Carnauba Wax #77 5/30/73

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A Monograph

Prepared for

Department of Health, Education and Welfare

Public Health Service

Food and Drug Administration

Rockville, Maryland

under contract FDA 72-101

by

Dr. Jon Villaume

and

Dr. William Goldman

FIRL Project No. 80G-C3378-01

May 30, 1973

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THE BENJAMIN FRANKLIN PARKWAY · PHILADELPHIA, PENNSYLVANIA 19103

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SUMMARY *

Pertinent biological studies treating Carnauba wax are unavailable in the published literature. Therefore, this monograph will be unusual in not including Biological data or Biochemical aspects sections.

The composition of Carnauba wax is fairly well established. It consists of ca 82% esters of normal saturated monofunctional acids and alcohols with another 15-16% as a free acid or alcohol of this same type. Thus, human processing should be essentially that of long chain monofunctional and saturated alcohols, acids and esters.

Oral toxicity data has not been reported. Although lack of data does not allow safety limits to be set, use in foods is quantitatively small and will not likely exceed 20 mg in a daily diet.

* In addition to standard periodical secondary sources, references listed in the secondary source bibliography on p. 12 have been searched.

CHEMICAL INFORMATION

CARNAUBA WAX

I. Nomenclature

A. Common name: Carnauba wax

B. Chemical name: none

C. Trade name: none

D. CAS Reg. No. MX8015869

II. Composition - Carnauba wax is derived from the leaves of the Carnauba palm. The composition is presented in Table I (taken from Kirk-Othmer Encyclopedia of Chemical Technology, 2nd ed., vol. 22, p. 159). These values are approximate and will vary with the age of the leaf, soil conditions and genetic differences among trees.

Table I. Approximate Composition of Carnauba Wax

Composition	Amount, %	Description
free acids	5.5	primarily C_{24}, C_{26}, C_{28} normal saturated monocarboxylic fatty acids
free alcohols	11	primarily C_{30}, C_{32}, C_{34} normal saturated monohydric primary fatty alcohols, C_{32} dominant (3)
hydrocarbons	1	primarily C_{27}, C_{29}, C_{31} normal saturated hydrocarbons
esters	82	
aliphatic, 40%		45.3% normal saturated monofunctional acids, primarily C_{24}, C_{26}, C_{28} with 54.7% normal saturated monofunctional primary alcohols, primarily C_{30}, C_{32}, C_{34}, with C_{32} dominant
ω-hydroxy[a] 13.2%		47.0% normal saturated monocarboxylic acids. 90% ω-hydroxy acids, primarily C_{22}, C_{24}, C_{26}, C_{28}, and 10% normal saturated monocarboxyl acids, primarily C_{24}, C_{26}, C_{28} with 53.0% normal saturated primary alcohols 90% monohydric alcohols, primarily C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, C_{34}, and 10% α-, ω-dihydric alcohols, (glycols[b]) C_{24}, C_{26}, C_{28}, C_{30}, C_{32}, C_{34}.
cinnamic aliphatic diesters (4), 28.8%		23.0% hydroxycinnamic acid, 5.8% *p*-methoxycinnamic acid, in each case esterified with α-ω glycols as described above
miscellaneous	0.5	a triterpene (5), plant pigments, etc

[a] Average mol. wt. 860. [b] The glycols were esterified with the monofunctional acid.

A study by gas-liquid chromatography has revealed the relative amounts of acids, alcohols and hydrocarbons, as illustrated in table II (ref. 17). Diols, hydroxacids and some long chain length alcohols are not included.

Table II

Normal saturated hydrocarbons, alcohols and acids in Carnauba wax

acids		alcohols		hydrocarbons	
carbon atoms	relative amount (%)	carbon atoms	relative amount (%)	carbon atoms	relative amount (%)
16	0.9	22	trace	24	3.2
18	2.5	24	trace	25	5.4
20	9.8	26	trace	26	8.3
22	10.3	28	2.3	27	13.3
24	47.4	30	13.7	28	16.0
26	9.4	32	69.5	29	16.0
28	13.8	34	14.5	30	12.3
30	5.5	36	trace	31	7.9
				32	6.6
				33	4.1
				other	6.5

III. Specifications

A. Food Chemicals Codex

Acid value: 2 - 10

Ester value: 75 - 85

Melting range: 82 - 86°

Unsaponifiable matter: 50 - 55%

Limits of impurities

Arsenic (as As): 3 ppm

Heavy metsls (as Pb): 40 ppm

Lead: 10 ppm

B. National Formulary, 13th ed.

not listed

C. The Pharmacopeia of the U.S.A., Sept. 1, 1970. 18th revision

Melting range: 81-86°

Residue on ignition: 0.25%

Heavy metals (as Pb): 40 ppm

Acid value: 2 - 7

Saponification value: 80 - 95

D. Chemical Grade

1. Fisher Chemical Co.

specifications not available

2. M C & B Manufacturing Chemists

specifications not available

IV. Description

A. General: It is hard and brittle, has a resinous fracture and ranges in color from light brown to pale yellow (Food Chemicals Codex).

B. Physical Properties

s.p. gr.: 1.0

melting range: 81 - 86°

solubility: insoluble in water; freely soluble in benzene, toluene and warm $CHCl_3$

C. Stability

stable in air

Analytical Methods

1. Separation of major fractions

Ref. 10

Dissolve a sample in $CHCl_3$. Dry a 13 cm long thin layer plate coated with silica gel. Cool, spot the plate with sample and develop the chromatogram with 3:1 trichloroethylene:$CHCl_3$in a tank saturated with the liquid. Running time is ca 1 hr. Dry the plate and spray with 0.05% Rhodamine B. Spots are red-brown under UV. Hydrocarbon, ester, hydroxy acid and alcohol fractions are separated from each other.

2. Separation of acids by column and paper chromatography

Ref. S1

Dissolve 0.5 g beeswax in 50 ml CS_2. Add to a silica gel column to remove hydrocarbons and elute the esters with 20:1 $CHCl_3$:ethanol. Saponify and dissolve the hydrosylate in a minimum of $CHCl_3$ for addition to an Al_2O_3 column. Remove the alcohols with 20:1 $CHCl_3$:ethanol and the acids with 10:1 $CHCl_3$:acetic acid.

Give the chromatograph paper prior treatment with the 230-240° b.p. petroleum fraction in petroleum ether and evaporate the solvent. As the moving medium employ 8:2.5:4:1.25 isopropanol:ethanol:acetic acid: water. After the separation dry the chromatogram and to develop the spots immerse it in copper acetate (15 ml saturated soln to 1 L water and soak the paper in saturated dithiooxamide, with 2 ml conc. NH_3/l added, for 20 min. Finally wash 5x with distilled water.

3. Separation of individual alcohols

Ref. 10

The alcohol fraction was separated and chromatographed as were the acids in no. 2 (ref. 56) above. Develop the spots of individual alcohols with 0.05% Rhodamine B.

4. Gas chromatography of acid, alcohol and hydrocarbon fractions

Ref. 17

Saponify 1 g of Carnauba wax using 50 ml 2 N KOH with gentle boiling for 3 hrs. Add the saponified mixture, 45 ml H_2O, water-alcohol and petroleum ether to a separatory funnel and separate the acid salts from alcohols and hydrocarbons. Acidify the mixture of Carnauba wax acids with HCl and methylate them. Add the alcohol plus hydrocarbon

mixture in petroleum ether to an alumina column. Flush off hydrocarbons with petroleum ether; then elute alcohols with 100:3 petroleum ether: methanol. Gas chromatograph the acid fraction on a 2 m column containing 20% Silicone 710 on 60-80 mesh brick at 300° using 5 l He/hr; chromatograph the hydrocarbons under identical conditions except use a flow of 3 l He/hr; and separate the alcohols on a 2 m column of 20% Silicone Rubber on 60-80 mesh Chromosorb W at 300° or 340° and 3 l He/hr.

5. Quality control tests

Acid value; Ester value; Melting range; Carnauba wax; Fats, Japan wax, rosin and soap; Heavy metals; Arsenic; Lead; and Saponification cloud tests are to be found on p. 170 of Food Chemicals Codex, 2nd ed.

Occurrence

A. Natural: Carnauba wax is derived from the fronds of the Brazilian wax palm (*Copernicia cerifera* Mart.). It can be cultivated in other areas outside of Brazil's rain forest but commercially significant quantities of wax are produced only when grown in this region. The fronds are harvested, dried, threshed and beaten to loosen the wax from the leaf surface, and finally shaken to remove the loosened wax scales, which are then melted and filtered (ref. 11).

B. In foods: used as a candy glaze and polish.

Consumer Exposure Information

Summary of possible daily intake of all Carnauba wax additives from NAS/NRC comprehensive GRAS survey, Oct., 1972, table 13A.

Possible daily intake in 2-65 yr age group, as mg Carnauba wax

Food Category	Average(1)	High A(2)	High B(3)
Baked Goods	0.62	0.92	0.96
Processed Fruit	1.77	3.76	10.65
Soft Candy	2.45	7.43	2.73
Confectionaries	0.04	0.11	0.06
Total	4.88	12.22	14.40

Possible daily intake in 0-5 mos. age group, as mg Carnauba wax

Food Category	Average(1)	High A(2)	High B(3)
Baked Goods	0.02	0.02	0.02
Processed Fruit	0.07	0.19	0.42
Soft Candy	0.08	0.84	0.09
Confectionaries	****(4)	0.01	****(4)
Total	0.17	1.06	0.53

Possible daily intake in 6-11 mos. age group, as mg Carnauba wax

Food Category	Average(1)	High A(2)	High B(3)
Baked Goods	0.11	0.23	0.18
Processed Fruit	0.78	1.94	4.66
Soft Candy	0.93	2.87	1.03
Confectionaries	0.01	0.03	0.02
Total	1.83	5.07	5.89

Possible daily intake in 12-23 mos. age group, as mg Carnauba wax

Food Category	Average(1)	High A(2)	High B(3)
Baked Goods	0.24	0.40	0.38
Processed Fruit	1.51	3.00	9.05
Soft Candy	1.48	3.93	1.64
Confectionaries	0.03	0.10	0.04
Total	3.26	7.43	11.11

(1) Usual additive use and mean consumption

(2) Usual additive use and high consumption

(3) Maximum additive use and mean consumption

(4) Level supplied by the respondents was considered to be incorrectly reported and could not be reconciled.

Summary of levels of Carnauba wax used in regular foods from NAS/NRC comprehensive GRAS survey (Table 2).

Food Category	No. of firms reporting	usual use (mean ppm)	max. use (mean ppm)
Baked goods	*	4.5	7.0
Processed fruit	*	15.0	9.0
Soft candy	13	422.3	470.0
Confectionaries	*	142.9	216.8

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80I

APPENDIX A

(full text copy of all
papers used in the preparation
of the monograph)

to

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Carnauba
10

Dünnschicht-Chromatographie auf dem Fettgebiet IX: Die Analyse der Esterwachse*

Von Prof. Dr. Dr. h. c. H. P. Kaufmann und Dr. B. Das
Aus dem Deutschen Institut für Fettforschung, Münster (Westf.)

Natürliche Wachse wurden mit Hilfe der Adsorptions-Chromatographie auf Dünnschicht-Platten in die einzelnen Stoffklassen getrennt. Die Identifizierung der getrennten Komponenten erfolgte durch entsprechende reine Vergleichssubstanzen. Die Trennung der homologen Reihen der Wachssäuren und Wachsalkohole erfolgte im Umkehrphasen-System, wobei es gelang, in beiden Reihen die einzelnen Homologen mit bis zu 36 C-Atomen zu trennen.

Thinlayer-Chromatography in the Field of Fats IX: Analysis of Wax Esters

Natural waxes were separated with the help of adsorption-chromatography on thinlayer plates into individual classes of components. The identification of the separated components was carried out with the corresponding pure substances. The separation of homologous series of wax acids and wax alcohols was done by reverse phase system, whereby it was possible to separate the individual homologes of both series with upto 36 carbon atoms.

La chromatographie sur plaque dans le domaine des lipides : Analyse des cires naturelles.

Des cires naturelles sont séparées en classes de matière individuelles à l'aide de la chromatographie d'adsorption sur plaques à couche mince. L'identification des composants se fait par les substances témoins pures correspondantes. La séparation des séries homologues des acides et alcools cireux a été effectuée dans le système en phases inversées, ce qui a permis de séparer dans les deux séries, les homologues contenant juqu'à 36 atomes de carbone.

Тонкослойная хроматография в области жиров. Сообщение IX: Анализ сложных эфирных восков.

Адсорбционной хроматографией на тонкослойных пластинках разделяют природные воска на отдельные классы веществ. Идентификация разделенных компонентов производят сравнением с соответствующими чистыми веществами. Разделение гомологичных рядов кислот и спиртов воска осуществляется методом обращения фаз. Этим путем удается определить в обоих рядах отдельные гомологи с числом С-атомов до 36.

Die systematische Analyse der Wachse ist im Hinblick auf deren Mannigfaltigkeit eine schwierige Aufgabe. Sie steckte bis in die jüngste Zeit noch in den Anfängen.

Die älteren Verfahren beruhten auf physikalischen Prüfungen und der Bestimmung der Kennzahlen, wie z. B. der Säurezahl und der Verseifungszahl. Die Herausgabe der ersten, speziell für Wachse ausgearbeiteten Methoden zur Bestimmung von Kennzahlen erfolgte 1930 von der „WIZÜFF" (*Wissenschaftliche Zentralstelle für Öl- und Fettforschung e. V.*, Berlin)[1]. In den letzten Jahren wurden außer den bekannten physikalischen Konstanten Glanz- und Glättezahl[2], Retentionseffekt, Pastenindex und Ölfixierungsvermögen[3] diskutiert. Bei der Darstellung der Säuren und Alkohole versuchte man vielfach durch fraktionierte Kristallisation, bei Säuren insbesondere über die Erdalkali-Salze, zum Ziel zu gelangen. Neuerdings trennt man das Gemisch der Alkohole und Säuren, nachdem letztere in die Methyl- oder Äthylester überführt worden sind, durch fraktionierte Destillation im Hochvakuum[4, 5, 6]. Bei einigen Wachsen gelang auch die Molekular-Destillation bestimmter Wachsester[7, 8]. Die Harnstoff-Addukte wurden ebenfalls zur Trennung der Ester herangezogen[9, 10, 11].

Durch Elementaranalyse, Molekulargewichts-Bestimmung, Schmelzpunkt sowie Säure- oder OH-Zahl identifizierte man die betreffenden Alkohole und Säuren[12]. Röntgenspektren und IR-Analysen lassen erkennen, ob eine langkettige Verbindung frei von Homologen vorliegt oder nicht[14, 15]. Auf Hand von IR-Spektren ist es auch möglich, die Kettenlänge unverzweigter Wachssäuren zu bestimmen, wenn die Säuren in hohem Reinheitsgrad isoliert werden[14, 16, 17, 18]. An neueren Untersuchungsmethoden seien noch die Ultrafiltration[19], die Mikroskopie[20] und die Harnstoff-Reaktivität[21] genannt.

Im Vordergrund der modernen Wachs-Analyse stehen die chromatographischen Verfahren. Sie sind berufen, nicht nur die Forschung zu fördern, sondern auch infolge ihrer Einfachheit in die Praxis überzugehen und überholte Methoden zu ersetzen. Zunächst gelang es, mit säulen-chromatographischen Methoden[22-32] sowohl die

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* Studien auf dem Fettgebiet. 300. Mitteilung: Vortrag anläßlich der DGF-Vortragstagung in Düsseldorf am 29. Oktober 1962.

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einzelnen Verbindungsklassen der Wachse als auch die Homologen der Wachssäuren zu trennen. Dann folgte die Papier-Chromatographie[22, 33–37]. Gegenstand der nachstehenden Untersuchungen ist die erstmals auf dem Wachs-Gebiet angewandte Dünnschicht-Chromatographie (DC). Mit ihrer Hilfe gelang es, die Wachse durch Adsorption auf nicht imprägnierten Platten in die verschiedenen Stoffklassen zu zerlegen sowie die Wachssäuren und Wachsalkohole mit bis zu 36 C-Atomen mit Hilfe der Umkehrphasen-Chromatographie zu trennen.

Trennung der Wachs-Komponenten in Stoffklassen

H. P. Kaufmann und *Z. Makus*[38] trennten mit Hilfe der Dünnschicht-Chromatographie Lipoid-Gemische in verschiedene Stoffklassen, wie Fettsäuren, Keto- und Hydroxysäuren, Aldehyde, Mono-, Di- und Triglyceride usw. Die in Wachsen vorkommenden langkettigen Komponenten machten infolge ihrer geringen Löslichkeit Abänderungen besonders im Hinblick auf das Fließmittel notwendig. Als besonders geeignet erwies sich hier ein Trichloräthylen-Chloroform-Gemisch im Verhältnis 3 : 1. Als Adsorptionsmittel diente Kieselgel G. Mit Hilfe dieses Systems ist es möglich, auch die in den Wachsen vorkommenden Stoffklassen, wie Kohlenwasserstoffe, normale Ester, Hydroxyester, Lactone, Alkohole, Hydroxyalkohole, Sterine, Wachssäuren usw., auf einer Platte zu trennen. Kohlenwasserstoffe haben den größten, freie Fettsäuren den kleinsten Rf-Wert. Die freien Fettsäuren bleiben in diesem System praktisch am Startpunkt. Bei Bienenwachs kann man bei Zimmertemperatur (ca. 22° C) arbeiten. Dagegen lassen sich Carnauba-, Schellack- und Wollwachs wegen ihrer geringeren Löslichkeit erst bei 42° C leicht trennen.

Arbeitsvorschrift

Als Adsorptionsmittel diente Kieselgel G für Dünnschicht-Chromatographie (Fa. *E. Merck*, Darmstadt). Die nach *E. Stahl*[39] beschichteten Platten wurden ca. 90 Min. im Trockenschrank bei 120° C getrocknet und in einem Exsikkator über P_2O_5 aufbewahrt. Alle benutzten reinsten Lösungsmittel wurden zusätzlich getrocknet, da der Wassergehalt einen sehr großen und nachteiligen Einfluß auf den Trennungsvorgang ausübt.

Die Wachse und die verschiedenen Vergleichssubstanzen wurden in Chloroform-Lösung (gegebenenfalls erwärmt) auf die markierten Startpunkte in einer Linie aufgetropft. Nach dem Verdunsten des Chloroforms wurde die Platte mit dem Fließmittel Trichloräthylen-Chloroform (3 : 1) entwickelt. Das Entwicklungsgefäß muß mit dem Fließmittel gesättigt sein. Zu diesem Zweck wurde es von innen mit Filtrierpapier bekleidet. Man kann die Kieselgel-Platte zum Auswaschen organischer Verunreinigungen vor dem Auftropfen einmal mit Benzol entwickeln und dann in einem Trockenschrank trocknen, wodurch Trennung und Anfärbung besser werden. Zur Entwicklung bei höherer Temperatur (42° C) wurde die Platte nach dem Auftropfen der Proben 4 bis 5 Min. bei 75° C erwärmt, um ein Auskristallisieren der aufgetropften Substanzen zu verhindern und um die Platte auf etwa gleiche Temperatur mit dem Fließmittel zu bringen. Dann wurde sie in die in einem Brutschrank befindliche Tennkammer eingestellt. Die Laufzeit betrug etwa 1 Std.

Nach dem Entwickeln des Chromatogramms — die Steighöhe soll etwa 13 bis 14 cm betragen — wurde das Fließmittel im Trockenschrank bei 100° C vollständig entfernt (ca. 30 Min.) und dann mit einer 0,05%igen wäßrigen Lösung von Rhodamin B besprüht. Man erhält im UV-Licht rotbraune Flecken auf gelbem Hintergrund.

Zur Identifizierung der einzelnen Flecke wurden folgende Vergleichssubstanzen nach der Methode von *H. P. Kaufmann* und *J. Pollerberg*[40] präparativ hergestellt: Stearyl-stearat, Stearyl-ricinolat, Stearyl-9,10-dihydroxystearat, Stearyl-3-keto-palmitat und Distearyl-sebazat, und zwar Stearyl-stearat aus Stearoylchlorid und Stearylalkohol mit Pyridin als Katalysator, und die übrigen Ester durch direkte Veresterung der Säuren mit entsprechenden Alkoholen unter Zusatz von p-Toluolsulfonsäure als Katalysator. Die Ester wurden über eine Aluminiumoxyd-Säule mit Benzol als Elutionsmittel von freien Fettsäuren gereinigt und mehrmals aus Äthanol umkristallisiert. Die Reinheit wurde dünnschicht-chromatographisch kontrolliert.

Abb. 1 zeigt schematisch die Dünnschicht-Analyse des Bienenwachses. Als Vergleichssubstanzen wurden aufgetropft: Wachssäuren, Ricinolalkohol, langkettige Wachsalkohole, Paraffin als Beispiel eines Kohlenwasserstoffes, Ester langkettiger Alkohole mit Wachssäuren, Hydroxyfettsäureester (Stearyl-ricinolat), Ester von Di-

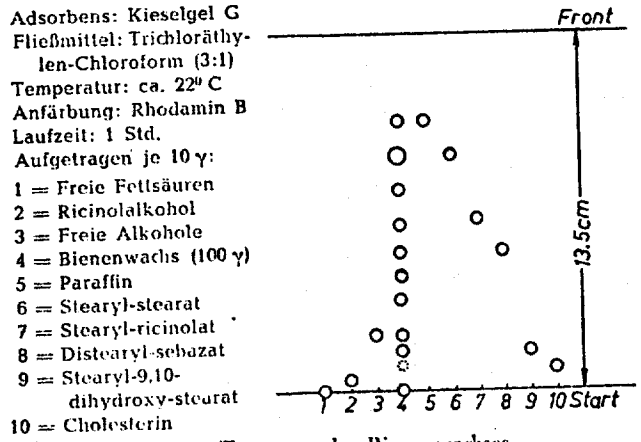

Abb. 1. Trennung des Bienenwachses

carbonsäuren mit Wachsalkoholen (Distearyl-sebazat) und Dihydroxyfettsäureester (Stearyl-9,10-dihydroxystearat) und Cholesterin. Beim Carnaubawachs (Abb. 2) wurden neben den bereits erwähnten Testsubstanzen auch noch Stigmasterin, Sitosterin und der Stearylester der 3-Keto-palmitinsäure als Vergleichssub-

[27] *G. Spengler* u. *E. Wöllner*, Fette · Seifen · Anstrichmittel **56**, 775 [1954].
[28] *G. Spengler* u. *G. Hanf*, Fette · Seifen · Anstrichmittel **57**, 474 [1955]; **59**, 509, 607 [1957].
[29] *G. Spengler* u. *E. Jantzen*, Fette · Seifen · Anstrichmittel **62**, 19 [1960]; **63**, 530 [1961].
[30] *L. J. N. Cole* u. *J. B. Brown*, J. Amer. Oil Chemists' Soc. **37**, 359 [1960].
[31] *W. Presting* u. *S. Jänicke*, Fette · Seifen · Anstrichmittel **62**, 81 [1960]; **63**, 49 [1961].
[32] *A. J. Howard* u. *D. Hamer*, J. Amer. Oil Chemists' Soc. **39**, 250 [1962].
[33] *H. P. Kaufmann* u. *B. Das*, Fette · Seifen · Anstrichmittel **63**, 614 [1961].
[34] *H. P. Kaufmann* u. *H. G. Kohlmeyer*, Fette · Seifen · Anstrichmittel **57**, 231 [1955].
[35] Diss. *J. Pollerberg*, Münster **1958**.
[36] *H. P. Kaufmann* u. *G. Kessen*, Hoppe-Seyler's Z. Physiol. Chem. **317**, 43 [1959].
[37] *H. P. Kaufmann* u. *B. Das*, Vortrag anläßlich der DGF-Vortragstagung in Hamburg, Oktober **1961**.
[38] Fette · Seifen · Anstrichmittel **62**, 1014 [1960].
[39] Pharmazie **11**, 633 [1956].
[40] Fette · Seifen · Anstrichmittel **64**, 908 [1962].

dann in einem Exsikkator über P_2O_5 aufbewahrt.

Imprägnierung

Die trockene Dünnschicht-Platte wurde vorsichtig in eine 3%ige Lösung von Tetradecan stand. in Petroläther (Sdp. 40° bis 60° C) eingetaucht. Nach dem Auftropfen der in Chloroform gelösten Substanzen (gegebenenfalls wurde die Lösung vor dem Auftropfen erwärmt) beließ man die Platte 20 Min. bei Zimmertemperatur. Man erwärmte sie 4 bis 5 Min. im Trockenschrank bei 70° C und entwickelte in einem Brutschrank bei 42° bis 43° C.

Fließmittel

Als Fließmittel diente ein Gemisch aus Isopropanol, Äthanol, Eisessig und Wasser im Verhältnis 8 : 3 : 4 : 1.3, das mit dem Imprägnierungsmittel bei 42° C gesättigt war. Nach dem Entwickeln des Chromatogramms wurden Fließ- und Imprägnierungsmittel im Trockenschrank entfernt, indem man die Kieselgur-Platte 1 Std. auf 170° C und die Gips-Platte 2½ Std. auf 90° bis 95° C erhitzte.

Anfärbung

Die Kieselgur-Platte kann mit Rhodamin B angefärbt werden. Eine bessere Anfärbung ergab das Besprühen mit einer 1%igen Lösung von α-Cyclodextrin in 30%igem Äthanol, Trocknen an der Luft und anschließende Behandlung mit Joddampf. Man erhielt weiße Flecken auf braunem Hintergrund. Eine stöchiometrische Anfärbung der Säuren auf der Gipsschicht erreichte man bei der Behandlung mit Kupferacetat und Rubeanwasserstoff[42], ähnlich wie bei der pc-Analyse. Die Platte wurde zu diesem Zweck zunächst mit dest. Wasser gewaschen. Dann tauchte man sie 12 Min. in eine Kupferacetat-Lösung (20 ml gesättigte Kupferacetat-Lösung in 1 l Wasser) und wusch anschließend 35 Min. mit fließendem Wasser, zum Schluß einige Male mit dest. Wasser, um den Überschuß von Kupferacetat zu entfernen, und legte die Platte dann 10 bis 15 Min. in eine 0.1%ige äthanolische Lösung von

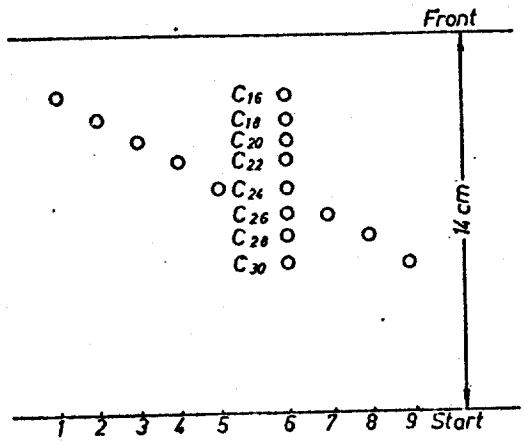

Abb. 5. Trennung gesättigter Wachssäuren

Trennung der stationären Phase: Gips
Fließmittel: Isopropanol-Äthanol-Eisessig-Wasser (8:3:4:1.3)
Imprägnierungsmittel: Petroleum-Fraktion vom Sdp. 240° bis 250° C
Temperatur: 42° bis 43° C
Laufzeit: 3 Std.
Anfärbung: Kupferacetat-Rubeanwasserstoff
Aufgetragen je 3 γ:

1 = C_{16}
2 = C_{18}
3 = C_{20}
4 = C_{22}
5 = C_{24}
6 = 1 bis 9 zusammen
7 = C_{26}
8 = C_{28}
9 = C_{30}

[42] *H. P. Kaufmann* u. *T. H. Khoe*, Fette · Seifen · Anstrichmittel 64, 81 [1962].

In Abb. 5 ist die Trennung reiner langkettiger Fettsäuren von C_{16} bis C_{30} schematisch dargestellt. Wie Abb. 6 zeigt, können mit Hilfe dieser Methode auch die Wachssäuren natürlicher Wachse analysiert werden. So enthält

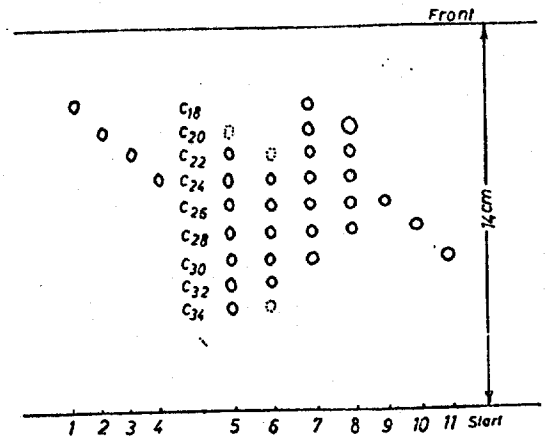

Abb. 6. Trennung der Wachssäuren des Bienen-, Carnauba-, Montan- und Sonnenblumenwachses

Bedingungen wie in Abb. 5
Aufgetragen je 3 γ:
1 = C_{18}
2 = C_{20}
3 = C_{22}
4 = C_{24}
5 = Bienenwachssäuren (in Methanol unlösliche Fraktion) (25 γ)
6 = Montanwachssäuren (20 γ)
7 = Carnaubawachssäuren (25 γ)
8 = Sonnenblumenwachssäuren (22 γ)
9 = C_{26}
10 = C_{28}
11 = C_{30}

Montanwachs Säuren von C_{22} bis C_{34}, Carnaubawachs von C_{18} bis C_{30} und Bienenwachs in der in Methanol unlöslichen Fraktion Säuren von C_{20} bis C_{34}. Im Sonnenblumenwachs konnten Säuren von C_{20} bis C_{24} nachgewiesen werden; dabei überwiegen Arachin- und Behensäure. Neben der von anderen Autoren bereits erwähnten Cerotin- und Montansäure fanden wir also noch Arachin-, Behen- und Lignocerinsäure. Diese Säuren wurden auch mit Hilfe des bereits früher beschriebenen Verfahrens papier-chromatographisch getrennt und auf photometrischem Wege quantitativ bestimmt (Tab. 1).

Tabelle 1

Säuren des Sonnenblumenwachses

Säuren	Gew.-%
Arachinsäure	43.9
Behensäure	26.6
Lignocerinsäure	10.3
Cerotinsäure	8.2
Octacosansäure	11.0
	100.0

Trennung der Wachsalkohole

Auch über die pc-Trennung langkettiger Alkohole haben *H. P. Kaufmann* und Mitarbb. bereits früher berichtet[31-37].

Sie wurden entweder nach ihrer Umsetzung mit Allylisocyanat zu Allylurethanen oder aber direkt unter Anwendung höherer Temperaturen getrennt. Bei dem letztgenannten Ver-

dann in einem Exsikkator über P_2O_5 aufbewahrt.

Imprägnierung

Die trockene Dünnschicht-Platte wurde vorsichtig in eine 2%ige Lösung von Tetradecan stand. in Petroläther (Sdp. 40° bis 60° C) eingetaucht. Nach dem Auftropfen der in Chloroform gelösten Substanzen (gegebenenfalls wurde die Lösung vor dem Auftropfen erwärmt) beließ man die Platte 20 Min. bei Zimmertemperatur. Man erwärmte sie 4 bis 5 Min. im Trockenschrank bei 70° C und entwickelte in einem Brutschrank bei 42° bis 43° C.

Fließmittel

Als Fließmittel diente ein Gemisch aus Isopropanol, Äthanol, Eisessig und Wasser im Verhältnis 8 : 3 : 4 : 1.3, das mit dem Imprägnierungsmittel bei 42° C gesättigt war. Nach dem Entwickeln des Chromatogramms wurden Fließ- und Imprägnierungsmittel im Trockenschrank entfernt, indem man die Kieselgur-Platte 1 Std. auf 170° C und die Gips-Platte 2½ Std. auf 90° bis 95° C erhitzte.

Anfärbung

Die Kieselgur-Platte kann mit Rhodamin B angefärbt werden. Eine bessere Anfärbung ergab das Besprühen mit einer 1%igen Lösung von α-Cyclodextrin in 30%igem Äthanol, Trocknen an der Luft und anschließende Behandlung mit Joddampf. Man erhielt weiße Flecken auf braunem Hintergrund. Eine stöchiometrische Anfärbung der Säuren auf der Gipsschicht erreichte man bei der Behandlung mit Kupferacetat und Rubeanwasserstoff[42], ähnlich wie bei der pc-Analyse. Die Platte wurde zu diesem Zweck zunächst mit dest. Wasser gewaschen. Dann tauchte man sie 12 Min. in eine Kupferacetat-Lösung (20 ml gesättigte Kupferacetat-Lösung in 1 l Wasser) und wusch anschließend 35 Min. mit fließendem Wasser, zum Schluß einige Male mit dest. Wasser, um den Überschuß von Kupferacetat zu entfernen, und legte die Platte dann 10 bis 15 Min. in eine 0.1%ige äthanolische Lösung von

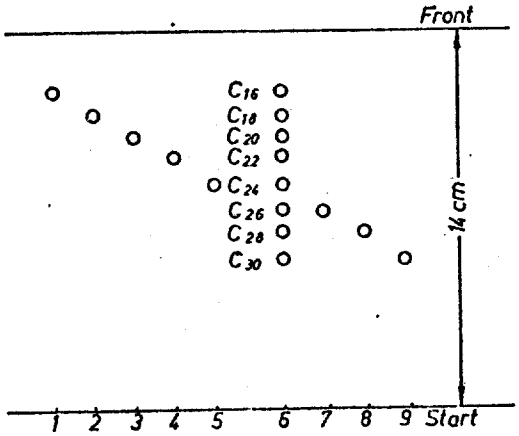

Abb. 5. Trennung gesättigter Wachssäuren

Trennung der stationären Phase: Gips
Fließmittel: Isopropanol-Äthanol-Eisessig-Wasser (8:3:4:1.3)
Imprägnierungsmittel: Petroleum-Fraktion vom Sdp. 240° bis 250° C
Temperatur: 42° bis 43° C
Laufzeit: 3 Std.
Anfärbung: Kupferacetat-Rubeanwasserstoff
Aufgetragen je 3 γ:

1 = C_{16}
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[42] *H. P. Kaufmann* u. *T. H. Khoe*, Fette · Seifen · Anstrichmittel 64, 81 [1962].

In Abb. 5 ist die Trennung reiner langkettiger Fettsäuren von C_{16} bis C_{30} schematisch dargestellt. Wie Abb. 6 zeigt, können mit Hilfe dieser Methode auch die Wachssäuren natürlicher Wachse analysiert werden. So enthält

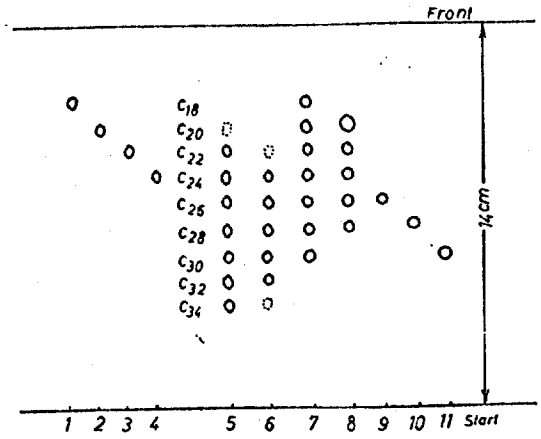

Abb. 6. Trennung der Wachssäuren des Bienen-, Carnauba-, Montan- und Sonnenblumenwachses

Bedingungen wie in Abb. 5
Aufgetragen je 3 γ:
1 = C_{16}
2 = C_{20}
3 = C_{22}
4 = C_{24}
5 = Bienenwachssäuren (in Methanol unlösliche Fraktion) (25 γ)
6 = Montanwachssäuren (20 γ)
7 = Carnaubawachssäuren (25 γ)
8 = Sonnenblumenwachssäuren (22 γ)
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Cerotinsäure	8.2
Octacosansäure	11.0
	100.0

Trennung der Wachsalkohole

Auch über die pc-Trennung langkettiger Alkohole haben *H. P. Kaufmann* und Mitarbb. bereits früher berichtet[31–37].

Sie wurden entweder nach ihrer Umsetzung mit Allylisocyanat zu Allylurethanen oder aber direkt unter Anwendung höherer Temperaturen getrennt. Bei dem letztgenannten Ver-

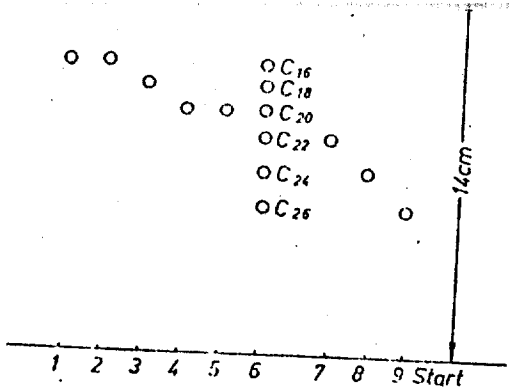

Abb. 7. Trennung synthetischer Wachsalkohole

Träger der stationären Phase: Kieselgur G
Fließmittel: Isopropanol-Äthanol-Eisessig-Wasser (8:3:4:2) mit Tetradecan stand. gesättigt
Imprägnierungsmittel: Tetradecan stand., 5 % in Petroläther
Temperatur: 42° bis 42.5° C
Laufzeit: ca. 2 Std.
Anfärbung: Rhodamin B
Aufgetragen je 4 γ:

1 = Cetyl-
2 = Oleyl-
3 = Stearyl-
4 = Arachidyl-
5 = Erucyl-
6 = 1 bis 9 zusammen
7 = Behenyl-
8 = Lignoceryl-
9 = Cerylalkohol

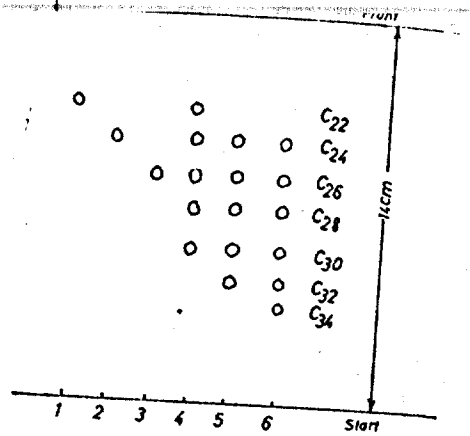

Abb. 8. Trennung der natürlichen Wachsalkohole

Fließmittel: Isopropanol-Äthanol-Eisessig-Wasser (8:3:4:1.5)
Die übrigen Bedingungen wie in Abb. 7
Aufgetragen je 4 γ:

1 = Behenylalkohol
2 = Lignocerylalkohol
3 = Cerylalkohol
4 = Alkohole des Sonnenblumenwachses (25 γ)
5 = Alkohole des Montanwachses (25 γ)
6 = Alkohole des Bienenwachses (25 γ)

fahren[37] wurden die Papiere mit einer Petroleum-Fraktion vom Sdp. 230° bis 240° C als 10%ige Lösung in Petroläther imprägniert und bei 42° C mit einem Gemisch von Isopropanol, Eisessig, Äthanol und Wasser im Verhältnis 8 : 4 : 3.5 : 1.6 als Fließmittel entwickelt. Nach der Entwicklung wurden die Chromatogramme 2 Std. bei 145° C getrocknet und anschließend mit Rhodamin B angefärbt.

Die dünnschicht-chromatographische Trennung langkettiger Alkohole gelingt mit einer ähnlichen Arbeitstechnik, wie sie bei der Trennung der Wachssäuren angewandt wurde. Sie erfolgte wieder im Umkehrphasen-System auf mit „Tetradecan stand." imprägnierten Platten und einem Gemisch von Isopropanol, Äthanol, Eisessig und Wasser im Verhältnis 8 : 3 : 4 : 2 als Fließmittel bei 42° C; die Laufzeit betrug 2.5 Std. Nach der Entwicklung wurden die Chromatogramme im Trockenschrank getrocknet und mit einer 0.1%igen Lösung von Rhodamin B besprüht. Auch hier traten kritische Partner auf, so z. B. Cetyl- und Oleylalkohol und Arachidyl- und Erucylalkohol. Abb. 7 zeigt schematisch die Trennung gesättigter und ungesättigter Wachsalkohole. In Abb. 8 ist die Trennung natürlicher Wachsalkohole mit den entsprechenden synthetischen Vergleichssubstanzen dargestellt. Montanwachs enthält Alkohole von C_{24} bis C_{32}, Bienenwachs von C_{24} bis C_{34} und Sonnenblumenwachs von C_{22} bis C_{30}. Damit wurden im Sonnenblumenwachs neben dem bereits in der Literatur erwähnten Ceryl- und Montanylalkohol noch Behenyl-, Lignoceryl- und Myricylalkohol nachgewiesen.

Die beschriebenen Versuche zeigen, daß die Dünnschicht-Chromatographie auch auf dem Gebiet der Wachs-Analyse mit Erfolg angewandt werden kann.

Untersuchungen auf dem Gebiet der thermopolymerisierten Pflanzenöle, II. Mitteilung

Von Dr. E. Fedeli, Dr. A. Valentini und Prof. Dr. G. Jacini

Stazione sperimentale Oli e Grassi, Milano

Bei der Untersuchung von thermopolymerisierten Pflanzenölen konnte festgestellt werden, daß sich die Polymeren anfangs ausschließlich durch Dimerisation bilden, während bei höheren Temperaturen und längerer Erhitzungszeit auch tetramere und höhermolekulare Produkte entstehen.

Etudes dans le domaine des huiles végétales thermopolymérisées. II.

L'examen des huiles végétales thermopolymérisées a permis de constater qu'au début, les polymères se forment exclusivement par dimérisation, cependant que des températures supérieures et une durée de chauffage prolongée occasionnent également la formation de tétramères et de produits macromoléculaires.

Studies in the Field of Heat-Polymerised Vegetable Oils. II.

In the investigations on the heat-polymerised vegetable oils it was established that in the beginning the polymeres are formed exclusively through the dimerisation, while at higher temperatures and longer heating periods also tetramers and higher molecular-weight products appear.

Исследования в области термополяризованных растительных масел. Сообщение II.

Исследованием термополяризованных растительных масел установливается, что сначала полимеры образуются исключительно димеризацией и что при высоких температурах и более продолжительном нагревании образуются также тетрамерные и более высокомолекулярные продукты.

THIN-LAYER CHROMATOGRAPHY IN THE FIELD OF FATS IX:

ANALYSIS OF WAX ESTERS

Prof. Dr. Dr. R. C. H. P. Kaufmann and Dr. B. Das
German Institute for Lipid Research, Munster (Westf.)

The systematic analysis of waxes is a difficult problem in view of their diversity. At present it is still in its infancy.

The older methods were based on physical tests and the determination of constants, as for example, the acid number and the saponification number. The first method worked out especially for the determination of constants for waxes was published in 1930 by the "WIZOFF" (Wissenschaftliche Zentralstelle fuer Oel-und Fettforschung e.v., Berlin)[1]. In later years in addition to the known physical constants, shine value and slip index[2], retention effect, paste index and oil absorption capacity[3] were discussed. With the preparation of the acids and the alcohols, many experiments were carried out by fractional crystallization with acids, especially the alkaline earth salts, in order to attain the objective. Recently, after separating a mixture of alcohols and acids, the latter were transformed into the methyl or ethyl ester by fractional distillation in a high vacuum[4,5,6]. In the case of several waxes, the molecular distillation of certain wax esters was successful[7,8]. The urea-adducts were also employed to separate the esters[9,10,11].

By elementary analysis, molecular weight determination and melting point, as well as the acid number or the base number, the respective alcohols and acids were identified[12]. X-ray spectroscopy[13] and IR analysis were used to determine whether a long chain compound free of homologs were present[14,15]. With IR spectra it is also possible to determine the length of the chain of unbranched wax acids, if the acids were isolated to a high degree of purity[14,16,17,18]. Ultrafiltration[19], microscopy[20] and urea-reactivity[21] have been designated as newer methods of investigation.

Chromatographic methods are in the foreground in modern wax analysis. They are replacing former methods not only because of their facilitation of research but also because of the ease of practical application. Mainly, column chromatographic methods[22-32] effect the separation of individual compound classes of waxes as well as the homologs of the wax acids. Paper chromatography[22,33-37] would be second. In the following experiments thin-layer chromatography is applied to the field of waxes for the first time. Using thin-layer with adsorption on non-impregnated plates, the waxes can be resolved into the various classes and the wax acids and wax alcohols with up to 36 carbon atoms can be separated by reverse phase chromatography.

SEPARATION OF THE WAX COMPONENTS INTO CLASS GROUPS

H. P. Kaufmann and F. Makus[38] using thin-layer chromatography separated a lipoid mixture into various class groups as fatty acids, keto acids, hydroxy acids, aldehydes, mono-, di-, and triglycerides and so forth. The long chain components occurring in the waxes, because of their slight solubility, cause variations especially from the viewpoint of the necessary mobile phase. Especially suitable here is a trichloroethylene-chloroform mixture in the ratio 3:1, using silica gel G. as the adsorbent. With this system it is also possible to separate on a plate the class groups occurring in the waxes, as, hydrocarbons, normal esters, hydroxy esters, lactones, alcohols, hydroxy alcohols, steroids, wax acids and so on. Hydrocarbons have the higher Rf values and free fatty acids have the lowest Rf values. In this system the free fatty acids remain practically at the starting point. Beeswax can be tested at room temperature (ca 22° C). Carnauba wax, shellac wax and wool wax, because of their lesser solubilities, separate easily at 42° C.

EXPERIMENTAL

Silica gel G (E. Merck, Darmstadt) was the adsorbent for thin-layer chromatography. According to E. Stahl[39] the layered plates were dryed in a drying chamber for approximately 90 minutes at 120° C and stored in a desiccator over P^2O^5. All pure reagents used were dried, since the water content produces a very great and detrimental influence on the separation process.

The waxes and the various reference standards in a chloroform solution (warmed if necessary) were droppered in series onto the marked starting point. Following the evaporation of the chloroform, the plate was developed with the mobile phase trichloroethylenechloroform (3:1). The developing tank must be saturated with the mobile phase. To attain this the inside of the tank was lined with filter paper. To wash out organic impurities, the silica gel plate can be developed with benzene before the spotting and then dried in a drying chamber, after which separation and coloring improve. For developing at a higher temperature (42° C), the plate was warmed for 4-5 minutes at 75° C after spotting the samples in order to prevent the test material from crystallizing out and in order to bring the plate to the same temperature as the solvent. The plate was then placed in an incubator. The flow time was approximately 1 hour.

Following the development of the chromatogram - the height of ascent was about 13 to 14 cm - the solvent was completely removed at 100° C (ca 30 minutes) in a drying chamber and was then sprayed with a 0.05% aqueous solution of Rhodamin B. Reddish brown spots against a yellow background were shown in UV light.

According to the method of H. P. Kaufmann and J. Pollerberg[40], the following reference standards were prepared with which to identify the original spots: stearyl stearate, stearyl ricinolate, stearyl-9, 10-dihydroxy stearate, stearyl-3-keto palmitate and distearyl sebacate. Stearyl stearate was prepared from stearoyl chloride and stearyl alcohol with pyridine as the catalyst and the remaining esters were prepared by direct esterification of the acids with the corresponding alcohols on addition of p-toluenesulfonic acid as the catalyst. The esters were removed from free fatty acids on an aluminum oxide column with benzene as the eluting agent. Repeated recrystallization from benzene followed. Purity was checked by thin-layer chromatography.

Fig I shows the thin-layer analysis of beeswax schematically. As reference standards the following were spotted: wax acids, ricinol alcohol, long chain wax alcohols, paraffin as an example of hydrocarbons, esters of long chain alcohols with wax acids, hydroxy fatty acid esters (stearyl ricinolate), esters of dicarboxylic acids with wax alcohols (distearyl sebacate) and dihydroxy fatty acid esters (stearyl-9,10-dihydroxy stearate) and cholesterol. In the case of carnauba wax (Fig. 2), reference standards of stigmasterin, sitosterin and the stearyl ester of 3-ketopalmitic acid were prepared. The esters of the keto acids are present in the waxes in small amounts and can be satisfactorily determined as yellow spots by thin-layer chromatography if the wax has been previously reacted with dinitrophenylhydrazine. The spots appear colored brown on spraying with methanolic KOH. Shellac wax and wool wax (Fig. 3) are somewhat more difficult to separate. By applying a mixture of trichloroethylene-chloroform in a 5:2 ratio as the solvent, wool wax can also be separated at room temperature. The thin-layer separation of the wax of sunflower seed (Fig. 4) indicated that this wax contains essentially the esters of long chain alcohols and acids with traces of some other material.

Concerning this analysis only two reports are to be found in the literature. According to A. Bareuther[12], recrystallization from petroleum ether, ethanol and chloroform yields ceryl cerotate. I Tsuchiya and coworkers[41], after fractional crystallization, determined the presence of octacosanyl octacosanate. The sunflower wax used in our investigations was obtained by wax extraction of the precipited oil in the wax-containing residue. To obtain an oil-free, pure wax, the mixture of wax and oil was extracted for several days with ether previously cooled from 0° to 2° C, then dissolving the extract in chloroform and filtering out impurities. The wax had a high melting point of 73.2° C, a saponification number of 85.7 and an iodine number of 1.4.

SEPARATION OF WAX ACIDS

The high molecular fatty acids and wax acids were separated paper chromatographically as mercury adducts of their allyl esters by H. P. Kaufmann and J. Pollerberg[22]. We recently reported a short method for direct paper chromatographic separation of the unchanged wax acids[33]. Contrary to this method the thin-layer chromatography offers a greater saving of time.

To identify the acids it was necessary to pretreat the total mixture of waxes. This was performed in the manner previously described[33]. The hydrocarbons were extracted from the mixture on a silica gel column.

The waxes were then saponified and the acids were separated from the alcohols on an Al_2O_3 column.

H. P. Kaufmann and Z. Makus[38] effected a separation of fatty acids of up to 22 carbon atoms by thin-layer chromatography using an undecane/acetic acid-acetonitrile system. In separating the high molecular wax acids it was shown to be necessary, with the waxes that have been mentioned, to work at higher temperatures and with a different solvent-system. A temperature of 42° C was especially suitable. A hydrocarbon fraction of boiling point 240° to 250° C (Tetradecane standard, Haltermann, Hamburg) was used as the stationary phase. A complete separation can be effected after developing the chromatogram by heating in a drying chamber at 170° C for 1 hour. As a carrier for the stationary phase, a silica gel plate can be used rather than a gypsum plate, and can later be washed in water after coloring, without any damage.

EXPERIMENTAL

As carrier for the stationary phase either silica gel G for thin-layer chromatography (E. Merck, Darmstadt) or gypsum[42] (from the same firm) was used. The coated silica gel G plates were dryed in a chamber at 120° C, while the gypsum plates were dryed at 90° to 95° C (ca 70 minutes). The plates were then stored in a desiccator over P_2O_5.

STATIONARY PHASE

The dry thin-layer plate was carefully immersed in a 5% solution of Tetradecane standard in petroleum ether (B.P. 40°-60°C). After spotting the chloroform solutions of the test materials (when necessary the test solution was warmed before spotting), the plates were allowed to stand at room temperature for 20 minutes. They were then warmed for 4-5 minutes in a drying chamber at 70° C and developed in an incubator at 42-43° C.

MOBILE PHASE

A mixture of isopropanol, ethanol, acetic acid and water in a ratio of 8:3:4:1.3, saturated with the stationary phase at 42° C, was used as the mobile phase. After developing the chromatogram, the stationary and mobile phases were removed in a drying chamber in which the silica gel plates were heated for 1 hour at 170° C and the gypsum plates were heated for 2.5 hours at 90° to 95° C.

COLORING

The silica gel plates can be colored with Rhodamin B. A better result is obtained by spraying with a 1% solution of α-cyclodextrin in 30% ethanol, air drying, and treating with iodine vapor. White spots on a brown background result. A stochiometric coloring of the acids on gypsum can be attained by treatment with copper acetate and dithiooxamide[42], similar to the paper chromatographic analysis. The plates were washed in distilled water. They were then immersed for 12 minutes in a copper acetate solution (20 ml of saturated copper acetate solution in 1 L% water), washed under running water for 35 minutes, washed several times with distilled water to remove the excess copper acetate, placed in a 0.1% ethanol solution of dithiooxamide for 10-15 minutes, and washed again with distilled water. Dark green spots against a white background are obtained.

In Fig. 5 the separation of pure, long chain fatty acids from C_{16} to C_{30} is schematically presented. As is shown in Fig. 6, this method can be used in the analysis of the wax acids of natural waxes. Montan wax contains acids from C_{22} to C_{34}, carnauba wax from C_{18} to C_{30} and beeswax contains acids of C_{20} to C_{34} in the methanol insoluble fraction. In sunflower wax acids from C_{20} to C_{28} can be determined: predominantly arachic and behenic acids. In addition to the cerotin and montan acids cited by other authors, we found arachic, behenic and lignoceric acids. These acids were separated by paper chromatography as described previously and were quantitatively determined photometrically (Tab.1).

TABLE 1

ACIDS OF SUNFLOWER WAXES

Acids	Weight %
Arachic acid	43.9
Behenic acid	26.6
Lignoceric acid	10.3
Cerotinic acid	8.2
Octacosanoic acid	11.0
	100.0

SEPARATION OF THE WAX ALCOHOLS

H. P. Kaufmann and coworkers have reported earlier on the paper chromatographic separation of the long chain alcohols[34-37].

They were separated either by their reactions with allyl isocyanate to allyl urethanes or separated directly by application of higher temperatures. In the latter named method[37], the paper was impregnated with a petroleum fraction of boiling point from 230° to 240° C (10% solution in petroleum ether). The development took place at 42° C using a mixture of isopropanol, acetic acid, ethanol and water in the ratio 8:4:3.5:1.6 as the mobile phase. After developing the chromatogram was dried for two hours at 145° C and then colored with Rhodamin B.

The thin-layer chromatographic separation of long chain alcohols was performed by a similar procedure, as was applied to the separation of the wax acids. This was effected in a reverse phase system with "Tetradecane standard" impregnated plates, with a mobile phase of isopropanol, ethanol, acetic acid, and water in the ratio 8:3:4:2. At a temperature of 42° C the flow time was 2.5 hours. After developing the chromatogram was dryed in a drying chamber and then sprayed with a 0.1% solution of Rhodamin B. Critical pairs occur here as: cetyl and oleyl alcohols and arachidyl and erucyl alcohols. Fig. 7 shows the separation of satruated and unsaturated wax alcohols schematically. The separation of natural wax alcohols with the corresponding synthetic reference compounds is presented in Fig. 8. Montan wax contains alcohols from C_{24} to C_{32}, beeswax from C_{24} to C_{34} and sunflower wax from C_{22} to C_{30}. In addition to the ceryl alcohol and montanyl alcohol mentioned in the literature, behenyl alcohol, lignoceryl alcohol and myricyl alcohol were determined in sunflower wax.

The experiments described prove that thin-layer chromatography can be applied with success to the field of wax analysis.

Adsorbent: Silica gel G
Solvent: Trichloroethylene-
Chloroform (3:1)
Temperature: ca 22° C

Color Agent: Rhodamin B

Flow time: 1 Hour

Amount applied (10 γ each)

1 = FREE FATTY ACIDS
2 = RICINOL ALCOHOL
3 = FREE ALCOHOL
4 = BEESWAX (100 γ)
5 = PARAFFIN
6 = STEARYL STEARATE
7 = STEARYL RICINOLATE
8 = DISTEARYL SEBACATE
9 = STEARYL-9,10-DIHYDROXY STEARATE
10 = CHOLESTEROL

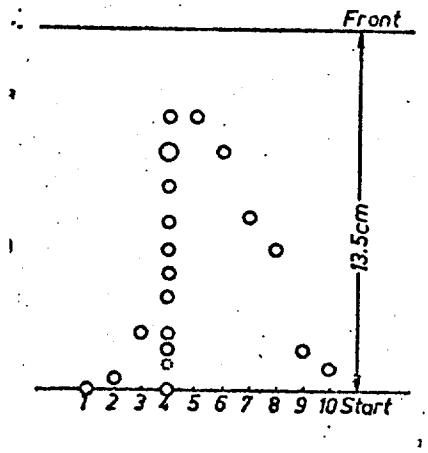

Fig. 1 SEPARATION OF BEESWAX

Temperature 42° C

All other conditions as in Fig. 1

Amount applied (10 γ each)

1. Free fatty acids
2. Ricinol alcohol
3. Free alcohols
4. Carnauba wax (120 γ)
5. Paraffin
6. Stearyl stearate
7. Stearyl ricinolate
8. Distearyl sebacate
9. Stearyl-3-keto palmitate
10. Stearyl-9,10-dihydroxy stearate
11. Stigmasterin
12. Sitosterin

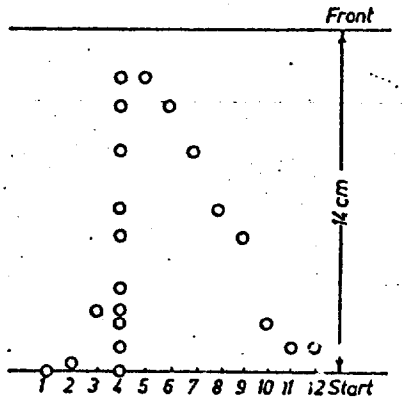

Fig. 2. SEPARATION OF CARNAUBA WAX

Conditions as in Fig. 2

Amount applied (10 γ each)

1. Free fatty acids
2. Ricinol alcohol
3. Free alcohols
4. Shellac wax (80 γ)
5. Wool wax (70 γ)
6. Paraffin
7. Cholesteryl palmitate
8. Stearyl stearate
9. Stearyl ricinolate
10. Distearyl sebacate
11. A 22 carbon atom lactone
12. Stearyl-9,10-dihydroxy stearate
13. Cholesterol
14. Stigmasterin

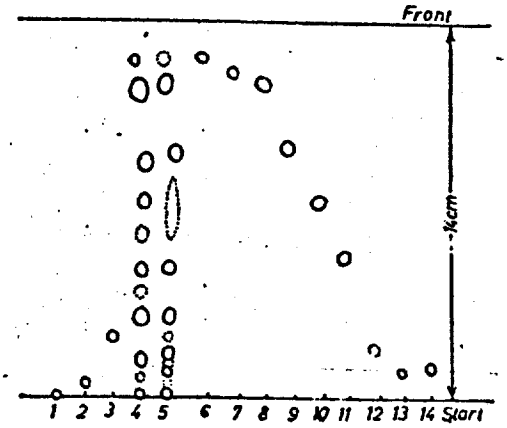

Fig. 3. SEPARATION OF SHELLAC WAXES AND WOOL WAXES

Conditions as in Fig. 2

Amount applied (10 γ each)

1. Fatty acids
2. Free alcohols
3. Sunflower wax (100 γ)
4. Paraffin
5. Stearyl stearate
6. Sitosterin

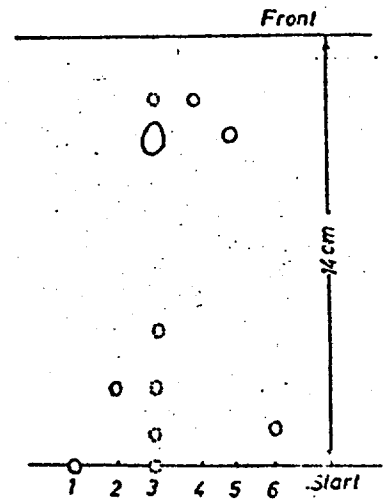

Fig. 4. SEPARATION OF SUNFLOWER WAXES

Carrier of the stationary phase: Gypsum
Mobile phase: Isopropanol-ethanol-acetic acid-water (8:3:4:1.3)
Petroleum fraction of boiling point 240° to 250°C
Temperature: 42° to 43°
Flow Time : 3 Hours
Coloring agent: copper acetate-dithiooxamide
Amount applied (3 γ each)

1. C_{16}
2. C_{18}
3. C_{20}
4. C_{22}
5. C_{24}
6. C_1-C_9
7. C_{26}
8. C_{28}
9. C_{30}

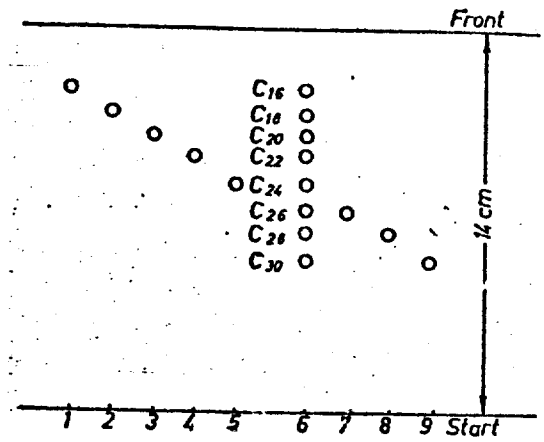

Fig. 5. SEPARATION OF SATURATED WAX ACIDS

Conditions as in Fig. 5.
Amount applied (3 γ each)

1. C_{18}
2. C_{20}
3. C_{22}
4. C_{24}
5. Beeswax acids (insoluble fraction (25 γ) in methanol)
6. Montan wax acids (20 γ)
7. Carnauba wax acids (25 γ)
8. Sunflower wax acids (22 γ)
9. C_{26}
10. C_{28}
11. C_{30}

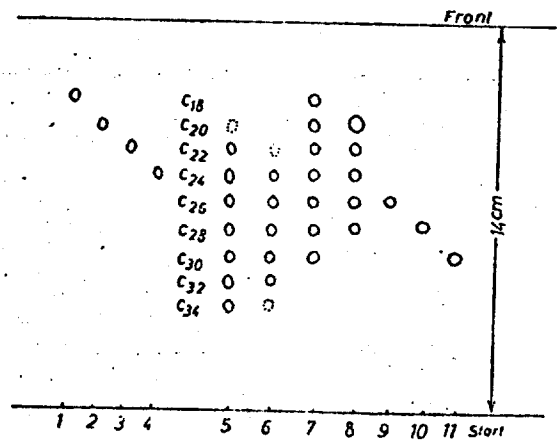

Fig. 6. SEPARATION OF THE WAX ACIDS OF BEESWAX, CARNAUBA WAX, MONTAN WAX AND SUNFLOWER WAX

Carrier of the stationary phase: Silica gel G
Mobile phase = Isopropanol-ethanol-acetic acid-water (8:3:4:2) saturated with tetradecane standard
Stationary phase: Tetradecane standard, 5% in petroleum ether
Temperature: 42° to 42.5° C
Flow time: ca 2 hours
Coloring agent: Rhodamin B
Amount applied (4 γ each)

1. Cetyl-
2. Oleyl-
3. Stearyl-
4. Arachidyl-
5. Erucyl-
6. C_1-C_9
7. Behenyl-
8. Lignoceryl-
9. Ceryl alcohol

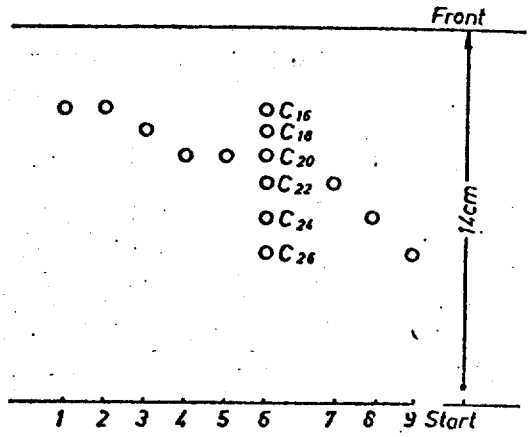

Fig. 7. SEPARATION OF SYNTHETIC WAX ALCOHOLS

Mobile phase: Isopropanol-ethanol-acetic acid-water (8:3:4:1.5)
Remaining conditions as in Fig. 7.
Amount applied (4 γ each)

1. Behenyl alcohol
2. Lignoceryl alcohol
3. Ceryl alcohol
4. Alcohols of sunflower waxes (25 γ)
5. Alcohols of montan waxes (25 γ)
6. Alcohols of Beeswaxes (25 γ)

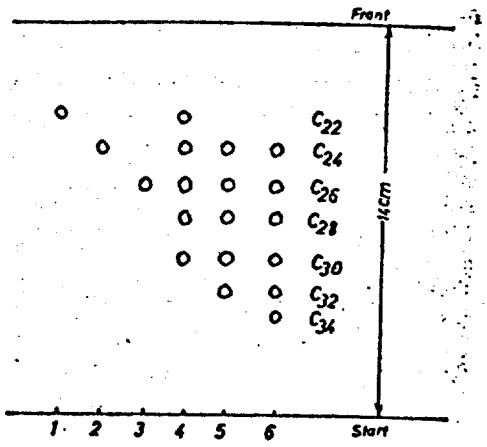

Fig. 8 SEPARATION OF NATURAL WAX ALCOHOLS

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Carnauba—Brazil's Wax Palm

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It is not altogether strange that some of the most unusual and useful plants thrive in fantastic climates and places on the face of the earth. It is in these diversified climates and geographical centers that Nature has worked tirelessly since the forming of the ameba in order to preserve life and establish a law which is known the world over as the survival of the fittest. She is continually shifting her fluid battlefronts as the elements attempt to erase the work of thousands of years. Entire mountain ranges disappear below the surfaces of the waters, causing ocean currents to change and climates to vary: sun spots appear and disappear, causing drought and heavy rainfall where rain has been normal.

In not only the plant but also the animal kingdom, life must evolute in order to be sustained. From the sun-scorched deserts where spiny cacti build up their resistance to heat and drought, to the dainty edelweiss in the Alps, plants must be "custom-built" in order to conform with Nature. Likewise from the darkest depths of the ocean where fish are equipped with electrical devices for seeing, to the cold white expanses of the arctic where giant polar bears grow thick layers of fat to protect against the cold and white fur to cut down visibility to the hunter's eye, animals must also be custom-built in order to survive.

It is in one of these strange climatic areas of the earth that a most useful tree species thrives and survives the unusual combination of too much and too little rainfall. This tree is known as the carnaúba palm (*Copernicia cerifera* Mart.) and, because of its many useful qualities, was given the name "The Tree of Life" by Humboldt, the famous naturalist and explorer.

The author has studied waxes for 12 years and has one of the most complete collections in existence. Since Pearl Harbor he has made four trips to Mexico and two to South America, the most recent being a 25,000-mile jaz entirely around that continent. A second article, discussing the chemical industry of South America, will appear in an early issue of Chemical and Engineering News.

Brazil is one of the most diversified countries in the world and therefore it is not too strange that the carnaúba palm grows in this great botanical garden spot. About half-way between where the mighty Amazon disgorges her turbulent waters past an island in her mouth as large as Belgium, and Natal, farthest eastern tip of South America, lie the Brazilian states of Maranhão, Ceará, Piauí, and Rio Grande do Norte. Although the carnaúba palm grows in other states in the northeast of Brazil, these four states are the leaders in the production of the most important vegetable wax in the world, carnaúba wax.

Although carnaúba wax is useful in our daily lives, and is used extensively in the industrial field, very little is known and has been written about the carnaúba palm and its production. A recent trip in the carnaúba-producing states of Brazil gave the writer opportunity to study and photograph the carnaúba industry as well as the people who are connected with it.

Brazil's past economy has woven a pattern of ups and downs hinged on vegetable products. First it was sugar; then, in 1839, Charles Goodyear discovered the vulcanization process and the great rubber boom of the Amazon forests was on. Brazil flourished and rubber became an important addition to her national income. Then, in 1876, an Englishman named Henry Wickham gathered some Hevea seeds and took them back to England, where he grew them in the Kew Gardens at London. They were then transplanted to the botanical gardens in Colombo, Ceylon. From Colombo came the first seeds to start the great rubber plantations in Malaya and Sumatra. Plantation rubber proved to be more productive than wild rubber and consequently the bottom fell out of the rubber industry in Brazil. Coffee has also played an important role in the economy of Brazil, but this has had competition in other parts of the world. The carnaúba wax palm has stood its ground since its discovery and has defied not only the greatest scientists to produce it synthetically but also other nations to grow it successfully. Seeds from the palm have been planted in other parts of the world having nearly the same latitude, particularly Ceylon, but little success has

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been attained. The palm will grow but does not produce wax. It is only the peculiar combinations of temperature, soil, long droughts, and extreme floods that produce wax-bearing carnaúba palms. Nature has trained the palm to form a protective coating of wax on its fronds to prevent excessive evaporation during the long droughts.

The carnaúba palm grows wild and in profusion throughout northeastern Brazil, growing best in a coastal strip between the Atlantic and 50 miles inland. Only in recent years has any attempt been made to cultivate and grow carnaúba in plantation style, probably because it is extremely slow-growing and does not become productive until about 10 years of age. It increases in its productivity of wax and is said to reach its maximum at 50 to 60 years. Unlike most palms, the carnaúba has a long life span and it is not uncommon for it to live 200 years. It grows to heights of from 50 to 60 feet. The carnaúba palm requires plenty of sunshine and air and dwarfs the other vegetation around it. It prefers to grow along the banks of rivers and lakes, but it is also found in small isolated groves from the damp lowlands along the coast to the forests of the interior. It is found on dry, sandy ground and resists the floods which sweep the countryside during the rainy season.

Collecting Carnaúba Fronds

The leaves or fronds are collected for wax production about 3 or 4 months after the rainy season ends. It is picturesque to see the carnaúba palm clippers traveling through the forests with tiny donkeys or burros laden with the freshly cut fronds. They are so covered with the fanlike fronds that many times the donkey is hardly visible, and it looks like a mound of Chinese fans being shoved along by the fierce winds which sweep through this belt of Brazil.

The fronds are cut by means of a small scythe-shaped knife lashed to the end of a long bamboo pole. An experienced clipper can cut around 3,000 fronds in a day. (It takes approximately 100 fronds to produce one pound of wax.) Clippers know exactly how many fronds can be cut from a tree without injuring the tree. Chemists who work with this wax should appreciate it more when they learn that each tree produces only an average of 6 ounces per year! Naturally, since the war the demand for carnaúba has increased greatly and clippers must travel farther into the interior in order to obtain the wax. This is partially the reason for the rise in price. Along the coastal areas clippers are paid around 10 cruzeiros, or 50 cents (U. S.) per 1,000 leaves clipped, which seems to be a fair wage in this section of Brazil. It is said that 35 to 40 million trees are worked annually, producing between 11,000 and 12,000 tons of carnaúba wax, or a total of 14,000 to 15,000 tons of all waxes, including uricury wax which also comes from a palm and has grown in importance since the war. The United States of America is Brazil's largest customer, taking nearly 70% of her exports.

After the leaves are clipped they are brought in to a central clearing in the carnaúba forest and laid out in neat rows on the ground to dry in the tropical sunshine for about 5 days, during which time they are turned over several times. Following this they are taken to small nearby houses for threshing. These houses contain posts 3 feet high with a V-shaped angle protruding from the end. In the notch of the V are several steel teeth. The fronds are whipped across the teeth two or three times in order to shred them and loosen the wax which covers the entire surface of the fronds and between the folds, then laid on the floor and beaten with special sticks to thresh the wax from them. They are then picked up and shaken and the fine fluffy wax powder floats down like sifting snow. When the wax threshers emerge from the houses, they look as though they had been exposed to a heavy frost, as the white powder covers them and clings to their eyelashes and eyebrows like frozen ice crystals. The warm tropical sun has nothing in common with frost, however.

After the wax has been threshed it is swept up, placed in bags, and taken to refineries. Sometimes crude refining is carried on at the threshing points. The wax is melted in kettles, poured into molds, and allowed to solidify, then broken up into blocks and bagged for export. In case it is refined, it is melted and forced through coarse cloth with the aid of wheel presses. No doubt the Brazilian refined wax will improve as soon as equipment can be obtained. Wax is refined in the United States by several processes, the most common being melting the wax, mixing in certain filter aids, and then forcing it through steam-heated filter presses. The flake wax is produced by running the molten refined wax over metal drums in thin layers, allowing it to solidify, and scraping it off with a doctor blade.

The various grades of carnaúba wax in Brazil are classified as follows: Primeira, Mediana, Caubype, Gorda Clara, Gordarosa, and Aromsa. In the U.S.A. these grades are known, respectively, as No. 1 Yellow, No. 2 Yellow, Caubype Light Yellow, North Country No. 2, North Country No. 3, and Chalky No. 3.

The light yellow grades come from the new fronds which are found at the top of the tree. The wax has not been subjected to the elements so long and consequently has not had a chance to pick up flying sand and dirt. The darker grades come from the older fronds and contain more impurities in the way of sand and dirt. The wax and dirt which remain after the Brazilian refining process are further refined by melting in boiling water, the wax rising to the top and the dirt settling to the bottom. This is classified as chalky wax. The chalky wax is extracted with boiling water and gets its chalky gray color from the process as well as from the impurities.

Most chemists know of the importance of the wax but few realize the other products which the carnaúba tree yields and the important part which it plays in the lives of the people who live in the carnaúba areas.

Carnaúba palm fronds are brought in from the forest

Spiny trunk of 10-year-old carnaúba palm

During the great droughts the tree actually sustains life.

Day after day at the predicted beginning of the rainy season the natives cast their eyes in the direction of the dry blue skies for the indication of one little rain cloud. They watch the streams for broken branches floating by which might indicate rains at the headwaters of the rivers. If it rains, a great celebration is held. If not, they prepare for an influx of population from the drought-stricken areas of the interior. (The Brazilian Government is alleviating this greatly by the construction of huge dams.) It is during these periods that the stately carnaúba palm rises up, shaking her fan-bedecked head in the swift winds, and seems to say, "Fear not, for I am the Tree of Life".

When everything else shrivels and seems to die, the carnaúba palm reaches its greatest productivity in wax. Cattle can live on the shoots of the young carnaúba palms which emerge from the baked earth. The tender hearts of the palm become a green vegetable or salad. The tree bears long clusters of edible fruit not unlike a date. The seed is crushed and a cooking oil is extracted. The shell around the nut is roasted and a coffeelike beverage is brewed. The pith of the tree contains a high starch content and a food similar to farina is made. The roots yield an extract and medicine for illness. The wood is burned and the ashes yield an alkali for home soap making. The wood of the tree is extremely hard and is impervious to insects and sea water. It is used in building the frames of native homes. The fronds of the palm are used to thatch their houses. Most of the natives in the carnaúba sections sleep in hammocks, and it is said that 80% of the people sleep in hammocks woven from carnaúba frond fibers. In addition to this they weave their hats, mats, baskets, and belts from the fibers. Their homes are swept with brooms made from the faulike fronds and candles made from the wax furnish light at night. Tons of fronds which have been discarded after the wax has been removed may play an important part in the new Brazil, for a high-grade paper has been made from it. Were it not for the lack of shipping bottoms and the distance from the paper mills in the south, the fibers might already be taking their place in the paper industry. Experiments by the Brazilian Government have shown that the straw is a possible source of cellulose. The discarded fronds, although it takes them a long time to disintegrate, are sometimes allowed to decay on top of the ground to enrich and mulch the soil for agriculture.

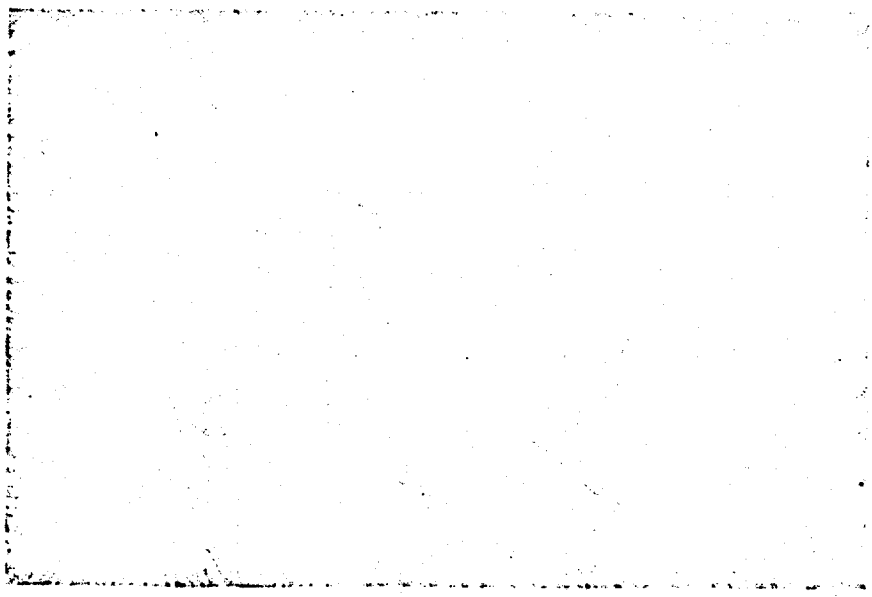
Brazil is sprawled out over half of South America and is thousands of square miles larger than the United States. Like the United States, the people vary in their customs and speech in different sections of the country. They have their gauchos, or cowboys of the south, the same as we. The Cariocas, as the people of Rio de Janeiro are known, and the Paulistas, of São Paulo, the great industrial center of South America, are other types. Farther up the coast lies Bahía, where the people are predominantly black. On the northeast shoulder the carnaúba country starts, and the Cearense from the State of Ceará are typical of this section. Despite flood and drought, these people are extremely healthy. As in the United States, Brazilians carry the strains of many bloods. The Cearense are descendants mostly of Portuguese, Indian, and some negro. Far from the thriving industrial centers of the south, these people live mostly from the products of the land. Carnaúba wax is an important industry in Ceará.

Other Industries

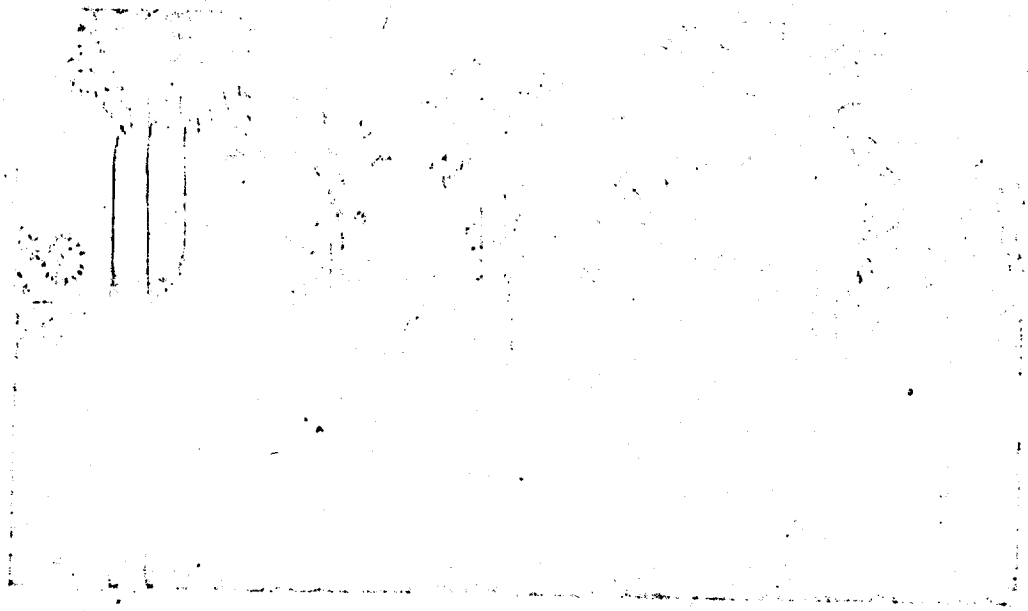
The oiticica oil industry is likewise gaining prominence. This drying oil, which is being used to replace tung oil, also owes its existence to the peculiar climatic conditions of Ceará. It is said the fruit or nut from the oiticica was the first incendiary bomb. Its discoverer was traveling in the interior and saw many small columns of smoke rising from the ground. Upon examination he found that the nut from the oiticica had caught fire, owing to spontaneous combustion from the high oil content.

Along the coast of the northeast of Brazil, the winds roll up the waters of the blue Atlantic, forming mighty waves which boom and crash against the palm-fringed sandy beaches. It is in these waters that still another occupation is carried on—sailing and fishing. The jangadeiros, as the sailors are known, perform one of the most dangerous types of sailing in the world. Their boats, jangadas, are made by fastening light ceiba logs together with wooden pegs and planting a huge sail in the center of the raftlike boat. This boat has no outrigger or rudder and is navigated by manipulation of the sail. These brave fishermen, who use only a hook and line for fishing, sail out into the open sea as far as 75 miles. It is colorful to see a line of them appear over the horizon late in the evening, crashing through the breakers, skillfully balancing the topheavy craft, and landing on the beach with the ease of a sled. The huge basket of fish which is lashed to the jangada is unloaded and the people rush down from the villages to buy fish for their evening meal.

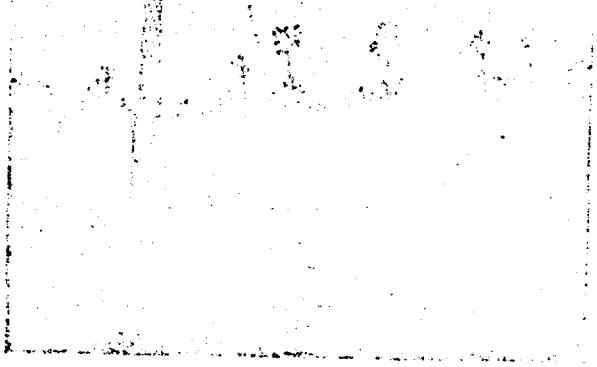
A great number of chapters could be



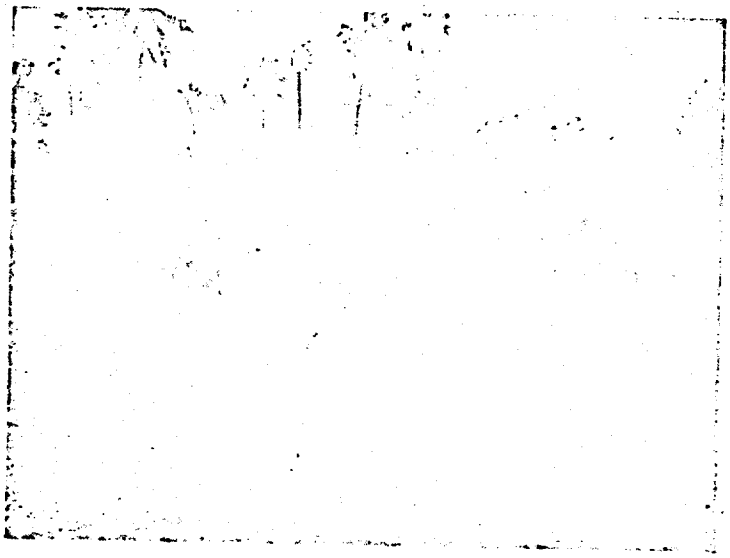
Author inspects carnaúba fronds left to dry in the sun before threshing.



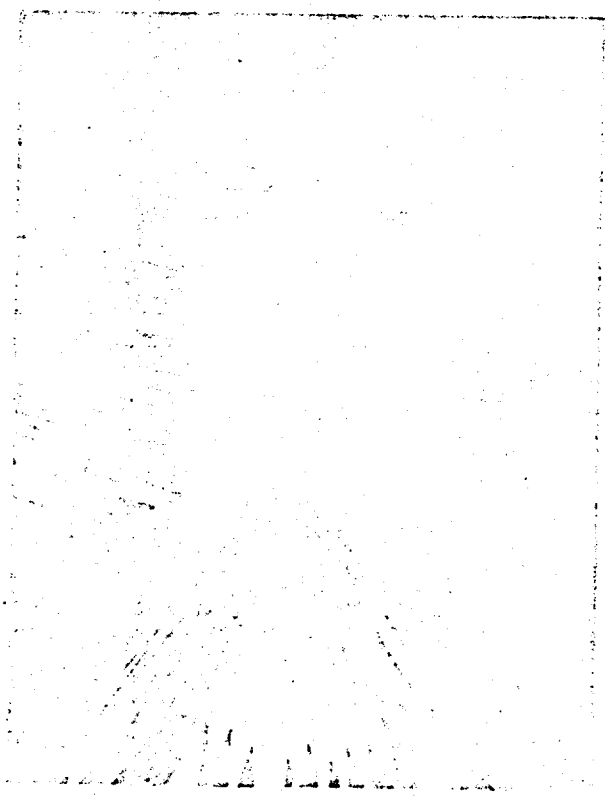
Typical landscape in the State of Ceará. Carnaúba palms grow along the edge of streams. Unlike most palms, it is not uncommon for them to have a life span as long as 200 years. They grow to heights of 50 and 60 feet.



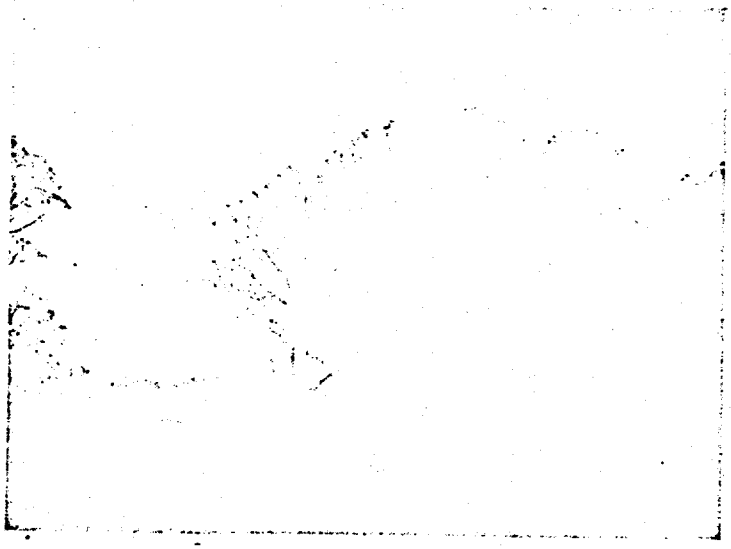
Clipping fronds. Note cluster of fruit at right. These are edible and not unlike a date. The shell is roasted to produce a coffeelike beverage, and the seed yields cooking oil.



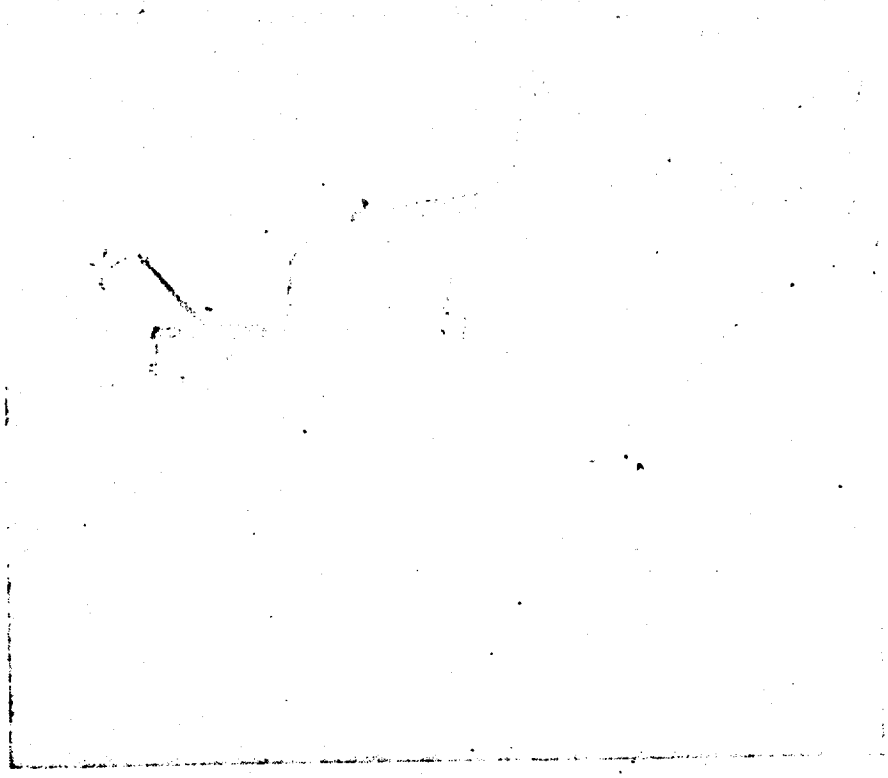
Loads of fronds brought in from the forest. It takes approximately 100 fronds to produce one pound of wax, and each tree produces only an average of 6 ounces per year; 35 to 40 million trees are worked annually.



Note strong chest and arm muscles, built up from years of clipping the palms with scythe-shaped knife lashed to a bamboo pole. Experienced clippers cut nearly 3,000 fronds a day.



Home of carnaúba wax worker. Since the war demand for carnaúba has greatly increased. Clippers must travel farther into the interior to obtain wax.



Part of the Brazilian jangadeiro's daily catch

written on the people and the various vegetable products which are of value in forming a favorable trade balance between the United States of Brazil and the United States of America. Great strides are being made in Brazilian industry. Although there are some factories in the north, industry centers around São Paulo and Rio de Janeiro. Streamlined buildings and factories grace the sky lines. It is said that São Paulo is the fastest growing city in the world. Already over 9,000 factories have come to life and are producing materials for a better Brazil!

Uses of Carnaúba Wax

It is in industry that carnaúba wax gains its greatest importance. Many factories scattered over the face of the earth owe their existence to its unusual properties. Its high melting point, non-tacky surface, hardness, compatibility with many chemical compounds, ability to take a high polish, and preservative qualities as a surface coating make it the most important wax in the chemical industry.

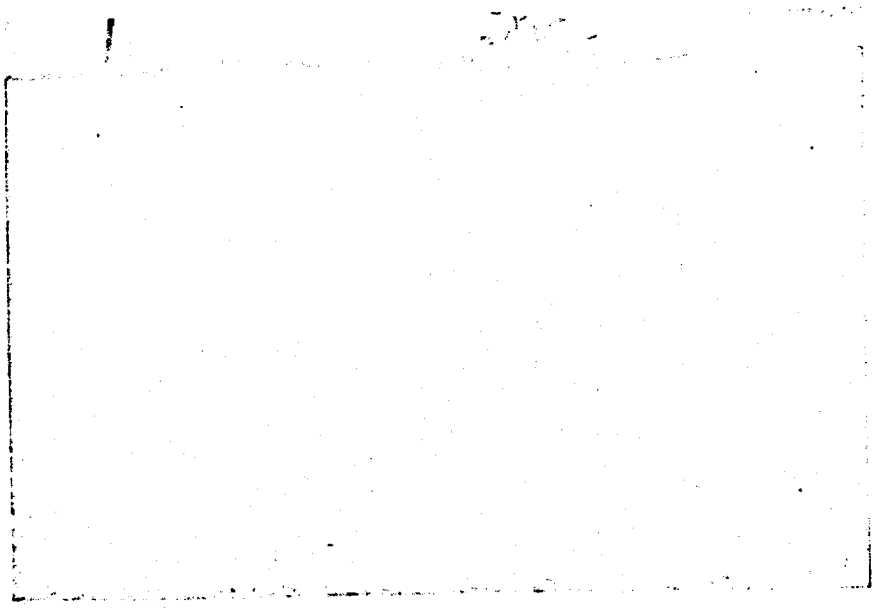
Outside of the surface finishing and polish industry, probably one of the greatest single contributions to American business is its use in carbon paper. An entire volume could be written on this phase alone. When transfer paper was first invented, it was a smeary, sticky mass of oils, greases, and lampblack, making only one or two copies at the most, and these were not very legible. The discovery that wax would harden and prevent carbon paper coatings from smutting revolutionized the carbon paper industry.

Carnaúba is by far the most important carbon paper wax. Its use in this industry depends upon scientific control. In addition to its hardening effect, it has many other important functions. It acts as a solvent for the basic dyes which are used as toners. It has a dispersing and wetting action on the pigments which gives flow to the molten ink. It has unusually high oil-retention properties—that is to say, more oil can be mixed with the wax than with any other wax, and the mix will still remain a solid. As much as 50% of 100-viscosity mineral oil can be added to the molten wax and when cooling the surface of the mix will be hard enough to cause a clean fracture when a spatula is forced into it. The wax causes high-grade typewriter and pencil carbons to give a sharp, clean copy. When the type of a business machine strikes the carbon paper, the carnaúba wax causes the coating to break off and release in a straight sharp fracture, thus giving neat, legible copies. If properly formulated, carbon papers have been known to give from 15 to 20 copies as against one or two in the original transfer coatings. Carnaúba's quick-setting properties and crystalline structure give the carbon paper chemist a diversified field from which to work. A high gloss or a flat finish may be obtained by manipulating temperatures and controlling the crystal growth of the carnaúba and other waxes in the formula. This allows the chemist to develop special carbons for the many types of business machines which have different strokes and pressures in their type.

It might be said that carnaúba wax and its important function in the carbon paper industry are the veins of business for, without duplicate copies, all business would bog down, including transportation, radio, and the press. Many special types of carbon paper have been developed since the United States' entry into the war. Carbons have been developed which will give a copy at 40° below zero, for use along the Arctic Circle or in high altitude bombers where the temperature drops to 30° to 40° below. Yet this same carbon can be used equally well in the hot dripping jungles of the South Pacific. To ask for a carbon coating which will conform to these specifications is like asking for a miracle, yet the carbon paper industry came through!

Stories on Uses of Carnaúba Wax

Stories such as this are not uncommon: A reconnaissance scout slips quietly through the jungle and comes upon a Japanese gun emplacement. Hurriedly he sketches the position and landmarks in an Army message book. A carbon copy



Carnaúba threshers. In background, heaps of fronds under carnaúba palm-thatched shelter

is made on feather-weight tissue paper and attached to the leg of a fast carrier pigeon. In a few hours our bombers are blasting the daylights out of the emplacement. As the frail wings of the pigeon whir through the thick air of the jungle, the carnaúba wax which is used in the special message book becomes of greatest importance. Thus, from the forests of Brazil "The Tree of Life" suddenly becomes "A Messenger of Death".

Like the fifty-odd carbon paper manufacturers in the U.S.A. who deserve a great deal of credit in the war effort, many other important industries which are users of carnaúba wax are playing their role in the grand strategy of the war.

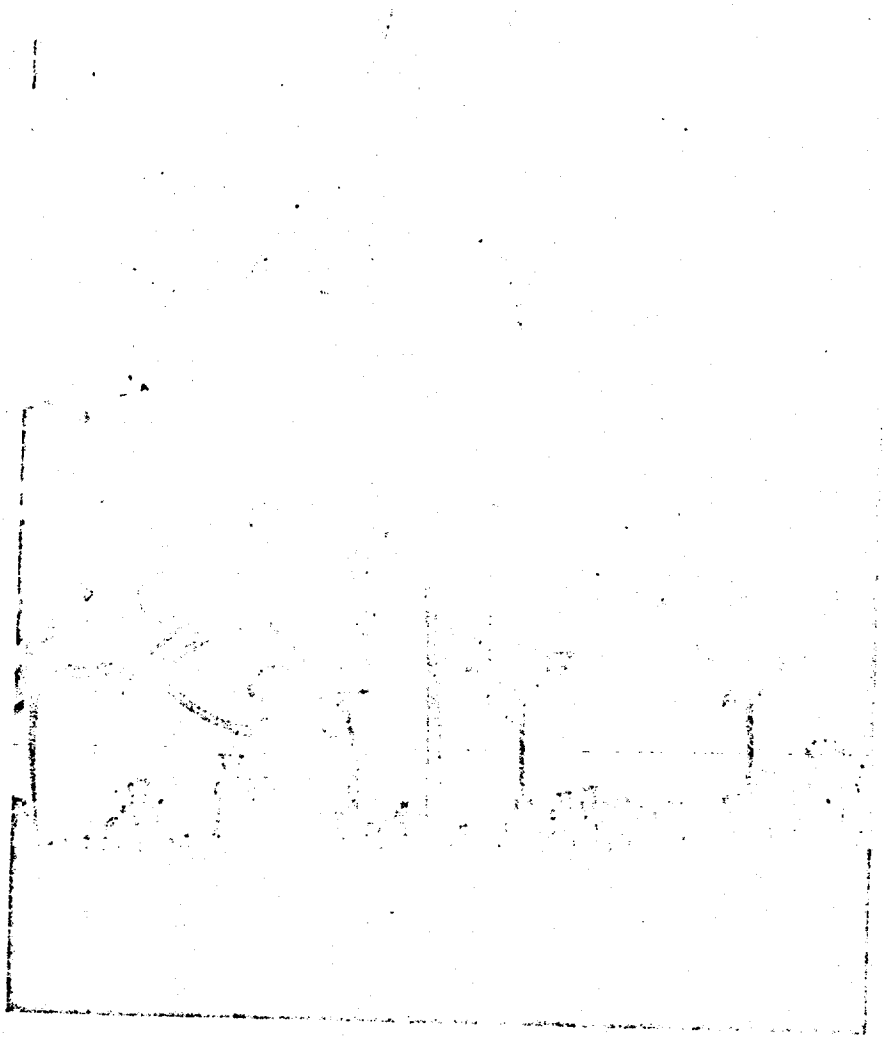
Many individual stories could be told of the use of carnaúba wax in the printing ink industry, paints, waterproofing, floor polishes, shoe polishes, insulation compounds, preservative finishes, the manufacture of phonograph master disks, lubrication operations, wax offset eliminators in printing, special emulsions, salves, ointments, cosmetics, crayons, shell coatings, military finishes, and scores of others.

Analysis of Carnaúba Wax

According to Green, the analysis of carnaúba wax is as follows:

Cerotic acid myricyl ester, myricil alcohol ($C_{29}H_{60}O$), ceryl alcohol ($C_{27}H_{56}O$, melting point 76° C., small amount), melissyl alcohol ($C_{30}H_{62}O$), unknown glycol ($C_{23}H_{52}O_2$) of uncertain formula. Phytosterin 0.5%, and a hydrocarbon, melting point 59° C.

Total hydrocarbons and alcohol, 54 to 55%; fatty acids 41 to 48%, chiefly carnaúbic acid ($C_{32}H_{67}COOH$), melting point 72.5° C.; small amount of cerotic acid ($C_{26}H_{53}COOH$), myricic acid, and an unknown hydroxy acid ($C_{31}H_{62}O_3$).



The edge of a carnaúba forest

The ash consists of silica, iron oxide, and sodium chloride.

Density at 15° C.	0.990-0.999
Melting point	80°-86° C.
Hardening point	76°-81° C.
Refractive index at 40° C.	1.472 n_D
Acetyl number	51
Iodine number	8-13.6
Acid number	0.3-10
Unsaponifiable	54-55%
Fatty acid	41-48%

Bradley Dewey to Receive Chemical Industry Medal

Bradley Dewey has been selected to receive the Chemical Industry Medal for 1944. This award is made annually by the American Section of the Society of Chemical Industry and the candidate to receive the medal is selected by the executive committee which constitutes the Medal Committee. The award is being made to Colonel Dewey for his work in colloid chemistry, especially as pertaining to rubber latex, and his accomplishment in administering the synthetic rubber program during the critical war period.

In 1911 he became Director of Research with the American Sheet and Tin Plate Co. In 1919, in association with Charles Almy, he organized the Dewey and Almy Chemical Co. He has been president of that company since its organization. In World War I, Bradley Dewey served in the Chemical Warfare Service of the U. S. Army; he was appointed First Lieutenant in April 1917 and by July 1918 had been promoted to Colonel in Charge of the Gas Defense Division. He was awarded the Distinguished Service Medal.

In September 1942 Colonel Dewey was appointed Deputy Rubber Director and since September 1943 has been Rubber Director. Under Colonel Dewey's administration and with his leadership there has been brought into being a synthetic rubber industry which has the capacity to produce synthetic rubber at a rate greater than 1,000,000 long tons a year. The Rubber Director recently resigned because he felt that the Production Executive Committee of the War Production Board, in that one of its functions is to coordinate the war effort, was the ideal body to present the manpower needs for the production of more heavy duty tires.

Nutrition Foundation

During the past two years $1,500,000 has been contributed to the Nutrition Foundation by 42 food and related manufacturers, $530,040 of which has been appropriated for 87 research grants in the science of nutrition.

A recommendation from the scientific director and the scientific advisory committee for publishing a Latin-American edition of *Nutrition Reviews* has been approved.

Borden Award in Nutrition

The American Institute of Nutrition will make this award in recognition of distinctive research by investigators in the United States and Canada which has emphasized the nutritive significance of the components of milk or of dairy products. The award will be made primarily for the publication of specific papers, but the judges may recommend that it be given for important contributions over an extended period of time.

The award may be divided between two or more investigators. Employees of the Borden Co. are not eligible for this honor.

The formal presentation will be made at the annual meeting of the American Institute of Nutrition at Cleveland, May 8, 1945. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee, Frederick J. Sture, Harvard Medical School, Boston, Mass., by January 15, 1945.

The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

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CARNAUBA
17

ETUDE PAR CHROMATOGRAPHIE EN PHASE GAZEUSE DES CONSTITUANTS DE LA CIRE DE CARNAUBA I.

Par Paul MAZLIAK.

■

Les recherches que nous poursuivons depuis plusieurs années sur la composition des cires de pomme nous ont conduit à mettre au point une micro-méthode d'analyse de ces cires (1) (2). En combinant la chromatographie d'adsorption (liquide-solide) et la chromatographie de partage en phase gazeuse (gaz-liquide), nous avons étudié les acides gras, les hydrocarbures et les alcools à longue chaîne entrant dans la composition de 500 mg de cire.

Pour voir si la méthode proposée était susceptible d'un emploi général dans l'analyse des cires végétales, nous avons appliqué la technique mise au point sur la cire de pomme à l'étude d'une cire végétale très différente. Nous avons choisi dans ce but une cire d'origine tropicale, recouvrant les feuilles du palmier brésilien *Copernicia cerifera* (MARTIUS) très communément connue sous le nom commercial de « cire de Carnauba ». La composition chimique de cette cire a déjà fait l'objet de nombreux travaux (STURKE (3), KOONCE et BROWN (4), MURRAY et SCHOENFELD (5) (6) (7) (8)) mais les analyses chimiques ont toujours été effectuées dans le passé à partir d'une grande quantité de produit naturel au départ (80 à 250 g). La méthode que nous avons utilisée devait nous permettre de retrouver, à partir d'une quantité beaucoup plus faible de cire, et beaucoup plus rapidement, les résultats déjà obtenus pour l'analyse des acides et des alcools; les hydrocarbures qui n'avaient jamais pu être séparés précédemment, ont été étudiés avec succès grâce à la méthode que nous avons employée.

Le tableau 1 présente quelques caractéristiques comparées de la cire de *Carnauba* et de la cire solide de pomme. Les données concernant la cire de *Carnauba* ont été empruntées à MURRAY et SCHOENFELD (8). Il est immédiatement visible que le point de fusion beaucoup plus élevé de la cire de *Carnauba* traduit une richesse beaucoup plus grande de cette cire en composés alcooliques, alors que le point de fusion plus bas de la cire de pomme traduit au contraire une grande richesse en hydrocarbures. Le point de fusion plus élevé de la cire de *Carnauba* traduit aussi un plus fort pour-

8
180-190

CARACTÉ

Point de fusion	
Insaponifiable	
Hydrocarbures	
n-Alcools	
Acides totaux	
Acides normaux	
ω-hydroxyacides	

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TABLEAU 1.

CARACTÉRISTIQUES COMPARÉES DE LA CIRE DE CARNAUBA ET DE LA CIRE DE POMME.

	Cire de Carnauba	*Cire de pomme*
Point de fusion	84° C	64° C
Insaponifiable	56 %	64 %
Hydrocarbures	0,5 %	43 %
n-Alcools	52,5 %	20 %
Acides totaux	17,6 %	20 %
Acides normaux	26,4 %	10 à 16 % (?)
ω-hydroxyacides et estolides		

centage des chaînes moléculaires très longues (à plus de 30 atomes de carbone). Ces deux facteurs (longueur des chaînes moléculaires et richesse en oxygène) rendent très difficile la vaporisation des constituants de la cire de *Carnauba*; l'analyse par chromatographie en phase gazeuse a tout de même pu en être faite, grâce à l'emploi de phases stationnaires spéciales supportant des températures très élevées.

PRÉPARATION DES FRACTIONS POUR LA CHROMATOGRAPHIE EN PHASE GAZEUSE.

Un gramme de cire commerciale jaune clair (fournie par les Etablissements Touzart et Matignon) est saponifié par 50 ml de potasse alcoolique (2N) pendant 3 h. à la température d'ébullition douce du mélange, sous un réfrigérant à reflux. Les savons formés sont dissous complètement en ajoutant 45 ml d'eau distillée à la liqueur de saponification et en poursuivant l'ébullition pendant une demi-heure. La séparation des savons et de l'insaponifiable se fait à la température du laboratoire, par partage des constituants entre le mélange polaire eau-alcool et de l'éther de pétrole apolaire, selon la méthode classique. Il faut cependant noter qu'une fraction des constituants de la cire n'est soluble, à cette température, dans aucune des deux phases; la densité de ces résidus solides les place dans la région inférieure de la phase éthérée et nous les avons recueillis avec l'insaponifiable. Cette fraction solide est sans doute constituée par des alcools à très longue chaîne moléculaire et des hydroxyacides, saponifiés ou non par le traitement précédent.

Les acides gras sont libérés des sels alcalins par l'acide chlorhydrique puis recueillis dans l'éther de pétrole. Ils sont ensuite transformés en esters méthyliques à l'aide d'acide paratoluène-sulfonique comme catalyseur. L'insaponifiable est chromatographié à la température du laboratoire sur une colonne d'alumine : une

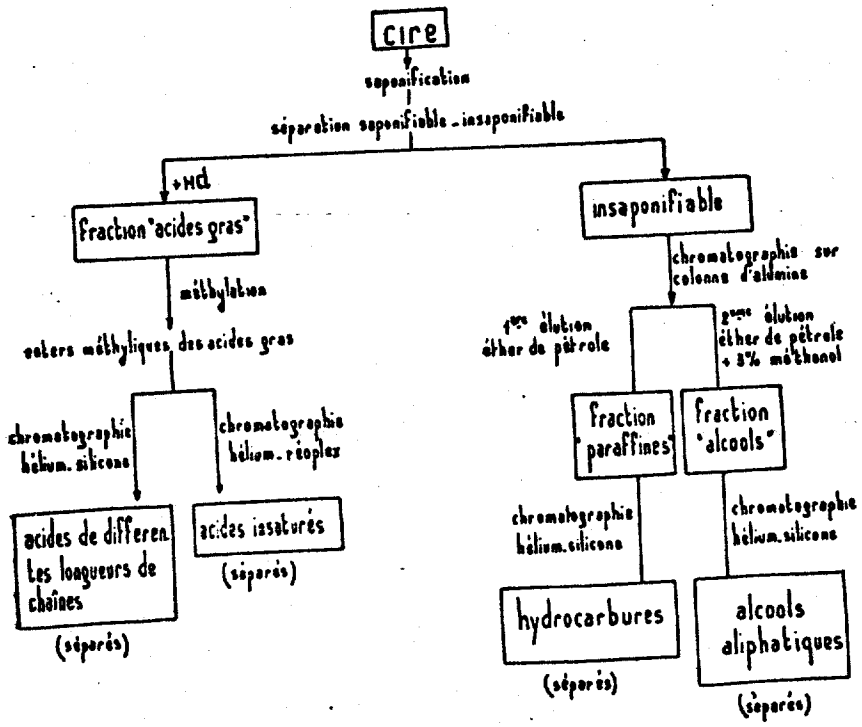

Fig. 1. — Procédés de préparation des fractions destinées à la chromatographie en phase gazeuse.

première élution par l'éther de pétrole seul permet de recueillir les *hydrocarbures;* une seconde élution par le mélange éther de pétrole-méthanol (100 : 3) donne les alcools. La Fig. 1 résume toute la suite des opérations sur lesquelles nous avons donné ailleurs tous les détails techniques (1). On obtient ainsi les mélanges d'esters méthyliques des acides gras, d'alcools et de paraffines prêts pour la chromatographie en phase gazeuse.

TABLEAU 2.

ACIDES GRAS NORMAUX DE LA CIRE DE CARNAUBA.

Les volumes de rétention corrigés sont exprimés relativement au V'_R de l'acide palmitique.

Longueur de la colonne : 2 m.
Gaz vecteur : hélium.
Phase stationnaire : graisse de silicone 710 (20 %) adsorbée sur de la brique (60-80 mesh).
Température : 300° C.
Débit du gaz : 51/h (suppression à l'entrée de la colonne : 860 g/cm² pression du gaz à la sortie égale à la pression atmosphérique).

Acides gras	Nombre d'atomes de carbone	log V' relatif mesuré sur le chromatogramme	log V'_R du témoin	pourcentages calculés	Pourcentages indiqués par MURRAY et SCHOENFELD (5)
ac. myristique	14	—	—0,18	—	—
ac. palmitique	16	0	0	0,9 %	—
ac. stéarique	18	0,30	0,30	2,5 %	3 %
ac. arachidique	20	0,54	0,50	9,8 %	11,5 %
ac. béhénique	22	0,74	0,72	10,3 %	9 %
ac. lignocérique	24	0,95	0,97	47,4 %	30 %
ac. cérotique	26	1,15	1,21	9,4 %	12 %
ac. montanique	28	1,35	1,42	13,8 %	16,5 %
ac. mélissique	30	1,56	1,63	5,5 %	7 %

Il faut noter qu'une fraction de l'insaponifiable, très vraisemblablement constituée de diols et d'hydroxyacides, reste sur la colonne d'alumine. Cette fraction est actuellement l'objet de nouvelles recherches et nous n'en parlerons plus par la suite.

ETUDE DES ACIDES GRAS NORMAUX.

Nous avons utilisé le chromatographe Jobin et Yvon muni d'un catharomètre à filament de tungstène porté à la même température que la colonne séparatrice. Les colonnes sont des tubes en acier inoxydable de 2 m de longueur sur 4 mm de diamètre intérieur. Les déterminations des différents corps séparés sont faites en comparant le volume de rétention corrigé du corps à celui du témoin pur. Nous avons utilisé deux sortes de témoins : des corps purs trouvés dans le commerce ou les corps précédemment identifiés dans la cire des pommes. Les volumes de rétention ont toujours été calculés relativement au volume de rétention d'un

composé bien défini, présent dans le mélange analysé. Les pourcentages des différents corps séparés sont calculés proportionnellement aux aires comprises sous les courbes représentant ces corps sur les chromatogrammes.

La Fig. 2 représente un chromatogramme des acides gras normaux saturés de la cire de *Carnauba*. Le tableau 2 donne les conditions expérimentales dans lesquelles a été obtenu ce chromatogramme ainsi que la liste des acides identifiés avec leurs pourcentages respectifs. Nous avons indiqué dans ce même tableau les

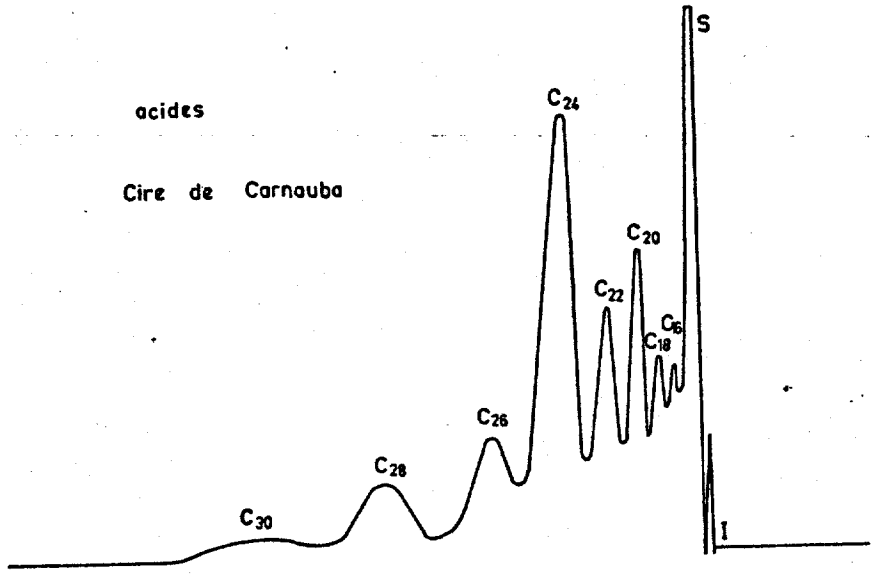

Fig. 2. — Chromatogramme des acides gras normaux de la cire de *Carnauba* (I : injection; S : solvant).

pourcentages trouvés par MURRAY et SCHOENFELD (5) après analyse par distillation fractionnée du mélange des esters méthyliques des acides. On constate que la chromatographie en phase gazeuse permet de retrouver tous les acides gras mis en évidence par les auteurs précédents, plus *l'acide palmitique* qui avait échappé à leurs investigations sans doute à cause de son très faible pourcentage. Les résultats quantitatifs sont en très bon accord avec ceux des auteurs australiens, si l'on tient compte surtout du fait que ce n'est pas le même échantillon de cire qui est analysé dans les deux cas.

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Fig. 3. — Chroma
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Etude des paraffines.

Peu de résultats ont été obtenus jusqu'à présent en ce qui concerne les hydrocarbures de la cire de *Carnauba* parce que ces corps ne forment qu'un très faible pourcentage de la masse totale des constituants (de 1 à 1,6 % selon Farcy (9)). Murray et Schoenfeld indiquent simplement qu'il s'agit d'un mélange complexe

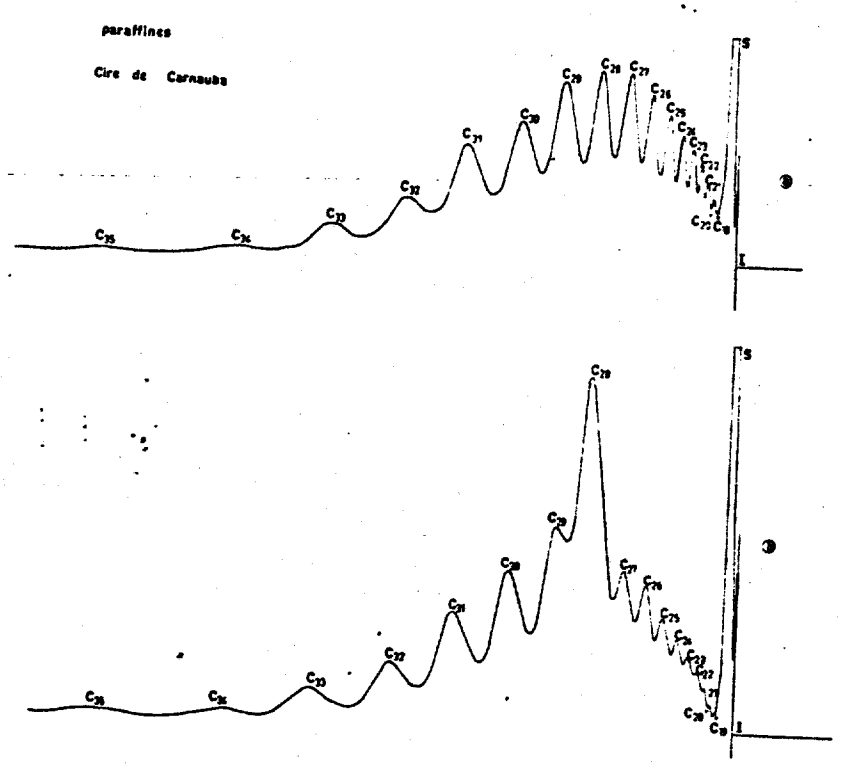

Fig. 3. — Chromatogrammes des paraffines de la cire de *Carnauba* (I : injection; S : solvant) *a*) chromatogramme des paraffines seules. — *b*) chromatogramme du mélange des paraffines enrichi d'octacosane synthétique.

comprenant sans doute essentiellement les hydrocarbures en C_{27}, C_{29} et C_{31} L'heptacosane doit être la paraffine la plus abondante selon Gottfried et Ulzer (10). La chromatographie en phase gazeuse nous a permis d'identifier dix-huit hydrocarbures (Fig. 3). Le tableau 3 en donne la liste. Les pourcentages de ces constituants ont été calculés de façon très approximative à cause de la position incertaine de la ligne de base des chromatogrammes.

Nous nous sommes assuré que nous avions bien affaire à des paraffines de différentes longueurs de chaîne en traçant, à l'aide des

TABLEAU 3.

HYDROCARBURES DE LA CIRE DE CARNAUBA.

Les volumes de rétention corrigés sont relatifs au V'_R du tétracosane.
Longueur de la colonne : 2 m.
Gaz vecteur : hélium.
Phase stationnaire : graisse de silicone 710 (20 %) adsorbée sur de la brique (60-80 mesh).
Température : 300° C.
Débit du gaz : 3 l/h (suppression à l'entrée de la colonne : 510/cm² pression du gaz à la sortie égale à la pression atmosphérique).

Hydrocarbures	Nombre d'atomes de carbone	log V'_R relatif mesuré sur le chromatogramme	log V'_R du témoin	Pourcentages calculés approximativement
nonadécane.	19	—0,57	—0,53	traces
eicosane	20	—0,46	—0,45	0,3 %
heneicosane	21	—0,34	—0,33	0,4 %
docosane	22	—0,21	—0,21	1,3 %
tricosane	23	—0,12	—0,12	2,3 %
tetracosane	24	0	0	3,2 %
pentacosane	25	0,10	0,10	5,4 %
hexacosane	26	0,21	0,21	8,3 %
heptacosane	27	0,31	0,31	13,3 %
octacosane	28	0,42	0,42	16,0 %
nonacosane	29	0,53	0,52	16,0 %
triacontane	30	0,63	0,61	12,3 %
hentriacontane	31	0,73	0,73	7,9 %
dotriacontane	32	0,81	0,83	6,6 %
tritriacontane	33	0,91	0,93	4,1 %
tetratriacontane	34	0,99	1,03	1,2 %
pentatriacontane	35	1,07	1,13	1,0 %

valeurs expérimentales, la courbe log $V'_R = f(n)$, n étant le nombre d'atomes de carbone dans le molécule et V_R le volume de rétention corrigé, relatif au V'_R du tetracosane. On peut voir sur la figure 4 que les points expérimentaux sont quasi parfaitement alignés :ceci signifie que l'on a bien affaire à une série d'homologues, d'après la loi de JAMES et MARTIN (11). En outre, pour nous assurer de la présence des paraffines renfermant un nombre pair d'atomes de carbone dans la molécule nous avons chromatographié un mélange formé des hydrocarbures de la cire plus une paraffine paire synthétique (l'octacosane par exemple, Fig. 3). Le « pic » correspondant à la paraffine paire ajoutée artificiellement apparaissait alors beaucoup plus grand sur les chromatogrammes. La présence de paraffines renfermant un nombre pair d'atomes de carbone dans leur

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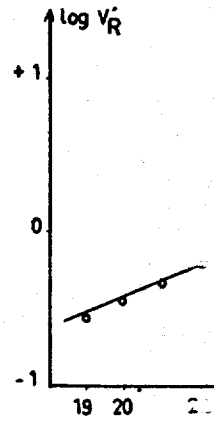

Fig. 4. — Courbe
rithmes des vol
lés relativement
de carbone (n)

Les volumes
V'_R de l'hex
Longueur de l
Gaz vecteur :
Phase stationn
80 mesh).
Température :
Débit du gaz :
(à 300° C : su
à 340° C : su
pression du g

molécule est ainsi clairement mise en évidence dans la cire de Carnauba. Nous avions déjà mis en évidence l'existence de telles paraffines dans la cire liquide de la cuticule des pommes en combinant la chromatographie en phase gazeuse et la spectrophotométrie infra-rouge (12). Ceci confirme l'hypothèse selon laquelle la biosynthèse des hydrocarbures des cires végétales se produirait autrement que par simple décarboxylation des acides gras saturés pairs. Un allongement de la chaîne moléculaire, atome de carbone par atome de carbone, paraît vraisemblable.

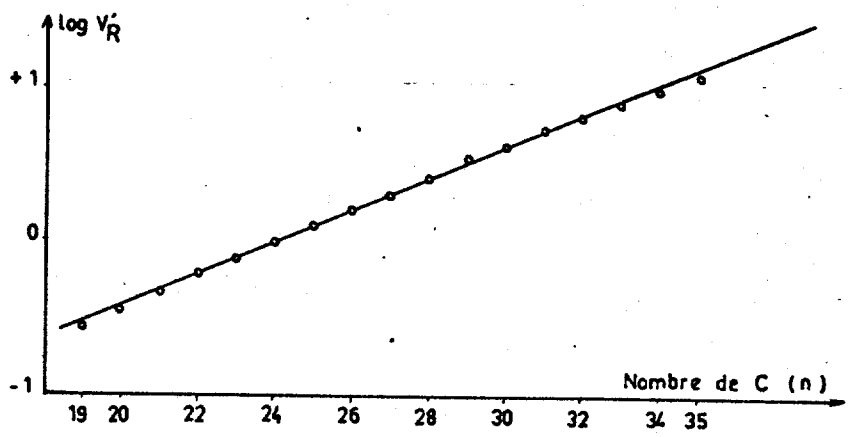

Fig. 4. — Courbe log $V'_R = f(n)$ obtenue en partant en ordonnées les logarithmes des volumes de rétention corrigés (V'_R), des hydrocarbures, calculés relativement au V'_R du tétracosane et en abcisses le nombre d'atomes de carbone (n) entrant dans la molécule d'hydrocarbures.

TABLEAU 4.

ALCOOLS DE LA CIRE DE CARNAUBA.

Les volumes de rétention corrigés sont exprimés relativement au V'_R de l'hexacosanol à 300° C et au V'_R de l'eicosanol à 340° C.
Longueur de la colonne : 2 m.
Gaz vecteur : hélium.
Phase stationnaire : Silicone Rubber (20 %) sur chromosorb W (60-80 mesh).
Température : 300 et 340° C.
Débit du gaz : 3,1 l/heure.
(à 300° C : suppression à l'entrée de la colonne : 1200 g/cm²
à 340° C : suppression à l'entrée de la colonne : 1100 g/cm²
pression du gaz à la sortie égale à la pression atmosphérique).

Alcool	Nombre d'atomes de carbone	à 300° C log V'_R mesuré sur le chromatogramme	à 300° C log V' du témoin	à 340° C log V'_R mesuré sur le chromatogramme	à 340° C log V'_R du témoin	Pourcentages calculés	Pourcentages indiqués par MURRAY et SCHOENFELD (6)
eicosanol	20	—	—0,72	—	0	—	
docosanol	22	—0,45	—0,44	—	—	traces	1 %
tetracosanol	24	—0,24	—0,21	—	—	traces	
hexacosanol	26	0	0	—	0,50	traces	4 %
octacosanol	28	0,18	0,19	0,66	0,66	2,3 %	5 %
triacontanol	30	0,36	0,37	0,845	0,835	13,7 %	19 %
dotriacontanol	32	0,54	0,58	1	1	69,5 %	51 %
tetratriacontanol	36	0,78	0,80	1,16	1,18	14,5 %	22 %
hexatriacontanol	36	—	—	1,40	1,38	traces	—

ETUDE DES ALCOOLS.

La vaporisation des corps oxygénés à longue chaîne moléculaire est très difficile. Pour pouvoir chromatographier toute la série des alcools aliphatiques primaires présents dans la cire de *Carnauba*, nous avons été obligé d'employer deux températures différentes. La Fig. 5 montre les chromatogrammes obtenus à 300 et 340° C, comparés avec un chromatogramme des alcools de la cire des pommes réalisé à 300° C. Huit alcools différents sont ainsi séparés. Le tableau 4 indique les conditions expérimentales réalisées et donne la liste des alcools identifiés avec leurs pourcentages respectifs. Dans le même tableau, nous avons fait figurer les pourcentages trouvés par MURRAY et SCHOENFELD par distillation fractionnée des dérivés acétylés des alcools (6). Tous les alcools décelés précédemment par ces auteurs sont retrouvés par chromatographie en phase gazeuse avec en plus des traces d'hexatriacontanol. Les résultats quantitatifs fournis par la chromatographie s'accordent très bien avec ceux qui ont été obtenus par la distillation fractionnée.

CONCLUSIONS.

Les résultats de nos expériences sur la cire des pommes d'une part et sur la cire de *Carnauba* d'autre part permettent d'assurer que la microméthode d'analyse proposée peut être utilement employée pour l'étude des constituants de cires végétales très diverses. La méthode est quantitative et possède en outre l'avantage d'être beaucoup plus rapide que toutes les méthodes antérieurement utilisées.

Laboratoire de Biologie végétale
Station du Froid
Bellevue C.N.R.S.

ALCOOL

Cire de

300° C

C_{34}

340° C

Fig. 5. — Chromat
différentes temper
des alcools de la
cools de la cire d
Carnauba à 340°

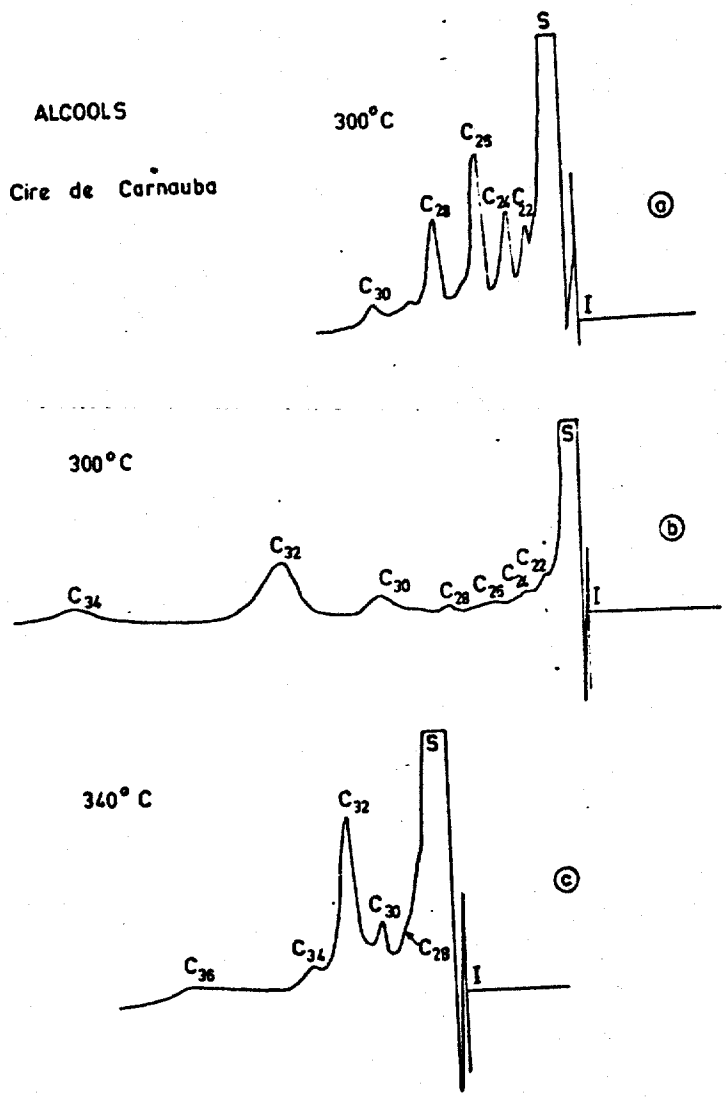

Fig. 5. — Chromatogrammes d'alcools des cires de pommes et de *Carnauba* à différentes températures (I : injection; S : solvant). — *a*) chromatogramme des alcools de la cire des pommes à 300° C. — *b*) chromatogramme des alcools de la cire de *Carnauba* à 300° C. — *c*) Chromatogramme de la cire de *Carnauba* à 340° C.

Résumé

La microméthode rapide de séparation, d'identification et de dosage des principaux constituants d'une cire végétale précédemment mise au point sur la cire cuticulaire des pommes est appliquée à la cire de *Carnauba*. Sont ainsi décelés les acides gras normaux de C_{16} à C_{30}, les paraffines paires et impaires de C_{19} à C_{35} et les alcools normaux de C_{20} à C_{36}. Les résultats quantitatifs sont en bon accord avec ceux obtenus dans le passé au moyen d'autres méthodes.

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NOTES

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CARNAUBA

GAS CHROMATOGRAPHIC STUDY OF THE CONSTITUENTS OF CARNAUBA WAX I.

By Paul Mazliak

The research that we have been conducting for the last several years on the composition of apple wax has led us to develop a micromethod for the analysis of waxes (1,2). By combining liquid-solid and gas phase chromatography we have studied the fatty acids, hydrocarbons and long-chain alcohols in a 500 mg wax sample.

To see if the proposed method could be used generally for the analysis of vegetable waxes we applied the technique developed for apple wax to an entirely different wax. We chose a wax from the tropics which covers the leaves of the Brasilian palm, Copernica cerifera (Martius), commonly called by the commercial name of Carnauba wax. The composition of this wax has already been the object of numerous studies (3-8) but at least 80-250 gm was used in all of these. The method that we have developed should allow us, with much smaller quantities and much more rapidly, to obtain the same results for the acids and alcohols; the hydrocarbons which could not be studied previously, gave successful results with the method that we have employed.

Table 1 presents some characteristics of Carnauba and apple waxes. The data concerning Carnauba wax have been borrowed from Murray and Schoenfeld (8). It is immediately obvious that the higher melting point of Carnauba wax indicates a greater amount of alcohols while the lower melting point of apple wax indicates that more hydrocarbon is present. The higher melting point of Carnauba wax also indicates that there is a greater percentage of very long chain molecules (30 C atoms or more). These two factors (chain length and oxygen richness) render the vaporization of Carnauba wax very difficult; the analysis by gas chromatography has been made though, due to the use of stationary phases which can withstand high temperature.

Table 1

Characteristics of Carnauba and apple waxes.

	Carnauba	Apple
Melting point	84°	64°
Unsaponifiable	56%	64%
Hydrocarbons	0.5	43
n-Alcohols	52.5	20
Total acids	-	-
n-Acids	17.6	20
Hydroxyacids and other	26.4	10-16

PREPARATION FOR GAS CHROMATOGRAPHY

A gram of clear yellow commercial wax (furnished by Touzart and Matignon) is saponified by 50 ml of 2 N alcoholic KOH for three hrs under reflux at the boiling point of the mixture. The soaps formed are completely dissolved by adding 45 ml of distilled water and the liquor is boiled for another half hour. The separation of the soap and unsaponifiable is done at room temperature by extraction using water-alcohol and petroleum ether, according to the usual procedures. It must be noted that at this temperature a fraction of the wax is insoluble in both phases; the density of these solid residues places them in the lower range of the ether soluble phase and we have collected them with the unsaponifiable. This fraction probably includes long chain alcohols and saponified or unsaponified hydroxyacids.

The fatty acids are liberated from the alkaline salt with HCl and then collected in petroleum ether. Next they are methylated with p-toluenesulfonic acid as catalyst. The unsaponifiable is column chromatographed on alumina at room temperature: one elution with petroleum ether allows the hydrocarbons to be collected; elution with 100:3 petroleum ether: methanol then gives the alcohols. Figure 1 resumes the necessary operations in the analysis for which we have given details elsewhere(1). The mixtures of methylated fatty acids, alcohols and hydrocarbons are now ready for gas chromatography.

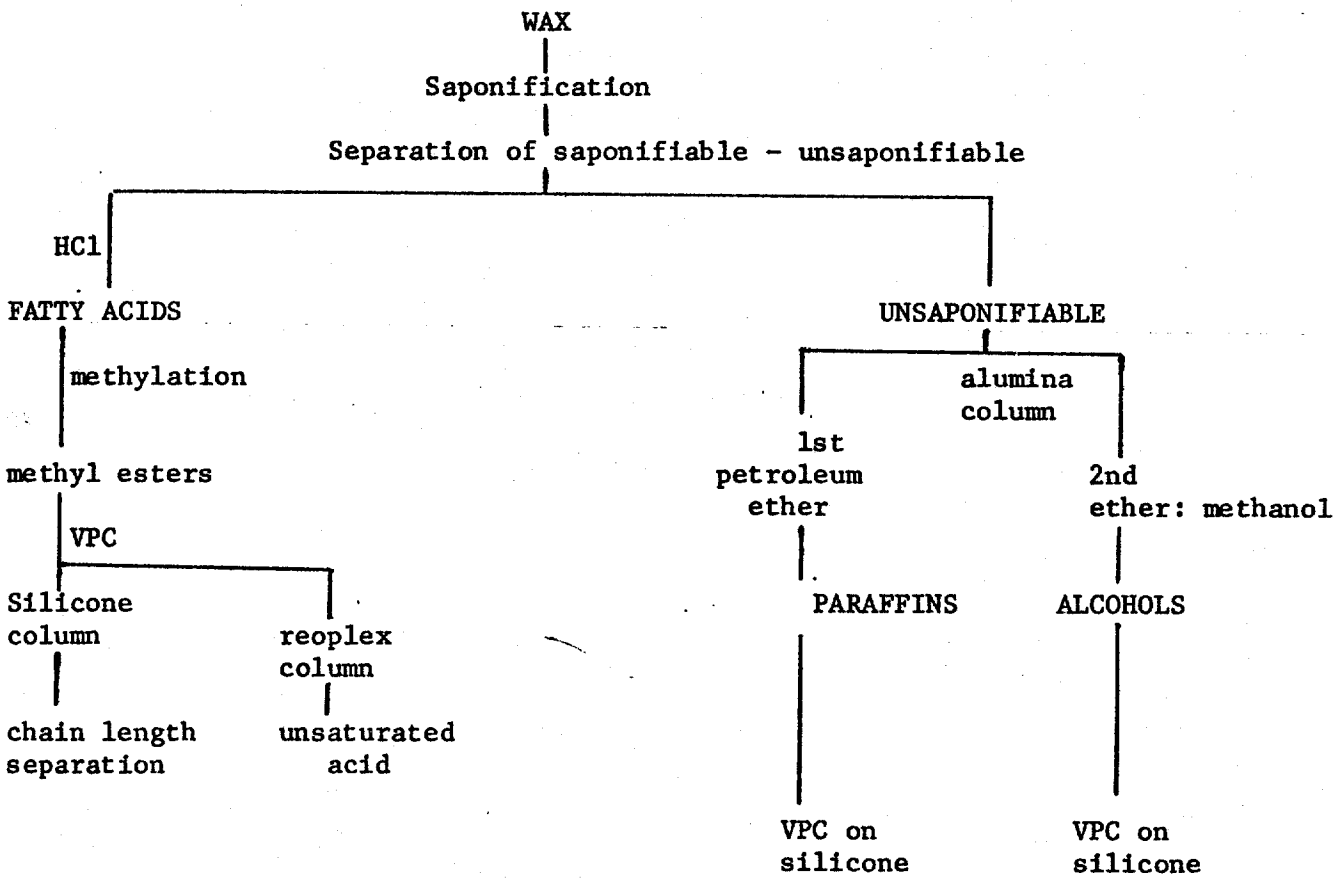

Figure 1 - Preparation of various fractions for gas chromatography

TABLE 2

NORMAL FATTY ACIDS OF CARNAUBA WAX

Corrected retention volumes (V_R) are expressed relative to palmitic acid

Column length: 2 m
Gas: helium at 5 liters/hour
Temperature: 300°
Stationary phase: 20% silicone oil 710 on 60/80 mesh brick

Fatty acid	Carbon Atoms	Log V'	log V	Calculated	Found by Ref. (5)
ac. myristique	14	-	-0,18	-	-
ac, palmitique	16	0	0	0,9%	-
ac. stearique	18	0,30	0,30	2.5%	3 %
ac. arachidique	20	0,54	0,50	9,8%	11,5%
ac. behenique	22	0,74	0,72	10,3%	9 %
ac. lignocerique	24	0,95	0,97	47,4%	30 %
ac. cerotique	26	1,15	1,21	9,4%	12 %
ac. montanique	28	1,35	1,42	13,8%	16,5%
ac. melissique	30	1,56	1,63	5.5%	7 %

It must be noted that a fraction of the unsaponifiable remains on the alumina column, probably constituting diols and hydroxyacids. This fraction is actually the subject of new research, of which we will speak later.

STUDY OF THE NORMAL FATTY ACIDS

We have employed a Jobin and Yvon chromatograph outfitted with a tungsten filament catharometer at the same temperature as the column. The columns are unoxydizable steel tubes 2 m long by 4 mm I. D. The determinations of the different compounds separated are made by comparing the corrected retention volume with that of a pure standard. We have commercial compounds or compounds identified in the analysis of apple wax as standards. The retention volumes have always been calculated relative to a well defined compound present in the mixture analyzed. The percentages are calculated proportional to the corresponding peak area for that compound on the chromatogram.

Figure 2 represents a chromatogram of the normal saturated fatty acids of Carnauba Wax. Table 2 gives the experimental conditions used as well as a list of the acids identified, with their percentages. We have also indicated the percentages found by Murray and Schonfeld (5) after fractional distillation of the methyl esters of the fatty acids. Gas chromatography permits one to identify all of the acids reported by these authors, plus palmitic acid, which they were unable to detect, probably because of its very low percentage. The quantitative results are in very good accord with these authors, especially if one takes into account the fact that different samples were analyzed in the two cases.

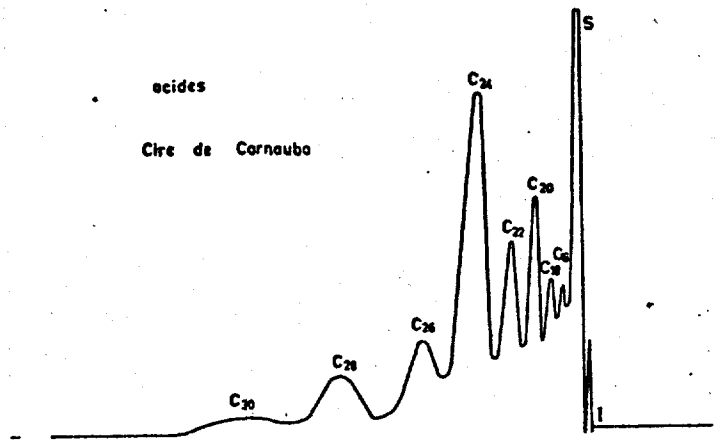

Fig. 2 - Chromatogram of the normal fatty acids of Carnauba wax (I=injection, S=solvent).

PARAFFIN STUDY

Up till now little data have been obtained on the hydrocarbons of Carnauba wax because they make up such a small percentage of the total mass of the constituents (1-6% according to Farcy (9)). Murray and Schoenfeld simply indicate that it is a complex mixture comprising essentially C_{27}, C_{29} and C_{31}. Hepatacosane must be the most abundant hydrocarbon according to Gottfried and Ulzer (10). Gas chromatography has allowed us to identify 18 hydrocarbons (Fig. 3). Table 3 lists them. The percentages given are very approximate because of uncertainty in the position of the base line of the chromatogram.

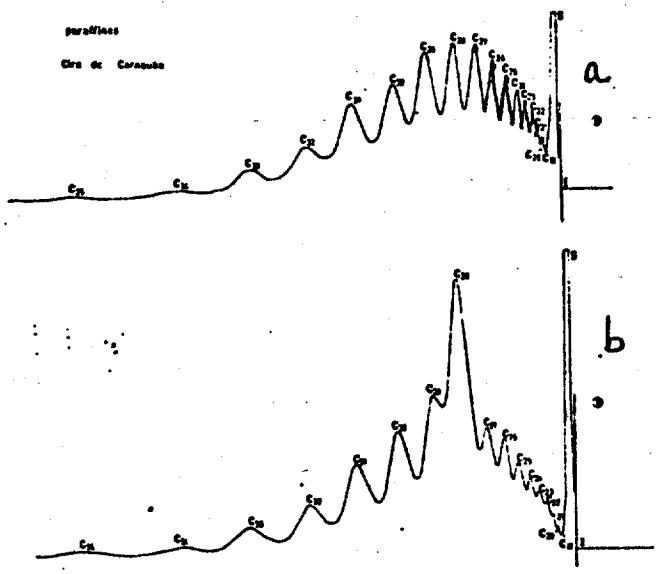

Fig. 3 - Chromatogram of Carnauba wax paraffins (I=injection, S=solvent). a) chromatogram of the paraffins alone. b) chromatogram of the paraffins enriched with octacosane.

Table 3

HYDROCARBONS OF CARNAUBA WAX

Retention volumes are relative to the V_R of tetracosane

Length: 2 m
Gas: helium at 3 liters/hr
Temperature: 300°
Stationary phase: 20% silicone oil 710 on 60/80 mesh fire brick

Hydrocarbon	Carbon atoms	Log V_R measured	Log V_R rel. to std.	Amount
Nonadecane	19	-0,57	-0,53	traces
eicosane	20	-0,46	-0,45	0,3 %
heneicosane	21	-0,34	-0,33	0,4 %
docasane	22	-0,21	-0,21	1,3 %
tricosane	23	-0,12	-0,12	2.3 %
tetracosane	24	0	0	3,2 %
pentacosane	25	0,10	0,10	5,4 %
hexacosane	26	0,21	0,21	8,3 %
heptacosane	27	0,31	0,31	13,3 %
octacosane	28	0,42	0,42	16,0 %
nonacosane	29	0,53	0,52	16,0 %
triacontane	30	0,63	0,61	12,3 %
hentriacontane	31	0,73	0,73	7,9 %
dotriacontane	32	0,81	0,83	6,6 %
tritriacontane	33	0,91	0,93	4,1 %
tetratriacontane	34	0,99	1,03	1,2 %
pentatriacontane	35	1,07	1,13	1,0 %

We are sure that we are dealing with hydrocarbons of different chain length from graphing log of experimental $V_R = f(n)$ [n is the number of carbon atoms and V_R is relative to tetracosane]. It can be seen from Fig. 4 that the experimental points are almost perfectly aligned. According to the law of James and Martin (11) this signifies that one is dealing with a series of homologues. Further, to complete the identification we have chromatographed a wax hydrocarbon mixture with known hydrocarbons added (octacosane for example, Fig. 3). The peak height of that compound then appears larger on the chromatogram. The presence of paraffins containing even numbers of carbon atoms is then clearly shown. We have already shown the existence of such paraffins in the liquid wax of the cuticle of apples by combining gas chromatography with infrared spectroscopy (12). This confirms the hypothesis according to which the biosynthesis of hydrocarbons of vegetable waxes takes place by a method other than simple decarboxylation of fatty acids with an even number of carbon atoms. A lenthening of the chain, carbon atom by carbon atom, appears probable.

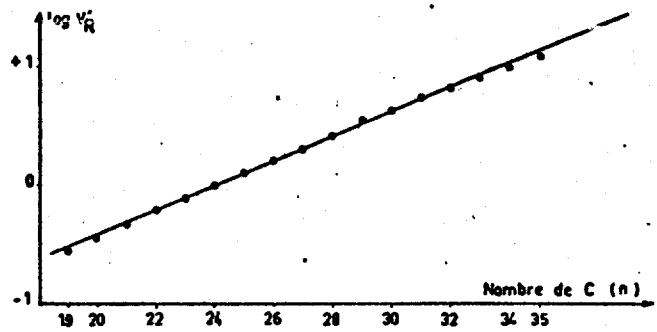

Fig. 4 - Graph of log V_R = f(n) obtained by plotting the corrected retention volumes relative to tetracosane on the ordinate and the number of carbon atoms hydrocarbon on the abcissa.

STUDY OF THE ALCOHOLS

The vaporization of oxygenated compounds of long chain length is very difficult. In order to chromatograph all the primary alifatic alcohols present in Carnauba wax we had to employ two different temperatures. Fig. 5 shows the results obtained at 300° and 340°, compared with a chromatogram of apple wax alcohols at 300°. Eight different alcohols have thus been separated. Table 4 indicates the experimental conditions and lists the alcohols identified with their percentages. In the same table we have given the values of Murray and Schonfeld obtained by fractional distillation of the acetylated alcohols (6). All the alcohols found previously have been identified in our study, and, in addition, traces of hexatriacontanol have been found by gas chromatogarraphy. The quantitative results obtained by gas chromatography are quite well in accord with those obtained by fractional distillation.

Table 4

ALCOHOLS OF CARNAUBA WAX

The retention volumes corrected are expressed relative to the V_R of hexacosanol at 300° and to the V_R of eicosanol at 340°.

Length: 2 m
Gas: helium at 3 liters/hr
Temperature: 300 and 340°
Stationary phase: 20% silicone rubber on Chromosorb W (60/80 mesh)

		300°		340°			
alcohol	carbon atoms	log V_R measured	Log V_R rel. to std.	Log V_R	V_R rel. to std.	%	% found by (6)
eicosanol	20	-	-0,72	-	0	-	1 %
docosanol	22	-0,45	-0,44	-	-	traces	
tetracosanol	24	-0,24	-0,21	-	-	traces	
hexacosanol	26	0	0	-	0,50	traces	4 %
octacosanol	28	0,18	0,19	0,66	0,66	2,3%	5 %
triacontanol	30	0,36	0,37	0,845	0,835	13,7%	19 %
dotriacontanol	32	0,54	0,58	1	1	69,5%	51 %
tetratriacontanol	36	0,78	0,80	1,16	1,18	14,5%	22 %
hexatriacontanol	36	-	-	1,40	1,38	traces	-

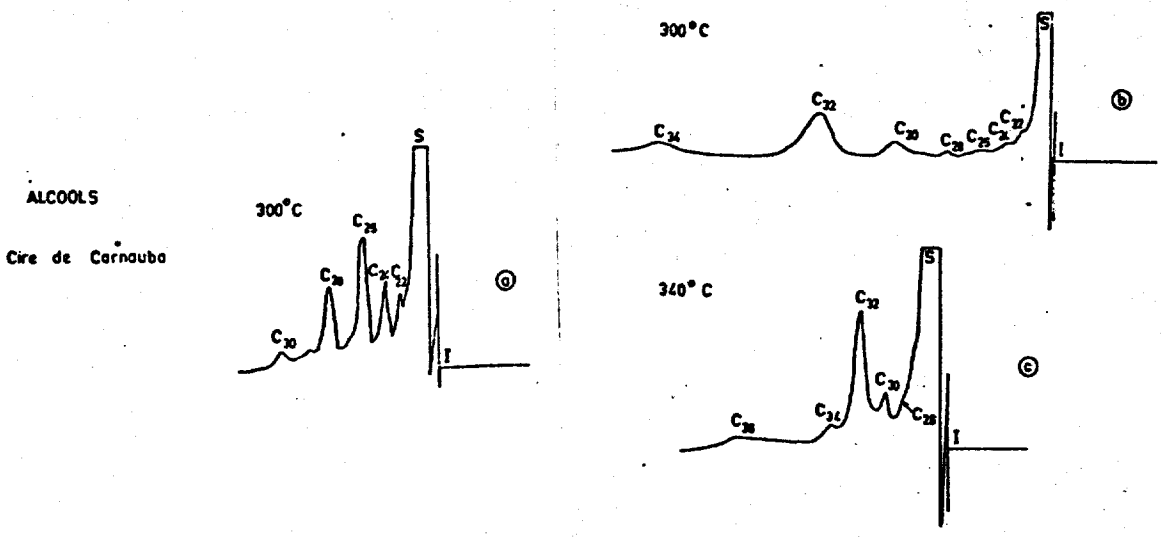

Fig. 5 - Chromatograms of alcohols of apple and Carnauba waxes at different temperatures (I = injection, S = solvent). a) chromatogram of apple wax at 300°. b) alcohols of Carnauba wax at 300°. c) Carnauba wax alcohols at 340°.

CONCLUSION

The results of our experiments on Carnauba wax and apple wax indicate that the microanalysis proposed may be employed for the study of very diverse vegetable waxes. The method is quantative and possesses the advantage of being much more rapid than previous methods.

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(6) Murray K. E. and Schoenfeld R. - Studies of waxes. III, the alcohols of Carnauba wax. J. Am. Oil Chemist's Society, 28, pp. 461-466 (1951).

(7) Murray K. E. and Schoenfeld R. - Studies of waxes. IX, the diols of Carnauba wax. Aust. J. Chem., 8 pp. 432-436 (1955).

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(9) Farcy L. - La cire de Candelilla. J. pharm. Chim., 22, pp. 174-175, (1920).

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in Form ihrer Methylester oder ihrer Bariumseifen durch Auswertung der trans-Bande bei 10.33 μ IR-spektrometrisch analysiert werden[19]. Als Beispiel sei hier die Bestimmung der Lage des Gleichgewichtes zwischen cis- und trans-Form bei der Isomerisierung der Palmitölensäure mit Stickoxyden beschrieben.

Eine kleine Probe Palmitolcinsäure-methylester wurde in der für die freie Säure beschriebenen Weise isomerisiert. Nach der Einwirkung der Stickoxyde auf den Ester (20 Min.) wurde in Petroläther aufgenommen, schnell mit Eis gewaschen und über wasserfreiem Natriumsul Das durch Abdampfen des Petroläthers gewo der cis-trans-isomeren 9.10-Hexadecensäuren w chlorkohlenstoff gelöst und IR-spektrometris analysiert. Die Auswertung nach dem Tange an Hand einer Eichkurve ergab einen trans-Geh

Zu den physikalischen Eigenschaften de dec eßlich auch ihr phi as ihrer Derivate

[19] E rt u. G. Mankel, 1 59].

S1

CARNAUBA

oleinsäure besitzt den gleichen Rf-Wert wie Me säure, Linolsäure, kurz, wie alle Fettsäuren mit de Wertzahl 14. Für die Ester gelten entsprechende hältnisse. So stimmen beispielsweise die Rf-Werte Palmitolcinsäure-methylester und Linolsäure-methyl in den Systemen Siliconöl/Aceton-Wasser (80 : 20) Undecan/Eisessig-Wasser (93 : 7)[20] überein. Die setzmäßigkeit gilt auch für die Glyceride. Tripalmit hergestellt durch direkte Veresterung der reinen

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Die Papier-Chromatographie auf dem Fettgebiet, XLVII. Mitteilung: Die qualitative und quantitative pc-Analyse der Wachssäuren*

Von Prof. Dr. Dr. h. c. H. P. Kaufmann und B. Das, M. Sc. (Tech.)

Aus dem Institut für industrielle Fettforschung, Münster (Westf.)

Es wird ein Verfahren zur pc-Trennung von geradzahligen Wachssäuren beschrieben. Mit Hilfe dieser Methode ist es möglich, Wachssäuren mit bis zu 36 Kohlenstoffatomen nachzuweisen. Nach diesem Verfahren wurden die Wachssäuren von Bienen-, Carnauba- und Montanwachs qualitativ und quantitativ untersucht.

La chromatographie sur papier dans le domaine des corps gras: L'analyse qualitative et quantitative par chromatographie sur papier des acides gras des cires

On décrit un procédé de séparation par chromatographie sur papier d'acides gras des cires à nombre de C pairs. Grâce à cette méthode il est possible de déterminer des acides ayant jusqu'à 36 atomes de carbone. On a étudié qualitativement et quantitativement à l'aide de ce procédé les acides des cires d'abeille, de carnauba et de montan.

Paper-Chromatography in the Field of Fats: Qualitative and Quantitative pc-Analysis of Wax Acids

A procedure for pc-separation of even numbered wax acids has been described. With the help of this method, it is possible to identify wax acids up to 36 carbon atoms. The wax acids of Bees-, Carnauba-, and Montan wax have been qualitatively and quantitatively investigated by this method.

Бумажная хроматография в области жиров: Качественный и количественный бумажнохроматографический анализ восковых кислот.

В работе описывается способ бумажнохроматографического разделения четных восковых кислот. Этим методом можно установить наличие восковых кислот с количеством атомов до 36. Метод применялся для качественного и количественного исследования восковых кислот воска пчел карнауба и монтана.

Während sich Gemische von Fettsäuren, die eine Kettenlänge bis zu 26 C-Atomen aufweisen, mit ausreichender Genauigkeit analysieren ließen[1], bereitete die direkte Trennung der Wachssäuren mit bis zu 36 Kohlenstoff-Atomen erhebliche Schwierigkeiten.

Daher führten *H. P. Kaufmann* und *J. Pollerberg* diese Säuren zunächst in die Allylester über und verbesserten auf diese Weise ihre Löslichkeitseigenschaften in einer für die pc-Analyse geeigneten Weise[2]. *S. Fieker* und *V. Hajek*[3] hydrophobierten das Papier mit Paraffin und entwickelten bei 85° C im System Paraffin-Essigsäure. Das Arbeiten bei höheren Temperaturen kompliziert die Arbeitsweise, und die permanente Imprägnierung erschwert die quantitative Auswertung[4].

Nach systematischen Versuchen mit verschiedenen Hydrophobierungsmitteln konnten wir feststellen, daß anstelle des vielfach verwendeten Undecans eine Petroleum-Fraktion vom Sdp. 230° bis 240° C bei 40° C angewandt, zur pc-Analyse dieser langkettigen Fettsäuren gut geeignet ist. Da sie durch Erhitzen des entwickelten Papyrogramms auf 150° C entfernt werden kann, hat sie alle Vorteile der temporären Imprägnierung. Als Fließmittel bewährte sich ein Gemisch von Isopropanol, Äthanol und Essigsäure. Bei diesem System ist die gegenseitige Löslichkeit von Fließmittel und stationärer Phase gegenüber Undecan herabgesetzt. Die Versuche wurden zunächst unter Verwendung gesättigter gerad-

* Studien auf dem Fettgebiet, 265. Mitteilung.

[1] *H. P. Kaufmann*, Analyse der Fette und Fettprodukte, Springer-Verlag, Berlin Göttingen Heidelberg 1958, Bd. I, S. 817; s. auch: Fette · Seifen · Anstrichmittel 62, 1, 153, 160 [1960].

[2] Fette · Seifen · Anstrichmittel 59, 815 [1957].

[3] Chem. Listy 52, 519 [1958].

[4] *H. P. Kaufmann* u. *Z. Makus*, Fette · Seifen · Anstrichmittel 62, 153 [1960].

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kettiger Säuren von C_{14} bis C_{30} (reinst) in der nachstehend beschriebenen Weise durchgeführt.

Imprägnierung

Das Papier der Firma *Schleicher & Schüll* Nr. 2043 b ausgew. wurde zunächst 15 Min. bei 110° C getrocknet und anschließend in eine Lösung von 10 % der Petroleum-Fraktion vom Sdp. 230° bis 240° C* in Petroläther (Sdp. 50° bis 70° C) eingetaucht. Dann preßte man zwischen Filterpapier-Bogen ab und ließ zur Entfernung des Petroläthers etwa 40 Min. bei Zimmertemperatur hängen. Der Imprägnierungsgrad soll weniger als 0.1 betragen. Dann wurden die zu untersuchenden Substanzen auf das Papier aufgetragen.

Entwicklung der Chromatogramme

Als Fließmittel diente ein Gemisch von Isopropanol (99.5 %), Äthanol (96 %), Essigsäure (99 bis 100 %) und Wasser im Verhältnis 8 : 2.5 : 4 : 1.25. Es wurde bei 42° C mit Petroleum (Sdp. 230° bis 240° C) gesättigt. Man entwickelte die Chromatogramme in einem Trockenschrank bei 42° bis 42.5° C. Die Laufzeit betrug etwa 14 Std.

Anfärbung

Zur Entfernung des Fließ- und Imprägnierungsmittels wurden die Chromatogramme im Trockenschrank 2 Std. auf 150° C erhitzt. Anschließend tauchte man sie in eine wäßrige Kupferacetat-Lösung (15 ml gesättigte Kupferacetat-Lösung in 1 l Wasser), wusch etwa 1¼ Std. in fließendem Wasser und zum Schluß dreimal mit dest. Wasser. Dann wurden die Chromatogramme 15 bis 20 Min. in eine wäßrige, gesättigte Lösung von Rubeanwasserstoff, zu der 2 ml konz. Ammoniak pro l zugegeben worden waren, getaucht und anschließend fünfmal mit dest. Wasser ausgewaschen. Diese Anfärbung war für die Analyse der Wachssäuren aus natürlichen Wachsen (s. weiter unten) ausreichend empfindlich. Sie konnte durch die sog. „potenzierte Anfärbung" mit Kupferacetat-Rubeanwasserstoff, wie sie *H. P. Kaufmann* und *E. Mohr*[5] bei der Sichtbarmachung der Fettsäuren mit Hilfe von Kupferacetat-Kaliumferrocyanid angewendet haben, verstärkt werden.

Wie Abb. 1 zeigt, wurden mit dieser Methode Trennungen mit gesättigten Wachssäuren erzielt. Wir wandten sie daher auf die Untersuchung der Wachssäuren von drei natürlichen Wachsen an, und zwar des Bienen-, Carnauba- und Montanwachses. Über ihren Gehalt an Wachssäuren, Unverseifbarem bzw. an Kohlenwasserstoffen gibt Tab. 1 Auskunft. Die papierchromatographische Untersuchung wird nachstehend beschrieben.

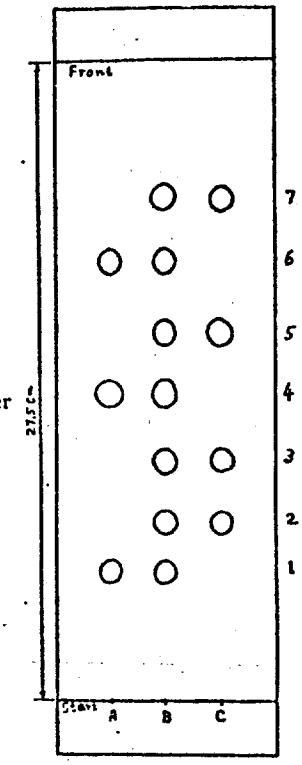

Abb. 1. Trennung gesättigter Wachssäuren

Aufgetragen: je 20 γ
A: C_{20}, C_{24}, C_{30},
B: C_{16}, bis C_{30},
C: C_{18}, C_{22}, C_{26}, C_{28}

Tabelle 1

Gehalt des Bienen-, Carnauba- und Roh-Montanwachses an Unverseifbarem, Kohlenwasserstoffen und Säuren (in Gew.-%)

	Bienenwachs		Carnaubawachs		Roh-Montanwachs	
	gef.	Literatur	gef.	Literatur	gef.	Literatur
Unverseifbares	54.0 **	54.0[6] bis 55.58[7]	53.1 ***	51.0 bis 56.0[8]	57.0 ***	—
Kohlenwasserstoffe	13.2 ****	11.0 bis 18.0[7, 10]	5.3 ****	5.7[9]	—	—
Säuren	44.3 **	45.0[11]	46.7	42.0 bis 50.0[8]	43.8	47.7 bis 52.3[12]

Bienen- und Carnaubawachs

Die Abtrennung der Kohlenwasserstoffe aus diesen Wachsen erfolgte nach den DGF-Einheitsmethoden[13] mit Hilfe einer Silicagel-Säule. Etwa 0.5 g Wachs wurden in 50 ml Schwefelkohlenstoff gelöst, auf eine Säule gegeben und mit 60 ml Schwefelkohlenstoff eluiert. Nachdem die Kohlenwasserstoffe abgetrennt worden waren, eluierten wir die Säuren und Ester mit einem Gemisch von 100 ml Chloroform-Äthanol

* Zu beziehen durch die Fa. *J. Haltermann*, Hamburg.

[5] Fette · Seifen · Anstrichmittel 60, 165 [1958].

** DGF-Einheitsmethode M-V 5 [57].

*** Nach dem Verfahren von *T. W. Findley* u. *J. B. Brown*, J. Amer. Oil Chemists' Soc. 30, 291 [1953].

**** DGF-Einheitsmethode M-V 6 [57]; Fette · Seifen · Anstrichmittel 59, 29 [1957].

[6] *S. D. Koonce* u. *J. B. Brown*, Oil and Soap 21, 231 [1944].

[7] *J. Lewkowitsch*, Analyst 33, 315 [1908].

[8] *T. W. Findley* u. *J. B. Brown*, J. Amer. Oil Chemists' Soc. 30, 291 [1953]; *K. E. Murray* u. *R. Schoenfeld*, J. Amer. Oil Chemists' Soc. 28, 461 [1951]; *W. B. Bunger* u. *F. A. Kummerow*, J. Amer. Oil Chemists' Soc. 28, 121 [1951].

[9] *T. W. Findley* u. *J. B. Brown*, J. Amer. Oil Chemists' Soc. 30, 291 [1953].

[10] *W. Fuchs* u. *A. de Jong*, Fette · Seifen · Anstrichmittel 56, 218 [1954]; *G. Spengler* u. *E. Wöllner*, Fette · Seifen · Anstrichmittel 57, 5 [1955].

[11] *A. H. Warth*, The Chemistry and Technology of Waxes, Reinhold Publ. Corp., New York 1956, 2. Aufl., S. 91.

[12] *J. Marcusson* u. *P. Lederer*, Chem. Umschau Gebiete Fette, Öle, Wachse, Harze 38, 253 [1931]; *G. Meyerheim*, Seifenfabr. 39, 366, 391 [1919]; *E. J. Fischer* u. *W. Presting*, Kleines Handbuch der Wachsindustrie, VEB Wilhelm Knapp-Verlag, Halle 1958, 3. Aufl., S. 96.

[13] DGF-Einheitsmethode M-V 6 [57]; s. auch *G. von Rosenberg*, Fette · Seifen · Anstrichmittel 59, 29 [1957].

(40:1)[11] und verseiften nach Abdampfen auf dem Wasserbad den Rückstand nach den DGF-Einheitsmethoden (4 Std.). Man filtrierte die ausgeschiedenen Säuren und Alkohole ab, trocknete und löste sie in möglichst wenig Chloroform, dem 5% Äthanol beigemischt worden waren. Diese Lösung wurde auf eine Al_2O_3-Säule (Al_2O_3 nach *Brockmann*; 15 g, 1.5 cm ⌀) gegeben und zunächst mit 60 ml Chloroform-Äthanol (20:1) eluiert. Das eingedampfte Eluat enthielt die von Säuren freien Alkohole. Anschließend eluierte man die Säuren mit 100 ml eines Gemisches von Chloroform-Essigsäure (10:1) bei +12° C.

Die aus Carnaubawachs gewonnenen Säuren waren reine Wachssäuren und konnten direkt zur pc-Analyse benutzt werden, während die Säuren aus Bienenwachs nur etwa 40% der eigentlichen Wachssäuren mit 20 und mehr Kohlenstoffatomen enthielten. Um diese abzutrennen, versetzte man das Gemisch mit heißem Methanol (98%ig) und ließ über Nacht stehen. Die auskristallisierten Wachssäuren wurden abfiltriert und mehrmals mit Methanol ausgewaschen. Zur pc-Analyse wurde eine 0.3%ige, auf 45° C erhitzte Chloroform-Lösung dieser Säuren aufgetropft. Durch die pc-Analyse wurde bewiesen, daß die in Methanol lösliche Fraktion (ca. 42%) keine Wachssäuren enthielt.

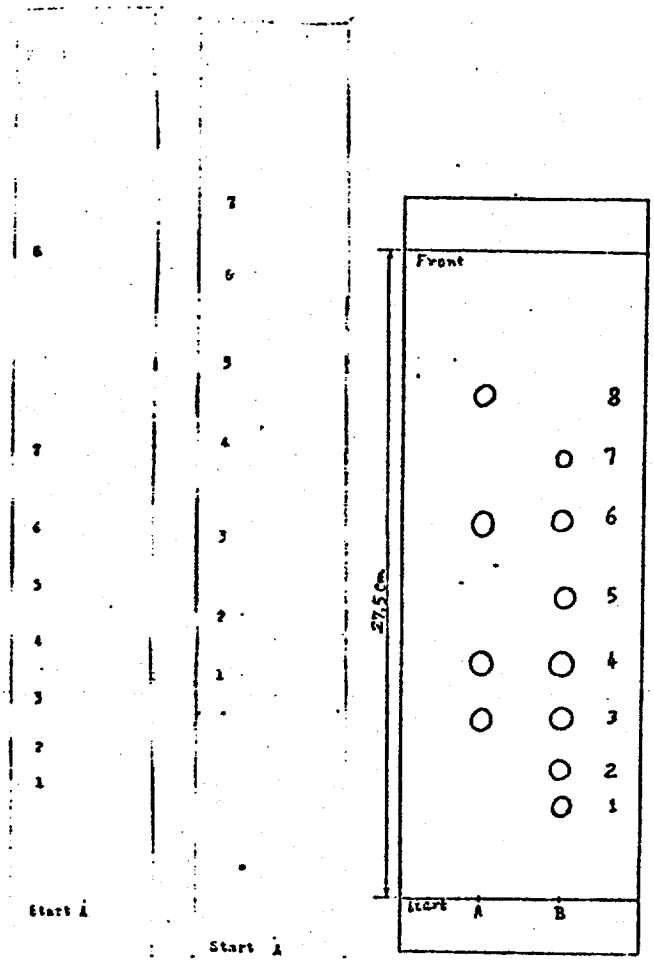

Abb. 2. pc-Trennung der Säuren des Bienenwachses
Aufgetragen: 125 γ
1 = C_{34} 5 = C_{26}
2 = C_{32} 6 = C_{24}
3 = C_{30} 7 = C_{22}
4 = C_{28} 8 = C_{18}
Bedingungen wie in Abb. 1

Abb. 3. pc-Trennung der Säuren des Carnaubawachses
Aufgetragen: 120 γ
1 = C_{30} 5 = C_{22}
2 = C_{28} 6 = C_{20}
3 = C_{26} 7 = C_{18}
4 = C_{24}
Bedingungen wie in Abb. 1

Abb. 4. Trennung der Roh-Montanwachssäuren
A = Vergleichs-Substanzen
B = Roh-Montanwachssäuren 90 γ
1 = C_{34} 5 = C_{26}
2 = C_{32} 6 = C_{24}
3 = C_{30} 7 = C_{22}
4 = C_{28} 8 = C_{20}
Bedingungen wie in Abb. 1

Montanwachs

Nach dem Verfahren von *T. W. Findley* und *J. B. Brown*[13] wurden 5 g Wachs mit NaOH verseift, getrocknet und im Soxhlet 100 Std. mit wasserfreiem Äther extrahiert. Danach destillierte man den Äther ab und extrahierte den Rückstand mit einem siedenden Gemisch von Methanol-Aceton (70:30). Nach dem Eindampfen wurden die Alkohole durch Umkristallisieren aus Aceton bei 5° C gereinigt. Der in der Soxhlet-Hülse befindliche Rückstand wurde nochmals 50 Std. mit Äther, dem einige ml konz. Salzsäure beigemischt waren, extrahiert und die Säuren nach dem gleichen Verfahren wie die Alkohole gewonnen. Anschließend reinigte man die Säuren über einer Al_2O_3-Säule, wie bei den Bienenwachssäuren bereits beschrieben wurde.

Das Ergebnis der pc-Trennung der Säuren des Bienenwachses zeigt Abb. 2, des Carnaubawachses Abb. 3 und des Roh-Montanwachses Abb. 4. Die quantitative Auswertung der Chromatogramme wurde photometrisch durchgeführt[16]. Bei der Auswertung sind gegebenenfalls vorhandene Isosäuren und ungeradzahlige Säuren nicht berücksichtigt. Die erhaltenen Werte verglichen wir mit den in der Literatur angegebenen (Tabb. 1 und 2), wobei

Tabelle 2

pc-Analyse der gesättigten Wachssäuren (in Gew.-%)

Säuren	Carnaubawachs gef.	Carnaubawachs Literatur[18]	Montanwachs* gef.	Bienenwachs gef.	Bienenwachs Literatur[19]
C_{18}	3.3	3.0	—	—	5.6
C_{20}	9.9	11.5	—	0.8	—
C_{22}	9.3	9.0	2.2	1.2	7.3
C_{24}	34.7	30.0	8.3	16.3	17.4
C_{26}	15.8	12.0	16.5	6.4	4.4
C_{28}	23.1	16.5	30.5	6.0	3.1
C_{30}	3.9	7.0	26.2	4.7	2.7
C_{32}	—	—	12.0	2.7	—
C_{34}	—	—	4.3	2.9	—
Palmitin- und C_{16}-Hydroxysäuren				59.0	59.5

wir uns darüber klar sind, daß bei derartigen Naturprodukten erhebliche Unterschiede der Zusammensetzung zu verzeichnen sind.

Die vorstehend beschriebenen Versuche zeigen, daß es gelingt, mit Hilfe eines bequem zu handhabenden Verfahrens die qualitative und quantitative pc-Analyse von Wachssäuren mit befriedigender Genauigkeit durchzuführen. Dagegen bedürfen die bisher bekannten Methoden zur Auftrennung in die verschiedenen Stoffgruppen der Wachse (Ester, freie Säuren und Alkohole, Kohlenwasserstoffe) einer eingehenden Bearbeitung. Darüber werden wir zu gegebener Zeit berichten.

[11] *J. Pollerberg*, Diss. Münster 1958.

[13] J. Amer. Oil Chemists' Soc. 30, 291 [1953].

[16] *A. Scher*, Fette · Seifen · Anstrichmittel 61, 855 [1959].

* *W. Fuchs* u. *R. Dieberg*[17] identifizierten die Säuren von C_{22} bis C_{34}; eine quantitative Bestimmung der Wachssäuren wurde bisher nicht durchgeführt.

[17] Fette · Seifen · Anstrichmittel 58, 826 [1956].

[18] *K. E. Murray* u. *R. Schoenfeld*, J. Amer. Oil Chemists' Soc. 30, 25 [1953].

[19] *H. P. Kaufmann* u. *J. Pollerberg*, Fette · Seifen · Anstrichmittel 59, 815 [1957].

PAPER CHROMATOGRAPHY IN THE FIELD OF FATS, XLVII: THE QUALITATIVE AND QUANTITATIVE pc-ANALYSIS OF WAX ACIDS

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While mixtures of fatty acids of chain length to 26 carbon atoms may be analysed with sufficient precision, the direct separation of wax acids having up to 36 carbon atoms cause considerable difficulties.

Therefore H. P. Kaufmann and J. Pollerberg converted these acids chiefly to their ally esters and by this improved their solubilities in a manner suitable for pc-analysis[2], S. Fieker and V. Hajek[3] used paraffin to render the paper water repellant and developed it at 85°C in a paraffin-acetic acid system. Working at higher temperatures complicated the procedure and permanent impregnation impeded the quantitative evaluation[4].

After systematic experiments with various moisture repellants, we determined that a petroleum fraction of boiling point 230° to 240°, used at a temperature of 40°C is suitable to the pc-analysis of these long chain fatty acids, in place of the much used undecane. Since by heating the paper chromatograms at 150°C, separation can be effected, this method has all the advantages of temporary impregnation. A mixture of isopropanol, ethanol, and acetic acid was verified as the mobile phase. In this system this reciprocal solubility of mobile phase and stationary phase in relation to undecane is precluded. The procedure was carried out chiefly on using straight chain acids from C_{18} to C_{30} (pure) according to the following description.

IMPREGNATION

Paper from the firm of Schleicker and Schull, No. 2043b was dried for 15 minutes at 110°C and then immersed in a solution of 10% petroleum fraction (B.P. 230-240°C)* in petroleum ether (B.P. 50°-70°C). The paper was then pressed between sheets of filter paper and hung for 40 minutes at room temperature to remove the petroleum ether. The rate of impregnation should be less than 0.1. The test materials were then applied to the paper.

DEVELOPMENT OF THE CHROMATOGRAMS

A mixture of isopropanol (99.5%), ethanol (96%), acetic acid (99-100%) and water in the ratio of 8:2.5:4:1:25, was used as the mobile phase. The chromatograms were then saturated with petroleum (B.P. 230°-240°C) at 42°C. Development was carried out in a drying chamber at 42°-42.5°C. The flow time was approximately 14 hours.

LOCATING REAGENT

To remove the mobile and stationary phases, the chromatograms were heated in a drying chamber for 2 hours at 150°C. They were then immersed in an aqueous copper acetate solution (15 ml of saturated copper acetate solution in 1 L of water), washed for approximately 1.25 hours in running water followed by washing three times with distilled water. The chromatograms were immersed for 15 to 20 minutes in an aqueous, saturated solution of dithiooxamide which had been added to 2 ml of concentrated ammonium hydroxide per L, and then washed five times with distilled water. This method of coloration was sufficiently sensitive for the analysis of the wax acids from natural waxes (see below). This can be strengthened by the so-called "potentiated coloration" with copper acetate-dithiooxamide as has been applied by H. P. Kaufmann and E. Mohr[5] in bringing out the fatty acids with the aid of copper acetate-potassium ferrocyanide.

TABLE 1

CONTENT OF UNSAPONIFIABLE HYDROCARBONS AND ACIDS (Weight %) OF BEESWAX, CARNAUBA WAX AND RAW MONTAN WAX.

	BEESWAX		CARNAUBA WAX		RAW MONTAN WAX	
	Found	Literature	Found	Literature	Found	Literature
Unsaponifiable	54.0**	54.0[6] 55.58[7]	53.1***	51.0 56.0[8]	57.0***	-
Hydrocarbons	13.2****	11.0 18.0[7,10]	5.3****	5.7[9]		
Acids	44.3**	45.0[11]	46.7	42.0 50.0[8]	43.8	47.7 52.3[12]

As Fig. 1 shows, a separation of saturated wax acids was achieved with this method. We applied it here to the examination of the wax acids of the three natural waxes beeswax, carnauba wax and montan wax. The information concerning the unsaponifiable and hydrocarbon content of the wax acids is given in Table 1. The paper chromatographic experiment will be described below.

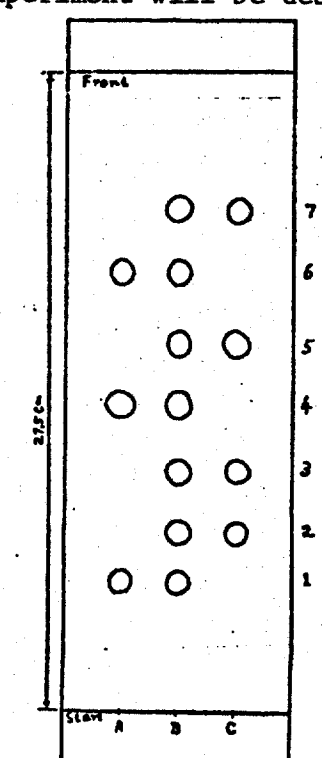
Fig. 1. Separation of Saturated Wax Acids

Amount applied (20 γ each)

A: C_{20}, C_{24}, C_{30}

B: C_{18} to C_{30}

C: C_{18}, C_{22}, C_{26}, C_{28}



BEESWAX AND CARNAUBA WAX

The separation of the hydrocarbons from these waxes was achieved following the DGF-Units method[13] with the aid of a silica gel column. Approximately 0.5 gm of wax was dissolved in 50 ml of carbon disulfide, run on a column and eluted with 60 ml of carbon disulfide. After the hydrocarbons had been separated, the acids and esters were eluted with a mixture of 100 ml of chloroform-ethanol (20:1)[14] and then saponified after evaporating the residue on a water bath, according to the DGF-Units method (4 hours). The acids and alcohols were then filtered, dried and dissolved in the least amount of chloroform, which had been mixed with 5% ethanol. This solution was run onto an Al_2O_3 column (Al_2O_3 according to Brockmann: 15 g, 1.5 cm inner diameter) and eluted with 60 ml of chloroform-ethanol solution (20:1). The evaporated eluate contained the alcohols free from acids. The acids were finally eluted with 100 ml of a mixture of chloroform-acetic acid (10:1) at 42°C.

The acids obtained from carnauba wax were pure wax acids and could be used directly in pc-analysis, while the acids from beeswax contained only approximately 40% of the true wax acids with 20 or more carbon atoms. To separate these hot methanol (98%) was added and the mixture was allowed to stand overnight. The crystallized wax acids were filtered and washed several times with methanol. A 3% chloroform solution, heated to 45°C, was used as the solvent for spotting these acids. It was demonstrated by pc-analysis that no wax acids were contained in the methanol soluble fraction (ca 42%).

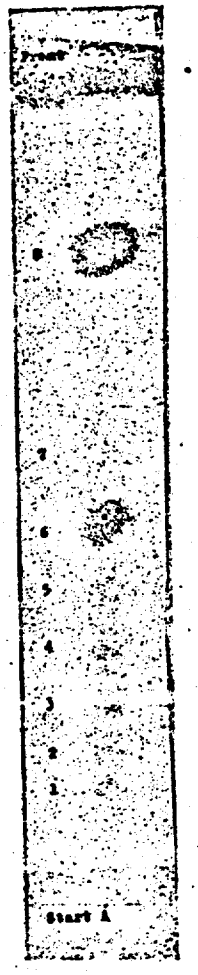
MONTAN WAX

According to the method of T. W. Findley and J. B. Brown[15], 5 gm of wax was saponified with NaOH, dried, and extracted for 100 hours in a Soxhlet with anhydrous ether. The ether was then distilled and the residue was extracted with a boiling mixture of methanol-acetone (70:30). After the evaporation the alcohols were purified by recrystallization from acetone at 5°C. The residue in the Soxhlet was again extracted for 50 hours with ether to which several ml of concentrated HCl had been added, and the acids were extracted by the same method as were the alcohols. The acids were purified on a Al_2O_3 column, as has been described for the beeswax acids.

TABLE 2

Pc-ANALYSIS OF THE SATURATED WAX ACIDS (Weight %)

	CARNAUBA WAX		MONTAN* WAX	BEESWAX	
Acids	Found	Lit[18]	Found	Found	Lit[19]
C_{18}	3.3	3.0	-	-	5.6
C_{20}	9.9	11.5	-	0.8	-
C_{22}	9.3	9.0	2.2	1.2	7.3
C_{24}	34.7	30.0	8.3	16.3	17.4
C_{26}	15.8	12.0	16.5	6.4	4.4
C_{28}	23.1	16.5	30.5	6.0	3.1
C_{30}	3.9	7.0	26.2	4.7	2.7
C_{32}	-	-	12.0	2.7	-
C_{34}	-	-	4.3	2.9	-
Palmitic and C_{16} - Hydroxy acids				59.0	59.5



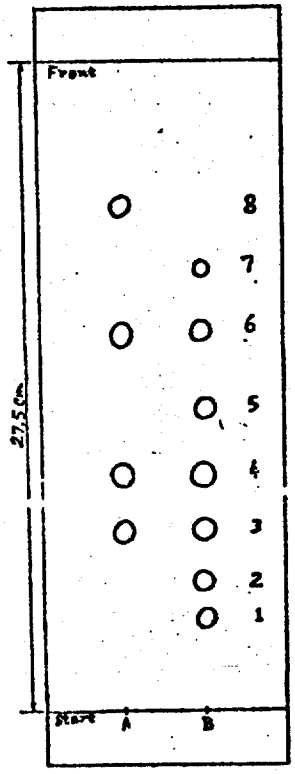


FIG. 2 FIG. 3 FIG. 4

pc-Separation of the acids of beeswax
Amount applied=125 γ

1=C_{34} 5=C_{26}
2=C_{32} 6=C_{24}
3=C_{30} 7=C_{22}
4=C_{28} 8=C_{16}

pc-Separation of the acids of carnauba wax
Amount applied=125 γ

1=C_{30} 5=C_{22}
2=C_{28} 6=C_{20}
3=C_{26} 7=C_{18}
4=C_{24}

Separation of Raw Montan Wax Acids
A= Reference Standards
B= Raw Montan Wax Acids 90 γ

1=C_{34} 5=C_{26}
2=C_{32} 6=C_{24}
3=C_{30} 7=C_{22}
4=C_{28} 8=C_{20}

CONDITIONS AS IN FIG. 1.

Fig. 2 shows the result of the pc-separation of the acids of beeswax, Fig. 3 shows carnauba wax and Fig. 4 shows raw montan wax. The quantitative evaluation of the chromatograms was carried out photometrically. If necessary in the evaluation, extant isoacids and those acids of uneven numbers of carbon are not considered. We compared the values found with those given in the literature (Tables 1 and 2), whereupon we have clarified that in cases of such natural products, considerable differences in the composition are to be noted.

The procedures described above show that the qualitative and quantitative pc-analysis of wax acids can be attained with satisfactory precision with the aid of a method conveniently implemented. We will report on this in time.

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