Report-Oil of Rue 9/6/72

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# REPORT

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OIL OF RUE

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# OIL OF RUE

## Summary

Oil of rue is the volatile oil obtained by steam distillation from the fresh blossoming plants. Ninety percent or more of the active components are the methyl heptyl and methyl nonyl ketones. According to FEMA its major use in foods is as a flavoring adjunct in beverages, ice cream, candy, and baked goods.

The oil is a powerful irritant when applied to the skin, producing burning, redness, and vesication (31) which probably arises from the ability of rue oil to quickly penetrate the skin. In studies on mice, for example, rapid percutaneous absorption through intact shaved abdominal skin was shown to occur with rue oil, while in contrast, no absorption occurred with such materials as anise oil, beechnut oil, and eugenol even after two hours (15).

When taken internally in large doses a variety of physiological disturbances may result, including "violent gastric pains and vomiting, prostration, confusion of mind, convulsive twitching, and in pregnant women, abortion" (31). The abortive effect of the oil is stated to arise from its direct action on the muscle fiber of the uterus. However, the placenta appears not to be a barrier to the oil since after administration to the adult some of it can be found in the placenta and in the embryo (18).

"In guinea pigs and rabbits large doses of commercial oil of rue produced dyspnea, diarrhea, torpor, sometimes hematemesis, and loss of weight. The most important lesions were granular fatty hepatitis and parenchymatous nephritis. The fact that these lesions were more marked in fetuses than in the mothers shows that the poison readily passes through the placenta" (19).

The medical uses for rue oil have included treatment of hysteria, colic, atonic amenhorrhea, menorrhagia, and worms (31). In regard to this last, the oil (from R. divaricata L.) has been found to have mild antihelmintic action when tested against vinegar eels, leeches, tubiflex worms, and ascarides. The toxicity for these species is apparently due to the methyl heptyl ketone component in the oil. The toxic threshold of the helmintes to this ketone is much lower than for the host-animal, the $LD_{50}$ for mice, for example, being 2,070 mg/kg (28).

When rue oil is injected into experimental animals in sufficient quantities to cause death, its toxic action seems to be system-wide, leading to congestion and lesions of all the important organs (4).

# OIL OF RUE

## Chemical Information

I. Nomenclature

A. Common name

Rue oil

B. Chemical name

(none)

C. Trade names (8)

Spanish Rue

French Rue

North African Rue

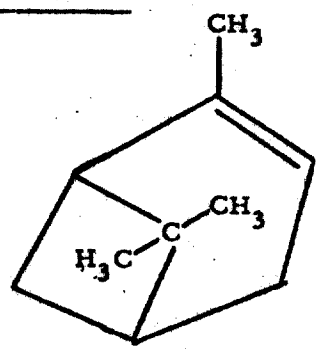
D. CAS Unique Registry Number

977051890

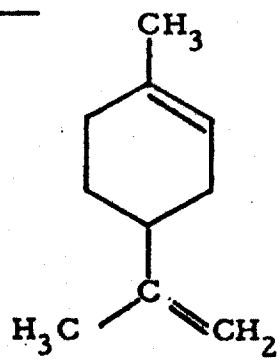
II. Empirical Formula & III. Structural Formula

Oil of Rue is a mixture of substances only some of which have been identified (8). These are:

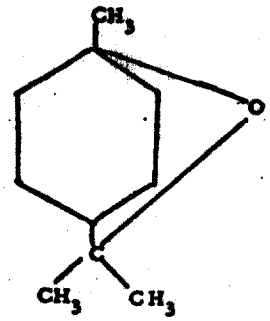
Pinene



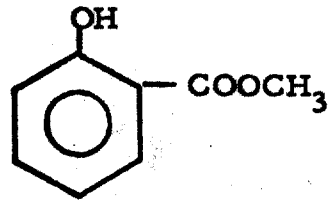

Limonene



**Cineole**




**Methyl salicylate**




**Methyl heptyl ketone**

$CH_3$-O-$C_7H_{20}$

**Methyl nonyl ketone**

$CH_3$-O-$C_9H_{26}$

The heptyl and nonyl ketones make up 90% or more of rue oil components, although the distribution of the two varies with the plant variety (8).

IV. Molecular Weight - of known components ( Handbook of Chemistry ):

| | |
|---|---|
| . methyl heptyl ketone | 142.24 |
| . methyl nonyl ketone | 170.29 |
| . pinene | 136.23 |
| . limonene | 136.23 |
| . cineole | 154.25 |
| . methyl salicylate | 152.14 |

V. Specifications

A. Chemical

(none)

B. Food Grade ( 6 )

Assay: Not less than 90 per cent of ketones, calculated as methyl nonyl ketone ($C_{11}H_{22}O$).

Limits of Impurities:

- Arsenic (as As). Not more than 3 ppm (0.0003%).
- Heavy metals (as Pb). Not more than 40 ppm (0.004%).
- Lead. Not more than 10 ppm. (0.001%).

C. Official Compendia

Food Chem. Codex. 1st Ed. 1966.

VI. Description

A. General Characteristics (6)

It is a yellow to yellow-amber liquid, having a characteristic fatty odor. It is soluble in most fixed oils and in mineral oil, but it is relatively insoluble in glycerin and in propylene glycol.

B. Physical Properties (6)

- Angular rotation. Between -1° and +3°.
- Refractive index. Between 1.4300 and 1.4400 at 20°.
- Solidification point. Between 7.5° and 10.5°.
- Specific gravity. Between 0.826 and 0.838.

Solubility in Alcohol: Soluble in 2 to 4 volumes of 70% alcohol, occasionally with opalescence or precipitation of solids (3).

Solubility (3) :

| | |
|---|---|
| • Benzyl Benzoate: | Soluble in all proportions. |
| • Diethyl Phthalate: | Soluble in all proportions. |
| • Fixed Oils: | Soluble in all proportions in most fixed oils. |
| • Glycerine: | Relatively insoluble. |

| | |
|---|---|
| . Mineral Oil: | Soluble in all proportions. |
| . Propylene Glycol: | Relatively insoluble. |

Stability (3) :

| | |
|---|---|
| . Alkali: | Relatively stable in the presence of weak alkali. |
| . Acids: | Relatively stable in the presence of weak organic acids. |

C. Stability in Containers (3)

Ship preferably in glass, tin-lined or galvanized containers.

Store preferably in tight, full containers in a cool place, protected from light.

## VII. Analytical Methods

. GLC ( 2 )

. Colorimetric ( 6 )

## VIII. Occurence and Levels found in:

A. Plants

Extracted from plants of the family Rutaceae, principally R. montana L., R. graveolens L., R. bracteosa L. (6).

When distilled freshly, 90 kg. of rue plants yield on the average 1 kg. of oil. On drying or on exposure to air for a few days, the yield of oil diminishes considerably, and 160 kg. of plant material may be required to give 1 kg. of oil ( 8 ).

B. Animals

(none)

C. Synthetics

(none)

D. Natural inorganic sources

(none)

# OIL OF RUE

## Biochemical Aspects and Biological Data

The pertinent biochemical and biological data found in the abstracts were deemed insufficient for inclusion into the usual monograph format.

# OIL OF RUE

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*(31) United States Dispensatory. 1960. XXV. Rue. 1831-1832.
J. B. Lippincott Company. Philadelphia, Pa.



*Uses.*—In recent years rose, if used at all, was employed for its agreeable odor.

*Storage.*—Preserve "in tight, light-resistant containers." *N.F. VIII.*

**Rosemary.** *Folia Anthos.*—"The leaves of *Rosmarinus officinalis* (Fam. *Labiatæ*)." *U.S.P. 1880.* Rosemary is a small evergreen shrub, native to southern Europe, with an erect stem, divided into many long, slender, ash-colored branches. The leaves are numerous, sessile, opposite, about 2.5 cm. long, rigid, linear, entire, obtuse at the summit, folded backward at the edges, of a firm consistence, smooth and green on the upper surface, whitish, woolly, and glandular beneath. They have a balsamic odor due to the presence of a volatile oil (see *Rosemary Oil*, Part I). In some countries they are employed as a condiment; they were formerly considered emmenagogue, but are not used in this country. Rosemary flowers are much sought after by the bees, and impart their characteristic flavor to the honey of the districts in which the plant abounds.

**Rotenone.**—This principle is found in a number of plants belonging to the family *Leguminosæ*, especially species of the genera *Derris, Lonchocarpus* and *Tephrosia.*

The root of *Derris elliptica* Benth., under various native names as *tuba, toefa, akar, toeba,* has been employed as an arrow poison by the natives of the Malay Peninsula and the East Indies, as well as for killing fish under water. From the root of this plant the natives prepared an active resin which they called *derrid.* In South America, the Indians along the Amazon River prepared from the *D. negrensis* Benth. a fish poison which they called *timbo;* in southern Asia the *D. uliginosa* Benth. (*D. trifoliata* Lour.) is used for the same purpose.

Various species of *Lonchocarpus* also are used as fish poisons by the natives of several South American countries; these are variously designated as *cube, barbasco, hairari* and *nekoe,* although the same names are sometimes given to fish-poisoning plants of other genera and families. The most important species are the *L. nicou* DC. (*stinkwood*) *L. urucu* Killip and Smith and the *L. violaceus* H. B. K. (*Robinia violacea* Jacq.).

The *Tephrosia virginiana* (L.) Pers. (*Cracca virginiana* L.) is known by a variety of colloquial names such as: *turkey pea, wild sweet pea, catgut, goat's rue, hoary pea, devil's shoestring.* It is native to dry sandy soil of the eastern United States. It is a foot or two high, with pubescent stems and leaves, and a handsome terminal raceme or panicle of yellowish-white, purple-tinged flowers. The roots, which are slender, long, and matted, are said to have been used by the Indians as a vermifuge, given in the form of decoction. *T. vogelii* Hook of Africa is used by the natives as a fish poison.

Clark (*J.A.C.S.*, 1943, 65, 27) found both rotenone and tephrosin as well as other related principles in the seeds of the *Milletia ferruginea* Hochst., an Abyssinian tree known popularly as the *berebera* tree.

All of the above and many related species of plants have been used, in one form or another, as insecticides. The powdered roots have been prepared as dusts; aqueous and organic-solvent extracts and even suspensions of powdered roots prepared; all of these have been found to be more or less effective against many classes of insects. A comprehensive report on the use of the *Lonchocarpus* species as insecticides was prepared by Roark (*Bull. Bur. Entomology*, U. S. Dept. Agriculture, March, 1936). Investigations revealed that four active principles, *rotenone* (*nicouline* or *tubotoxin*), *deguelin, toxicarol* and *tephrosin* may be present in the various roots, although sometimes one or more of these constituents may be absent. Of these, rotenone is the most important and best-known. It occurs as white, odorless crystals, melting at 163°; it is insoluble in water but dissolves in alcohol, acetone, chloroform, and many other organic liquids. The empirical formula of rotenone is $C_{23}H_{22}O_6$; its structure has been shown to be that of an optically active dihydrobenzofuran system having an isopropenyl side chain connected to the dihydropyrone radical (*J.A.C.S.*, 1932, 54, 810). Rotenone has been synthesized. Deguelin is isomeric with it; tephrosin and toxicarol are hydroxy derivatives of deguelin. The chemistry of the several principles is discussed by Clark (*J.A.C.S.*, 1932, 54, 3000, and in earlier papers); the extraction and separation of rotenone, deguelin and tephrosin are described also by Clark (*J.A.C.S.*, 1943, 65, 27).

Rotenone is extremely poisonous to insects and to fish, but only slightly toxic to mammals. Haag (*J. Pharmacol.*, 1931, 43, 193) found the intravenous lethal dose for dogs was about 0.5 mg. per Kg., but that by mouth 600 times this dose was required to kill. Small doses, when injected intravenously, had a stimulating effect on the respiration, but fatal quantities kill by paralyzing the respiratory mechanism.

Rotenone, when applied to the skin, produces only an occasional mild irritation; it is not absorbed by the skin. Inhalation of finely powdered rotenone or derris can be dangerous, for it has been shown that finely divided rotenone is about six times as poisonous as coarse crystals. The fatal dose of rotenone is probably very large, having been estimated to be about 200 Gm. Since rotenone acts as a gastric irritant and also stimulates the emetic center after absorption, swallowed material is likely to be removed before poisoning occurs. Chronic ingestion may produce necrosis of cells in the central and midlobular areas of the liver (Lehman, *Bull. N. Y. Acad. Med.*, 1949, 25, 382).

According to Ginsburg (*New Jersey Agricul. Bull.*, Circ. 273, 1933) rotenone is decomposed by sunlight, losing its toxicity after a few days' exposure.

Rotenone itself, as well as preparations of the roots from which it may be obtained, is widely used as an insecticide. Williams *et al.* (*South. Med. Surg.*, 1941, 103, 199) used a 2 per cent lotion in the treatment of the dermatitis caused by "chiggers" (*Trombidii*) with excellent results; a 10 per cent emulsion has been employed in treating scabies.

According to the researches of van Hasselt (*Arch. internat. pharmacodyn. therap.*, 1911, 21, 242), closely allied, physiologically, to derrid is *pachyrhizid,* from the *Pachyrhizus angulatus* Rich. This drug is a powerful poison to the central nervous system, especially the respiratory center, and in larger doses causes slowing of the pulse by a direct action of the heart muscle. [V]

**Rue.** *Ruta. Herb of Grace. Countryman's Treacle.*—The U.S.P. 1870 recognized the leaves of the *Ruta graveolens* L. (Fam. *Rutaceæ*). It is a low, shrubby plant, with several shrubby branching stems, which, near the base, are woody and covered with a rough bark, but in their ultimate ramifications are smooth, green, and herbaceous. The leaves are fleshy, 2- to 3-pinnatifid, the flowers yellow, and disposed in a terminal branched cyme. The plant is a native of the south of Europe, locally established in pastures in the United States and cultivated in our gardens. The leaves have a strong, disagreeable odor, especially when rubbed. Their taste is bitter, hot, and acrid. The fresh leaves may inflame and even blister the skin. This irritant property depends chiefly on a volatile oil, which is contained in glandular hairs, apparent over the whole aerial surface of the plant. They contain also the coloring principle rutin.

Rue yields a very small proportion of a yellow or greenish volatile oil (*Oleum Rutæ, U.S.P. 1880*), which becomes brown on aging. The oil has the

strong unpleasant odor of the plant, and an acrid taste. It consists largely of methyl-nonyl-ketone, $CH_3.CO.C_9H_{19}$, with small amounts of other ketones, esters, and phenols.

*Uses.*—Rue is said to have been used by the ancients as a condiment. In modern times it has been employed in treating hysteria, worms, colic, and atonic amenorrhea and menorrhagia. Its medicinal activity depends upon its volatile oil, which is a powerful local irritant, causing, when applied to the skin persistently, burning, redness, and vesication, and when taken internally in large doses, violent gastric pains and vomiting, prostration, confusion of mind, convulsive twitching and, in pregnant women, abortion. It has been used in Europe for the production of criminal abortion, in a number of cases with fatal results. Prochnow (*Arch. internat. pharmacodyn. therap.*, 1911, 21, 314) demonstrated, however, that it has only a feeble stimulating influence on the uterus. Patoir (*Compt. rend. soc. biol.*, 1938, 127, 1324) found post-mortem, in the lower animals, degeneration of the liver and parenchymatous nephritis. The abortive action seems to be due more to its general toxicity than to any specific effect on the uterus.

**Ryanodine.**—From extracts of the stem and root of various *Ryania* species (Fam. *Flacourtiaceæ*) fractions possessing insecticidal activity have been prepared by several investigators. Extracts of *Ryania speciosa* Vahl. (*Ryaxia pyrifera* [L. C. Rich.] Vitt.) were found to be very active and promising. From these Rogers *et al.* (*J.A.C.S.*, 1948, 70, 3086) isolated an alkaloid, called *ryanodine*, having approximately 700 times the insecticidal activity of the stem wood from which it is obtained. Ryanodine is soluble in water, alcohol, acetone, ether and chloroform.

Pharmacological studies of ryanodine by Pick and Tullius (*Arch. internat. pharmacodyn. therap.*, 1951, 86, 121) showed that the alkaloid, when tested on isolated frog abdominal muscle, produces a gradually increasing contraction of the muscle which progresses to an irreversible rigidity; while the effect is enhanced by the addition of acetylcholine, ryanodine does not inhibit true cholinesterase. It was concluded that the site of action of ryanodine is on the muscle proteins or on the myogenic juncture of the motor end plates.

From the studies of Procita *et al.* (*J. Pharmacol.*, 1952, 106, 411) it is apparent that ryanodine is extremely toxic to mammals. The intraperitoneal $LD_{50}$ dose for white rats is in the range of 300 to 350 micrograms per kilogram of body weight; the animals exhibit signs of depression and air hunger within 10 minutes after an injection and die following anoxic convulsions. The dog appears to be extremely sensitive to ryanodine, an intravenous dose of 100 micrograms per kilogram of body weight usually being fatal.

**Sabadilla.** *Cevadilla.*—The dried ripe seeds of *Schœnocaulon officinale* (Schlecht.) A. Gray [*Asagræa officinalis* (Cham. and Schlecht.) Lindl.], were recognized by the B.P. of 1885. At one time cevadilla was erroneously believed to be derived from *Veratrum Sabadilla* Schiede. The sabadilla grows in the Andes of Mexico and Guatemala and Venezuela. It is also cultivated. The fruit is a capsule having three locules in each of which there are from three to four seeds. The seeds are elongated, pointed at each end, flat on one side and convex on the other, somewhat curved, 5 to 8 mm. long, wrinkled, slightly winged, black or dark brown on the outside, whitish within, hard, inodorous, and of an exceedingly acrid, burning, and bitter taste. The powdered drug is sternutatory.

Sabadilla seed usually yields, according to Poetsch and Parks (*J. A. Ph. A.*, 1949, 38, 522), from 3 to 6 per cent of total alkaloids and from 15 to 20 per cent of fixed oil. The alkaloids include *cevadine* (*crystallized veratrine*), *cevine* (*sabadinine*), *veratridine* (*amorphous veratrine*), *cevadilline* (*sabadilline*), *sabadine*, and *sabatine*. Cevine is an alkanolamine having the formula $C_{27}H_{42}NO_8$, and is obtainable also from veratrum viride; cevadine is the tiglic acid ester of cevine and veratridine is the veratric acid ester of the same base (but see discussion under *Constituents* of *Veratrum Viride*). For further information concerning these alkaloids see Poetsch, Hennig *et al.* (*ibid.*, 1949, 38, 522, 525; 1951, 40, 168).

Of these alkaloids the most important, from the medical standpoint at least, appear to be cevadine and veratridine, the other alkaloids being much less active physiologically. Cevadine and veratridine occur also in veratrum viride (*q.v.*).

Two acids have also been found in sabadilla—the *sabadillic* or *cevadic acid* of Pelletier and Caventou, and the *veratric acid* of Merck, which Koerner showed to be dimethylprotocatechuic acid.

Under the title *Veratrina* (*veratrine, veratria*) the N.F. V recognized a mixture of alkaloids, said to be mainly cevadine, veratridine and cevine (Mendez and Montes, *loc. cit.*). This mixture was described as follows: "Veratrine is a white, or grayish white, amorphous powder, odorless, but causing intense irritation and sneezing when even a minute quantity reaches the nasal mucous membrane. *Great caution must be used in handling it.* It is slightly hygroscopic. One Gm. of Veratrine dissolves in 1760 cc. of water, 2.8 ml. of alcohol, 0.7 cc. of chloroform, and in 4.2 cc. of ether at 25° C.; also in 1345 ml. of water at 80° C.; insoluble in petroleum benzin." *N.F. V.*

Cevadilla is an acrid, drastic emetocathartic, sometimes acting with great violence, and in overdoses capable of producing fatal effects.

Probably because of its unfortunate name it has been assumed by some that the mixture of alkaloids called "veratrine" represents the therapeutic virtues of veratrum. Its physiological action, however, differs from that of veratrum, and if it possesses any therapeutic usefulness it is certainly not for the conditions for which veratrum is employed.

Veratrine (the mixture of alkaloids) is an intense local irritant and locally somewhat anesthetic. Rubbed upon the skin it produces a sensation of warmth and a peculiar tingling. When taken orally it produces a sense of acrimony, and in large doses causes violent vomiting, serous purging with intense burning in the mouth and throat and general muscular weakness. It is a powerful muscle poison and if given in sufficient dose in the lower animals it causes an extraordinary prolongation of the contraction of the striated muscles; the time required for relaxation of voluntary muscles may be 20 or 30 times as long as normal. A similar influence is exerted upon the ventricular muscles, at least in the frog, but the slowing of the heart rate seen in warm-blooded animals seems to be due chiefly to stimulation of the cardio-inhibitory center. Death in mammals is generally due to depression of the respiration. (See also under *Veratrum Viride*, Part I.)

The only justifiable therapeutic use of veratrine is as an anodyne counterirritant in neuralgias and various forms of arthritis. Even for this purpose it has no advantages sufficient to offset the dangers of systemic poisoning which might follow from its absorption. The N.F. V recognized an oleate (*Oleatum Veratrinæ*) containing 2 per cent of the alkaloids and an ointment (*Unguentum Veratrinæ*) containing 4 per cent.

Sabadilla dusts and sprays have recently been found very effective insecticides against the squash bug, cabbage worms, and loopers.

Unterschiede zwischen den Gruppen 2 und 3 ergeben sich insofern, als nach Anwendung des ME vor Fibrin-Implantation die Exzisionsstücke schon vom 5. Tage an, besonders aber vom 8. Tag an und später, deutlich verstärkte bindegewebige Abgrenzungen zeigten. In der Mitte lag mehr unverändertes Fibrin als in der 2. Gruppe (Abb. 5).

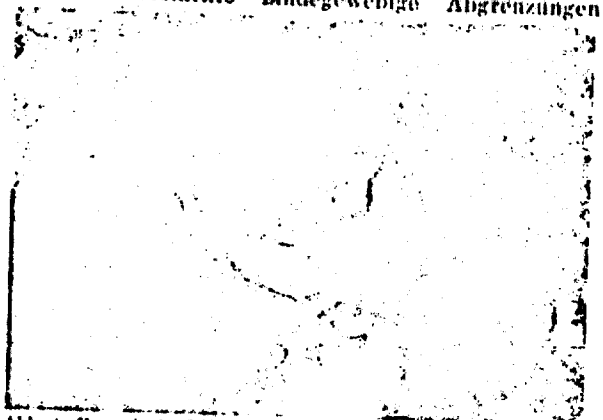

Abb. 4: Versuchstier VII a 17/37. Fibrin-Implantat nach 20tägiger Einwirkung von insgesamt 5 cm³ ME. Färbung: Haematoxylin-Eosin. Vergrößerung: Ölimmersion 1 : 900 4fach linear.

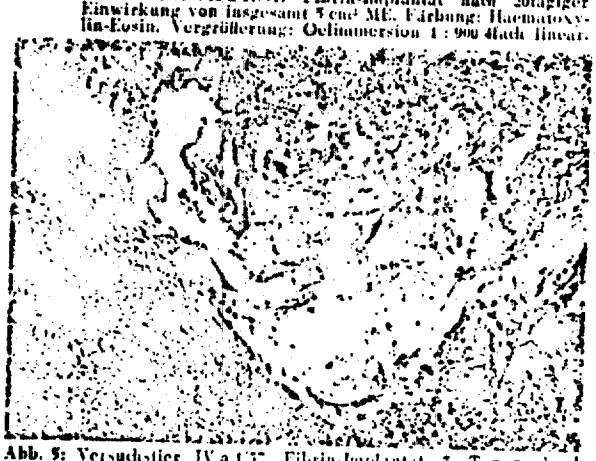

Abb. 5: Versuchstier IV a 1/37. Fibrin-Implantat. 3. Tag p. impl. 3 Tage vor Fibrin-Implantation 5 × 0,25 cm³ ME. Färbung: Haematoxylin-Eosin. Vergrößerung: 1 : 90 4fach linear.

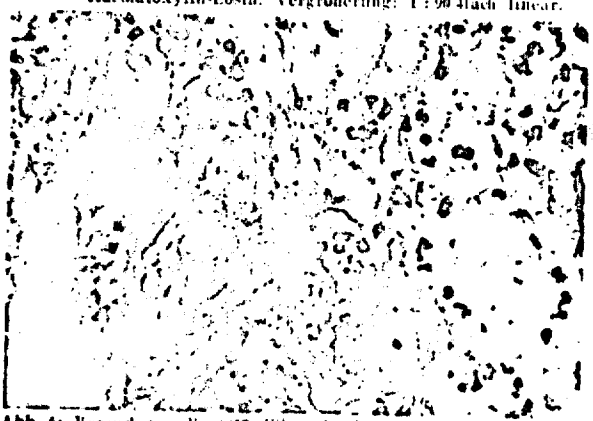

Abb. 6: Versuchstier V a 8/37. Fibrin-Implantat 3 Tage p. impl. Vor Fibrin-Implantation 5 × 0,25 cm³ ME. Färbung: van Gieson. Vergrößerung: 1 : 500 4fach linear.

Umgeben wurde das Implantat von einer Zone netzartig ausgeprägter, zugleich auch in der Struktur veränderter restierender Fibrin-Partien, in und neben der viele, z. Teil überalterte leukocytäre Zellen lagen. Neben den Leukocyten fielen Erythrocyten auf, sowie große, blaßblau gefärbte Zellen. Anschließend waren zur Peripherie hin mehr Leukocyten und weniger histiocytäre Zellelemente, Klasmatocyten und Fibrocyten, sowie überalterte hypersegmentierte Leukocyten und Fibrin-Reste nachweisbar (Abb. 6).

Die Ergebnisse dieser Arbeit lassen eine hemmende Wirkung des ME erkennen, wenn dieser auf ein durch Fibrin-Implantation bereits hervorgerufenes Granulationsgewebe trifft. An sich hätte man nach den in unserer ersten Arbeit mitgeteilten Ergebnissen einen Summationseffekt erwarten dürfen: einmal durch die Fibrin-Implantation, die zu einer Zellproliferation führt und dann durch den ME, der in einem Wundgebiet, das nicht durch Fibrin-Implantation verändert ist, ebenfalls eine Zellproliferation auslöst. Demnach kommt es durch Applikation des ME offenbar bei zellarmen Geweben zu einer Stimulierung, bei zellreichen Geweben eher zu einer Hemmung der Zellproliferation. Die Veränderungen des Gewebes verlaufen bei Fibrin-Implantation unter Einwirkung des ME zum Teil anders, als bei alleiniger Fibrin-Implantation. Um das eingelagerte Fibrin bildet sich eine Abgrenzungsschicht aus bindegewebigen Zellen und extrazellulärem Hyalin. Das abgegrenzte Fibrin bleibt fast unverändert liegen. Der in unserer ersten Arbeit beschriebene fermentative Abbau des Fibrins durch die proteolytische Tätigkeit der Leukocyten tritt hier an Bedeutung zurück. Eher werden die oben beschriebenen Zellen mit Einschlüssen im Protoplasma erkennbar, die von der Peripherie zum Zentrum des Implantates vordringen. Über die zur Klärung dieser Vorgänge durchgeführten histiochemischen Untersuchungen werden wir später berichten.

**Zusammenfassung:**

Nach Feststellung des stimulierenden Einflusses eines aus aktivierten Kälbermilzen gewonnenen Extraktes auf zellarmes mesenchymales Gewebe von Meerschweinchen wurde der Einfluß dieses Extraktes auf das durch Implantationen von Fibrin hervorgerufene junge, zellreiche Bindegewebe untersucht. Dabei ergab sich, daß der Milz-Extrakt die durch Fibrin-Implantation angeregte Bildung von Bindegewebe hemmt. Dieser Befund führt zu der Annahme, daß der Milz-Extrakt an zellarmen Geweben eine Stimulierung, an zellreichen Geweben dagegen eine Hemmung der Zellproliferation hervorruft.

**Summary:**

*Experimental Studies on the Effect of Spleen Extract on the Formation of New Connective Tissue induced by Fibrin Implantation*

Extracts obtained from activated calves' spleens show stimulating influence on mesenchymatous tissues with few cells of guinea-pigs. Subsequently the influence of the a. m. extract on young cellular connective tissue produced under the influence of fibrin implantations was investigated. The extract was found to inhibit the formation of connective tissue induced by fibrin implantations. These findings indicate that the spleen extract causes stimulation in tissues devoid of cells and inhibition of cellular proliferation in cellular tissues.

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# Percutane Resorption von ätherischen Ölen und ihren Inhaltsstoffen

Von Fr. Meyer und E. Meyer

Die Ansicht, daß ätherische Öle percutan aufgenommen werden können, ist sehr verbreitet (v. Czetsch-Lindenwald u. Schmidt-La Baume 1930, 1936). Für eine Reihe dieser Stoffe kann die Möglichkeit der percutanen Resorption auf Grund der vorliegenden Literatur auch als erwiesen betrachtet werden; für viele andere dagegen fehlen bisher exakte experimentelle Unterlagen.

Pfaffrath konnte 1931 zeigen, daß Essenzen von Thymian, Eucalyptus und Terpentin nach percutaner Applikation in der Ausatmungsluft und in geringem Maße auch im Urin nachweisbar sind. Moncorps, Schmidt u. Tholey (1937) konnten Guajacol nach äußerlicher Anwendung in Form einer Salbe im Harn nach-

weisen. Macht (1938) fand, daß ätherische Öle aus Zimtrinde, Fenchel, Betula, Zitrone, Orange, Anis, Pfefferminz, Thymian, *Rosa geranium*, *Caryophyllus aromaticus*, *Gaultheria procumbens* u. a. nach percutaner Einwirkung von 1 cm³ Bewußtlosigkeit und tödliche Vergiftungen bei Mäusen hervorrufen können. Einige von ihnen, z. B. Anethol, verwendete er mit Erfolg als Trägersubstanzen. Verschiedene Alkaloide, die als solche oder in Baumwollsamen- und Olivenöl percutan nicht aufgenommen werden (u. a. Morphin, Strychnin, Aconitin, Atropin, Eserin und Curare) konnte er auf diese Weise zur Wirkung bringen. Bürgi (1942) untersuchte zusammen mit Stähli ebenfalls die Resorption ätherischer Öle und fand, daß Campher, Öl. *Eucalypti*, *Thymi*, *Citri*, *Terebinthinae*, *Rosmarini*, *Bergamottae*, *Pini*, *Juniperi* und *Lavendulae* nach äußerlicher Anwendung mit Hilfe des Bürgi'schen Apparats in der Ausatmungsluft von Kaninchen nachweisbar sind. Im Gegensatz zu den Untersuchungen von Macht (1938) waren in denen von Bürgi (1942) orale und pulmonale Aufnahme sicher ausgeschlossen, die behandelte Hautpartie war begrenzt und die resorbierende Oberfläche damit konstant. In neuerer Zeit stellten Valette u. Cavier (1945 a, b) Untersuchungen über die Resorption ätherischer Öle an und fanden besonders günstige Werte für α-Pinen und Eucalyptol. Da Eucalyptol weniger flüchtig ist als α-Pinen, wurde es als Vehikel besonders empfohlen. Die genannten Autoren konnten z. B. Dihydrofollikulin (1946 a), Testosteron (1946 b), Desoxycorticosteron (1947 a), synthetische Oestrogene (1948 a) und Progesteron (1948 b) in einer Mischung von Äthanol und Eucalyptol in verhältnismäßig großer Menge percutan zur Aufnahme bringen.

In Ergänzung der Befunde von Valette und seiner Schule haben wir die uns zugänglichen ätherischen Öle und ihre Inhaltsstoffe in unsere vergleichenden Untersuchungen über die Permeabilität der Haut einbezogen und erwartungsgemäß große Unterschiede in der Resorptionsgeschwindigkeit gefunden.

Ein Teil der untersuchten ätherischen Öle entstammte einer mehr als 10 Jahre alten Sammlung unseres Instituts. Es ist daher möglich, daß z. B. infolge Polymerisation die Viscosität einiger Stoffe gegenüber frischer Ware gestiegen und die Resorptionsgeschwindigkeit daher zu klein gefunden wurde.

## Methodik

Wird eine Flüssigkeit von der Haut resorbiert, so besitzt sie auch die Funktion einer Trägersubstanz. Eine inkorporierte oder eine darin gelöste Verbindung gelangt mit ihr zusammen zur Aufnahme und kann so die erfolgte Resorption anzeigen. Als solcher Indikator ist Eserin besonders gut geeignet (Vogel, R., 1899; Valette, G. et Cavier, R., 1936), weil es charakteristische, leicht registrierbare Wirkungen auf die quergestreifte Muskulatur besitzt. Die Latenz vom Aufbringen auf die Haut bis zum Eintritt der Eserin-Wirkung auf die periodisch gereizte Kaumuskulatur von Mäusen wurde als Maß für die Resorptionsgeschwindigkeit verwendet. Kontakt- bzw. resorbierende Oberfläche waren 2.3 cm² rasierter Bauchhaut von 230 männlichen Tieren. Die Eserin-Konzentration betrug 0.27%, bezogen auf die Base. (Näheres zur Methode vgl. Meyer u. Kerk, 1959).

## Ergebnisse

In Tab. 1 sind einige Ergebnisse mit aliphatischen ätherischen Ölen und ihren Bestandteilen dargestellt. In dieser ersten, chemisch nahe verwandten Gruppe von Verbindungen mit 10—14 C-Atomen wurde Geranylformiat (34 min) am schnellsten aufgenommen. Es folgten Geranylpropionat (38 min), Linalylacetat (52 min), Geranylbutyrat (53 min), Geranylacetat (58 min) und Citral (63 min). Geraniol und Linalool wurden binnen 2 h nicht in erkennbarer Menge resorbiert. Allerdings fehlte nach s.c. Injektion von 10γ Eserin, gelöst in Geraniol, ein Anstieg der Hubhöhe, so daß eine Inaktivierung des Eserins (Inkompatibilität) oder eine antagonistische Beeinflussung des Eserin-Effektes nicht sicher ausgeschlossen werden konnten. Beim Linalool schien sich die tertiäre OH-Gruppe ungünstig auf die Resorption auszuwirken.

Tab. 1

| Trägersubstanz für Eserin (0,27%ige Lösung) | min bis zum Anstieg der Hubhöhe (arithm. Mittel) | Zahl der ausgewerteten Versuche |
|---|---|---|
| Citral | 63 | 5 |
| Geraniol | negativ | 4 |
| Geranylacetat, frisch | 58 | 5 |
| Geranylacetat, dickflüssig | negativ | 6 |
| Geranylformiat | 34 | 6 |
| Geranylpropionat | 38 | 5 |
| Geranylbutyrat | 53 | 5 |
| Linalool | negativ | 5 |
| Linalylacetat | 52 | 7 |
| Citronellal, dickflüssig | negativ | 6 |
| Öl. *Rutae* | 27 | 6 |
| Valeriansäurediaethylamid | negativ | 3 |
| Menthylvalerianat | 43 | 3 |

Daß die Viskosität für die percutane Resorption eine große Rolle spielt, geht daraus hervor, daß wir ein altes, dickflüssiges Geranylacetat mit negativem Erfolg prüften, während eine frische, dünnflüssige Charge bereits nach 58 min in erkennbarer Menge resorbiert war. Auch der Versuch mit dickflüssigem Citronellal verlief negativ.

Oleum *Rutae*, dessen Hauptbestandteil Methylnonylketon ist und das somit in die aliphatische Reihe gehört, wird sehr gut resorbiert. Unter den geschilderten Versuchsbedingungen fanden wir einen Mittelwert von 27 min. Während Valeriansäurediaethylamid in der auf 2 h begrenzten Versuchsdauer nicht in erkennbarer Menge zur Resorption (3 Tiere) gelangte, wurde Menthylvalerianat offenbar trotz seiner großen Kettenlänge relativ schnell aufgenommen.

In Tab. 2 ist das Ergebnis der Untersuchung von alicyclischen Verbindungen, Terpenen und solchen ätherischen Ölen zusammengefaßt, deren Hauptbestandteil sich in diese Gruppen einordnen läßt. Mit Ausnahme von zwei Stoffen, dem festen Terpinhydrat und einer alten, dickflüssigen Qualität des Terpineols (Terpinolen) kamen alle Verbindungen dieser Gruppen zur Aufnahme, z. T. schon nach erstaunlich kurzer Zeit.

Tab. 2

| Trägersubstanz für Eserin (0,27%ige Lösung) | min bis zum Anstieg der Hubhöhe (arithm. Mittel) | Zahl der ausgewerteten Versuche |
|---|---|---|
| Terpinhydrat 5% in Cyclohexan | 16 | 6 |
| Terpinhydrat 10% in Propanol | negativ | 6 |
| Terpineol*) | 33 | 5 |
| Terpineol, dickflüssig | negativ | 7 |
| Terpinylacetat | 50 | 6 |
| Limonen | 43 | 6 |
| Carvon | 33 | 5 |
| Thymen = 1-Pinen | 22 | 6 |
| Fenchon | 45 | 6 |
| Fenchylacetat | 54 | 5 |
| Bornylacetat | 65 | 6 |
| Ol. *camphoricum* | 39 | 6 |
| Ol. *Pini sibiric.* | 53 | 5 |
| Ol. *Terebinthinae* | 62 | 5 |
| Ol. *Tanaceti* | 38 | 5 |
| Ol. *Sabinae* | 48 | 5 |
| Ol. *Mentae pulег.* | 29 | 6 |
| Ol. *Eucalypti* | 31 | 5 |
| Ol. *Menthae pip.* | 38 | 5 |

*) frisches Handelspräparat.

Die Resorption einer 5%igen Terpinhydrat-Lösung in Cyclohexan war 16 min nach äußerlicher Applikation erkennbar. Sie erfolgte praktisch mit der gleichen Geschwindigkeit wie Cyclohexan allein (vgl. Meyer, Meyer u. Kerk, 1959). Eine 10%ige Lösung des genannten Stoffes in Propanol dagegen kam nicht zur Aufnahme. Sein Zusatz beschleunigt die Resorption von Propanol (vgl. Meyer u. Kerk, 1959) somit nicht. Terpinhydrat verhält sich hiernach in bezug auf die percutane Resorption ziemlich indifferent.

Terpineol kam trotz seiner tertiären OH-Gruppe verhältnismäßig schnell zur Aufnahme (33 min). Ihre Veresterung mit Essigsäure verlangsamte die Resorption statt sie zu beschleunigen, wie nach den geschilderten, mit Linalool und Linalylacetat erhobenen Befunden (vgl. Tab. 1) vielleicht zu erwarten gewesen wäre. Der für Terpinylacetat errechnete Mittelwert betrug 50 min.

Limonen (43 min) und das in Kümmel- und Dill-Öl reichlich vorkommende Carvon (33 min) wurden dagegen ziemlich schnell resorbiert.

Bei den Terpenen und den terpenhaltigen ätherischen Ölen war eine Abhängigkeit der percutanen Resorption von der chemischen Konstitution nicht zu erkennen.

Thymen = 1-Pinen wurde in dieser Reihe besonders gut resorbiert (22 min). Es folgten Fenchon (45 min), Fenchylacetat (54 min) und Bornylacetat (65 min). Trotz naher chemischer Verwandtschaft wurden Bornylacetat und Campher (leichtes Campher-Öl) mit

sehr unterschiedlicher Geschwindigkeit aufgenommen (39 und 65 min).

Ol. *Pini sibirici*, das vorwiegend Bornylacetat, l-Pinen und Santen enthält, sowie Ol. *Terebinthinae* mit verhältnismäßig hohem α-Pinengehalt kamen nur langsam zur Resorption. Die Mittelwerte von je 5 Versuchen betrugen 55 und 62 min.

Für Ol. *Tanaceti* mit seinem hohen Thujon-Gehalt und das diesem chemisch sehr ähnliche Verbindungen (Sabinol und Sabinolacetat) enthaltende Ol. *Sabinae* fanden wir etwas günstigere Werte: 38 und 48 min.

Die schnelle Resorbierbarkeit des Ol. *Menthae pulegiae* (29 min) wird auf das zu 80% darin enthaltene Pulegon zurückzuführen sein. Das außerdem im Puleyöl enthaltene Menthon und Menthol, sowie l-Limonen und Dipenten sind ebenfalls percutan resorbierbar. Ihr verhältnismäßig geringer Anteil indessen spricht dafür, daß sie von untergeordneter Bedeutung sind. Ol. *Eucalypti* enthält neben Eucalypten (α-Pinen), Pinocarveol, Butter-, Valerian- und Capronsäurealdehyd vorwiegend (ca. 75%) Eucalyptol, dessen schnelle cutane Aufnahme Valette u. Cavier (1945) vor uns gefunden hatten. (Über die lokale Verträglichkeit vgl. Oettel, 1936).

Die percutane Resorbierbarkeit von Ol. *Menthae piperitae*, das neben Menthon, verschiedenen Menthonestern und Terpenen 30—50% Menthol enthält, ist mit Ol. *Pini sibirici* oder Ol. *Terebinthinae* vergleichbar.

Wie aus Tab. 3 hervorgeht, in der aromatische Inhaltsstoffe von ätherischen Ölen zusammengefaßt sind, werden Carvacrol, Anethol, Ol. *Anisi*, Eugenol und Iso-Eugenol nicht oder nur sehr langsam von der Haut resorbiert. Für Safrol war die percutane Aufnahme dagegen binnen 2 h eindeutig nachweisbar (Mittelwert 72 min; über die verschiedene lokale Verträglichkeit vgl. Oettel, 1936). Erstaunlich ist die geringe Latenzzeit für Cumin-Öl (28 min), das aus p-Cymol, wenig α- und β-Pinen, Dipenten, β-Phellandren, viel Cuminaldehyd und Cuminalkohol besteht. Da Cuminalkohol und Cuminaldehyd (Mittelwerte von 87 und 47 min) nicht besonders schnell aufgenommen werden, scheint p-Cymol für die Resorbierbarkeit des Cuminöls in erster Linie verantwortlich zu sein. Ol. *Petroselini* (68 min) und Ol. *Thymi* (82 min) wurden nur verhältnismäßig langsam, Cinnamein und der sehr viscöse Zimtaldehyd nicht in erkennbarer Menge resorbiert.

Tab. 3

| Trägersubstanz für Eserin (0,25%ige Lösung) | min bis zum Anstieg der Hubhöhe (arithm. Mittel) | Zahl der ausgewerteten Versuche |
|---|---|---|
| Carvacrol | negativ | 6 |
| Anethol | " | 6 |
| Ol. *Anisi* | " | 5 |
| Eugenol | " | 6 |
| Iso-Eugenol | " | 6 |
| Safrol | 72 | 6 |
| Cuminöl | 28 | 5 |
| Cuminalkohol | 87 | 5 |
| Cuminaldehyd | 47 | 6 |
| Ol. *Thymi* | 82 | 5 |
| Ol. *Petroselini* | 68 | 6 |
| Cinnamal = Zimtaldehyd | negativ | 3 |
| Cinnamein = Zimtsäurebenzyläther | " | 5 |

Einige ätherische Öle, die sich wegen ihrer Inhaltsstoffe den bisher geschilderten Gruppen nicht ohne weiteres zuordnen lassen, sind in Tab. 4 zusammengestellt.

Außer Ol. *Galangae* und Pfefferöl werden sie sehr viel langsamer als die in Tab. 1—3 genannten Öle resorbiert. Die ermittelten Werte liegen bei 1 h (Ol. *Juniperi* 59 min) oder weit darüber (Ol. Balsam. *Copaiv.* 92 min). Für Ol. *Calami*, *Fagi äther.*, *Pimentae*, *Origan. cretic.* und *Spicae* sowie für Patchuli-, Tolubalsam- und Ajowan-Öl ist bei einer Beobachtungsdauer von 2 h eine Resorption nicht erkennbar.

Zur Prüfung der percutanen Toxizität wurden die relativ gut resorbierbaren Verbindungen aus den Tab. 1 bis 4 unter den gleichen Bedingungen — jedoch ohne Eserin-Zusatz — 4 h lang auf die Haut von je 2 Mäusen appliziert. Das Ergebnis dieser orientierenden Kontrolluntersuchungen ist in Tab. 5 zusammengefaßt.

Tab. 4

| Trägersubstanz für Eserin (0,25%ige Lösung) | min bis zum Anstieg der Hubhöhe (arithm. Mittel) | Zahl der ausgewerteten Versuche |
|---|---|---|
| Ol. *Calami* | negativ | 5 |
| Pfefferöl | 38 | 5 |
| Ol. *Galangae* | 33 | 6 |
| Patchuliöl | negativ | 4 |
| Ol. *Fagi äther.* | " | 6 |
| Tolubalsam-Öl | " | 4 |
| Ol. *Copaivae* | 92 | 5 |
| Ol. *Pimentae* | negativ | 6 |
| Ol. *Origani cretici* | " | [illegible] |
| Ajowan-Öl | " | [illegible] |
| Ol. *Spicae* | " | [illegible] |
| Ol. *Juniperi* | 59 | [illegible] |

Tab. 5

| Trägersubstanz | Körpergewicht in g | | Exitus letalis nach h | | Befund nach 4 h | |
|---|---|---|---|---|---|---|
| Citral | 15 | 16 | 1/2 | 1 1/2 | — | — |
| Geranylacetat | 16 | 16 | 4 | — | — | o. B. |
| Geranylformiat | 13 | 19 | — | — | o. B. | o. B. |
| Geranylpropionat | 19 | 18 | — | 4 | o. B. | — |
| Geranylbutyrat | 26 | 19 | — | — | o. B. | o. B. |
| Linalylacetat | 15 | 17 | — | 2 | o. B. | — |
| Ol. *Rutae* | 25 | 25 | 4 | — | — | o. B. |
| Menthylvalerianat | 18 | 26 | 3 | — | — | o. B. |
| Terpineol | 12 | 18 | 2 1/2 | — | — | o. B. |
| Terpinylacetat | 16 | 19 | — | — | o. B. | o. B. |
| Limonen | 20 | 14 | — | — | o. B. | o. B. |
| Carvon | 18 | 21 | — | — | o. B. | o. B. |
| Thymen | 21 | 17 | — | 3 1/2 | o. B. | — |
| Fenchon | 18 | 19 | — | — | o. B. | o. B. |
| Fenchylacetat | 18 | 24 | — | — | o. B. | o. B. |
| Bornylacetat | 22 | 20 | — | — | o. B. | o. B. |
| Ol. *camphoricum* | 21 | 26 | — | — | o. B. | o. B. |
| Ol. *Menthae pulegiae* | 17 | 16 | — | — | o. B. | o. B. |
| Ol. *Tanaceti* | 16 | 17 | — | 4 | o. B. | — |
| Ol. *Sabinae* | 17 | 19 | — | — | o. B. | o. B. |
| Ol. *Eucalypti* | 19 | 16 | — | 2 | o. B. | — |
| Ol. *Pini sibirici* | 17 | 11 | — | — | o. B. | o. B. |
| Ol. *Terebinthinae* | 18 | 23 | — | 1 1/2 | o. B. | — |
| Safrol | 24 | 19 | 3 | — | — | o. B. |
| Cuminöl | 21 | 13 | 3 | — | — | o. B. |
| Cuminalkohol | 23 | 19 | — | 4 | o. B. | — |
| Cuminaldehyd | 20 | 20 | — | — | o. B. | o. B. |
| Ol. *Petroselini* | 24 | 16 | — | — | o. B. | o. B. |
| Pfefferöl | 13 | 19 | — | — | o. B. | o. B. |
| Ol. *Galangae* | 26 | 22 | — | 2 | o. B. | — |

Hiernach sind ätherische Öle im Kontakt mit der äußeren Haut nicht indifferent. Eine Abhängigkeit der percutanen Toxizität von der Resorptionsgeschwindigkeit ließ sich jedoch nicht feststellen.

## Zusammenfassung

Ätherische Öle und ihre Inhaltsstoffe werden von der Haut mit sehr unterschiedlicher Geschwindigkeit resorbiert. Eine vergleichende Untersuchung der Resorptionsgeschwindigkeit an der unverletzten, rasierten Bauchhaut von Mäusen ergibt Werte von 0 bzw. $< 0{,}5$ mm³ bis zu 2—4 mm³/cm²·h. Verhältnismäßig schnell werden Geranylformiat, Geranylpropionat, Terpineol, Carvon und Thymen sowie Rauten-Öl, Poley-Öl, Eucalyptus-Öl, Cumin-Öl und Ol. *Galangae* aufgenommen.

Für 22 der insgesamt 36 untersuchten Stoffe ist innerhalb der auf 2 h limitierten Beobachtungsdauer eine Aufnahme nicht erkennbar, u. a. für Geraniol, Linalool, Carvacrol, Anethol, Eugenol, Iso-Eugenol, Cinnamal und Cinnamein, für Ol. *Anisi*, Ol. *Calami*, Ol. *Fagi* äther., Ol. *Pimentae* und Ol. *Spicae*.

Ätherische Öle und ihre Inhaltsstoffe werden somit von der Haut weder schlechthin und ohne Ausnahme noch im Vergleich zu anderen Stoffen besonders bevorzugt aufgenommen.

Bei großen Kontaktflächen ist eine resorptive Schädigung nicht ausgeschlossen.

**Summary**

***Percutaneous Absorption of Ethereal Oils and their Ingredients***

Ethereal oils and their ingredients are absorbed by the skin with varying velocity. Comparative investigations of the absorption velocity by the intact, shaved abdominal skin in mice showed values between O or <0.5 mm³ and 2—4 mm³/cm² per hour. The absorption was found to be comparatively rapid in geranylformiate, geranylpropionate, terpineol, carvon, thymene, ol. *rutae* poley oil, eucalyptus oil, cumin oil, and ol. *galangae*. In 22 out of 56 substances tested, no absorption was found within the period of observation, which was limited to 2 hrs. Among these were geraniol, linalool, carvacrol, anethol, eugenol, iso-eugenol, cinnemal, cinnamein, ol. *anisi*, ol. *calami*, ol. *fagi* ether, ol. *pimentae*, and ol. *spicae*. Ethereal oils and their ingredients are not absorbed by the skin without exception, neither is the absorption especially favoured, as compared with other solvents. Damage through absorption may occur if the contact areas are large.

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# Zur antibakteriellen Wirkung ätherischer Öle

Von M. Kienholz

Die medizinische Anwendung ätherischer Öle ist vielfältig. Sie erstreckt sich auf ihre Verwendung als Analgetica, Antiphlogistica, Phlogistica, Sedativa, Excitantien, Expectorantien, Carminativa usw.

Die ätherischen Öle werden durch Wasserdampfdestillation aus Blättern, Blüten und Früchten der verschiedensten Pflanzen gewonnen. Die Zusammenfassung in eine Gruppe verdanken sie ihrer Funktion als Geruchsstoffe von Pflanzen, einigen gemeinsamen physikalischen Eigenschaften und der Art ihrer Gewinnung, denn chemisch handelt es sich um ganz verschiedene Substanzen. Viele aus Pflanzen gewonnene ätherische Öle sind aus Einzelkomponenten zusammengesetzt, doch häufig ist die empirisch gefundene Wirkung eines durch Destillation gewonnenen Öles nur einem Teil der Komponenten zuzuschreiben. Diese Erkenntnis führte durch Eliminierung nicht wirksamer Bestandteile zur Herstellung wirkungsvollerer Präparate. Auch versuchte man, alle zur erfolgreichen Bekämpfung einzelner Krankheiten erwünschten Wirkungen durch Mischung ätherischer Öle verschiedener Eigenschaften in einem Präparat zu vereinen.

Ein solches aus einzelnen ätherischen Ölen zusammengesetztes Präparat ist das Baokang-Öl*). Seine gute Wirkung bei Erkältungskrankheiten veranlaßte uns, die antibakteriellen Eigenschaften dieses Gemisches verschiedener ätherischer Öle zu prüfen.

Im Baokang-Öl sind folgende Substanzen enthalten: Ol. *Menthae piperitae*, Ol. *Eucalypti*, Ol. *Macidis*, Ol. *Foeniculi*, Ol. *Coriandri*, Ol. *Melissae indicum*, Ol. *Cupressi*, Ol. *Juniperi*, Ol. *Majorani*, Ol. *Rosmarini*, Ol. *Thymi*, *Zingiberis*, d-Campher und Aromatien.

Es wird empfohlen, das Baokang-Öl bei Erkältungskrankheiten entweder auf Würfelzucker getropft langsam im Munde zergehen zu lassen oder zum Inhalieren einige Tropfen davon auf ein Taschentuch zu bringen.

Entsprechend dieser Applikationsweisen führten wir Versuche durch, bei denen das Öl im flüssigen und im gasförmigen Zustand mit Bakterien in Berührung gebracht wurde.

Die antibakterielle Wirkung prüften wir gegen *Coli-*, *Lactis aerogenes-*, *Proteus-* und *Pyocyaneus*-Bakterien, pathogene und apathogene Staphylokokken, haemolysierende und nicht haemolysierende Streptokokken, Hefe, Heubazillen und Diphtheriebakterien. Von den Bakterienarten wurden mehrere Stämme verschiedener Provenienz untersucht.

*) Das Baokang Öl wurde uns freundlicherweise von der Frenon GmbH, Werne a. d. Lippe, zur Verfügung gestellt.

## Keimhemmende Wirkung

Die keimhemmende Wirkung des Baokang-Öls wurde wie folgt geprüft:

### Methodik

Mit Traubenzuckerbouillon stellten wir eine geometrisch abfallende Verdünnungsreihe her, so daß jedes folgende Röhrchen jeweils nur die Hälfte der Öl-Konzentration des vorangegangenen enthielt. Im allgemeinen begannen wir mit einer Konzentration von 10 000 γ Öl/cm³ Nährflüssigkeit. Das Baokang-Öl ließ sich in Traubenzuckerbouillon leicht suspendieren. Jedes Röhrchen enthielt 5,0 cm³ einer entsprechenden Öl-Verdünnung. Die eingeimpfte Keimzahl wurde nephelometrisch bestimmt, um bei Wiederholungsversuchen vergleichbare Werte zu haben. Die mit den verschiedenen Bakterienarten beimpften Öl-Verdünnungsreihen wurden bei 37° C 24 h bebrütet. Nach dieser Zeit lasen wir diejenige Verdünnung ab, welche die Keime in ihrem Wachstum total zu hemmen vermochte und bestimmten die Trübungswerte in den übrigen Verdünnungsröhrchen.

### Ergebnisse (Tab. 1)

Tab. 1: Die bakteriostatische Wirkung von Baokang-Öl

| Bakterienart | Ölmenge in γ/cm³ Nährflüssigkeit |
|---|---|
| *Coli*-Bakterien | 2500 |
| *Proteus*-Bakterien | 1250 |
| Staphylokokken | 625 |
| Streptokokken | 625 |
| Hefe | 625 |
| Heubazillen | 625 |
| Diphtheriebakterien | 1250 |

Von den geprüften gramnegativen Keimen wurden *Lactis aerogenes*- und *Pyocyaneus*-Bakterien selbst durch 10000 γ Öl/cm³ Nährflüssigkeit in ihrem Wachstum nicht beeinträchtigt. *Coli*-Bakterien hingegen zeigten bei einer Konzentration von 2500 γ Öl/cm³ Nährflüssigkeit kein Wachstum. Grampositive Bakterien reagierten empfindlicher auf das Baokang-Öl. So wurden pathogene und apathogene Staphylokokken, sowie haemolysierende und nicht haemolysierende Streptokokken bereits durch eine Konzentration von 625 γ Öl/cm³ Nährflüssigkeit in ihrem Wachstum gehemmt. Die gleiche Konzentration vermochte auch das Wachstum der Hefe und Heubazillen zu verhindern, während Diphtheriebakterien bei 1250 γ Öl/cm³ Nährflüssigkeit nicht wachsen konnten. Die Bestimmung der Trübung ergab, daß in den Röhrchen, welche die Hälfte bis 1/32 der das Wachstum total hemmenden Öl-Konzentration enthielten, die Werte unterhalb derer des Kontrollröhrchens lagen. In den Röhrchen mit noch niedrigeren Öl-Konzentrationen konnte nach 24 h Bebrütung im Vergleich zur Kontrolle kein Unterschied im Wachstum festgestellt werden.

Durch Messung der Trübungswerte in bestimmten Zeitabständen vom Beginn der Bebrütung ab, konnte der Einfluß der verschiedenen Öl-Konzentrationen auf das Wachstum der Bakterien gut veranschaulicht werden. Dabei zeigte sich, daß mit steigender Öl-Konzentration

# VAPOR PHASE CHROMATOGRAPHY IN THE IDENTIFICATION OF CERTAIN ESSENTIAL OILS IN BIOLOGIC MATERIALS

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Among the volatile products of a complex chemical nature comprised under the generic denomination of essential oils there are some which are of interest in toxicology because of their pharmacological activity; the oils of Sabina, Tuja and Ruta are among these, in that they are used as abortives. Their search in biological matter constitutes, still today, a particularly difficult undertaking: the variety of their constitution and the lack of an up to date method are the most obvious reasons (1,2,3,4).

Gas chromatography, which has opened new horizons and new possibilities to the study of mixtures of volatile substances, can, when suitably adapted, be of valuable assistance in the solution of the problem. As is known, various procedures are used for the detection of the separate components by this modern technique; the most widespread method today is based on the measurement of thermal conductivity of the transporting gas, but one may also resort to light absorption, to interferometry, to the measurement of the surface potential, to mass spectrometry or to spectrophotometry of the $CO_2$ of combustion of the component vapors (5,6,7). Whatever method is used, it seems evident that the mere determination of the coefficient of distribution by measuring the time of retention does not constitute -especially in biological matter- a reliable criterion because the case is not infrequent of different compounds having the same retention time on different columns. A method which has the advantage of combining speed of execution with accuracy of results is that which uses the recognition of the functional groups present in the separate molecules, by means of suitably chosen reagents. The eluated fractions can be collected either with the apparatus proposed by Walsh and Merritt (8) or more simply with a 5-6 mm diameter glass tube connected to the outlet hole of the transport gas, ground at the end to receive a hypodermic

needle with which the fraction is bubbled in a small microcone 3.5 cm high x 1 cm diameter. Even taking account of the influence of the various genetic and environmental factors on the composition of the essential oils of many plants (9) it can be assumed that most of these can be identified on the basis of the simultaneous interpretation of a certain chromatographic pattern and of the presence of certain compounds contained in a high percentual quantity.

In the particular case of the essential oil extracted from Juniperus Sabina the two compounds present and to be identified are d-sabinene and sabinile acetate; in the case of Thuja occidentalis, the alpha and the beta tujone; while for Ruta graveolens, the methylnonylketone and the methylheptyl ketone. For each of the above compounds we chose a reaction which provided a sensitivity comprised between 20 and 40 Mg.

Experimental part

From the top of young Juniperus Sabina L. branches we obtained, by distillation in vapor current, an essence which showed the following physical and chemical characteristics under analysis:

| | |
|---|---|
| Specific gravity at 15°C | 0.925 |
| Refractive at 20°C | 1.4790 |
| Saponification number | 125 |

The fractionation by gas chromatography of the volatile components of the essence was done on Celite C 22 column (30-60 Mesh) containing approximately 20% of polyethyleneglycol 400. The apparatus used, Fractovap Model B of the firm C. Erba, was provided with an indicating system consisting of a heat conducting cell and a continuous line electronic recorder. The other experimental characteristics are:

| | |
|---|---|
| Column length | 2 m |
| Temperature of thermostatic chamber | 120 ° |
| Temperature of the evaporator | 200 ° |
| $H_2$ gas transport | $H_2$ |
| Pressure 0.5 kg/cm$^2$ - Flow 6 lit/hr | |
| Paper speed | 1/4" per minute |

From the chromatogram obtained it was possible to observe the presence, in addition to small quantities of limonene and pinene, d-sabinene and sabinyl acetate which were confirmed by collecting the fractions expelled at the time of maximum response of the recorder, by means of an appropriate device, in the following reagents:

1) for d-sabinene: 10 drops of concentrated sulfuric acid plus one drop of 37% formalin; coloration wine-red. Sensitivity 20 µg (Le Rosen's reagent 10).

2) for sabinyl acetate: the eluate fraction is bubbled in 10 drops of phosphoric acid (d. 1.8). Then the acetates are determined in the solution according to the technique suggested by Feigl, with the reagent composed of potassium iodide and iodate (11). Sensitivity: 20 µg.

Our essential oil of Thuja occidentalis L. had the following chemical and physical characteristics:

| | |
|---|---|
| Density at 15° | 0.920 |
| Refractive index at 20° | 1.458 |
| Ether number | 20 |

This was subjected to gas chromatographic analysis under the identical experimental conditions as specified above; alpha and beta-thujone was accomplished by bubbling the fraction in 10 drops of petroleum ether to which were added 2 drops of bromine. The solvent was then evaporated to obtain the corresponding tribromide in the form of a reddish residue (12). Sensitivity: 40 µg.

Ruta graveolens L., a biennial plant of the Rutacea family, contains in its green parts an essential oil composed of more than 90% of a Mixture of methylnonyl ketone and methylheptyl ketone in addition to small quantities of pinene and limonene. Our sample of essence of Ruta graveolens had the following chemical and physical characteristics:

| | |
|---|---|
| Specific gravity at 15° | 0.840 |
| Refractive index at 20° | 1.430 |

Content of ketones calculated as methylnonyl ketone (Hydroxylamine method): 93.10%.

From the chromatogram obtained and shown in Fig. 3 it is easy to observe that the percentages of the respective components remain within the limits mentioned in the latest tests.

The chemical confirmation of the components was performed by collecting the eluated fractions in a suitable reagent consisting of:

1) For methylnonyl ketone: 10 drops of 95% alcohol containing 1 drop of 5% solution of sodium nitroprussiate and 1 drop of 30% aqueous solution of sodium hydrate. After waiting two minutes 1 drop of glacial acetic acid is added: brownish coloration. Sensitivity: 10 µg (13).

2) For methylheptyl ketone: 10 drops of dinitrophenylhydrazine reagent prepared according to Allen (14). Sensitivity: 20 µg.

Analysis of the essences of Sabina, Ruta and Thuja in biological matter

First the essences are isolated by means of distillations in vapor current in the presence of excess water and in acid medium with tartaric acid. The distillate obtained is then subjected to successive concentration according to the technique proposed by Stewart & Stolman (15) so as to obtain not more than 20-35 cc of solution.

The essence is then extracted treatment with successive portions of ether. The ether solution obtained is concentrated at ambient temperature to a volume of 2-3 cc.

Not more than 30 µl are used each time for the chromatographic detection and subsequent chemical identification.

Fig. 4 presents the chromatogram obtained from a biological sample containing traces of essences of Juniperus Sabina and of Thuja occidentalis.

The results obtained fully confirm the good possibilities offered by gas chromatography for the analysis of volatile substances in biological matter.

(For Figures and Bibliography see original publication)

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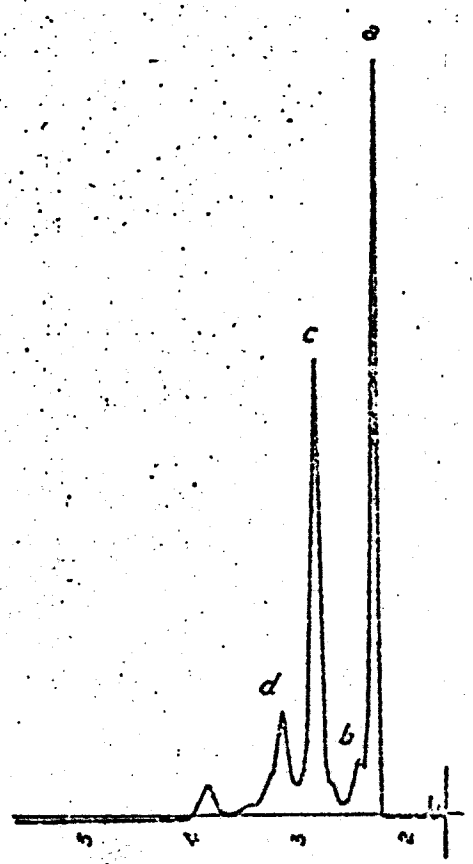


Fig. n. 1

a = *d-sabinene;* b = *α-pinene;* c = *sabinil acetato;* d = *limonene.*

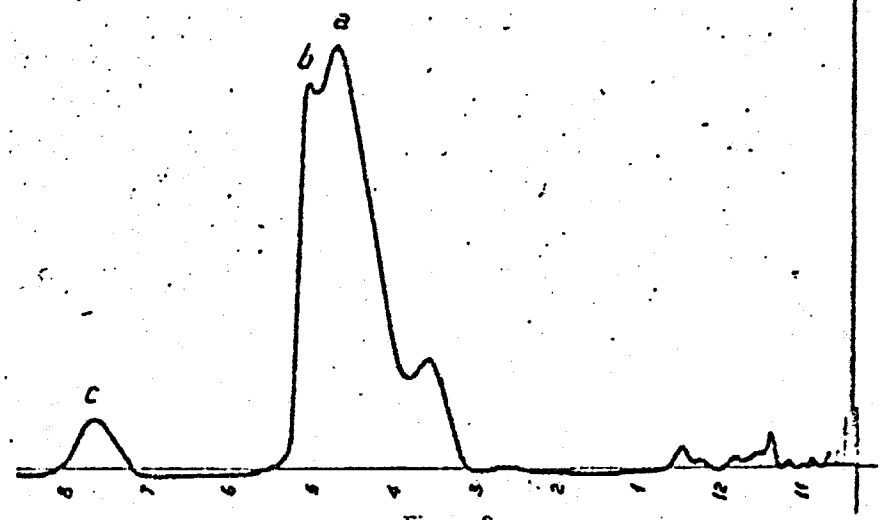


Fig. n. 2

a = *α-tujone;* b = *β-tujone;* c = *borneolo.*

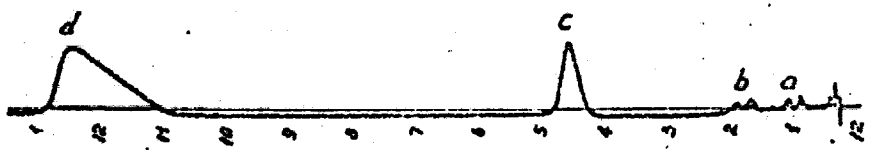


Fig. n. 3

a = *pinene;* b = *limonene;* c = *metileptilchetone;* d = *metilnonilchetone.*

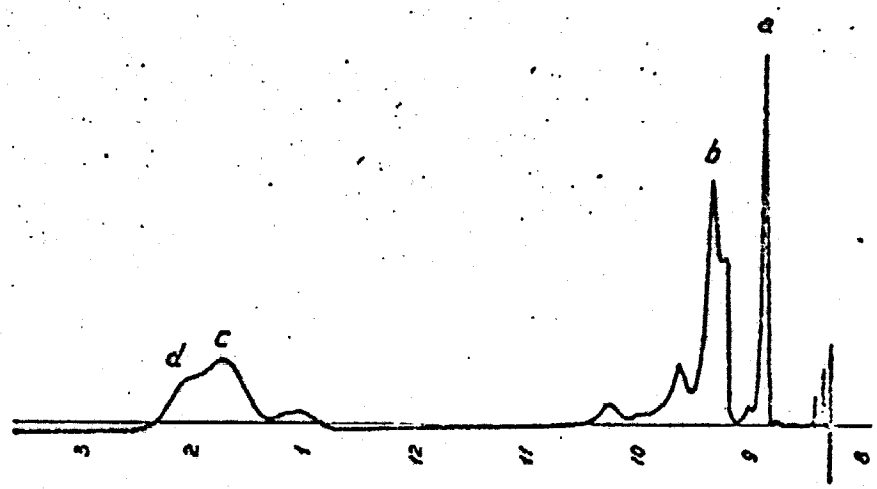


Fig. n. 4

a = *d-sabinene;* b = *sabinil acetato;* c = *α-tujone;* d = *β-tujone.*

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Sont encore à prendre en considération : des lavements au lait légèrement salé, des injections intra-veineuses glucosées hypertoniques (12,5 à 20 p. 100) : 100 à 250 centimètres cubes, puis des toniques cardiaques ainsi que la lobéline (0 gr. 01). En cas de menace d'asphyxie (par tuméfaction) : trachéotomie.

Telles seraient les lignes de conduite à envisager au cours du traitement d'une intoxication par le permanganate, sur lequel des précisions manquaient jusqu'ici.

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## DE LA RUE COMME ABORTIF ET POISON

PAR

Madame le Dr Marie PAPAVASSILIOU et le Dr C. ELIAKIS.

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### I

*Ruta graveolens L.* de la famille des rutacées est une plante très répandue dans tous les Pays méridionaux de l'Europe. En France, elle est connue sous les noms de R. commune, R. des Jardins, Herbe de Grâce, R. fétide ou puante pour son odeur forte et désagréable. Connue depuis la plus haute antiquité, elle est citée d'abord par Homère. Ainsi « Μῶλυ », c'est-à-dire rue sauvage donna Mercure à Ulysse pour le sauver de la puissance magique de Circé. Dioscoride après, donne des renseignements curieux sur les qualités de la rue sauvage et commune, tous les deux médicaments emménagogues, diurétiques efficaces contre l'inflammation pulmonaire, la dyspnée, la goutte et les frissons des fièvres intermittentes. La rue, de plus, est un antidote incomparable des poisons et est encore citée de même par Plutarque, Pline et Galien. Les Arabes ont utilisé

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la rue contre toute maladie et les Chinois recommandent les fleurs de *ruta augustifolia* contre l'épilepsie.

Mais dans toutes les époques jusqu'à nos jours la rue fut principalement un abortif populaire et c'est sous cette qualité que la science du XIXe siècle connut et étudia la plante.

D'après une étude chimique de la rue faite en 1811 par Mähl, elle contient : une substance azotée précipitable par la tannine, acide malique, amidon, gomme, chlorophylle et une essence huileuse jaunâtre d'une odeur aromatique et fétide. Weiss, plus tard, trouva dans les feuilles de la rue un glycoside la rutine inactif, et l'acide rutinique.

Le principe actif est l'huile qui a p.s 0,84, indice de réfraction à 15° 1,4639 et bout à 228° C. Il se compose en majeure partie de méthyl monylcétone ($C_{22}O_{22}O_2$) d'un hydrocarbure de la formule $C_{20}H_{16}$ et d'un isomère de borneol. Il est oxydable par l'acide nitrique en acide acétique et pellargonique. Le chlore attaque vivement l'essence en formant de l'acide chlorydrique. Il forme avec l'iode des solutions gélatineuses et avec le bisulfite de sodium des cristaux. Il réduit rapidement à chaud le nitrate d'argent ammoniacal.

Hamelin, à la suite d'une série d'expériences, a constaté que la rue, par son essence, provoque aux animaux du laboratoire : une irritation du tube digestif surtout vers le pylore et le commencement de l'intestin grêle. La température baisse progressivement jusqu'au collapsus. La respiration et le pouls se ralentissent par la suite. Les phénomènes nerveux se présentent par une narcose et rarement par des convulsions (comme il a été une seule fois constaté chez le lapin). *Mais l'action principale du médicament est l'avortement* provoqué aux animaux pleines, fait pendant la période du collapsus, avec congestion ou anémie des organes génitaux.

Le docteur Hélie aussi qui eut l'occasion d'étudier

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plusieurs cas d'avortement dus à la rue, chez les femmes signale à peu près les mêmes symptômes : congestion de la muqueuse stomacale, vomissements, ralentissement du pouls qui devient petit, refroidissement de la peau, salivation avec gonflement de la langue ou convulsions, paralysie, avortement.

Le mécanisme d'avortement par la rue, est mal connu. S'agit-il d'un phénomène secondaire par suite d'un dérangement général de l'organisme maternel où il se produit grâce à la congestion d'abord du tube digestif et par la suite de la matrice par un mécanisme réflexe?

Hamelin prétend que la plante a une action spécifique sur l'utérus par le système nerveux d'une façon directe ou indirecte. La rue sous forme de poudre de feuilles, d'infusion ou d'essence éthérée, prise à doses fortes et souvent répétées, pour causer l'avortement, provoque une intoxication grave, qui se déclare par des vomissements opiniâtres, d'abord glaireux qui deviennent bilieux, sanguinolents, par la suite des douleurs à l'épigastre, salivation abondante, soif ardente. Délire, somnolence, collapsus. Le pouls devient lent et petit ou fréquent et irrégulier. Douze ou vingt-quatre heures après l'ingestion du médicament les douleurs de la délivrance commencent. Fréquemment, l'issue est fatale pour la femme. L'autopsie ne montre pas des lésions caractéristiques et la recherche de l'essence de rue s'effectue dans le contenu stomacal et intestinal par une distillation à vapeurs d'eau, d'après Dragendorff.

## II

La rue, est un médicament populaire assez répandu en Grèce. Récemment nous avons constaté deux fois l'essence mélangée à l'essence de la sabine dans des viscères des femmes ayant subi l'avortement. Étant donné que depuis trente ans, il nous a été impossible de

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trouver des observations à ce sujet, nous avons jugé nécessaire de nous occuper de quelques points de vue médico-légaux et toxicologiques susceptibles d'intéresser l'expert.

I. L'expert peut-il confirmer la présence de l'essence de rue retirée des viscères et par quels moyens?

II. La présence de la sabine et d'apiol peut-elle influencer la recherche de la rue?

III. L'expert peut-il constater la présence de la rue en faisant ses recherches sur le produit de la conception dans le cas où d'autres pièces à conviction manquent?

Comme il est connu, l'essence de la rue n'a des réactions chimiques spécifiques d'une façon absolue. Il faut alors se servir de moyens plus sûrs, tels que la recherche de l'indice de réfraction. Cette recherche a, de plus, l'avantage de n'avoir besoin que d'une ou deux goutelettes d'essence facile à retirer des viscères, pour la plupart des cas. Nous avons utilisé ce moyen de recherche avec des résultats satisfaisants dans les deux cas d'intoxication que nous décrivons ci-dessous. Dans le premier cas une jeune femme prit une infusion de plantes abortives pour se débarrasser de son enfant. L'avortement s'en suivit, mais la femme succomba. Les viscères envoyées au laboratoire afin d'y être analysées ils étaient l'estomac, la rate, les reins, l'utérus, et une partie du foie. Elles ne présentaient aucune lésion microscopique. Réduits en pulpe, nous les avons sousmis à une distillation à vapeurs d'eau, excepté une partie que nous avons utilisée pour les recherches des poisons caustiques, organiques, métaux et métalloïdes, d'ailleurs sans aucun résultat positif. Le distillat agité avec du pétrole léger, donna après l'évaporisation du solvant, des goutelettes huileuses qui donnaient :

| | |
|---|---|
| Les réactions chimiques de la rue et de la sabine. . | très nettes. |
| Les réactions chimiques de l'apiol . . . . . | négatives. |
| Indice de réfraction à 15°. . . . . | 1,4645 |

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Étant donné que l'indice de réfraction est pour la rue 1,4639 et pour la sabine 1,475, nous conclûmes plutôt qu'il s'agissait d'un mélange de deux huiles avec prédominance de l'essence de rue.

Dans le second cas il s'agit aussi d'un avortement après absorption d'infusions de plantes. On nous a envoyé les viscères (morceaux de l'estomac, intestin grêle, foie, cœur et utérus).

L'huile retirée donnait :

| | |
|---|---|
| Indice de réfraction à 15° . . . . . | 1,466 |
| Réactions chimiques de rue et de sabine . . . . . | positives. |

De l'indice de réfraction, nous résumons que nous étions en présence d'un mélange d'huiles.

Une étude comparative entre l'essence de rue, l'huile essentielle de sabine, l'apiol jaune et verte, faite avec des produits étalon (Maison Schimmel) relativement aux diverses réactions chimiques de ces corps avec le même réactif, nous a donné les résultats suivants :

| RÉACTIF | ESSENCE DE RUE | ESSENCE DE SABINE | APIOL JAUNE | APIOL VERTE |
|---|---|---|---|---|
| $HNO_3$ fumant. | Rouge-cerise foncé | Rouge-cerise immédiat | Jaune-brun | Vert-olive |
| $H_2SO_4$c. | Rouge-orange | Rouge vif | Rouge-brun | Brun |
| $H_2SO_4 + feCl_3$. | Rouge-violacé | Rouge-cerise | » | » |
| HCC alcoolisé. | Néant | Rose | Néant | Néant |
| $H_2SO_4$ alcoolisé. | Rose | Rose | » | » |
| Acide picrique à chaud. | Néant | Néant | » | » |
| $NaHSO_3$ | Cristaux en abondance | Cristaux rares | » | » |
| Nitrate d'argent ammoniacal | Rien | Réduction rapide | » | » |

De ce qui précède, il ressort que l'apiol jaune et verte ne présentent aucune réaction chimique semblables à celles de rue et de sabine, dont plusieurs réactions sont à peu près identiques. L'essence de rue à cause de son caractère cétonique réduit le nitrate d'argent ammo-

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niacal et ceci est un signe caractéristique. Mais en toxicologie nous ne pouvons en profiter de cette propriété. Peut-être d'autres substances volatiles provenant des viscères peuvent aussi réduire le nitrate d'argent ammoniacal. Ainsi dans les cas où on se trouve en présence d'un mélange d'apiol, rue et sabine, la présence d'apiol n'entrave pas la recherche des autres, tandis que la présence de la sabine seule rendra le résultat douteux.

Enfin pour voir si le placenta est perméable à l'essence de rue, et, par conséquent, s'il est possible à l'expert de confirmer l'avortement per l'analyse simple du fœtus, nous avons administré par os à une cobaye de 750 grammes pleine se trouvant vers la fin de sa gestation, pendant trois jours, 12 gouttes d'essence de rue ajoutées à une certaine quantité d'huile. Le matin du troisième jour, l'animal commença à avoir des douleurs, elle se tordait et criait. Cinq heures après il accoucha trois petits cobayes vivants, et après une demie-heure fut l'expulsion des délivres, dans lesquels nous retrouvâmes l'essence de rue (après distillation) par détermination de l'indice de réfraction.

A une seconde cobaye (de 840 gr.) se trouvant en commencement de la gestation nous avons donné per os 12 gouttes d'essence en quatre jours. L'animal avorta et les fœtus expulsés d'une façon prématurée n'avaient subi aucune macération susceptible de nous induire à penser à une mort intrautérine.

La distillation des embryons avec les placentas donna des gouttes huileuses de l'essence.

Un examen pharmacodynamique de la rue exécuté en même temps dans le Laboratoire de pharmacie expérimentale de l'Université d'Athènes par M. G. Logaras, notre collaborateur à ce sujet, a montré que la rue provoque : Des contractions vives de l'utérus isolée du cobaye plongé dans une solution de 50 centimètres cubes de tyrode avec 5 centimètres cubes d'infusion de

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la plante, modification du rythme cardiaque de la grenouille (d'après Straub) et abaissement considérable de la tension artérielle chez les chats au-dessus d'une quantité de 2 centimètres cubes d'infusion.

### *Conclusion*

D'après ce que nous avons exposé la rue continue actuellement d'être un abortif assez répandu en Grèce. L'avortement est provoqué par l'action directe de la rue sur la fibre musculaire utérine. La substance active de la plante est l'huile essentielle qui se trouve dans tous les organes des animaux avortés et peut s'identifier avec certitude dans les cas où cela est possible, seulement par la détermination de l'indice de réfraction de l'huile extraite des viscères, du placenta et de l'embryon grâce à la perméabilité placentaire.

(*Laboratoire de Médecine Légale et de Toxicologie de l'Université d'Athènes*. Directeur : M. le Prof. Dr. J. Géorgiadis.)

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## SUR LES VARIATIONS DE LA TENEUR EN ALCOOL DE DIVERS LIQUIDES ORGANIQUES ET VISCÈRES, APRÈS INGESTION D'ALCOOL SUIVIE DE SUBMERSION DANS L'EAU DOUCE. ÉTUDE EXPÉRIMENTALE. DÉDUCTIONS MÉDICO-LÉGALES

PAR

MM. Pierre LANDE, Pierre DERVILLÉE et Jean GODEAU.

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Parmi les différentes questions qui peuvent être posées au médecin-expert procédant à l'autopsie d'un cadavre de noyé non putréfié, il faut envisager celle-ci :

(Portuguese publication, pp. 506-510)

Department of Legal Medicine - "Oscar Freire" Institute

Director: Prof. Flaminio Favero

APIOL, SAVIN, RUE

(Experimental Studies)

By Flaminio Favero, Hilario Veiga de Carvalho, Elisa Novah

Apiol, savin and rue have been regarded as emmen agogues and for that purpose they are prepared for therapeutic use. Besides that indication, they serve also as an abortive for those who believe to be needing it.

However, these substances are nothing other than dreadful toxins. The emmena gogic action is merely a uterine hemorrhage, equivalent in that region to hemorrhages of the digestive tract, and is a more advanced degree of the generalized congestion observed after they are taken. It is not a real catamenial discharge. which is why these substances cannot be classified as emmenagogues, as we have said. On the other hand, they are not abortive substances either. As toxins they can kill the embryo, but they have no effect whatever on the mechanism of i expulsion. The abortive effect is indirect, brought about by intoxidation. Tha is the only way through which the components of that deadly triad act.

As there were interesting aspects to be studied, both from the toxicological and from the anatomo-pathological point of view, already related in previous reports, we believe it to be of interest to describe our observations in this pape in which we state our experience in condensed form.

.....

III. Rue

Rue (Ruta graveolens, family of the rutaceae) is found on the market as an essence and also as powder, the latter being rarely used in medicine.

The people use a decoction of the leaves and stem of the plant, all parts of which contain the active principle, an essential oil, which accounts for the properties of rue.

It is a greenish yellow essence, soluble in alcohol, ether, benzine, chlorofo xylol, and insoluble in water.

It is not as toxic as the essence of savin. Tests made on white rats gave us the following results:

1. Rat of 18 g. Hypodermic injection of 1/2 cc of essence at 1 p.m. Died during the night;

2. Rat of 19 g. Intraperitoneal injection of 1/2 cc. As after 20 minutes the symptoms were getting worse, we injected, 40 minutes after the first injection, another 1/2 cc, whereupon it died within 23 minutes;

3. Rat of 15 g. Intraperitoneal injection of 1/2 cc. When all symptoms had appeared, we tried to inject, in that state, 1 cc of morphine hydrochloride in t proportion of 1 mmg per 100, but it died 1 hour later;

4. Rat of 19 g. Intraperitoneal injection of 0.25 cc. It died 1 minute later, but from abdominal hemorrhage, not from intoxication;

5. Rat of 20 g. Intraperitoneal injection of 1 cc. Died after 1 hour and 27 minutes;

6. Rat of 20 g. Intraperitoneal injection of 2 cc. Died after 42 minutes;

7. Rat of 25 g. Intraperitoneal injection of 1/2 cc. It withstood another 1/2 cc on two subsequent days; in the afternoon of that day, the alterations in respiration and running appeared. The next day, at 10 a.m., it died.

Symptoms. - Immediate intense elimination through the respiratory passages, causing a foam in the animals' mouth, the smell being then perceptible; irregula running, with increasingly quick movements, loss of equilibrium, and finally cessation of running even with insistent stimuli; contraction of the limbs; dyspnea; rattling respiration, at increasingly long intervals; spasm of the resp atory muscles; taquicardia, marked cyanosis.

Pathological anatomy. - The injuries described as pertinent to the action of rue are stated, according to the authors, as phenomena of so-called "gastro-enteritis" or "inflammation of the digestive tube", which are more or less intense. In our experimental observations, the picture is a different one, as will be seen below, different even from what we have observed for savin and from what has been described for apiol.

Our observations may be outlined as follows:

1. Lung: This organ shows considerable congestion. Besides, one notes the presence of whitish, hardened lumps over the section surface. Microscopically they are a condensation of the parenchyma, the alveoles being filled with endothelioid cells, as can be seen in figure 8. These nodules were located preferentially around the bronchi (especially in the hilus) and under the pleural lobe. In cases of more prolonged intoxication, these nodules go into necrosis, with the coagulation type.

2. Heart: The central organ of circulation shows appreciable hyperemia. Apart from this picture, the microscopic examination revealed the existence of regressive alterations of the muscular elements, with symptoms of granulose degeneration, to the point of hyalinization in the more advanced stages (see fig. 9).

3. Stomach and intestines: We noted only discreet congestion, which never reached a marked degree.

4. Liver: In addition to moderate congestion, we found under microscopic analysis, regression of the hepatic cells, such as turbid swelling, and in the cases surviving longest, micro-vacuolization of said cells (see fig. 10) and eve necrosis; the latter we never observed en masse or in blocks, but in scattered cells in the groups in which the micro-vacuolization was most intense.

5. Kidney: Congestion was evident in this organ. Being predominantly glomc ular, it sometimes formed small nuclei of hemorrhagic suffusion, which were concentrated in the medullar zone. On the side of the cells of the renal tubules, especially in their secreting portion, turbid swelling was seen under the microscope.

6. Spleen: Under the microscope this organ clearly showed intense congestic with diffusion of red blood cells. When the intoxication was of a more chronic nature, this consistent picture was accompanied by nuclei of necrosis, of the appearance of coagulation necrosis, framed by a reaction areola (see fig. 11).

7. Ovary: In this organ we found only a discreet congestion.

8. Uterus: Of identical form, the possibility of recording congestion of thi organ was doubtful. In one of the females, which was pregnant, we observed he

rrhagic suffusion of the mucus and clear phenomena of the death of the fetus, which, however, had not been expelled (see fig. 12). Thus exposed to intoxicatio this animal did not resist as much as the others, which were subjected to a more violent intoxication.

9. Testicles and seminal vesicles: We found no symptom in these organs, not even that of a discreet congestion.

The lesions just described call for some considerations. In fact, it is found that the organs most attacked were the lungs. In them severe damage is seen, as was to be expected, as this is where most of the poison is eliminated, as we observed for these essences. Then also the heart and spleen are heavily affected the former showing a severe myocardosis and the latter possibly reaching a state of necrosis. In the other organs the predominant symptom is congestion, and in the genital apparatus even this is discreet.

Reactions - With sulfuric acid: 1 drop of essence of rue, plus 6 of sulfuric acid - Red-brown coloration. Wait 3 minutes, add 1 cc of alcohol. Coloration light pink, opalescent. After a while the essence separates at the edges, with greenish color.

With Frohde's reagent (sodium molybdate - 0.10 g : sulfuric acid - 100 cc) - red-brown coloration, more intense than with pure sulfuric acid.

By addition of alcohol, 3 minutes later, dirty yellowish color which soon disappears.

With Mandelin's reagent (ammonium vanadate 1 part, sulfuric acid 100 parts) brownish color, somewhat reddish, soon becoming more pronounced, oily droplets separating out from the start. The coloration disappears when 1 cc of alcohol is added.

With chloral hydrate - nothing.

With hydrochloric acid in alcohol - nothing.

With picric acid - nothing.

With bromine water - nothing.

With sodium nitroprussiate - 0.10 g and sulfuric acid 100 cc, red color (our contribution).

With fuming nitric acid - coloration not very pronounced.

Research. - In an analysis of organic material, this was reduced to paste, much water was added, and it was distilled by direct heat with intense cooling, the process being continued until some drops fallen from the condenser no longer gave a positive reaction.

We obtained better results by operating in this way than by steam distillatio

The distillate is collected in a flask with tap. After adding ether, it is vigorously agitated for about 20 minutes, then allowing the liquids to separate. If the quantity of ether is not sufficient, it will be somewhat emulsified. In that case, more is added and the product is agitated again. After the two liquid layers have formed, they are separated through the tap, the ether being received in a dish or crystallizer. It must have dissolved the entire essence, thus removing it from the water which accompanied it during the distillation. We performed on it one of the reactions to see if it was indeed free from the principle in question. In case of absence, it is discarded. But if the reaction is positive, treat with ether, agitate, separate, adding this ether to the first. The liquid will thus be completely drained of essence.

Left standing until the next day, the ether evaporates, the rue being liberated.

We dissolved the droplets in alcohol, measured the volume, and took one part of it for analysis.

Quantitative analysis - preparation of the standard solution. There were taken 0.4 cc of essence, adding alcohol to make 100 cc. Thus each cc contains 4 thousandths cc of rue.

As the red color obtained with Frohde's reagent is more intense than that fro sulfuric acid, we decided to use this reagent (0.10 g of sodium molybdate in 100 cc of sulfuric acid).

Technique. - Take 4 cc of the standard, dilute in another 2 cc of alcohol and add 4 cc of reagent. Because of the dilution, the color is only rose yellow.

With the solution to be determined, one tries to obtain the same or a similar color, by varying its volume and always bringing up to the total of 6 cc with

alcohol, and using the same 4 cc of reagent.

Here we must take into account the correction indicated for savin.

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au contraire le bleu ne s'accumule que dans le bourrelet cathodique.

Le cas particulier enregistré microphotométriquement par Mlle Veil (2) correspond au 3°, ce qui indique que le gel employé par cet auteur était alcalin (par rapport au point isoélectrique). On reconnaît dans son tracé le liseré hypercoloré séparant la zone décolorée (voisine de l'anode) du gel primitif (vers la cathode) et l'accumulation du colorant dans le bourrelet cathodique.

*(Laboratoire de physique de la Faculté de médecine, Ph. Fabre.)*

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## Note sur l'action de l'essence de Rue sur l'organisme animal,

par André et Gérard Patoir et H. Bédrine.

Poursuivant notre étude expérimentale sur la toxicité des abortifs végétaux, dont nous avons apporté ici-même, en juillet 1936, les premiers résultats, nous nous sommes attachés à administrer à des animaux de laboratoire des préparations contenant de la Rue.

Nous avons utilisé l'essence de Rue, préparation commode que nous avons donnée par ingestion. Le choix de cette voie s'explique par le souci de nous rapprocher le plus possible des conditions d'absorption toxi-médicamenteuse. Nous utilisons donc la sonde de Nelaton qu'il est relativement facile de faire passer dans l'œsophage des animaux communément employés.

Les doses varient de 250 à 300 gouttes pour des cobayes de 250 à 300 gr. et de 500 à 600 gouttes pour des lapins de 2.000 gr. à 2.500 gr. Les résultats expérimentaux et histologiques obtenus ne sont pas superposables aux quantités ingérées. Ceci nous semble dû à ce fait que l'essence de Rue n'est pas un produit chimiquement défini et nous rappelons que le produit du commerce est un mélange de *Ruta graveolens*, *Ruta montana* et *Ruta bracteosa*, dont les concentrations en méthylnonylcétone et méthylheptylcétone ne sont pas les mêmes.

Sur 10 animaux, 3 sont morts rapidement en 7 à 9 jours ; quatre, intoxiqués un peu plus lentement, étaient encore vivants au bout de 20 jours et furent sacrifiés ; trois survécurent et ne parurent pas souffrir ultérieurement de leur intoxication (les trois étaient gravides).

Les animaux présentaient de la dyspnée, de la diarrhée, de la torpeur, parfois une hématémèse et maigrissaient rapidement.

Les lésions histologiques intéressent le foie et surtout le rein : hépatite granulo-graisseuse assez discrète d'une part ; néphrite

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parenchymateuse du type épithélial très accentuée d'autre part. Il nous faut ajouter à cela une congestion intense intéressant tous les parenchymes.

L'intoxication des femelles gravides nous a montré que l'avortement n'était pas obtenu sans difficultés et que ce résultat n'était atteint qu'au prix d'une intoxication profonde ; qu'enfin l'organisme du fœtus était lui aussi considérablement lésé par l'agression toxique que n'arrête pas le filtre placentaire. L'hépato-néphrite est plus intense chez le fœtus que chez la mère.

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## Action toxique de l'essence de Sabine et de l'Armoise sur l'organisme,

par André et Gérard Patoir et H. Bédrine.

Les techniques d'intoxication sont les mêmes que celles utilisées dans nos travaux précédents. Nous administrons de l'essence de Sabine commerciale, mélange de *Juniperus sabina tamariscifolia* et *cupressifolia*.

Les constituants principaux en sont nous le rappelons : 1°) les produits qui passent au-dessous de 195° : un aldéhyde ou cétone isolé par Schimmel et des terpènes (pinène, sabinène et terpinène) ; 2°) les produits qui passent entre 195 et 235° : le sabinol, alcool secondaire terpénique cyclique à l'état pur ou éthérifié ; 3°) au-dessus de 235° le cadinène, sesquiterpène bicyclique.

Nous avons fait ingérer à la sonde en gomme des doses variant de 60 à 300 gouttes pour les cobayes et de 300 à 500 gouttes chez les lapins. Ici encore il n'y a pas parallélisme entre le degré des lésions et la dose absorbée.

Treize animaux ont été ainsi traités : sept ont succombé au milieu d'un tableau de toxémie progressive : pâleur, dyspnée, hématurie ; signes de gastro-entérite, cachexie. Deux ont été sacrifiés au bout de 8 et 10 jours. Cinq enfin survécurent un certain temps.

L'Armoise, connue depuis la plus haute antiquité pour ses vertus emménagogues, voire abortives, a fait également l'objet de quelques-unes de nos recherches expérimentales. La voie d'administration est toujours la même. Nous nous sommes servis de deux préparations pharmaceutiques, la tisane (5 gr. de produit pour un litre d'eau), l'extrait mou aqueux de feuilles (Codex 1884), d'usage plus facile. Les doses sont ici à peu près constantes, 25 à 40 gr. d'extrait mou, l'infusion de feuilles apparaissant trop peu toxique.

Sur huit animaux intoxiqués de la sorte trois moururent à 13,

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DEPARTAMENTO DE MEDICINA LEGAL — INSTITUTO "OSCAR FREIRE"
Diretor: Prof. FLAMINIO FAVERO

# APIOL, SABINA, ARRUDA
(Estudos experimentais)

por

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O apiol, a sabina e a arruda teem tido foros de emenagogos e com tal fim são apontados ao uso terapêutico. Além dessa indicação, oferecem também o papel abortivo para quem dele julgue necessitar.

Sem embargo, não são essas substâncias outra coisa do que tóxicos temíveis. A ação emenagoga seria apenas uma hemorragia uterina, equivalente nesse distrito às hemorragias do tubo digestivo e seria um gráu mais avançado da congestão generalisada que se observa pela sua propinação. Não seria um fluxo catamenial verdadeiro, o que importa em não classificar aquelas substâncias como emenagogas, como já o vimos assinalando. Por outro lado, também não são substâncias abortivas. Como tóxicas que são, podem matar o embrião, mas não teem poder algum sobre o mecanismo da sua expulsão. O efeito abortivo é indireto, mediato à intoxicação. Esta é o único meio por que agem os componentes daquela tríade nefasta.

Como havia aspectos interessantes a estudar, quer do ponto de vista toxicológico, quer do ponto de vista anatomopatológico, já referidos em notas anteriores, achámos de interêsse compendiar as nossas observações neste trabalho em que condensamos a nossa experiência.

## I — APIOL

O apiol foi retirado por BLANCHET e SELL das sementes de persil e chamado, a princípio, cânfora de persil. Distilando-se essas sementes com água, o apiol é arrastado, formando longas agulhas

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finas, solúveis no alcool, no eter, acetona, benzina, éter acético, e insolúveis na água.

Deriva da alilbenzina ao ser tratada pelo alilapionol, sendo o éter metilênico e dimetílico do alilbenzeno :

Aquecido à ebulição com KOH alcoólica, transforma-se num isômero : o isoapiol.

O $H^2SO^4$ concentrado o dissolve com coloração vermelha.

Pela oxidação crônica, dá o aldeído apiólico ($C^{10}H^{10}O^5$).

No comércio, é encontrado em 3 formas : o apiol cristalizado, o apiol verde, que deve seu colorido à presença de clorofila, e o apiol amarelo que se deriva do verde ao ser tratado pelo carvão animal e litargírio, desembaraçando-o, dêste modo, da clorofila e matérias graxas.

*Sintomatologia.* — A intoxicação apiólica oferece como quadro as vertigens, cefaléias, mal-estar, polineurite, hepato-nefrose, abolição dos reflexos tendinosos, albuminúria, às vezes hematúria. Por via da intoxicação, o abortamento às vezes se processa.

*Anatomia patológica.* — As lesões que se observam nos animais intoxicados pelo apiol apresentam-se predominantemente instaladas no fígado e nos rins e a degeneração celular é a sua manifestação primitiva ; observam-se todos os gráus da degeneração, até à necrose da célula : inchação turva, microvacuolização, esteatose e lise total ; os núcleos também oferecem os quadros típicos da regressão nuclear. Quer as células hepáticas, quer as células dos túbulos renais, são elas sede dêstes processos degenerativos. E, como é o quadro hepático e renal o que domina na intoxicação apiólica, é justificável, sob êste aspecto, que seja denominado aquele quadro de hépato-nefrose apiólica (fig. n.° 1). Para os demais órgãos da economia, nada nos foi dado observar de maior monta ; anotemos, tão sòmente, a congestão que às vezes era generalizada mas que sempre se manifestava para o lado dos pulmões. Os órgãos genitais internos não apresentavam nenhuma eleição.

*Dosagem.* — L. Payen, do Laboratório de Medicina Legal de Lille, faz a dosagem partindo do apiol cristalizado, mediante sua transformação em ácido oxálico e depois oxalato de cálcio que é solubilizado pelo $H^2SO^4$ ao quinto, doseando o ácido oxálico obtido, a quente, pelo permanganato N/10.

Aproveita-se, assim, o fato do apiol, ao ser tratado pelo $HNO^3$, se oxidar, dando ácido oxálico.

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Em nossas experiências, não tendo sido possível adquirir o apiol cristalizado, trabalhámos com o apiol verde.

E' solúvel no álcool, éter, benzina, xilol, clorofórmio, acetona, e insolúvel em água.

O ácido azótico o oxida dando ácido oxálico.

Sua solução alcoólica, tratada pela água de cloro até turvação persistente e depois por algumas gotas de amoníaco, toma coloração vermelha tijolo fugaz (A. Jorisson).

Sendo a única reação corada a do $H^2SO^4$, resolvemos aproveitá-la para a dosagem colorimétrica. Com o apiol, êsse ácido puro dá uma coloração vermelha de sangue. Em solução alcoólica, o colorido vai variando segundo a riqueza em apiol.

Preparámos a solução padrão dissolvendo Ogr., 2842 de apiol em 100 cc. de alcool etílico. Desta solução, tomámos 2 cc., completando o volume a 4 com alcool e tratámos então por um excesso de de ácido sulfúrico puro — 6 cc. Se a côr for intensa, em vez de 2cc. toma-se 1 cc. ou mesmo décimos de centímetro cúbico, aferindo depois, sempre, com álcool, o volume a 4 cc.

Da solução cujo teor se procura, tomam-se 4 cc. para 6 de ácido sulfúrico puro, reduzindo-os, caso seja preciso, mas aferindo o volume a 4 cc. com alcool. Assim, estaremos operando nas mesmas condições da solução padrão.

Conseguidas côres iguais ou próximas, levam-se os líquidos ao colorímetro e, obtida a média de várias leituras, estabelece-se a regra de 3 inversa.

Tratando-se dum processo colorimétrico, é rápido e pouco dispendioso.

## II — Sabina

O óleo essencial de sabina é retirado da Juniperus Sabina, da família das coníferas. Além doutras qualidades, teria a de emenagogo enérgico, devendo ser administrado com certa reserva pois que doses superiores a 5 ou 10 gotas podem determinar intoxicações que, nem sempre seguidas de abortamento, podem, por vezes, determinar a morte da paciente.

*Sintomas.* — Seus sintomas de intoxicação consistem em inflamação do tubo digestivo, vómitos, diarréia, aceleração do pulso e, algumas vezes, *trismus* e tétano. Por injeção de 5 décimos de centímetro cúbico do óleo essencial em rato branco de 23 gr. 500, pudemos verificar ânsia de vómitos e hemorragia bucal, tendo-se

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dado a morte no prazo de 2 minutos. Por injeção, pela mesma via, de 3 décimos de cc. em rato de 21 gr. 500, verificámos ânsia de vómitos, perturbação na marcha logo de início, perda de equilíbrio, dispnéia intensa e fortes tremores. Por injeção de 25 centésimos de cc. em rato de 18 gr. 700, verificámos excitação, dispnéia depois de 7 minutos, grande titubeação na marcha, tremores, tendo o animal, neste estado, resistido por espaço de 4½ horas, quando o sacrificámos por estrangulamento.

Essas propriedades tóxicas da sabina são devidas ao óleo essencial, muito solúvel no alcool e éter — o sabinol.

*Anatomia patológica.* — As lesões descritas na intoxicação pela essência de sabina cifram-se em congestão dos órgãos da pequena bacia ou, então, na assim chamada gastro-enterite.

Nas nossas verificações, ressaltou desde logo à observação uma congestão generalizada a todos os órgãos. Como em seguida faremos menção, numerosos órgãos da economia são atingidos, distanciando-se, assim, a sabina do seu companheiro habitual de malefícios, o apiol, em que, não teem sido observadas lesões ao nível doutros órgãos, além do fígado e do rim. Como fenômeno interessante a que inicialmente se deve fazer referência, foi observada, em dois dos animais em experiência, a presença dum franco transudato peritoneal.

Vejamos o que se observou nos órgãos predominantemente atingidos :

1 — *Pulmão :* a víscera pneumática apresentou-se, como os demais órgãos, séde duma notável congestão ; além dêsse caráter, por assim dizer, geral, verifica-se, também, uma condensação acentuada do seu parênquima ; esta fenomenologia pulmonar assim tão acentuada, parece-nos correr por conta da eliminação da essência que julgamos relevante pela via alveolar.

2 — *Estômago :* no estômago, como via de regra, no tubo gastro-intestinal, foi observada congestão intensa da parede, mormente da túnica mucosa, por vezes acompanhada de forte hemorragia, com descamação dos estratos superficiais, como o demonstra a fig. n.º 2.

3 — *Intestino :* como ind'agora o dissemos, é a congestão intensa o quadro predominante, geralmente secundado por hemorragias francas (fig. n.º 3).

4 — *Rim :* no rim, além da constante congestão, verificam-se estados regressivos para o lado dos túbulos, mormente secretores,

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chegando-se frequentemente à observação da necrose; a esteatose degenerativa não foi observada e cremos ser a explicação da sua ausência o fato de havermos intoxicado os animais de forma, em geral, aguda, indo, pois, os elementos celulares imediatamente à necrose; a figura n.º 4 dá-nos quadro demonstrativo.

5 — *Fígado:* os mesmos fenômenos regressivos apontados para o rim, são encontrados no fígado, notando-se extensas áreas de necrose; aqui, igualmente, não nos foi dado observar a esteatose e a razão de sua falta deverá ser a mesma anteriormente apontada; a necrose instala-se por áreas, mais ou menos extensas, ficando entre elas ilhotas de parênquima mais ou menos conservado, o que também ocorre com os espaços de KIERNAN (fig. n.º 5).

6 — *Utero:* o útero é um órgão severamente atingido pela congestão; ela póde permanecer nêsse estado, quando as doses são pequenas mas, também, pode ir à hemorragia franca com disarquitectonia quasi total de sua parede e, mormente, de sua mucosa (fig. n.º 6), quando as doses se elevam.

7 — *Testículo:* como não ha especificidade na ação da sabina, pareceu-nos interessante observar o que se passaria para o lado dos testículos, então quando a indicação absurdamente tida como terapêutica estaria afastada; a mesmíssima congestão, de igual intensidade, esteve presente no quadro microscópico (fig. n.º 7).

8 — *Cápsula suprarrenal:* também êste órgão merece atenção especial pois que, além da já abundantemente citada congestão, temos também sob a vista fenômenos regressivos para o lado das células da sua medular, atestados, afóra as modificações cromáticas e estruturais do protoplasma, pelas alterações nucleares (acentuada picnose); talvez se deva atribuir a indiferença, a quietude dos animais no início das experiências ou nas intoxicações de caráter mais crônico, a essas alterações regressivas das células da medular suprarrenalínica.

*Pesquisa.* — Sendo uma essência o princípio ativo, a sua pesquisa se faz por distilação. Como é alto o seu ponto de ebulição, 155 a 161°, todos os que dela se teem ocupado indicam a distilação pelo vapor d'água que facilita o carreamento dos vapores da da sabina.

Entretanto, a distilação nestas condições, além de ser grandemente retardada, aumenta muito o volume do distilado, o que dá em consequência uma diluição extrema de doses às vezes milesi-

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mais da essência. Por isso, em nossos trabalhos, depois de reduzir a polpa o material e passá-lo para o balão distilador, juntámos, para uns 25 grs. dêle, 500 cc. de água distilada e, em geral, recolhemos de 350 a 400 e poucos cc. Nesta altura, gotas tomadas do distilado não davam mais nenhuma das reações indicadas na pesquisa da essência de sabina. Esta distilação foi, portanto, feita a fogo nú e forte, para favorecer o desprendimento da essência, e com refrigeração intensa para não se perderem os vapores.

Conseguido o distilado, exgotâmo-lo completamente pelo éter comum, verificando sempre que o líquido a ser rejeitado não desse reação positiva para a sabina. Na separação da essência do resto do distilado, abandonámos, de início, o éter de petróleo, visto dar, também, reação com o ácido sulfúrico puro. Dentre os vários líquidos extratores que poderiam ser indicados, apenas encontrámos o éter comum não reagindo àquele ácido. Aconteceu, às vezes, do éter ficar turvo (pela emulsão). Nêstes casos, adicionámos mais éter o que fazia desaparecer a emulsão. Separado o éter, recebíamo-lo em cápsula de vidro e abandonavamos à temperatura ambiente até evaporação completa. O resíduo (às vezes constituído por pequenas gotas, às vezes por um pouco de líquido quando o éter passava emulsionado) era tratado pelo alcool, passando-se agora o líquido para um vaso graduado e lavando-se várias vezes a cápsula com álcool. O volume total era anotado.

*Reações.* — Tratando-se duma essência, é de inestimável préstimo o odôr do distilado, que deve ser desagradável e forte para o nosso caso. A essência de sabina não tem reações peculiares; todas elas são as reações das essências em geral.

Deixando de lado as diversas reações experimentadas com o óleo essencial puro e depois com o distilado, vamos nos deter mais demoradamente na ação do ácido sulfúrico, que melhores resultados nos forneceu. Com a essência pura, êste ácido produz uma coloração vermelho-alaranjada. Em soluções, essa tonalidade varia até ao amarelado muito claro, segundo as percentagens do princípio ativo. Obtivemos positividade desta reação com solução alcoólica de óleo de sabina na proporção de 0cc. 025%; a 0 cc. 01% obtivemos traços. Tratando o óleo essencial puro pelo ácido sulfúrico e após uns minutos pelo alcool, a coloração desaparece quasi completamente. Em se tratando, porém, de solução, e esta feita no alcool, a coloração no momento de encontro das diver-

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sas substâncias é rósea ou alaranjada e acentua-se, logo a seguir, o alaranjado. Mesmo efeito apontamos para o éter comum.

Como num caso de análise as vísceras podem estar adicionadas de agentes conservadores, estudamos a ação de diversos dêles sobre a reação e concluímos serem todos prejudiciais à sua positividade. Para o formol, por ser o mais comum, adicionâmo-lo à matéria que levava sabina e distilámos.

Para os outros, tomámos 1 cc. da solução alcoólica de sabina a 0gr.43993%, juntámos 1 cc. do conservador, 2 cc. de alcool, 2 cc. ácido sulfúrico.

Os resultados foram os seguintes:

*Formol* — Comunica uma tonalidade levemente pardacenta e pequena turvação.

*Sublimado.* — A princípio, coloração alaranjada, depois pardacenta e turva.

*Cloreto de zinco.* — Alaranjado. Aos poucos, tonalidade pardacenta e turva.

*Clorofórmio.* — Róseo mais intenso que a reação normal e límpido.

*Metanol.* — Róseo mais claro que a reação normal.

Nestas condições, estando as vísceras adicionadas dêstes conservadores, serão falsos os resultados da análise.

*Doseagem.* — Diante do silêncio dos autores quanto à possibilidade da doseagem da sabina, procurámos obtê-la. E foi excelente o resultado.

Estudada cuidadosamente a reação do ácido sulfúrico, resolvemos aplicá-la à doseagem colorimétrica da sabina, para o que se faz necessária a solução padrão.

Já mencionámos atraz: 1.º) que o éter altera levemente a coloração produzida pelo ácido sulfúrico e sabina; 2.º) que a extração da sabina do distilado é feita pelo éter.

Ao envez de preparar a solução padrão dissolvendo simplesmente o óleo essencial no alcool, tomámos 0gr.43993 de óleo, dissolvemos em 100 cc. de alcool, juntámos 20 cc. de éter comum, agitámos muito bem para haver boa mistura, e abandonámos, nas mesmas condições que as dos casos de pesquisa, à evaporação espontânea, ao ambiente, até o volume retornar a 100 cc. Assim, o efeito do éter se teria feito sentir no padrão. Tomámos, então, 4 cc. da solução em análise e juntámos 2 cc. de acido sulfúrico. Segundo

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a intensidade da côr, diminuíamos, às vezes, a quantidade de material e aferíamos sempre o volume a 4 cc. com alcool.

Com o padrão, íamos variando as proporções e completando sempre 4 cc. com o alcool, juntando, então, 2 cc. de ácido sulfúrico. Obtidas as côres próximas, levámos ao colorímetro e, depois de várias leituras, procedíamos ao cálculo.

*Causas de êrro.* — Durante a pesquisa, após a extração pelo éter, êste deve ser evaporado. Ora, sendo a sabina volátil, a evaporação do éter por longas horas deve favorecer muito a sua evaporação. Deixámos, então, numa cápsula, quantidade conhecida de sabina adicionada de éter. Uma vez evaporado êste, verificámos a perda de pêso. Em média, uma evaporação de 15 horas com a temperatura máxima de 17.° e mínima de 14°., produz uma perda de 0gr.07354 ao ambiente, em cápsula medindo 85 × 85 mms., cifra esta que deverá ser juntada ao resultado final. As condições ambientes (temperatura, gráu higrométrico do ar) e a superfície de evaporação farão variar esta cifra. Então, lembramos a idéia de, para os casos em que o distilado indique pelo seu odor tratar-se de sabina ou nos casos de análise determinada de sabina, deixar-se também evaporar, nas mesmas horas, quantidade conhecida do óleo essencial adicionado de éter. A diferença de pêso verificada nesta experiência deverá ser adicionada ao resultado da análise.

## III — Arruda

A arruda (Ruta graveolens, família das rutaceas) é encontrada no comércio sob a forma de essência e também em pó, sendo êste raramente usado em medicina.

O vulgo emprega a decoção das folhas e caule da planta, tendo, todas as suas partes, o princípio ativo que é um óleo essencial, ao qual se devem as qualidades da arruda.

E' uma essência amarelo-esverdeada, solúvel no alcool, éter, benzina, clorofórmio, xilol e insolúvel em água.

Não é tão tóxica quanto a essência de sabina. Experiências feitas em camondongos brancos deram-nos os seguintes resultados:

1.° — Camondongo de 18 grs. Injeção hipodérmica de meio cc. de essência, às 13 hs. Morreu durante a noite;

2.° — Camondongo de 19 grs. Injeção intra-peritoneal de meio cc. Como depois de 20 minutos os sintomas fossem recru-

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descendo, injetámos, após 40 minutos da 1.ª injeção, mais meio cc. tendo então morrido no prazo de 23 minutos +.

3.º — Camondongo de 15 grs. Injeção intraperitoneal de meio cc. Uma vez instalados todos os sintomas, tentámos a sua volta ao estado a *quod*, injetando 1 cc. de clorhidrato de morfina na proporção de 1 mmg. por 100, mas morreu 1 hora depois;

4.º — Camondongo de 19 grs. Injeção intraperitoneal de 25 centésimos de centímetro cúbico. Morreu 1 minuto depois, mas por hemorragia abdominal e não por intoxicação;

5.º — Camondongo de 20 grs. Injeção intraperitoneal de 1 cc. Morreu depois de 1 hora e 27 minutos;

6.º — Camondongo de 20 grs. Injeção intraperitoneal de 2 cc. Morreu após 42 minutos;

7.º — Camondongo de 25 grs. Injeção intraperitoneal de meio cc. Suportou mais meio cc. em 2 dias seguidos; à tarde dêste dia apareceram as alterações da respiração e da marcha. No dia imediato, às 10 horas da manhã, morreu.

*Sintomas.* — Forte e imediáta eliminação pelas vias respiratórias, o que provoca coceira no focinho dos animaizinhos, percebendo-se logo o cheiro; marcha irregular, tornando-se os movimentos cada vez menos ágeis, perda de equilíbrio e, afinal, cessação da marcha mesmo com estímulos insistentes; contratura dos membros; dispnéia; respiração estertorosa, espaçando cada vez mais; espasmos da musculatura respiratória; taquicardia; cianose acentuada.

*Anatomia patológica.* — As lesões descritas como pertinentes à ação da arruda cifram-se, segundo os AA., em fenômenos da assim chamada "gastro-enterite" ou "inflamação do tubo digestivo", fenômenos êsses mais ou menos intensos. Nas nossas observações experimentais, outro é o quadro, como abaixo se verá, distanciando-se, mesmo, do que temos observado para a sabina e daquele que tem sido descrito para o apiol.

Decalquemos as nossas observações:

1. — *Pulmão:* êste órgão apresenta uma notável congestão; além dêste fenômeno, notou-se a presença de nódulos esbranquiçados e endurecidos, semeados pela superfície de corte; microscopicamente, êles correspondem a uma condensação do parênquima, apresentando-se os alvéolos cheios de células de tipo endotelioide, como bem se observa na figura n.º 8. Êsses nódulos situavam-se

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preferivelmente em redor dos brônquios (máxime no hilo) e sob o folheto pleural. Nos casos de intoxicação mais prolongada, êsses nódulos entram em necrose, com o tipo de coagulação.

2. — *Coração:* o órgão central da circulação apresenta-se sede duma apreciável hiperemia; afóra êste quadro, o exame microscópico revelou a existência de alterações regressivas dos elementos musculares, com quadros de degeneração granulosa, atingindo, nos estádios mais avançados, aos de hialinização (observe-se a fig. n.° 9).

3 — *Estômago e intestinos:* anotámos, tão sòmente, discretos quadros de congestão, que nunca alcançaram o gráu de notória evidência.

4. *Fígado:* além da congestão, que não é de grande amplitude, tivemos sob as vistas, à análise microscópica, manifestações regressivas das células hepáticas, tais como a inchação turva e, nos casos de sobrevida mais dilatada, a micro-vacuolização das ditas células (veja-se a figura n.° 10) e, mesmo, a necrose; esta última, nunca a observámos em massa ou em blocos, mas, sim, em células esparsas nos grupos em que a micro-vacuolização era mais intensa.

5. — *Rim:* nêste órgão foram evidentes os quadros de congestão; esta, que era predominantemente glomerular alcançava, por vezes, a formar pequenos núcleos de sufusão hemorrágica, que se localizavam predominantemente na zona medular; para o lado das células dos túbulos renais, mormente na sua porção secretora, notou-se a existência, ao microscópio, de inchação turva.

6. *Baço:* êste órgão mostrou, com grande clareza ao exame microscópico, uma intensa congestão, com difusão de hemátias; êsse constante quadro acompanhou-se quando a intoxicação era de caráter mais crônico, de núcleos de necrose, assumindo o aspecto da de coagulação, e que se margeavam por uma aréola de reação (veja-se a figura n.° 11).

7. — *Ovário:* nêste órgão não encontrámos mais que uma discreta congestão.

8. — *Utero:* de idêntica forma, era de dúvida a possibilidade de se registar congestão dêste órgão. Num dos animais fêmeas, que se encontrava grávido, observou-se sufusão hemorrágica da mucosa e fenômenos claros de morte do produto da concepção,

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sem que, contudo, tivesse havido a expulsão do mesmo (veja-se a figura n.º 12) Êste animal, assim expôsto à intoxicação, não resistiu tanto quanto os demais, que foram submetidos a uma intoxicação mais violenta.

9. — *Testículos e vesículas seminais:* nêstes órgãos não tivemos a oportunidade de registar qualquer fenômeno, nem mesmo o duma discreta congestão.

As lesões observadas e agora descritas impõem algumas considerações. De feito, verifica-se que os órgãos mais castigadamente atingidos foram os pulmões; nêles se observaram lesões graves o que era, aliás, de esperar, visto serem êles sede de eliminação preponderante do tóxico, como seguidamente temos observado para estas essências. Afóra êles, aparecem-nos, desde logo, o coração e o baço, duramente atingidos, naquele dando lugar a uma miocardose grave e nêste chegando a estabelecer-se até o quadro da necrose. Nos demais órgãos, é a congestão o quadro que domina e, no aparelho genital, é ela, assim mesmo, discreta.

*Reações* — Pelo ácido sulfúrico: 1 gota de essência de arruda, mais 6 de ácido sulfúrico — Coloração vermelho-pardacenta. Esperar 3 minutos, juntar 1 cc. de alcool. Coloração róseo-clara, opalescente. Depois dalgum tempo a essência se separa pelas bordas, com coloração esverdeada.

Pelo reativo de Frohde (Molibdato de sódio – 0,gr.10: ácido sulfúrico – 100cc.) — coloração vermelho-pardacenta mais intensa que com o ácido sulfúrico puro.

Por adição de alcool, 3 minutos depois, coloração amarelada-suja que desaparece aos poucos.

Pelo reativo de Mandelin (Vanadato de amônio – 1 parte e ácido sulfúrico — 100 partes) coloração pardacenta, levemente avermelhada, que aos poucos vai se pronunciando, separando-se, desde o início, gotículas oleosas. Desaparece a coloração pela adição de 1 cc. de alcool.

Pelo hidrato de cloral — nada.

Pelo ácido corídrico alcoolizado — nada.

Pelo ácido pícrico — nada.

Pela água de bromo — nada.

Pelo nitroprussiato de sódio — 0,gr.10 e ácido sulfúrico 100 cc.., coloração vermelha (contribuição nossa).

Pelo ácido azótico fumegante — coloração pouco pronunciada.

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*Pesquisa.* — Num caso de verificação em material orgânico, reduz-se êste a pasta, junta-se grande volume de água, e distila-se a fogo nú com intensa refrigeração, prolongando-se o processo até que umas gotas caídas do refrigerante não deem reação positiva.

Obtivemos melhores resultados operando assim do que fazendo a distilação em presença de corrente de vapor de água.

O distilado é recolhido num funil (em forma de bola) com torneira. Junta-se éter, agita-se energicamente uns 20 minutos, deixa-se que os líquidos se separem. Se a quantidade de éter não for suficiente, êle ficará um pouco emulsionado. Faz-se, nesta hipótese, nova adição e agita-se outra vez. Formadas as duas camadas líquidas, separam-se pela torneira, recebendo o éter numa capsula ou cristalizador. Êle deve ter dissolvido toda a essência, retirando-a, assim, da água que a acompanhou na distilação. Com esta, experimentamos uma das reacções para ver se de fato está isenta do princípio em procura. Em caso de ausência, será rejeitada ; sendo, porém, positiva a reação, trata-se por éter, agita-se, separa-se. reunindo êste éter ao primeiro. Nestas condições, o líquido sofrerá um exgotamento completo da essência.

O éter, deixado de um dia para outro, evapora-se, ficando a arruda em liberdade.

Dissolvemos as gotículas em alcool, medimos o volume, e dêste tomamos uma parte para doscar.

*Doseagem — preparo da solução padrão.* — Foram tomados 4 décimos de centímetro cúbico de essência e completados 100 cc. com alcool. Cada cc. contém, pois, 4 milésimos de cc. em arruda.

Sendo o vermelho fornecido pelo reativo de Frohde mais intenso que o do ácido sulfúrico, alvitrámos pelo emprêgo dêste reativo (0,gr. 10 de molibdato de sódio em 100 cc. de ácido sulfúrico).

*Técnica.* — Tomam-se 4 cc. do padrão, diluem-se em mais 2 cc. de alcool e juntam-se 4 cc. do reagente. O colorido, dada a diluição, é apenas amarelo-rosado.

Com a solução a doscar, procura-se obter colorido igual ou próximo, variando o seu volume e aferindo sempre o total a 6 cc. por meio do alcool, e usando os mesmos 4 cc. do reagente.

Aqui, teremos de levar em conta a correção indicada para a sabina.

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## Resumo

Os AA. estudaram experimentalmente, em animais de laboratório, os sintomas que surgem pela absorção do apiol, da sabina e da arruda, e que são: marcha irregular, tremores, perda de equilíbrio, cessação da marcha, contratura dos membros, dispnéia, respiração estertorosa, espasmo da musculatura respiratoria, náuseas, hemorragia bucal, taquicardia, cianose acentuada. Passando à parte química, depois de rápido apanhado sobre as reações do apiol, sabina e arruda, detiveram-se os AA. na sua doseagem colorimétrica, servindo-se de soluções padrões dos três produtos, que usaram puras ou diluidas em alcool, conforme os casos, e usando como reagente do apiol e da sabina o ácido sulfúrico puro e da arruda o reativo de Frohde. Aproveitaram também para fazer o estudo dos quadros anatomopatológicos em que foram patentes a congestão generalizada a todos os órgãos e a degeneração de vários órgãos parenquimatosos; as hemorragias uterinas eram uma manifestação de intoxicação idêntica às hemorragias do tubo gastrointestinal e não poderão ser, na mulher, uma verdadeira menorragia. Das três essências estudadas, o apiol é o que poupa mais a economia, assestando-se as suas lesões apenas no rim e no fígado. Os pulmões foram sempre atacados, como via de eliminação das essências que são. Em geral, todos os órgãos sofrem os malefícios dos tóxicos estudados. Os AA. concluem pela ação sòmente tóxica das substâncias estudadas. Documentam o seu trabalho com 12 microfotos.


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## DESCRIPÇÃO DAS FIGURAS

*Folha 1*

**Fig. 1** — Hepatonefrose apiólica: em A, veem-se células hepáticas em diversos gráus de degeneração; em B, esteatose dos túbulos renais (Sudan III). Microfoto. Obj. 40 x, oc. perip. 8 x.

**Fig. 2** — Lesão do estômago na intoxicação pela sabina: vê-se a mucosa congesta e hemorragia.

**Fig. 3** — Lesão do intestino na intoxicação pela sabina: vê-se congestão da mucosa e hemorragia.

**Fig. 1** — Apiolic hepatonephrosis.

**Fig. 2** — Gastric hemorrhage and congestion, produced by apiol.

**Fig. 3** — Intestinal hemorrhage and congestion, produced by apiol.

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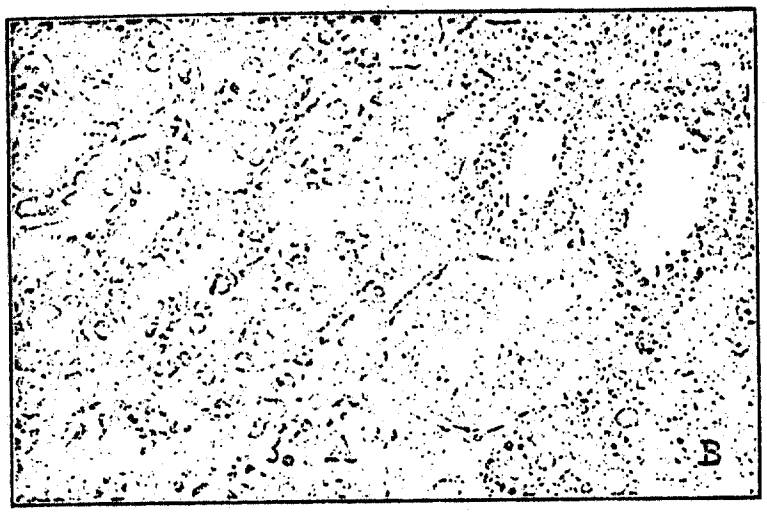


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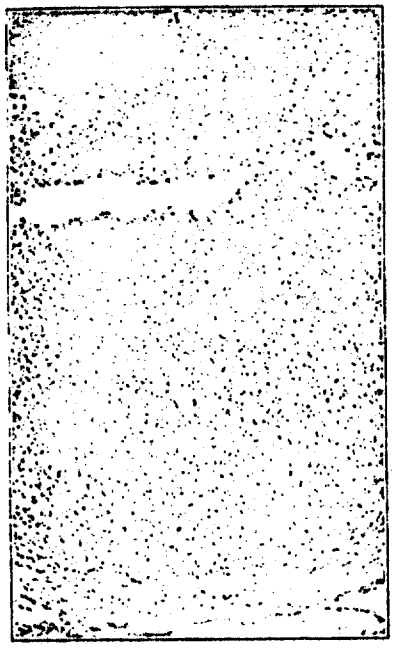

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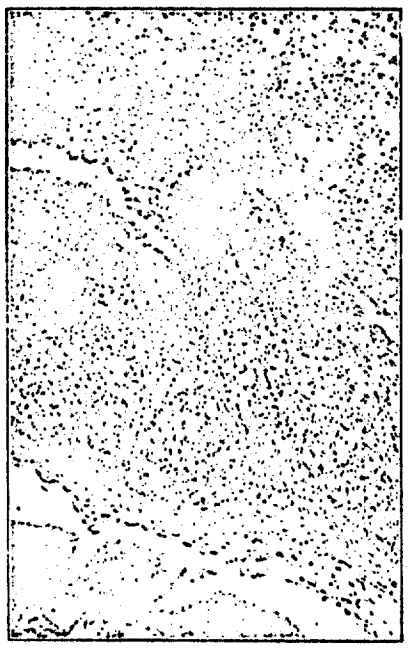

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*Folha II*

Fig. 4 — Lesão do rim na intoxicação pela sabina: vê-se necrose das células tubulares.

Fig. 5 — Lesão do fígado na intoxicação pela sabina: veem-se áreas de necrose.

Fig. 6 — Lesão do útero na intoxicação pela sabina: vê-se notável hemorragia com disarquitectonia da parede.

Fig. 4 — Renal epithelium necrosis, produced by savin.

Fg. 5 — Liver cells necrosis, produced by savin.

Fig. 6 — Uterine hemorrhage, produced by savin.

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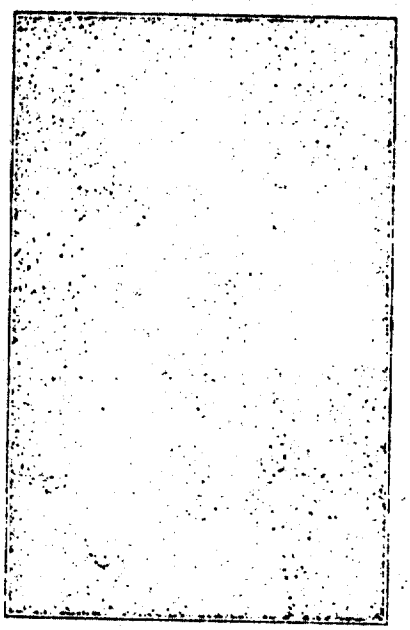

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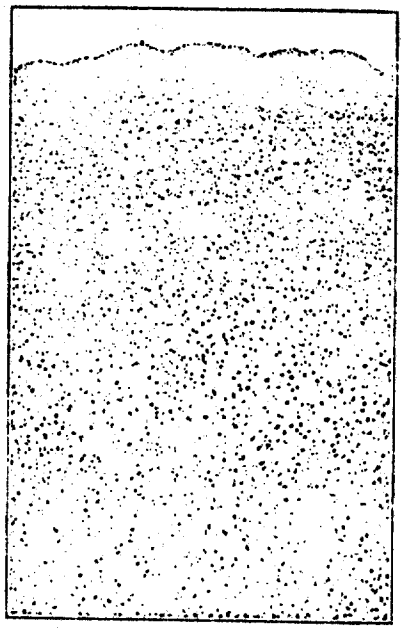

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*Folha III*

Fig. 7 — Lesão do testículo na intoxicação pela sabina: vê-se congestão intensa do órgão.

Fig. 8 — Lesão do pulmão na intoxicação pela arruda: vê-se um nódulo de invasão dos alvéolos por células de tipo endotelioide.

Fig. 7 — Testicle congestion, produced by savin.

Fig. 8 — Invasion nodule of endothelial-like cells.

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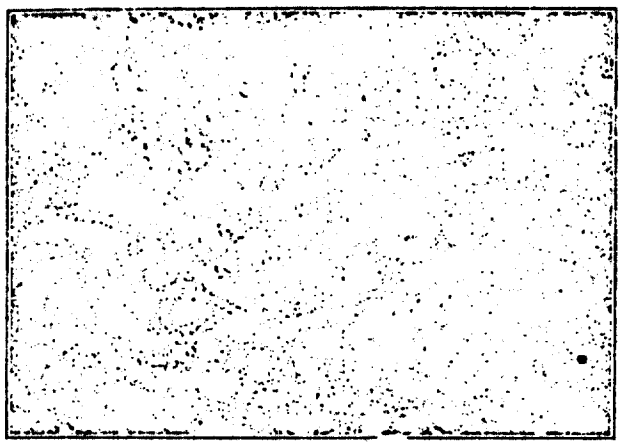

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*Folha IV*

**Fig. 9** — Lesão dos elementos miocárdicos na intoxicação pela arruda: vê-se hialinização dum elemento muscular do coração.

**Fig. 10** — Lesão do fígado na intoxicação pela arruda: vê-se microvacuolização das células hepáticas.

**Fig. 9** — Muscle hyalinization produced by rue.

**Fig. 10** — Microvacuolization of liver cells, produced by rue.

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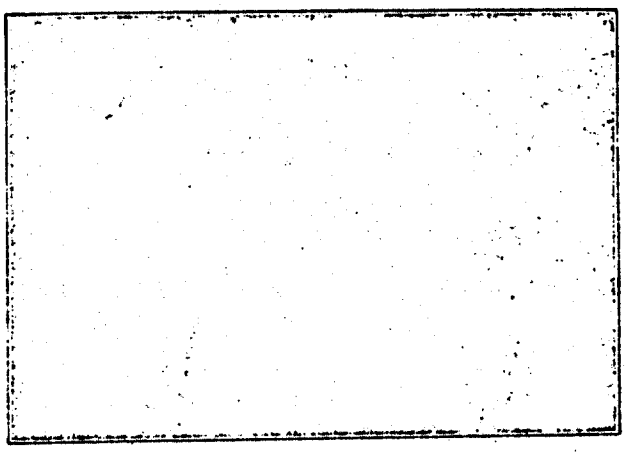

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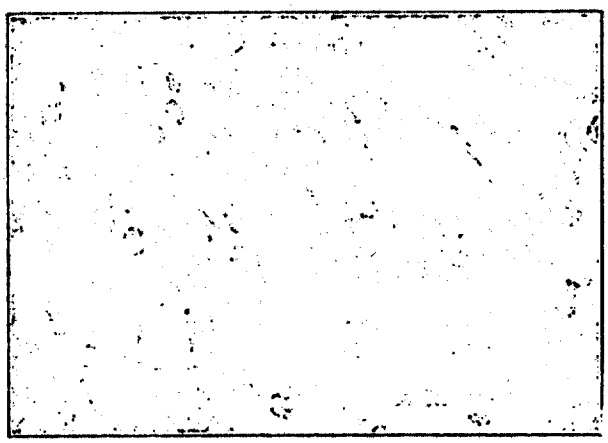

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*Folha V*

**Fig. 11** — Lesão do baço na intoxicação pela arruda: vê-se área de necrose cercada por aréola de reação.

**Fig. 12** — Lesão do útero grávido na intoxicação pela arruda: vê-se, ao centro, o embrião; na mucosa, observa-se sufusão hemorrágica; os envólucros embrionários estão descolados e o embrião não apresenta caracteres de vitalidade.

**Fig. 11** — Spleen necrosis, surrounded by reaction, produced by rue.

**Fig. 12** — Hemorragic suffusion of the decidua, produced by rue.

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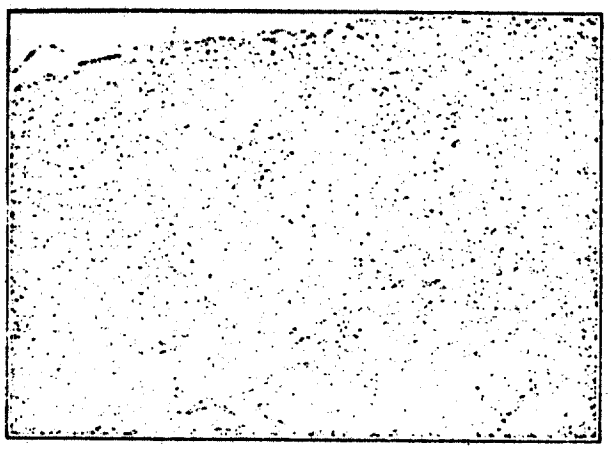

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## Summary

The A. A. studied experimentally, in animals of laboratory, the symptoms produced by the absorption of apiol, savin and rue which are : irregular march, tremblings, equilibrium failure, stopping of the march, limbs contracture, dyspnéa, stertorous breathing, breathing musculature spasm, nauses, bucal hemorrhage, tachycardia, marked cyanosis. On the chemical field after a short survey of the chemical reactions of apiol, savin, and rue, they treated closer with the colorimetric dosage, using standard solutions of the three substances, pure or diluted in alcohol, according to the cases, the pure sulfuric acid, serving as reagent for the apiol and savin and for rue, the FROHDE's reactive.

Tha anatomo-pathological features were : generalized congestion of every organ and degeneration of several parenchymatous organs ; the uterine hemorrhages were toxic manifestations as well as the gastro-intestinal ones, and were not a true menorrhagie. Of the three studied essences, the apiol is the less harmful, attaining only the kidney and liver. The lungs were ever harmed because they are the way of elimination.

The AA. concluded that the action of the studied substances are merely toxic.

(German publication, vol. 9, No. 8, pp. 516-519)

From the Pharmacological Institute, University of Hamburg; Director: Professor Dr.med. G. Malorny

## Percutaneous Resorption of Essential Oils and Their Ingredients

By Fr. Meyer and E. Meyer

It is widely held that essential oils can be absorbed percutaneously (v. Czetsch-Lindenwald and Schmidt-LaBaume, 1950, 1956). For a number of these substances the possibility of percutaneous resorption may be regarded as proven on the basis of the existing literature, but for many others exact experimental dat are still missing.

Pfaffrath was able to show in 1934 that after percutaneous application essenc of thyme, eucalyptus and turpentine are demonstrable in exhaled air and in some slight degree also in urine. Moncorps, Schmidt and Tholey (1937) were able to demonstrate guaiacol in urine after external use in the form of an ointment. Macht (1938) found that essential oils from cinnamon bark, fennel, betula, lemon, orange, anise, peppermint, thyme, Rosa geranium, Caryophyllus aromaticus, Gaultheria procumbens and others can cause, after percutaneous action of 1 cc, unconsciousness and lethal intoxication in mice. He successfully used some of the e.g. anethole, as vehicles. Several alkaloids which as such or in cottonseed an olive oil are not absorbed percutaneously (among them morphine, strychnine, aconitine, atropine, eserine and curare) he was thus able to make effective. Bürgi (1942) jointly with Stähli also examined the resorption of essential oils and found that camphor, Oleum Eucalypti, Thymi, Citri, Terebinthinae, Rosmarini, Bergamottae, Pini, Juniperi and Lavendulae are detectable, after external applica tion, in the exhaled air of rabbits by means of Bürgi's apparatus. In contrast the studies of Macht (1938), in those of Bürgi (1942) oral and pulmonal absorpti were certainly excluded; the treated skin portion was limited and the resorbing surface was thus constant. Recently Valette and Cavier (1945 a, b) made experiments about the resorption of essential oils and found particularly favorable values for alpha-pinene and eucalyptol. As eucalyptol is less volatile than

alpha-pinene, it is especially recommended as vehicle. The authors referred to were able to cause for example dihydrofolliculin (1946 a), testosterone (1946 b) desoxycorticosterone (1947 a), synthetic estrogens (1948 a) and progesterone (194 b) in a mixture of ethanol and eucalyptol to be absorbed percutaneously in relatively large quantity.

Supplementing the findings of Valette and his school, we have included in our comparative studies on the permeability of the skin the essential oils accessible to us and their ingredients and have found great differences in the rate of resorption, as expected.

Some of the tested essential oils came from a collection of our Institute more than 10 years old. It is possible, therefore, that due to polymerization, for instance, the viscosity of some substances was higher than in fresh material and the rate of resorption was therefore found too low.

Method

If a liquid is resorbed by the skin, it functions also as vehicle. A compound incorporated or dissolved therein is absorbed together with it and thus can indicate that resorption has taken place. Eserine is especially suitable as such an indicator (R. Vogel, 1899; G. Valette and R. Cavier, 1951) because it has characteristic, easily recordable effects on striated muscle. The latency between application on the skin and occurrence of the eserine effect on the periodically stimulated chewing muscles of mice was used as a measure of the rate of resorption. The contact or resorbing surfaces were 2.2 $cm^2$ of shaved abdominal skin of 250 male animals. The eserine concentration was 0.25% referred to the base. (For further details of the method cf. Meyer & Kerk, 1959).

Results

In Table 1 are shown some results with aliphatic essential oils and their constituents. In this first, chemically closely related group of compounds with 10-14 C atoms, geranyl formate was absorbed fastest (34 min). Then followed geranyl propionate (38 min), linalyl acetate (52 min), geranyl butyrate (55 min), geranyl acetate (58 min) and citral (63 min). Geraniol and linalool were not r

sorbed within 2 hours in perceptible quantity. However, after subcutaneous injection of 10 gamma of eserine, dissolved in geraniol, there was no increase in the response, so that inactivation of the eserine (incompatibility) or an antagonistic influence on the eserine effect could not be ruled out with certainty. Fo linalool the tertiary OH group seemed to have an adverse effect on the resorptior

Table 1

| Vehicle for eserine (0.25% solution) | Min. to start of rise (arithmetic mean) | Number of tests evaluated |
|---|---|---|
| Citral | 63 | 5 |
| Geraniol | negative | 4 |
| Geranyl acetate, fresh | 58 | 5 |
| Geranyl acetate, viscous | negative | 6 |
| Gernayl formate | 34 | 6 |
| Geranyl propionate | 38 | 5 |
| Gernayl butyrate | 55 | 5 |
| Linalool | negative | 5 |
| Linalyl acetate | 52 | 7 |
| Citronellal, viscous | negative | 6 |
| Ol. Rutae | 27 | 6 |
| Valeric acid diethylamide | negative | 3 |
| Menthyl valerianate | 43 | 5 |

That the viscosity plays an important part in percutaneous resorption is evident from the fact that we tested with negative result an old, thick geranyl acetate, while a fresh, fluid charge was resorbed already after 58 minutes in per ceptible quantity. Also the test with viscous citronellal was negative.

Oleum Rutae, whose main constituent is methylnonyl ketone and which therefore belongs in the aliphatic series, is resorbed very well. Under the described test conditions we found a mean of 27 min. While during the test period limited to 2 h valeric acid diethylamide was not resorbed in perceptible quantity (3 animals),

menthyl valerianate was evidently absorbed relatively quickly despite its great chain length.

Table 2 summariezes the result of the testing of alicyclic compounds, terpene: and those essential oils whose main constituent can be classified in these group: With the exception of two substances, solid terpin hydrate and an old, viscous grade of terpineol (terpinolene), all compounds of these groups were absorbed, i part after a surprisingly short time.

Table 2

| Vehicle for eserine (0.25% solution) | Min. to start of rise (arithmetic mean) | Number of tests evaluated |
|---|---|---|
| Terpin hydrate 5% in cyclohexane | 16 | 6 |
| Terpin hydrate 10% in propanol | negative | 6 |
| Terpineol (*) | 33 | 5 |
| Terpineol, viscous | negative | 7 |
| Terpinyl acetate | 50 | 6 |
| Limonene | 43 | 6 |
| Carvone | 35 | 5 |
| Thymene = l-pinene | 22 | 6 |
| Fenchone | 45 | 6 |
| Fenchyl acetate | 54 | 5 |
| Bornyl acetate | 65 | 6 |
| Ol. camphoricum | 39 | 6 |
| Ol. Pini sibirici | 55 | 5 |
| Ol. Terebinthinae | 62 | 5 |
| Ol. Tanaceti | 38 | 5 |
| Ol. Sabinae | 48 | 5 |
| Ol. Mentae puleg. | 29 | 6 |
| Ol. Fucalypti | 31 | 5 |
| Ol. Menthae pip. | 58 | 5 |

(*) Fresh commercial product

The resorption of a 5% terpin hydrate solution in cyclohexane was recognizabl 16 minutes after external application. It took place practically at the same speed as cyclohexane alone (cf. Meyer, Meyer & Kerk, 1959). A 10% solution of this substance in propanol, however, was not absorbed. Its addition, therefore, does not accelerate the resorption of propanol (cf. Meyer & Kerk, 1959). This means that with respect to percutaneous resorption terpin hydrate is rather indifferent.

Terpineol was absorbed relatively quickly (33 min), despite its tertiary OH group. Its esterification with acetic acid made the resorption slower instead o faster, as might have been expected from the described findings obtained with linalool and linalyl acetate (cf. Table 1). The mean calculated for terpinyl acetate was 50 min.

Limonene (43 min) and carvone (35 min), which occurs abundantly in caraway an dill oil, were resorbed fairly quickly.

In the terpenes and the terpene-containing essential oils, a dependence of percutaneous resorption on the chemical constitution was not seen.

Thymene = l-pinene was resorbed particularly well in this series (22 min). Th followed fenchone (45 min), fenchyl acetate (54 min) and bornyl acetate (65 min) Despite a close chemical relationship, bornyl acetate and camphor (light camphor oil) were absorbed at greatly different speed (39 and 65 min).

Ol. Pini sibirici, which contains predominantly bornyl acetate, l-pinene and santene, as well as Ol. Terebinthinae with a relatively high alpha-pinene conte were resorbed but slowly. The mean values of 5 tests each were 55 and 62 min.

For Ol. Tanaceti with its high thujone content and for Ol. Sabinae which contains compounds chemically very similar thereto (sabinol and sabinol acetate) we found somewhat more favorable values: 38 and 48 min.

The rapid resorption of Ol. Menthae pulegiae (29 min) seems to be attributabl to the 80% of pulegone contained therein. Also the menthone and menthol further contained in pulegol, as well as l-limonene and dipentene, are resorbable percutaneously. But their relatively small proportion suggests that they are of mino importance. Ol. Eucalypti contains, in addition to eucalyptene (alpha-pinene),

pinocarveol, butyric, valeric and caprioic acid aldehyde, predominantly (ca. 75% eucalyptol, the rapid cutaneous absorption of which Valette & Cavier (1945) had found before us. (Concerning local tolerance cf. Oettel. 1936).

The percutaneous resorbability of Ol. Menthae piperitae, which contains 30-50% menthol in addition to menthone, various menthone esters and terpenes, is comparable with Ol. Pini sibirici or Ol. Terebinthinae.

Table 3

| Vehicle for eserine (0.25% solution) | Min. to start of rise (arithmetic mean) | Number of tests evaluated |
|---|---|---|
| Carvacrol | negative | 6 |
| Anethole | " | 6 |
| Ol. Anisi | " | 5 |
| Eugenol | " | 6 |
| Iso-eugenol | " | 6 |
| Safrol | 72 | 6 |
| Cumin oil | 28 | 5 |
| Cumin alcohol | 87 | 5 |
| Cumin aldehyde | 47 | 6 |
| Ol. Thymi | 82 | 5 |
| Ol. Petroselini | 68 | 6 |
| Cinnemal = Cinnamon aldehyde | negative | 3 |
| Cinnamein = Cinnamic acid benzyl ether | " | 5 |

As can be seen from Table 3, in which are listed the aromatic ingredients of essential oils, carvacrol, anethole, Ol. Anisi, eugenol and iso-eugenol are not resorbed by the skin, or only very slowly so. For safrol, instead, the percutaneous absorption was clearly demonstrable within 2 h (mean value 72 min; concerning the different local tolerance cf. Oettel, 1936). Surprising is the short latency period for cumin oil (28 min), which consists of p-cymol, little alpha- and beta-pinene, dipentene, beta-phellandrene, much cuminaldehyde and cumin alcohol. Since cumin alcohol and cuminaldehyde (mean values of 87 and 47 min) are not ab-

sorbed particularly quickly, it seems that p-cymol is primarily responsible for the resorbability of cumin oil. Ol. Petroselini (68 min) and Ol. Thymi (82 min) were resorbed relatively slowly, cinnameine and the very viscous cinnamon aldehy not in perceptible quantity.

Some essential oils which cannot readily be classified in the groups describe until now because of their ingredients are listed in Table 4.

Table 4

| Vehicle for eserine (0.25% solution) | Min. to start of rise (arithmetic mean) | Number of tests evaluated |
|---|---|---|
| Ol. Calami | negative | 5 |
| Pepper oil | 38 | 5 |
| Ol. Galangae | 33 | 6 |
| Patchouli oil | negative | 4 |
| Ol. Fagi ether. | " | 6 |
| Tolubalsam oil | " | 4 |
| Ol. Copaivae | 92 | 5 |
| Ol. Pimentae | negative | 6 |
| Ol. Origani cretici | " | 6 |
| Ajowan oil | " | 6 |
| Ol. Spicae | " | 5 |
| Ol. Juniperi | 59 | 6 |

Except for Ol. Galangae and pepper oil, they are resorbed much more slowly than the oils named in Tables 1 to 3. The values found are 1 hour (Ol. Juniperi 59 min) or much more (Ol. Balsam. Capaiv. 92 min). For Ol. Calami, Fagi aether., Pimentae, Origani cretici and Spicae as well as for patchouli, tolubalsam and ajowan oil, resorption is not detectable during the observation period of 2 h.

To test the percutaneous toxicity, the relatively well resorbing compounds fro Tables 1 to 4 were applied under the same conditions - but without eserine addition - on the skin of 2 mice each, for 4 hours. The result of these orientative control tests is summarized in Table 5.

It shows that essential oils in contact with the epidermis are not indifferen

A dependence of the percutaneous toxicity on the rate of resorption was, however not ascertainable.

Table 5

| Vehicle | Body weight in g | | Exitus letalis after h | | Finding after 4 h | |
|---|---|---|---|---|---|---|
| Citral | 15 | 16 | ½ | 1½ | - | - |
| Geranyl acetate | 16 | 16 | 4 | - | - | no f |
| Geranyl formate | 12 | 19 | - | - | no f. | no f |
| Geranyl propionate | 19 | 18 | - | 4 | no f. | - |
| Geranyl butyrate | 26 | 19 | - | - | no f. | no f |
| Linalyl acetate | 13 | 17 | - | 2 | no f. | - |
| Ol. Rutae | 25 | 25 | 4 | - | - | no f |
| Menthyl valerianate | 18 | 26 | 3 | - | - | no f |
| Terpineol | 12 | 18 | 2½ | - | - | no f |
| Terpinyl acetate | 16 | 19 | - | - | no f. | no f |
| Limonene | 20 | 14 | - | - | no f. | no f |
| Carvone | 18 | 21 | - | - | no f. | no f |
| Thymene | 21 | 17 | - | 3½ | no f. | - |
| Fenchone | 18 | 19 | - | - | no f. | no f |
| Fenchyl acetate | 18 | 24 | - | - | no f. | no f |
| Bornyl acetate | 22 | 20 | - | - | no f. | no f |
| Ol. camphoricum | 21 | 26 | - | - | no f. | no f. |
| Ol. Menthae pulegiae | 17 | 16 | - | - | no f. | no f. |
| Ol. Tanaceti | 16 | 17 | - | 4 | no f. | - |
| Ol. Sabinae | 17 | 19 | - | - | no f. | no f. |
| Ol. Eucalypti | 19 | 16 | - | 2 | no f. | - |
| Ol. Pini sibirici | 17 | 11 | - | - | no f. | no f. |
| Ol. Terebinthinae | 18 | 25 | - | 1½ | no f. | - |
| Safrol | 24 | 19 | 3 | - | - | no f. |
| Cumin oil | 21 | 13 | 3 | - | - | no f |

Table 5 continued.

| | | | | | | |
|---|---|---|---|---|---|---|
| Cumin alcohol | 23 | 19 | - | 4 | no f. | - |
| Cumin aldehyde | 20 | 20 | - | - | no f. | no f |
| Ol. Petroselini | 24 | 16 | - | - | no.f. | no f |
| Pepper oil | 15 | 19 | - | - | no f. | no f |
| Ol. Galangae | 26 | 22 | - | 2 | no f. | - |

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Summary

(given in English in original publication, p. 519)

Literature

(requires no translation, see p. 519)

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Translated by Carl Demrick Associates, Inc./LH/db

Bei großen Kontaktflächen ist eine resorptive Schädigung nicht ausgeschlossen.


## Summary

### *Percutaneous Absorption of Ethereal Oils and their Ingredients*

Ethereal oils and their ingredients are absorbed by the skin with varying velocity. Comparative investigations of the absorption velocity by the intact, shaved abdominal skin in mice showed values between O or <0.5 mm³ and 2—4 mm³/cm² per hour. The absorption was found to be comparatively rapid in geranylformiate, geranylpropionate, terpineol, carvon, thymene, ol. *rutae* poley oil, eucalyptus oil, cumin oil, and ol. *galangae*. In 22 out of 56 substances tested, no absorption was found within the period of observation, which was limited to 2 hrs. Among these were geraniol, linalool, carvacrol, anethol, eugenol, iso-eugenol, cinnemal, cinnamein, ol. *anisi*, ol. *calami*, ol. *fagi* ether, ol. *pimentae*, and ol. *spicae*. Ethereal oils and their ingredients are not absorbed by the skin without exception, neither is the absorption especially favoured, as compared with other solvents. Damage through absorption may occur if the contact areas are large.


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(French publication, pp. 993 - 999)

**RUE AS ABORTIVE AND POISON**

by Dr. Marie Papavassilious and Dr. C. Eliakis

I.

Ruta graveolens L. of the Rutaceae family is a plant widespread in all countries of southern Europe. In France it is known under the names of R. commune, R. des Jardins, Herbe de Grâce, R. fétide or stinking, owing to its strong and unpleasant odor. Known since the remotest antiquity, it is first mentioned by Homer. Thus Mercury gave "Μῶλυ", i.e. wild Rue to Ulysses to save him from the magic power of Circe. Later on Dioscoride gives strange information concerning the qualities of common and wild Rue, both emmenagogic medicines, effective diuretics against pulmonary inflammation, dyspnea, gout and the shivers of intermittent fevers. Moreover Rue is an incomparable antidote for poisons and is again mentioned even by Plutarch, Pliny and Galen. The Arabs used Rue for any illness and the Chinese recommend the flowers of ruta augustifolia against epilepsy.

But in all ages until our time Rue was principally a popular abortive and it is for this quality that the science of the XIXth century knew and studied the plant.

According to a chemical study of Rue made in 1811 by Mähl, it contains: a nitrogenous substance which can be precipitated by tannin, malic acid, starch, gum, chlorophyll and a yellowish oily essence having an aromatic and fetid odor. Weiss later found in the leaves of Rue a glycoside, inactive rutine and rutinic acid.

The active principle is the oil, which has a specific gravity of 0.84, a refractive index at 15° of 1.4639 and boils at 228°C. It consists for the most part of methylnonyl ketone ($C_{22}O_{22}O_2$), of a hydrocarbon of formula $C_{20}H_{16}$ and of an isomer of borneol. It can be oxidized by nitric acid, in acetic and pellargonic acid. Chlorine sharply attacks the essence, forming hydrochloric acid. With iodine it forms gelatinous solutions and with sodium bisulfite it forms crystals. Under heat it rapidly reduces ammonium silver nitrate.

After a series of experiments Hamelin observed that Rue, owing to its essence, causes in laboratory animals: an irritation of the digestive tract particularly towards the pylorus and the beginning of the small intestine. The temperature decreases progressively until collapse. Respiration and pulse slow down in consequence. The nervous phenomena are represented by a narcosis and rarely by convulsions (as was observed only once in the rabbit). But the principal action of the medicament is abortion caused in pregant animals and occurring during the period of collapse, accompanied by congestion or anemia of the genital organs.

Doctor Hélie, who also had the opportunity to study several cases of abortion in women due to Rue, mentions more or less the same symptoms: congestion of the stomach mucus, vomiting, slowing down of the pulse, cooling of the skin, salivation with swelling of the tongue or convulsions, paralysis, abortion.

The mechanism of abortion through Rue is not well known. Is it a secondary phenomenon due to a general upsetting of the maternal organism in which it occurs owing to the congestion first of the digestive tube and then of the womb by a reflex mechanism?

Hamelin claims that the plant has a specific action on the uterus through the nervous system, either directly or indirectly. Rue in the form of powdered leave infusion or ether essence, taken in strong and frequently repeated doses to cause abortion, causes a serious intoxication manifested by stubborn vomiting, at first glairy and then bilious, bloody, following pains in the epigastrium, abundant salivation and burning thirst. The pulse becomes slow and weak or frequent and irregular. Twelve or twenty-four hours after ingestion of the medicament, labor pains start. Delivery is frequently fatal for the woman. Autopsy shows no characteristic lesions, and the test for the essence of Rue is perfomed on the contents of the stomach and intestines by means of water vapor distillation, according to Dragendorff.

II.

Rue is a popular medicament fairly widespread in Greece. Twice recently we have observed the essence mixed with essence of sabine in the viscera of women having undergone abortion. In view of the fact that it had been impossible for u

to make direct observations on this subject for the past thirty years, we conside ed it necessary to become involved with certain medical-legal and toxicological points apt to be of interest to the expert.

I. Can the expert confirm the presence of essence of Rue withdrawn from th viscera, and by what means?

II. Can the presence of sabine and apiol influence the test for Rue?

III. Can the expert observe the presence of Rue by conducting his tests on the product of conception in the absence of other evidence?

As is known, essence of Rue does not have absolutely specific chemical reactions. One must then use more reliable means, such as checking the refractive index. This test, moreover, has the advantage of requiring but one or two drops of essence, easily withdrawn from the viscera, in most cases. We used this analysis with satisfactory results in the two cases of intoxication described below. In the first case a young woman took an infusion of abortive plants in order to rid herself of her child. Abortion ensued, but the woman died. The viscera sent to the laboratory for analysis were the stomach, the spleen,the kidneys, the uterus and part of the liver. These exhibited no microscopic lesio Reduced to pulp, we then subjected them to water vapor distillation, except for a part which we used for detection of caustic, organic poisons, metals and metalloids, all with no positive result. The distillate agitated with light petroleum yielded after evaporation of the solvent, oily droplets which gave:

| | |
|---|---|
| Chemical reactions of Rue and Sabine | very clear |
| Chemical reactions of apiol | negative |
| Refractive index at 15° | 1.4645 |

Inasmuch as the refractive index for Rue is 1.4639, and for Sabine 1.475, we concluded that this was rather a mixture of two oils with a preponderance of essence of Rue.

The second case also involved abortion after an infusion of plants. The visco were sent to us (pieces of the stomach, small intestine, liver, heart and uterus).

The oil withdrawn yielded:

| | |
|---|---|
| Refractive index at 15° | 1.466 |
| Chemical reaction of Rue and Sabine | positive |

From the refraction index we assumed we were in the presence of a mixture of oils.

A comparative study between essence of Rue, essential oil of Sabine, yellow and green apiol, done with standard products (Schimmel firm) relative to the various chemical reactions of these products with the same reagent gave us the following results:

| Reagent | Essence of Rue | Essence of Sabine | Yellow apiol | Green apiol |
|---|---|---|---|---|
| $HNO_3$, fuming | Dark cherry red | Immediate cherry red | Yellow-brown | Olive-green |
| $H_2SO_4$ c | Red-orange | Bright red | Red-brown | Brown |
| $H_2SO_4 + FeCl_3$ | Red-violet | Cherry red | " | " |
| Alcohol HCC | Nil | Pink | Nil | Nil |
| Alcohol $H_2SO_4$ | Pink | Pink | " | " |
| Hot picric acid | Nil | Nil | " | " |
| $NaHSO_3$ | Abundant crystals | Rare crystals | " | " |
| Ammoniacal silver nitrate | Nothing | Rapid reduction | " | " |

From the above it appears that yellow and green apiol produce no chemical reaction similar to those of Rue and Sabine, several reactions of which are almost identical. Essence of Rue, in view of its ketonic nature, reduces the ammoniacal silver nitrate, and this is a characteristic sign. But in toxicology we cannot take advantage of this property. It may be that other volatile substances coming from the viscera can also reduce the ammoniacal silver nitrate. Thus in the case of a mixture of apiol, Rue and Sabine, the presence of apiol does not hinder the test for the others, while the presence of Sabine alone renders the result doubtful.

Finally, in order to see if the placenta is permeable to the essence of Rue, and consequently if it is possible for the expert to confirm abortion by simple

analysis of the fetus, we administered by mouth, to a 750 gram pregnant guinea pig in the final stage of gestation, for three days, 12 drops of essence of Rue added to a certain quantity of oil. On the morning of the third day the animal began to have pains; it writhed and cried. Five hours later it delivered three live baby guinea pigs and after one half hour the afterbirth was expelled, in which we found the essence of Rue (after distillation) by determination of the refractive index.

To a second guinea pig (weighing 840 grams) in the early stage of gestation we administered by mouth 12 drops of essence over four days. The animal aborted and the prematurely expelled fetuses had undergone no maceration likely to lead us to think of intrauterine death.

Distillation of the embryos with the placentas yielded oily drops of the essence.

A pharmacodynamic examination of the Rue, performed at the same time in the Laboratory of Experimental Pharmacy of the University of Athens by M. G. Logaras, our co-worker on this project, showed that Rue causes sharp contractions of the isolated uterus of the guinea pig immersed in a solution of 5 cubic centimeters of infusion of the plant, modification of the cardiac rhythm of the frog (after Straub) and a considerable decrease in blood pressure in cats above a quantity of 2 cubic centimeters of infusion.

CONCLUSION

According to what we have described, Rue still continues to be a fairly widespread abortive in Greece. Abortion is caused by the direct action of Rue on the uterine muscular fiber. The active substance of the plant is the essential oil which is found in all the organs of the aborted animals and which can be identified with certainty, in the cases where this is possible, only by the determination of the refractive index of the oil extracted from the viscera, the placenta and the embryo, owing to the placentar permeability.

(Laboratory of Legal Medicine and Toxicology of the University of Athens. Director: Prof. Dr. J. Géorgiadis).

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Translated by Carl Demrick Associates, Inc./ARB/db

(French publication, pp. 1324-5)

NOTE ON THE ACTION OF THE ESSENCE OF RUE ON THE ANIMAL ORGANISM

by André and Gérard Patoir and H. Bédrine

Continuing our experimental study on the toxicity of vegetal abortives, the first results of which were presented here in July 1936, we administered preparations containing Rue (Ruta) to laboratory animals.

We used essence of Rue, a convenient preparation which was administered by ingestion. The choice of this route is explained by our concern to approximate as nearly as possible the conditions of toxi-medicamental absorption. We therefore use the Nelaton probe, which is fairly easy to insert in the esophagus of commonly used animals.

The doses vary from 250 to 300 drops for guinea pigs weighing 250 to 300 gr., and from 500 to 600 drops for rabbits weighing 2000 to 2500 gr. The experimental and histological results do not match the quantities ingested. We believe this is due to the fact that essence of Rue is not a chemically defined product and we point out that the commercial product is a mixture of Ruta graveolens, Ruta montana and Ruta braceteosa, whose concentrations in methylnonyl ketone and methylheptyl ketone are not the same.

Of 10 animals, 3 died quickly within 7 to 9 days; four, intoxicated a little more slowly, were still alive after 20 days and were sacrificed; three survived and did not seem to suffer further from their intoxication (these three were pregnant).

The animals had dyspnea, diarrhea, torpor, hematemesis and lost weight rapidly.

The histological lesions involve the liver and particularly the kidney; fair granulo-fatty hepatitis in one part; very pronounced parenchymatose nephritis of the epithelial type in the other part. To this must be added an intense congestion involving the entire parenchyma.

The intoxication of the pregnant females showed us that the abortion was not obtained without difficulties and that this result was achieved only at the cost of a deep intoxication; finally the organism of the fetus was also considerably

damaged by the toxic aggression which is not stopped by the placental filter. Hepatonephritis is more intense in the fetus than in the mother.

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# Testing of a less known essential oil of the Croatian coastal region

(28)

By B. Srepel

Aetherol. Rutae divaricatae

Ruta divaricata Ten. /R. graveolens L. var. divaricata Engler, R. graveolens subsp. divaricata Ten. (Gams.)/ occurs as a native plant mainly on the karst heaths around the Adriatic Sea. Its morphological characteristics are the yellowish green, egg-shaped, almost odorless leaves, distinctly dentellated corona petals and only superficially split capsules (1). The essential oil is found localized in schizolysigenous oil-containing cells (2, 3).

The plant material used for the studies was collected in the surroundings of Bakar and Crikvenica during the blooming period of the plant.

## 1. Determination of the oil content

The content of essential oil was determined in an apparatus with reflux distillation (4). Four drug samples were tested. The air-dry plant parts contained the quantities of essential oil shown in Table 1.

Table 1.

Content of essential oil in individual organs of Ruta divaricata Ten. in ml/100 g drug.

| Drug sample No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Leaves | 0.22 | 0.18 | 0.15 | 0.17 |
| Stems | 0.03 | 0.03 | 0.03 | 0.03 |
| Unripe fruits | 4.7 | 4.5 | 3.6 | 3.6 |

## 2. Testing of the properties of the essential oils

From the drug samples of the herb R. divaricata Ten. we prepared by steam distillation sufficient quantities of essential oils to be able to determine the properties. All four oils had a light yellow color and a balsamic-aromatic odor which was not similar to that of methyl-n-nonyl ketone (MNK). In UV light the oils showed only a faint violet-whitish fluorescence. The physical and chemical values of the tested oils appear in Table 2.

Table 2

Properties of the essential oils

| Oil No. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| d 15/15 | 0.9150 | 0.8804 | 0.8903 | 0.8798 |
| alpha $^{D}_{20}$ | + 1.0° | 0° | + 3.0° | + 4.0° |
| $n^{D}_{20}$ | 1.4600 | 1.4515 | 1.4722 | 1.4553 |
| Solidification point | + 4.7° | + 6.5° | + 0.5° | + 3.5° |
| Solubility in vol. of 70% ethanol | 1:4-4.5* | 1:4-4.5* | 1:4-4.5* | 1:4-4.5* |
| Acid number | 10.9 | 10.4 | 11.7 | 12.4 |
| Ester number | 40.1 | 36.7 | 30.6 | 42.7 |
| % ketone as MNK | 58.9 | 59.7 | 54.9 | 63.6 |

(*) with exclusion of wax-like substances

## 3. Some identity reactions of the oils

a) The essential oils of all above-ground plant parts of R. divaricata gave - when diluted with ethanol - after addition of a few drops of 2% iron chloride solution, an intensive, blood red color.

b) With diazotized sulfanilic acid the oils gave a yellow color changing to orange.

c) The reaction for azulene with E.P. reagent proved negative with all tested oils.

## 4. Paper chromatographic test of the carbonyl compounds

From the carbonyl compounds of the essential oils 2,4-dinitrophenyl hydrazone (5,6) were prepared and analyzed by descending separation on a Whatman #1 paper impregnated with kerosene, using 80% ethanol (7). The test substances were similarly produced 2,4-dinitrophenyl hydrazones of MNK, of methyl-n-heptyl ketone (MH and of methyl-n-amyl ketone. The paper chromatographically separated 2,4-dinitrophenyl hydrazones are visible in daylight as yellow to orange spots.

All tested oils of R. divaricata showed the presence of MNK (Rf 0.14), whose identity was confirmed by the melting point and mixed melting point of the 2,4-

dinitrophenyl hydrazone isolated from the chromatograms and purified. The essential oil of the stems contained, in addition to MNK, also MHK and another carbonyl compound not identified.

5. Pharmacodynamic testing of the oils

Tested in vitro on vinegar eels, leeches, tubifex worms and ascarids, and compared with MNK, emulsions of the oils prepared with gum arabic showed only a slight anthelmintic effect.

The toxicity of the oils was determined on white mice (8). For the oil $DL_{50}$ g/kg was 2.07 (P = 0.05, error limit 1.77 - 2.41), for MNK, 3.88 (P = 0.05, error limit 3.48 - 4.34).

(For literature references see p. 110)

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Translated by Carl Demrick Associates, Inc./LH/db

*Tabelle 1.*

**Gehalt an äther. Öl in einzelnen Organen der Ruta divaricata Ten. in ml/100 g Droge**

| Drogenmuster Nr. | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Blätter | 0,22 | 0,18 | 0,15 | 0,17 |
| Stengel | 0,03 | 0,03 | 0,03 | 0,03 |
| Unreife Früchte | 4,7 | 4,5 | 3,6 | 3,6 |

## 2. *Prüfung der Eigenschaften der ätherischen Öle*

Von den Drogenmustern des Krautes der R. divaricata Ten. bereiteten wir durch Wasserdampfdestillation genügende Mengen ätherischer Öle, um ihre Eigenschaften bestimmen zu können. Alle 4 Öle hatten eine lichtgelbe Farbe und einen balsamisch-aromatischen Geruch, der demjenigen des Methyl-n-nonylketons (MNK) nicht ähnlich war. Im UV-Licht zeigten die Öle nur eine schwache violett-weissliche Fluoreszenz. Die physikalischen und chemischen Werte der geprüften Öle befinden sich in der Tabelle 2.

*Tabelle 2.*

**Eigenschaften der äther. Öle**

| Öl Nr | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| d 15/15 | 0,9150 | 0,8804 | 0,8903 | 0,8798 |
| $\alpha_D^{20}$ | +1,0° | 0° | +3,0° | +4,0° |
| $n_D^{20}$ | 1,4600 | 1,4515 | 1,4722 | 1,4553 |
| Erstarrungspunkt | +4,7° | +6,5° | +0,5° | +3,5° |
| Löslichkeit in Vol. 70%-igen Äthanols | 1:4—4,5* | 1:4—4,5* | 1:4—4,5* | 1:4—4,5* |
| Säurezahl | 10,9 | 10,4 | 11,7 | 12,4 |
| Esterzahl | 40,1 | 36,7 | 30,6 | 42,7 |
| % Ketone als MNK | 58,9 | 59,7 | 54,9 | 63,6 |

* unter Ausscheidung wachsähnlicher Substanzen

## 3. *Einige Identitätsreaktionen der Öle*

a) Die ätherischen Öle aller oberirdischen Pflanzenteile der R. divaricata gaben – mit Äthanol verdünnt – nach Zusatz von einigen Tropfen 2%-iger Eisenchloridlösung eine intensive, blutrote Farbe.

b) Mit der diazotierten Sulphanylsäure gaben die Öle eine gelbe Farbe die ins Orange überging.

c) Die Reaktion auf Azulene mit E. P.-Reagens fiel bei allen geprüften Ölen negativ aus.

## 4. *Papierchromatographische Prüfung der Carbonylverbindungen*

Aus den Carbonylverbindungen der ätherischen Öle wurden 2,4-Dinitrophenylhydrazone hergestellt[5, 6] und auf einem mit Kerozen impregnierten Whatman Nr. 1 Papier mit 80%-igem Äthanol absteigend getrennt.[7] Als Testsubstanzen dienten auf gleiche Weise hergestellte 2,4-Dinitrophenylhydrazone des MNK, Methyl-n-heptylketons (MHK) und Methyl-n-amylketons. Die papierchromatographisch getrennten 2,4-Dinitrophenylhydrazone sind bei Tageslicht als gelb bis orange gefärbte Flecke sichtbar.

Alle geprüften Öle der R. divaricata zeigten die gegenwart von MNK (Rf 0,14), dessen Identität durch den Schmelzpunkt und Mischschmelzpunkt des aus den Chromatogrammen isolierten und gereinigten 2,4-Dinitrophenylhydrazons bestätigt wurde. Das ätherische Öl der Stengel enthielt ausser MNK auch MHK und noch eine weitere, nicht identifizierte Carbonylverbindung.

## 5. *Pharmakodynamische Prüfung der Öle*

Mit Gummi arabicum hergestellte Emulsionen der Öle zeigten in vitro auf Essigaalen, Blutegeln, Tubifex-Würmern und Askariden geprüft, und mit MNK verglichen, nur einen geringen anthelmintischen Effekt.

Die Toxizität der Öle wurde an weissen Mäusen bestimmt.[8] Für das Öl betrug $DL_{50}$ g/kg 2,07 (P = 0,05, Fehlergrenze 1,77–2,41), für MNK 3,88 (P = 0,05, Fehlergrenze 3,48–4,34).

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