-dimethyl-2-oxazolidinethione.

[h] Cyclizes to (−)-5-phenyl-2-oxazolidinethione.

[i] Cyclizes to (+)-4-methyl-2-oxazolidinethione.

[j] Volatile with steam.

[k] Volatile with steam but may decompose.

[l] Name proposed in agreement with Dr. M. G. Eitlinger.

Table 4. Molecular Rotations of Sulphoxide *iso*Thiocyanates of Natural Origin, Some Derivatives and Related Compounds.

Formula	M. p.	$[M]_D$[a]	Solvent	Conc. g./100 ml.	References
$CH_3SO(CH_2)_3NH_2$	27°	— 143°	EtOH	1.0	(55, 54)
$CH_3SO(CH_2)_3NCS$ (iberin)		— 120°	EtOH		(55)
$CH_3SO(CH_2)_3NHCSNHC_6H_5$	137°	— 138°	EtOH	1.9	(75)
$CH_3SO(CH_2)_3NHCSNHCH_2C_6H_5$	108°	— 138°	EtOH	2.0	(75)
$CH_3SOCH{=}CH(CH_2)_2NCS$ (sulphoraphene)		— 238°	EtOH	1.4	(119)
$CH_3SOCH{=}CH(CH_2)_2NHCSNH_2$[b]	219°	— 138°	H_2O	1.1	(119)
$CH_3SOCH{=}CH(CH_2)_2NHCSNHC_6H_5$	121°	— 280°	$CHCl_3$	1.0	(119)
$CH_3SO(CH_2)_4NH_2$		— 169°	MeOH	0.4	(121)
$CH_3SO(CH_2)_4NCS$ (sulphoraphane)		— 140°	$CHCl_3$	1.2	(121)
		— 117°	$CHCl_3$	2.1	(108)
$CH_3SO(CH_2)_4NHCSNHC_6H_5$	145°	— 146°[c]	EtOH	1.8	(108)
$CH_3SO(CH_2)_5NCS$ (alyssin)[d]		— 132°	$CHCl_3$	4.0	(75)
$CH_3SO(CH_2)_5NHCSNH_2$	106°	— 167°	H_2O	2.0	(75)
$CH_3SO(CH_2)_5NHCSNHC_6H_5$	126°	— 176°	EtOH	2.1	(75)
$CH_3SO(CH_2)_5NHCONHC_6H_5$	102°	— 147°	EtOH	0.8	(75)
$CH_3SO(CH_2)_5NHCSNHCH_2C_6H_5$	104°	— 175°	EtOH	1.2	(75)
$CH_3SO(CH_2)_5NHCONHCH_2C_6H_5$	106°	— 182°	EtOH	0.9	(75)
$CH_3SO(CH_2)_8NHCSNH_2$	88°	— 190°	EtOH	1.0	(66)
$CH_3SO(CH_2)_8NHCSNHC_6H_5$	139°	— 148°	$CHCl_3$	0.8	(66)
$CH_3SO(CH_2)_8NHCONHC_6H_5$	121°	— 186°	EtOH	1.3	(66)
$CH_3SO(CH_2)_9NHCSNH_2$	104°	— 175°	EtOH	2.1	(77)
$CH_3SO(CH_2)_9NHCSNHC_6H_5$	122°	— 200°	EtOH	0.6	(77)
$CH_3SO(CH_2)_9NHCSNHCH_2C_6H_5$	119°	— 226°	MeOH	0.4	(77)
$CH_3SO(CH_2)_9NHCONHCH_2C_6H_5$	121°	— 190°	EtOH	1.0	(77)
$CH_3SO(CH_2)_{10}NHCSNH_2$	92°	— 181°	EtOH	1.0	(83)
$CH_3SO(CH_2)_{10}NHCSNHC_6H_5$	139°	— 163°	EtOH	0.4	(83)
$CH_3SO(CH_2)_{10}NHCONHCH_2C_6H_5$	114°	— 190°	EtOH	1.2	(83)

[a] All rotations reported were determined at 15–25°.

[b] The author found that the reported compound (119) can hardly be authentic sulphoraphenethiourea.

[c] The value reported is a result of a microdetermination carried out in the author's laboratory with a specimen of natural provenance, kindly furnished by Dr. Ž. PROCHÁZKA.

[d] Non-analyzed specimen.

References, pp. 169–176.

Table 5. The Occurrence of *iso*Thiocyanates or Their Parent Glucosides in Species of the Family *Cruciferae*.

R, root material; G, green parts; S, seeds. **Bold** figures in the column "Compounds" refer to the individual mustard oils listed in Table 3, p. 159; the other figures in the same column indicate the number of unspecified mustard oils observed. I and PC in the column "Evidence" denote isolation and paper chromatography, respectively.

Plant	Part	Compounds	Evidence	References
Aethionema cordifolia DC.	G	**1**	PC	(*38*, *133*)
A. pulchella	S	**2**	PC	(*38*, *133*)
A. saxatile	S	**2**	PC	(*139*, *151*)
Alliaria officinalis ANDRZ.	R	**5**	I	(*152*)
		5, **20**, (*24*)	PC	(*14*)
	G	**5**	I, PC	(*38*, *151*)
		5, **20**	PC	(*14*)
	S	**5**	I, PC	(*14*, *38*, *152*)
Alyssum alpestre	S	**1**	PC	(*38*)
A. Arduini	S	**2**	PC	(*38*, *133*)
A. arenarium	S	**1**, **2**	PC	(*38*, *133*)
A. argenteum	S	**1**	PC	(*38*)
A. argenteum ALL.	S	**2**, **15**, **16**	PC, I	(*139*, *140*, *151*)
A. argenteum (ALL.) VITM.	S	**16**, (**15**)	I	(*75*)
A. Benthami	S	**5**	PC	(*139*, *151*)
A. Bornmulleri HAUSKNECHT	S	**1**, **6**	PC	(*38*, *75*, *133*)
	G	**1**	PC	(*139*, *151*)
A. Borzaeanum NYÁR.	S	**15**, **16**	PC	(*75*)
A. calycinum L.	S	**1**	PC	(*38*)
	G	**1**	PC	(*139*, *151*)
A. corymbosum BOISS.	S	**3**, **6**, **15**, (**16**)	PC	(*38*, *75*, *133*)
A. maritimum LAM.	S	**16**	PC	(*75*)
A. montanum L.	S	**1**, **6**, **15**, **16**	PC	(*38*, *75*)
A. orientale ARD.	S	**6**, **7**, **16**	PC	(*75*)
A. ovirense A. KERN.	S	**15**, **16**	PC	(*75*)
A. pedemontanum	S	**2**	PC	(*38*)
A. rostratum STEV.	S	**1**	PC	(*139*, *151*)
A. saxatile L.	S	**3**	PC	(*139*, *151*)
	S	**6**, **7**, **15**, **16**	PC	(*75*)
	G	**3**	PC	(*14*)
A. saxatile L. var. *citrinum*	S	**6**, **15**, **16**	PC	(*75*)
A. sinuatum L.	S	**7**, **15**, **16**	PC	(*75*)
A. Wulfenianum	S	**1**, **2**	PC	(*38*, *133*)
Arabis albida	S	**1**	PC	(*38*)
A. alpestris	S	**3**	PC	(*38*, *133*)
A. alpina L.	S	**1**, **2**	PC	(*38*, *139*, *152*)
	S	**18**	I	(*77*)
	G	**3**	PC	(*139*, *151*)

(Table 5, continued.)

Plant	Part	Compounds	Evidence	References
Arabis aubrietoides BOISS. ...	S	1	PC	(38)
	G	4	PC	(139, 151)
A. bellidifolia JACQ.	S	2	PC	(38, 133)
	G	6	PC	(139, 151)
A. colinsii FERNALD	S	6	PC	(87)
A. corymbiflora VEST.	G	2	PC	(139, 151)
A. hirsuta SCOP.	G	2	PC	(139, 151)
	S	17	I	(66)
A. holboellii HORNEM.	G	5	PC	(139, 151)
A. procurrens WALDST. et KIT.	S	3	PC	(38, 133)
	G	2	PC	(139, 151)
A. pumila JACQ.	G	4	PC	(139, 151)
A. retrofracta (GRAHAM) RYDBERG	S	5	PC	(87)
A. rochinensis	S	3	PC	(38, 133)
A. rosea DC.	S	3, 4	PC	(38, 133)
	G	1	PC	(139, 151)
A. soyeri RENT.	S	1	PC	(38, 133)
Armoracia lapathifolia GILIB..	R	5	I	(146, 152)
		5, 24	I, PC	(14, 47, 144)
	G	5, 24	PC	(14)
Aubrietia columnifera GUSSM.	G	3, 4, 22	PC	(81, 139, 151)
A. deltoidea DC.	G	22	I	(81)
	S	21, 22	PC	(81)
A. erubescens GRISEB.	S	21, 22	PC	(81)
A. hybrida HORT.	G	22	PC	(81)
A. intermedia HELDR. et ORPH.	S	21, 22	PC	(81)
Barbarea arcuata (OPIZ.) REICHB.	S	1 (?), 24	PC	(72)
B. intermedia BOR.	S	24	PC	(72)
B. praecox R. BR.	S	24	I	(32, 35)
B. stricta FRIES	G	1	PC	(139, 151)
	S	1	PC	(139, 151)
B. vulgaris R. BR.	G	1	PC	(14, 38, 131)
	R	5, 24	PC	(14)
	S	24, 29	I	(79)
Berteroa incana (L.) DC.	G	2, 3, 4	PC	(15, 38, 139, 151)
	S	2, 3	PC	(38, 139, 151)
	S	15, 16	I	(91)
	R	1	PC	(15)
Biscutella auriculata L.	G	2	PC	(139, 151)
	S	2	PC	(38, 133)
B. laevigata L.	G	2	PC	(139, 151)
	S	1, 2	PC	(38, 133, 139, 151)

References, pp. 169–176.

(Table 5, continued.)

Plant	Part	Compounds	Evidence	References
Brassica cernua THUNB.	S	?		(152)
B. dichotoma ROXB.	S	?		(152)
B. glauca ROXB.	S	?		(152)
B. integrifolia O. E. SCHULZ	S	?		(152)
B. juncea CZERN. et COSS.	S	5	I	(118)
	R	24	PC	(15)
	G	5, 6, 7, 24	PC	(15)
B. napus L.	S	6, 7, 9 (or 12), 21, 24, 27	I, PC	(89)
	R	27	I	(4)
B. nigra KOCH	R	5, 24	PC, I	(14, 144)
	G	5 (24)	I	(14)
	S	5 (24)	I	(152)
B. oleracea L. var. *capitata* L.	R	24	I	(144)
B. oleracea var. hort.	S	5, 6, 7, 24	PC	(13, 53)
	S	5, 27	I	(4, 127)
	G	5, 6	PC	(53)
	G	(5, 6, 8), 27	I	(1, 100)
	G	9, 12	I	(109)
B. pseudojuncea	S	5, 6	PC	(53)
B. rapa L.	S	(6, 7, 24), 27	PC, I	(4, 13)
	R	27, 24	I	(4, 144)
Bunias erucago L.	S	2, 3	PC	(38, 133, 139, 151)
B. orientalis L.	S	2, 3	PC	(38, 133, 139, 151)
	G	2	PC	(38, 131)
	R	1, 2	PC	(15, 131)
Cakile maritima SCOP.	S	5	PC	(72)
	S	7	PC	(14)
Camelina dentata (WILLD.) PERS.	S	19	PC	(83)
C. microcarpa ANDRZ.	S	19	PC	(83)
C. sativa CRANTZ	S	19	I	(83)
Capsella bursa pastoris (L.) MEDIC.	S	5	I	(152)
Cardamine amara L.	S	4	PC	(38, 131)
	G	4	I	(152)
C. flexuosa WITH.	G	1	PC	(139, 151)
C. graeca L.	S	6, 20	PC	(72)
C. hirsuta L.	G	?	I	(152)
C. pratensis L.	R	2 or 3	PC	(38)
	G	4, 2	I, PC	(38, 152)
	S	4	PC	(38, 131)

(Table 5, continued.)

Plant	Part	Compounds	Evidence	References
Cheiranthus Allioni hort.	S	3	PC	(139, 151)
C. cheiri L.	S	10	I	(123)
C. Kewensis	S	2	PC	(139, 151)
Cochlearia anglica (L.) ASCH. et GRB.	G	3	I	(69)
	S	3, 4, 28	PC	(82)
C. danica L.	S	3, 4, 28	PC	(82)
C. officinalis L.	S	3, 4, 28	I	(82)
Conringia orientalis (L.) DUMORT	S	28	I	(82, 141)
Coronopus didymus (L.) SM.	G	20	I	(101)
Crambe maritima L.	S	5, 2	PC	(38, 72)
	G	1	PC	(139, 151)
Dentaria digitata LAMARCK	G	1	PC	(139, 151)
D. enneaphylla L.	G	3	PC	(139, 151)
Diplotaxis erucoides (L.) DC.	S	5	PC	(38, 133)
D. muralis (L.) DC.	R	5	PC	(38)
	G	5	PC	(38, 131)
	S	5	PC	(72, 131)
D. tenuifolia (L.) DC.	R, G	11	I	(17)
Draba aizoides	S	2	PC	(38, 133)
D. aizoon WALLENB.	S	1	PC	(139, 151)
D. borealis DC.	S	4, 20 (?)	PC	(72)
D. Haynaldi STUR.	S	1	PC	(139, 151)
D. incana L.	S	(5, 6), 2	PC	(38, 72, 133)
	G	1	PC	(139, 151)
D. norvegica GUNN.	S	3	PC	(38, 133)
	G	2	PC	(139, 151)
D. pyrenaica	S	3	PC	(38, 133)
D. repens	S	2	PC	(38, 133)
Erica sativa MILL.	G	11	PC	(15)
	S	11, 2	I, PC	(38, 73)
E. sativa LMK.	S	2	PC	(139, 151)
	G	2	PC	(139, 151)
Erucastrum gallicum (WILLD.) O. E. SCHULZ	S	5, 6	PC	(72)
E. Pollichii SCH. et SP.	G	1, 24	PC	(14, 139, 151)
Erysimum alpinum	S	1	PC	(38)
E. arcansanum NUTT.	S	10	PC, I	(123, 131)

References, pp. 169–176.

(Table 5, continued.)

Plant	Part	Compounds	Evidence	References
Erysimum cheiranthoides L.	S	5	PC	(72)
	G	4	PC	(139, 151)
E. helveticum	S	2	PC	(38, 133)
E. nanum Boiss. et Hohen.	S	10	PC	(131)
E. ochroleucum DC.	S	10, 25	PC	(78)
E. pachycarpum hort. fil. et Thoms.	S	2, 5	PC	(38, 133, 139, 151)
	G	2	PC	(139, 151)
E. Perofskianum Fisch. et May	S	1 (?), 5	PC	(72, 139, 151)
	S	14	I	(126)
E. pumilum DC.	S	1, 3, (10, 25)	PC	(38, 78, 133, 139, 151)
	G	2	PC	(139, 151)
E. rupestre DC.	S	3, (10, 25)	PC, I	(78, 139, 151)
E. strictissimum L.	S	3	PC	(139, 151)
E. vincoleucum	S	2	PC	(38, 133)
Eutrema wasabi Maxim.	R, G	4, 5	I	(103)
Farsetia clypeata	R	4 or 5, 11	PC	(38, 73)
	G	1	PC	(38)
	S	1	PC	(38)
Hesperis matronalis L.	G	1–2, 5, 3	PC	(38, 139, 151)
	S	3 ?, 5, 4, 11	PC	(38, 73, 139, 142, 151)
Hutchinsia alpina R. Br.	G	2	PC	(139, 151)
	S	24	PC	(72)
Iberis amara L.	R	9, 2	PC	(15, 38)
	G	9	PC	(38)
	S	9	I	(136)
	G	9	PC	(38)
I. sempervirens L.	S	8, 11	I	(85)
	G	5	PC	(14, 15)
I. umbellata Dunnetti L.	S	2	PC	(139, 151)
Isatis tinctoria L.	S	2, 6	PC	(38, 72, 133, 139, 151)
	G	1	PC	(139, 151)
Kernera saxatilis Medicus	S	2	PC	(38, 133)
	G	3, 4	PC	(139, 151)
Lepidium campestre R. Br.	S	21, 2	PC	(38, 133, 139, 151)
	G	21	PC	(38)
	R	2	PC	(15)
L. densiflorum Schrad.	S	20	PC	(72)
L. Draba L.	G	3, 12	PC	(38, 108, 132, 139, 151)

(Table 5. continued.)

Plant	Part	Compounds	Evidence	References
Lepidium graminifolium L.	R, G	20	PC	(15)
L. latifolium L.	S	2	PC	(38, 133)
	G	2, 8	PC	(14, 38, 133)
L. Menziesii DC.	S	2	I	(56)
L. ruderale L.	S	20	PC, I	(38, 40, 133)
	G	20	PC	(15, 38, 133)
	R	20	PC	(15)
L. sativum L.	S	20	I	(35, 40)
	G	20	PC	(38)
L. virginicum L.	S	20	PC	(72)
Lunaria annua L.	S	3, 4, 3	PC	(14, 72)
L. biennis MNCH. (= *L. annua* L.)	S	3, 15	I, PC	(69, 91)
L. rediviva L.	S	15	PC	(91)
Malcolmia maritima R. BR.	S	10, 16	I	(76, 90)
Matthiola annua R. BR.	S	1, 2, 3, 11	PC	(38, 69, 73, 139, 151)
	G	2, 24	PC	(14, 38)
M. bicornis DC.	S	13	I	(138)
M. fenestralis (L.) R. BR.	S	1 (?)	PC	(72)
M. incana R. BR.	S	1	PC	(139, 151)
Nasturtium officinale R. BR.	S	2, 24	PC	(38, 72, 133)
	G	2, 24	PC, I	(14, 35, 38, 131)
Parrya menziesii	S	1, 3	PC	(38, 133, 139, 151)
Raphanus raphanistrum L.	G	3	PC	(38, 152)
R. sativus L. var. *alba*	R	13	I	(119)
	S	1, 2, 1 (?), 3	PC	(38, 72, 133)
R. sativus L. var. *nigra*	R	3	PC	(139, 151)
	S	2	PC	(139, 151)
R. sativus L. var. *radicula* PERS.	S	5 (trace)	PC	(72)
Rapistrum perenne ALL.	G	1, 6	PC	(72, 139, 151)
R. rugosum (L.) ALL.	G, S	10	I	(40)
Rorippa amphibia (L.) BESS.	S	2	PC	(38)
R. silvestris (L.) BESS.	R	2	PC	(15)
Schiverekia Dörfleri	G	2	PC	(139, 151)
Sinapis alba L.	R	24	I	(144)
	S	21	I	(94, 114)
S. arvensis L.	S	3, 2, 5	PC, I	(38, 133, 152)
	G	[illegible]		(152)

References, pp. 169 - 176.

(Table 5, continued.)

Plant	Part	Compounds	Evidence	References
Sisymbrium austriacum JACQ.	S	3, 30	PC, I	(67)
S. cheiranthoides ET. et W.	S	?	I	(99)
S. Loeselii L.	G	1	PC	(38)
S. officinalis (L.) SCOP.	S	(?), 1	I, PC	(38, 99, 131)
	G	1	PC	(38, 131)
S. sophia L.	S	5	PC	(72)
	G	2	PC	(15)
S. strictissimum L.	S	2, (3, 4)	PC	(38, 72, 133)
Thlaspi alpestre L.	S	1, 2	PC	(38, 133, 139, 151)
T. arvense L.	S	1, 5	PC	(38, 40, 72, 133, 153)
T. perfoliatum L.	S	3	PC	(139, 151)
	R, G	2	PC	(15)
T. rotundifolium GAUDIN	G	1	PC	(139, 151)
Vesicaria graeca RENT.	G	3	PC	(139, 151)
V. sinuata POIR.	S	11	PC	(73)
V. utriculata L.	S	11	PC	(73)
Vogelia paniculata HORNEM.	G	1	PC	(139, 151)

Table 6. The Occurrence of *iso*Thiocyanates or Their Parent Glucosides in Families Other than *Cruciferae*.

The symbols are those used in Table 5, p. 161.

Plant	Part	Compounds	Evidence	References
1. *Capparidaceae*				
Capparis spinosa L.	S	1	I	(63, 152)
	G	1	PC	(15)
Cleome arabica L.	S	1 + unknown	PC	(72, 84)
C. arborea BSS.	S	1 + unknown	PC	(84)
C. gigantea L.	S	1 + unknown	PC	(84)
C. graveolens RAFIN.	S	1 + unknown	PC	(84)
C. monophylla L.	S	1 + unknown	PC	(84)
C. speciosissima DEPPE	S	1 + unknown	PC	(84)
C. spinosa JACQ.	S	2 (1 + unknown)	PC, I	(84, 130, 151)
C. viscosa L.	S	1 + unknown	PC	(84, 152)
C. trachysperma (TORR. et GRAY) PAX et K. HOFFM.	S	1 + unknown	PC	(84)
Gynandropsis gynandra (L.) BRIQ.	S	1	PC	(72, 84)
G. pentaphylla DC.	S	unknown	I	(152)

(Table 6, continued.)

Plant	Part	Compounds	Evidence	References
2. *Caricaceae*				
Carica papaya L.	S	20	I	(21)
3. *Euphorbiaceae*				
Jatropha multifida L.....	Latex	20 (?)	I (?)	(29)
Putranjiva Roxburghii WALL.	S	3, 4, unknown	I	(110)
4. *Limnanthaceae*				
Limnanthes douglasii R. BR.	S	23	I	(22)
5. *Moringaceae*				
Moringa pterygosperma GAERTN.	R	"20"	I	(98)
6. *Phytolaccaceae*				
Codonocarpus cotinifolius (DESF.).....	G	4, (20?)	I	(6)
7. *Plantaginaceae*				
Plantago major L. (?) ...	G	13	PC	(107)
8. *Resedaceae*				
Reseda alba L.....	R	24	PC	(15)
R. lutea L.....	R	(?), 20	PC	(14, 152)
	G	(?)	I	(152)
R. luteola L.....	R, G, S	(?)		(124)
	R	2, 24	PC	(14, 38)
	R, G	1	PC	(131)
	G, S	29	I, PC	(80)
R. odorata L.....	R	24	I	(5)
	S	1, 2, 1 (?)	PC	(72, 139, 151)
9. *Salvadoraceae*				
Salvadora oleoides DEN...	S	20	I	(105)
10. *Tropaeolaceae*				
Tropaeolum majus L.....	S	20	I	(34)
	G	20	I	(34)
T. peregrinum (canariense)	S	3, 4	PC	(72)

References, pp. 169—176.

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The Effect of Thiocyanate in Nutrition on the Iodine Content of Cow's Milk

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With 1 figure in 3 details and 5 tables

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When the claim (1) that milk produced on *Cruciferae* plants has an antithyroid effect has been investigated in our institute in recent years, special attention has been paid to goitrogenic substances in these plants and in milk (2). GMELIN and VIRTANEN (3) showed that the thiocyanate ion (SCN^-) which causes „cabbage goitre" is formed in crushed *Brassica* plants enzymically from the precursors glucobrassicin (4) and its N_1-methoxy derivative, neoglucobrassicin (5). These precursors were found in different *Brassica* species and their structures were elucidated. The thiocyanate formed when the cow is fed on *Brassica* plants is secreted partly into the milk, but its concentration in milk does not rise essentially above 1 mg% even when very large doses of thiocyanate are used (6). VILKKI et al. (7) have shown that this level does not yet cause any disturbances in the uptake of iodine by the thyroid gland of man. In feeding

experiments of long duration it has not caused goitre in rats, either (2). This is true provided that the lack of iodine in the nutrition is not so great that even a small disturbance in iodine uptake would lead to the development of goitre.

VIRTANEN, KREULA, and KIESVAARA (8) found that also the strong antithyroid compound, L-5-vinyl-2-thiooxazolidone (goitrin), – which is formed in many *Brassica* fodder plants when these are crushed (9) – is transferred to milk from the rumen of the cow in so minute amounts that it is of no practical importance.

Even though the amount of thiocyanate which is formed from a large amount of cabbage or rutabaga does not make milk goitrogenic, it may have an indirect unfavourable effect on the iodine metabolism of the animal. In addition to a disturbed iodine uptake by the thyroid gland, it has been observed that the iodine content in milk produced on different *Brassica* species has decreased while the thiocyanate content has increased. On the other hand, surprisingly high iodine contents have been observed in milk after the feeding of thiocyanate (10) has been discontinued. These observations led to the question how thiocyanate affects the iodine content of cow's milk. In order to elucidate this question, three cows were fed 1.5 g of SCN^- as the potassium salt daily during one week and 3.0 g daily during the next week. These amounts of thiocyanate correspond to the amounts which cows take in when *Brassica* fodder plants are fed in abundance. The milk samples were analysed for both iodine and thiocyanate.

Experimental

Methods

A modification of the method of BARKER et al. (11) was used with slight alterations for the determination of the total iodine in milk and fodder. Using this modification, VILKKI (12) had earlier determined the iodine contents of the most common foodstuffs in Finland, especially milk. 0.25–1.0 ml of milk or 50–300 mg of ground fodder, 1 ml of 10% zinc sulphate and 1 ml of 5 N potassium hydroxide were measured into glass tubes. The mixture was dried for 18 hours at 110 °C and then for 2 hours at 150 °C, after which it was heated at 600 ° for 2 hours. The ash was dissolved in 5 ml of 0.08 N potassium arsenite. 3 ml of the clear centrifuged liquid was acidified with 1 ml of 7 N sulphuric acid which contained 10% (w/v) sodium chloride and warmed to 37 °. 1 ml of 0.01 N cerium ammonium sulphate was added and the optical density of the solution was measured against water with a Klett-Summerson colorimeter (filter 42) 6 and 12 minutes after the addition. The amount of iodide catalysing the reduction of Ce (IV) was obtained by comparing the difference of the corresponding transmission values with those of the samples after the addition of 0.04 μg of iodide.

The determination of the thiocyanate in the milk was performed according to BARKER's (13) method. The protein in 10 ml of milk was precipitated with 10 ml of 20% trichloroacetic acid, 1 ml of 5% (w/v) iron (III) nitrate in 2.5% (v/v) nitric acid was added to 5 ml of the filtrate, and the colour was measured with the KLETT-SUMMERSON colorimeter (filter 50). A solution containing 5 ml of the filtrate and 1 ml of water was used as a blank. The thiocyanate content of the sample was obtained by comparing the results with a standard curve.

The arrangement of the feeding experiment

The feeding experiment was carried out with three Ayrshire cows with a fairly equal milk production on the Joensuu farm during the indoor feeding period 7.2.–12.3.1962.

Aino,	5 years old, calved on January	14, 1962
Ella II,	3 „ „ „ „ September	25, 1961
Elo II,	4 „ „ „ „ August	31, 1961

The average rations and the average iodine contents of the different feeds given during the experiment are shown in Table 1.

Table 1.
The rations and iodine contents of the different feed components used in the experiment

Feed ration/day	Iodine, µg/kg fodder	Iodine, mg, in daily ration
25 kg maize-AIV-silage	190	4.8
5 „ crushed oats	45	0.2
3 „ hay	115	0.3
3 „ straw	350	1.1
40 l water	15	0.6
	Total	7.0 mg

The feeding experiment was continued during five periods of one week. Additional portions of potassium iodide and thiocyanate, mixed with a small amount of crushed oats, were given daily at 2–2.30 p. m. to each cow as shown in Table 2.

Table 2. Intake of additional KI and KSCN in different test periods

Week	Days	KI mg	KSCN g	Cows
1st	1st–5th	0	0	all three
2nd	6th–12th	13.1*	0	„
3rd	13th–19th	13.1	2.5**	„
4th	20th–26th	13.1	5.0***	„
5th	27th–33rd	13.1	0	Elo II
5th		0	0	Aino and Ella II

* 10 mg I^-
** 1.5 g SCN^-
*** 3.0 g SCN^-

Table 3. The average milk production in different test periods

Cow	Week	Morning milk kg/day	Evening milk kg/day	Production kg/day
Aino	1st	8.1	7.1	15.3
	2nd	7.7	6.5	14.1
	3rd	7.7	6.5	14.1
	4th	7.0	6.1	13.1
	5th	7.3	6.2	13.5
Ella II	1st	7.4	6.3	13.7
	2nd	7.1	5.3	12.4
	3rd	6.2	4.7	10.9
	4th	6.2	5.2	11.5
	5th	5.6	4.7	10.3
Elo II	1st	7.6	6.2	13.8
	2nd	7.2	5.7	12.9
	3rd	6.4	5.1	11.5
	4th	5.7	4.8	10.6
	5th	5.8	4.7	10.5

The cows were milked in the morning at 6–6.30 and in the evening at 5–5.30. Determinations of iodine and thiocyanate were carried out on each milk sample. The average milk production of the cows during the different periods is shown in Table 3.

Results

The results presented in Fig. 1 and Tables 1 and 5 show that the continuous feeding of thiocyanate effectively decreased the iodine content of the milk of each cow although the contents revealed individual differences.

The thiocyanate (SCN^-) contents of the milk during the experiment are seen in Table 4.

Table 4. Secretion of thiocyanate into milk

Cow	The average SCN^- content of milk, mg/l				Secretion of SCN^- into milk during the test, % of given amount
	Before SCN^- feeding 1st–12th days	1.5 g SCN^-/day 14th–19th days	3.0 g SCN^-/day 23rd–26th days	After SCN^- feeding 31st–33rd days	
Aino	1.7	6.2	8.1	1.9	3.6
Ella II	3.4	6.8	9.3	2.2	2.2
Elo II	3.5	6.3	8.1	3.4	2.0

A daily ration of 1.5 g of SCN^- per cow raised the thiocyanate concentration in the milk in 24 hours to 0.6 – 0.7 mg % independently of the initial level. With a double ration, the thiocyanate content of Ella II's milk rose to 0.9 mg%, and that of Aino's and Elo II's milk temporarily to 1.2 mg %, from which level it soon decreased to an average level of 0.8 mg %. During the whole experiment the total secretion in Aino's milk was almost twice (3.6% of the given amount of SCN^-) that in the other cows (2.0–2.2% of the given amount). The thiocyanate concentration, which was usually higher in the evening milk than in the more abundant morning milk, decreased rapidly to the initial level after the feeding of thiocyanate was discontinued. The decrease was somewhat more rapid than in the previous experiments of VIRTANEN and GMELIN (6) in which no additional iodide was given.

The changes that occurred simultaneously in the iodine content of the milk are seen in Fig. 1.

During the first week, when the cows received 7 mg of iodine per day in the fodder, the iodine content of the milk amounted to about 10–20 μg per l, which corresponded to 2.6–4.1% of the daily iodine intake. The daily addition of 10 mg of I^- in the second week increased the iodine content of the milk to 90–175 μg per l, which corresponded to 7.4–13.2% of the total iodine fed. In the third week, the iodine content of the milk decreased abruptly already after the first dose of thiocyanate (1.5 g of SCN^- per 24 hours) and fell to a minimum on the second or third day (3.1–4.4% of the total iodine secreted into the milk), rose then almost to the maximum of the previous iodide period, but began to decrease again in two test cows after 3–4 days. A double dose of thiocyanate (3 g of SCN^-) caused the iodine content to decrease to a relatively stable level which corresponded to the minimum level obtained already with the first dose (1.5 g) or to about $^1/_3$ of the concentration reached before the thiocyanate period (3.0–4.3% of the total iodine secreted into the milk). The average amounts of iodine secreted into milk during the experimental periods are shown in Table 5.

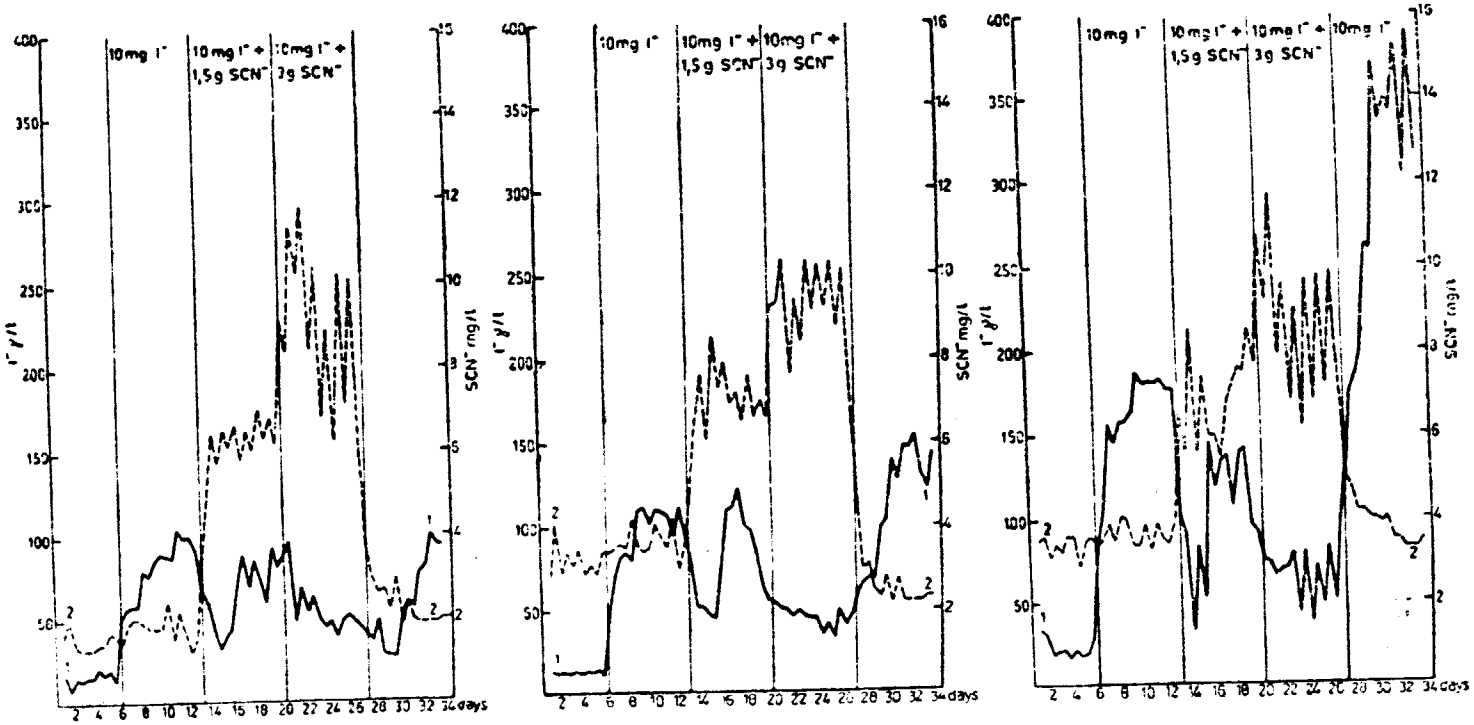

Fig. 1. The effect of a continuous feeding of iodide and thiocyanate on the iodine (1) and thiocyanate (2) content of the milk. The amounts of the daily doses of iodide and thiocyanate (SCN^-) are given in the upper edge of the Figure in connection with each test period.

The discontinuation of the thiocyanate feeding caused a remarkable change. Although iodide was no longer given to two cows, the iodine content of the milk rose so high that it either reached or exceeded considerably the high iodine level that prevailed before the feeding of thiocyanate when the cows received 10 mg of I^- per day. It was 5–11 times the iodine level of the milk in the initial period of the experiment before the feeding of iodide (Table 5 and Fig. 1, Aino and Ella II). The feeding of iodide was continued to one of the cows (Elo II) during the last period when thiocyanate was no longer given; the iodine content of the milk increased to twice the content in the second week of the experiment when the cow received the same quantity of iodide but no thiocyanate (Fig. 1, Elo II). Irrespective of whether the cow took in only the 7 mg of iodine present in the fodder, or an additional 10 mg of I^-, on the average 20% of iodine fed was secreted into the milk on the sixth day after the thiocyanate feeding had been discontinued. The average secretion of iodine 4–7 days after the thiocyanate feeding ended varied from 15 to 21%.

Discussion

All the feeding experiments (those reported in this paper and four others not described) have shown that the feeding of thiocyanate to cows strongly decreases the iodine content of the milk. The results suggest an active mechanism which concentrates iodine in the mammary gland of the cow. BROWN-GRANT (14) and POTTER et al. (15) have established this kind of mechanism in gnawers and its prevention with thiocyanate in injection experiments with ^{131}I and SCN^-. GARNER et al. (16) observed in their feeding experiments with cows that the secretion of radioiodine into milk was prevented by 10 g of sodium thiocyanate which according to our observations greatly exceeds the amount of thiocyanate which the cow can get daily even

Table 5.

Daily doses of I^- and SCN^- (lower part of the table), and the average secretion of iodine into milk (upper part of the table)

Cow	1st week		2nd week		3rd week		4th week		5th week	
	2nd–5th days		8th–12th days		15th–19th days		22nd–26th days		30th–33rd days	
	% of dose	µg/l	% of dose	µg/l	% of dose	µg/l	% of dose	µg/l	% of dose	µg/l
Aino	3.5	16	7.7	99	5.7	79	3.7	49	15.0	7 6
Ella II	2.6	13	7.4	103	5.5	86	3.0	44	19.6	139
Elo II	4.1	21	13.2	175	7.6	113	4.3	68	21.1	347
I^- (mg) given in 24 h	7.0		17.0		17.0		17.0		7.0 (17.0 Elo)	
SCN^- (mg) given in 24 h	0		0		1.5		3.0		0	

when it is fed large quantities of *Brassica* plants. In our experiments the effect has been achieved with quantities corresponding to those cattle receive normally. The mechanism of iodide uptake by the mammary gland is obviously very sensitive to the effect of thiocyanate, more sensitive than the mechanism of iodide uptake by the thyroid gland. Also the fact that REINEKE (17) recently succeeded in achieving a similar result with a goat in injection experiments, using a quantity of thiocyanate which did not yet inhibit iodide uptake by the thyroid gland, supports this view.

Because thiocyanate prevents the secretion of iodine into milk, it protects cows from getting goitre when the iodine content of their feed is low. On the other hand, the low iodine content of milk is harmful from the point of view of human nutrition.

The finding that when the feeding of thiocyanate was discontinued, the iodine content of milk increased within a few days to a value 2 to 11 times as high as that attained with the corresponding iodine doses before thiocyanate was fed is especially striking. It seems at first sight as if iodine had accumulated somewhere else in the organism during the feeding of thiocyanate and was secreted into the milk after the influence of thiocyanate had ceased. However, it must be taken into consideration that even in the last experimental period the amount of iodine secreted into milk was only about 20% of the quantity given. A hypothesis of an accumulation of iodine in the organism during the thiocyanate feeding is thus not necessary to explain the enormous rise in the iodine level of the milk when thiocyanate was omitted. It is more probable that the feeding of thiocyanate activates a mechanism in the organism which stimulates the iodide uptake by the mammary gland. As a result of this activation, the secretion of iodine into milk rises when thiocyanate is no longer given. The fluctuations in the iodine content of the milk when a lower dose of thiocyanate (1.5 g of SCN^-/cow) was given during the third feeding period also support this assumption. A sharp decrease in the iodine content was followed by a strong increase and then a decrease. Only when 3.0 g of thiocyanate was fed daily to a cow, did the low level remain constant.

As we have found so far, the iodide uptake by the mammary gland is very similar to that of the thyroid gland. When thiocyanate inhibits the iodide uptake by the mammary gland, the capacity of the gland to take up iodide is stimulated. This state of stimulation continues even after the feeding of thiocyanate is stopped, and it is the cause for the exceptionally high iodine content of the milk when thiocyanate is removed from the food. It is possible that the results presented in this paper are the first hints of an analogy between the mammary gland and the thyroid gland regarding the uptake of iodide.

According to previous investigations in this laboratory, the continuously increasing use of *Cruciferae* plants in the feeding of cows, especially in the northern areas, does not make the milk goitrogenic. The results presented above reveal an indirect danger: the feeding of *Cruciferae* plants decreases the iodine content of milk greatly. This effect must be taken into consideration particularly in a country like Finland where milk is the most important source of iodine in food (12). For this reason the general use of iodized salt in human nutrition should be emphasized more than ever before. The use of iodine-containing salt mixtures in cattle feeding in order to eliminate possible disadvantages caused by thiocyanate in the fodder is also recommended.

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Summary

When cows are continuously fed amounts of potassium thiocyanate corresponding to the quantity formed from 15–30 kg of the marrow kale or rutabaga per day (1.5 and 3.0 g of SCN^-, respectively), the thiocyanate concentration in the milk rises and the iodine content of the milk decreases. When the feeding of thiocyanate is discontinued, the iodine content of the milk rises much above the initial level during a few days. The results suggest that this is due to a mechanism that regulates the uptake of iodide by the mammary gland.

Zusammenfassung

Wenn die Kühe kontinuierlich mit solchen Mengen von Kaliumrhodanid gefüttert werden (resp. 1.5 und 3.0 g SCN^-), welche der aus 15–30 kg Markstammkohl oder Kohlrübe pro Tag entstehenden Quantität entsprechen, steigt die Konzentration von Rhodanid in der Milch unter gleichzeitiger Senkung des Jodgehalts. Wenn die Fütterung von Rhodanid nicht mehr fortgesetzt wird, steigt der Jodgehalt stark über das Anfangsniveau während ein paar Tagen. Die Resultate weisen darauf hin, daß dies auf einen Mechanismus zurückzuführen sei, der die Jodidaufnahme der Milchdrüse regelt.

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