Chromatographic Isolation of the Original Constituents of Natural Waxes. The Composition of Ouricuri Wax[1]

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THE CLASSICAL METHOD of investigating natural waxes involves disruptive attack by saponification, followed by examination of the fragments. From these results it has been concluded that natural waxes are mainly mixtures of esters of high molecular weight, normal acids, and alcohols. Identification of these fragments has been an important objective in this field (1,2,3,4,5). So far very few attempts have been made to determine the original components of natural waxes; most of these attempts have only demonstrated their complex nature and have shed little light on the composition.

Findley and Brown (6) used molecular distillation in conjunction with functional group analysis to investigate a number of waxes. They were successful with some, but others, *e.g.*, ouricuri wax, were considerably altered by the distillation. Chromatographic procedures have been developed for separating a mixture of synthetic "wax-like" compounds (7), the unhydrolyzed constituents of beeswax (8), and also Montan wax (9). However, because of differences in solubility, these procedures are not directly applicable to most of the plant waxes. A qualitative isolation of wax esters, after an 85-stage crystallization, has been reported (10), but this technique is unsuited to a comprehensive study of any given wax.

This paper reports the chromatographic separation of a mixture of synthetic, allegedly waxlike compounds and of the unhydrolyzed constituents of spermaceti and ouricuri waxes. An estimate of the original composition of ouricuri wax is given.

Materials

Activated alumina, 80–200 mesh, was obtained from the Fisher Scientific Company and from M. Woelm *anionotrophic, pH 4, mesh 80–200* (supplied by the Standard Scientific Supply Corporation).

Silica gel, Davison (mesh size through 200) was used throughout.

Heptane, 99 moles per cent pure, obtained from Phillips Petroleum Company, was dried and redistilled before use.

Glacial acetic acid was dehydrated by refluxing one liter of the acid with 50 ml. of acetic anhydride for 3 hrs. and was redistilled.

Dotriacontane was prepared by reacting cetyl bromide with metallic sodium in anhydrous ether: m.p. 69.5°C. (reported 70°); C, 85.20%; H, 14.69% (calcd. C, 85.24; H, 14.75).

Stearic acid was obtained by purification of hystrene (T-97), supplied by the Atlas Powder Company, by twice recrystallizing from acetone at 0°: neut. eq., 285.0 (calcd. 284.5) iodine value, 0.3.

Octadecanol. Crude octadecanol, supplied by Eastman-White, was recrystallized three times from 95% ethanol and once from methanol: m.p. 57.8°; hydroxyl value, 208.8 (calcd. 207.5).

Octadecyl stearate was prepared by the method of Hilditch and Paul (11): sap. value 104.8 (calcd. 104.5).

Stearone, supplied by Armour and Company, was twice recrystallized from 50% heptane-acetone (ketone eq. 505; calcd. 506.9).

Spermaceti wax was obtained from Orr, Brown, and Price Drug Company.

Ouricuri wax was supplied and authenticated by S. C. Johnson and Son Inc.

Analytical Methods

The isolated wax fractions were analyzed by the following modified procedures of Findley and Brown (6).

Hydroxyl Value. The sample (0.1–0.5 g.) and 4 ml. of the acetylating reagent [a mixture of 1 part of acetic anhydride in 3 parts of pyridine, with 0.4% water added, as recommended by Hawke (12)] were placed in a 125-ml. Erlenmeyer flask, which was then connected to a 75-cm. air condenser; the joint surfaces were wet with a drop of pyridine to ensure a good seal. After refluxing for 90 min. and cooling, 10 ml. of 85% aqueous pyridine were added. The mixture was warmed for 2 min., and 35 ml. of benzene or chloroform were added. The excess acetic acid was titrated against N/2 alcoholic KOH with phenolphthalein as indicator. The titration was corrected for the amount of alkali consumed by the free acid in the sample.

Acid and Ester Value. Acid and ester values were done separately to permit the use of aqueous N/10 alkali for acid value determinations. *Acid value*—The sample was dissolved in 10 ml. of warm benzene, then 5 ml. of neutral alcohol were added. The flask was allowed to cool before titrating with 0.1 N aqueous alkali, using phenolphthalein as indicator. The desirable end-point was reached when the solution remained pink for at least 3 min. *Ester value*—The sample was heated under reflux for 90 min., with a mixture of 5 ml. of benzene and 10 ml. of an approx. N/2 solution of KOH. The excess alkali was then titrated against 0.5 N HCl from a microburette. The acid value is the number of milliequivalents of base required to neutralize the acids present in the sample, divided by the sample weight; the ester value is the difference between the saponification value and the acid value. *Carbonyl value*—This was determined by the method described previously (6). The carbonyl value is obtained by dividing the number of milliequivalents of base consumed in neutralizing the liberated hydrochloric acid by the sample weight. The hydroxyl value is obtained similarly except that the number of milliequivalents of base equivalent to the acetic acid required to acetylate the sample is divided by the sample weight.

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Molecular weights were determined by the Rast method of melting-point depression of camphor. These values are subject to considerable error but are of sufficient accuracy to afford important information on fractions.

The concentration of all functional groups is expressed in "milliequivalents per gram" of sample. This method of expression provides an easy means of comparison and also affords greater facility in calculating mean molecular weights. For monofunctional compounds the mean molecular weight (M.M.W.) is obtained by dividing the number of milliequivalents per gram into 1,000.

Separation Methods and Results

Alumina has been used successfully for the separation of fatty acid esters (13,14,15), but some degree of hydrolysis was observed when applied to waxes (13,16) and total hydrolysis when applied to diacetyl toxicarol (17).

Chromatographic separations in the experiments reported below were carried out in a glass column, 5.0 cm. in diameter. The top of the column was fitted with a ground-glass joint 24/40, into which could be inserted a suitably sized reservoir (usually a 1-liter R.B. flask), modified to fit into the column. Frequently regenerated adsorbent was used, and this was prepared as follows. The sample was washed thoroughly with methanol and with water, then heated in a muffle furnace at 600°C. for 5 hrs. and washed with acidulated water until the supernatant attained the desired pH. The supernatant liquid was decanted, and the saturated alumina was placed in a drying oven at 105° for varying lengths of time, depending upon the adsorptive strength required. A standard method to determine the absorptive activity of alumina is given by Brockman and Schodder (18). This procedure was modified by us to provide more rigid control of the adsorbent. The amount of alumina used in the standard test was decreased to 5.5 g. in a 1.3-cm. diameter column, but the same series of dyes was maintained. Our designation of Grade 2 is given to alumina on which p-methoxy-azobenzene (lower) would just separate from Sudan yellow. Grade 4 alumina permits the complete removal of p-methoxy-azobenzene but leaves Sudan yellow as a band beginning 4 cm. from the top of the column.

Initially an effective procedure was developed for separating a mixture of known compounds similar to those allegedly present in natural waxes. The adsorbent was alumina, (Fisher) 600 g. washed free of alkali with 1% HCl, and reactivated by heating at 105°C. for 10 hrs. to produce Grade 2. Heptane removed hydrocarbon, 1% ether in heptane eluted octadecyl stearate and stearone together, and 1% propanolic heptane removed octadecanol (Table I). When stearic acid was added to this mixture, it was eluted as the final component by 1% acetic acid in heptane.

Fractionation of spermaceti wax was then undertaken before proceeding to the more complex plant waxes. When spermaceti wax was chromatographed on fresh alumina of Grade 2, 8.5% of the ester was destroyed, and equivalent increases in the amount of acids and alcohols were recovered. However, when chromatographed on regenerated alumina, separations free of disruption resulted.

Table II shows F_8 as the eluate from 1,400 ml. of propanolic heptane. We found that it was possible to introduce propanolic heptane at this point to remove the remainder of the esters without the danger of bringing down any of the alcohols. The latter functional group requires three liters more of propanolic heptane before it appears in the effluent. Similarly 1% acetic acid was introduced before complete elution of alcohols.

TABLE I

Separation of Known Compounds on Alumina

[Mixture contained dotriacontane 2.8019 g., octadecyl stearate 2.6200 g., stearone 1.1562 g., octadecanol 2.6228 g., yield 99.0% by weight.]

Eluant	Volume, ml.	Elution rate, ml./hr.	Recoveries			
			Hydrocarbon, wt.%	Ester, wt.%	Ketone, wt.%	Alcohol, wt.%
C_7H_{16}	3,600	300	99.2	.....	.....	.....
Et_2O : C_7H_{16} 1 99	4,000	500	.....	99.2	98.1	.....
C_3H_7OH : C_7H_{16} 1 99	5,000	600	.....	.....	.....	99.0

The elution procedure used in separating synthetic compounds was followed for spermaceti wax; however, with this wax, heptane removed the bulk of the ester fraction.

Ouricuri wax was considerably altered when chromatographed on alumina similar in grade to that used for the separation of spermaceti wax. Preliminary experiments showed that a) the separation had to be conducted at 50° to keep the wax soluble in heptane, b) Grade 4 [3] alumina, *vide infra*, was a satisfactory adsorbent on which to chromatograph ouricuri wax, and c) to attempt a complete separation of all the main functional groups on one column of this adsorbent would involve impractical amounts of alumina and solvent. Consequently the separation was carried out in two stages, first a relatively coarse resolution on alumina, and second, a refined one on silica gel.

The wax, 15 g., was dissolved in 1,500 ml. of heptane and transferred to the standard column, containing 450 g. of alumina (Woelm) Grade 4, and maintained at 50° by a heating coil of resistance wire. At this temperature the use of 1% ether-heptane was discontinued. The first five fractions were eluted in order by heptane: 1% propanolic heptane; 5% propanolic heptane; benzene-ethanol 2:1; and 1% acetic acid in heptane. The column was then washed free of the residue with two eluants: two liters of 5% acetic acid in benzene-ethanol 2:1 (eluate a); and one liter of 5% acetic acid in benzene-ethanol 1:1 (eluate b). The wax which was recovered from these two eluates was contaminated with inorganic matter. The two fractions were purified and rechromatographed as follows.

The solids obtained from a) were warmed with benzene to dissolve the wax only, and the benzene solution was then decanted. A final wash of the inorganic residue with a small quantity of ethanol removed all the wax. The combined ethanol and benzene wax solution was chromatographed on a 120-g. column of alumina, Grade 4, (3.4-cm. in diameter). Three fractions were obtained; the first (F_6) was eluted by 0.5% acetic acid in benzene, the second (F_7) was eluted by 5% acetic acid in benzene, and the third (F_8) was eluted by benzene-ethanol-acetic acid 50:25:2.5. The latter still contained inorganic matter. The wax portion was separated from impurities in

[3] To prepare Grade 4 from regenerated alumina, the adsorbent, 450 g., saturated with water was heated in a 1,000-ml. beaker for 8 hrs. at 105°.

TABLE II

Separation of Spermaceti Wax on Alumina

Fraction	Eluant	Eluant volume, ml.	Recovery, wt.%	Ester		Alcohol		Acid	
				m.Eq./g.	Total m.eq.	m.Eq./g.	Total m.eq.	m.Eq./g.	Total m.eq.
F_1	C_7H_{16}	500	8.2	1.96	3.04	.....	.....	.....	.....
F_2	C_7H_{16}	500	30.7	2.10	12.20	.....	.....	.....	.....
F_3	C_7H_{16}	500	16.4	2.20	6.82	.....	.....	.....	.....
F_4	C_7H_{16}	500	9.5	2.18	3.94	.....	.....	.....	.....
F_5	C_7H_{16}	500	9.9	2.19	4.10	.....	.....	.....	.....
F_6	C_7H_{16}	500	4.1	2.23	1.73	.....	.....	.....	.....
F_7	Et_2O : C_7H_{16} 1 99	1500	9.7	2.20	4.03	.....	.....	.....	.....
F_8	C_3H_7OH : C_7H_{16} 1 99	1400	6.8	2.24	2.89	.....	.....	.....	.....
F_9	C_3H_7OH : C_7H_{16} 1 99	3000	2.6	.....	.....	3.69	1.82	.....	.....
	ACOH : C_7H_{16} 1 99	500							
F_{10}	ACOH : C_7H_{16} 1:99	6000	1.2	.....	.....	.....	.....	3.36	0.77
Total		.....	.....		38.75		1.82		0.77
Spermaceti wax		.....	.....	2.13	40.33	0.10	1.89	0.037	0.70

Yield 99.1% by weight. Elution rates: F_1 300 ml./hr.; F_2–F_8 500 ml./hr.; F_9–F_{10} 600 ml./hr.

the way previously described and rechromatographed on silica gel by eluting with 5% acetic acid in benzene. The resulting eluant consisted entirely of resins.

The solids from b) were purified by dissolving the mixture in benzene and passing the wax solution and impurities through a column (3.4 cm. in diameter) of silica gel (Davison, mesh size through 200), and eluting with 5% acetic acid in benzene-ethanol 1:1. The recovered fraction (F_9) was free of contaminants and was also resinous. These results are summarized in Table III.

Further Separation of F_1, F_2, F_3. Fraction (F_1), was a yellowish, white plastic solid. An aliquot, 2.7225 g., was dissolved in 300 ml. of heptane and chromatographed on the standard silica gel column (120 g.). The first subfraction F_1P_1 consisted of hydrocarbons, melting range 57°–71°. The second eluate, F_1P_2, was a yellowish, white crystalline solid with melting range 82°–87°; it was simple ester, *i.e.*, contained no other functional group. The third subfraction, F_1P_3, was similar in appearance to the second, melting range 82°–118.5°. The last eluate, F_1P_4, though insufficient for complete analysis, gave a relatively high hydroxyl value and was considered to be free alcohols brought down by imperfect separation in F_2. These results are summarized in Table IV.

Fraction F_2 was green in color. An aliquot, 3.2772 g., was dissolved in 75 ml. of chloroform and chromatographed on silica gel. Experience with the chromatographic separation of beeswax constituents (8) suggested that these eluants would give a more efficient separation of the constituents of F_2. The resulting subfractions were as follows. F_2P_1 was yellow in color, m.p. 86°–87°, and had only traces of hydroxyl material present. F_2P_2 was dark green and of softer consistency than the preceding subfraction, m.p. 76–79°. F_2P_3, m.p. 86–87°, was eluted by chloroform: acetic acid 50:50:2. The M.M.W. found was 670 and calculated from ester value was 690. The results of this separation are given in Table V.

Fraction F_3. An aliquot, 1.0896 g., of this light-brown brittle material was chromatographed on silica gel with chloroform as solvent and developer. A wide diffuse band fluorescent in u.v. light moved slowly down the column. The total eluate recovered was 1.0453 g., m.p. 82°–83°, ester value 2.34 m.Eq./g., and hydroxyl value 1.66 m.Eq./g. The M.M.W. found, 839, was almost twice the value predicted from the ester value, 427. *Fraction F_4* was not further chromatographed.

A Calculation of the Approximate Composition of Ouricuri Wax

Hydrocarbons. Subfraction F_1P_1 (Table IV) consists entirely of hydrocarbons and represents 1.3% of the original wax.

Simple Esters. Simple esters were the main constituents of subfractions F_1P_2 and F_1P_3 (Table IV)

TABLE III

Separation of Ouricuri Wax on Alumina and Silica Gel

Fraction	Eluant	Eluant volume, ml.	Wt.% of wax	Ester		Alcohol		Acid		Mol. wt. (found)
				m.Eq./g.	Total m. Eq.	m.Eq./g.	Total m. Eq.	m.Eq./g.	Total m. Eq.	
F_1	C_7H_{16}	3600	22.1	1.07	3.56	.....[a]	.....[a]	.....	.....	N.D.[b]
F_2	C_3H_7OH : C_7H_{16} 1 : 99	3000	28.2	1.37	5.79	1.42	6.00	0.06	0.25	N.D.
	C_3H_7OH : C_7H_{16} 1 : 19	500								
F_3	C_3H_7OH : C_7H_{16} 1 : 19	2000	12.1	1.65	2.99	1.68	3.05	0.16	0.29	730
F_4	C_6H_6 : EtOH 2 : 1	800	2.0	2.68	0.82	1.81	0.55	0.16	0.05	725
F_5	AcOH : C_7H_{16} 1 : 99	1500	8.7	0.00	.....	0.15	0.19	2.02	2.64	480
F_6	AcOH : C_6H_6 0.5 : 99.5	600	3.0	1.64	0.76	0.87	0.40	0.96	0.44	650
F_7	AcOH : C_6H_6 1 : 19	1000	5.4	2.80	2.25	1.57	1.26	0.47	0.38	1950
F_8	C_6H_6 : EtOH : AcOH 50 : 25 : 2.5	750	4.8	3.78	2.73	2.13	1.54	4.55	3.28	N.D.
F_9	C_6H_6 : EtOH : AcOH 50 : 50 : 5	2000	10.0	2.66	3.99	2.27	3.41	2.66	3.99	N.D.
Total recovered		.....	96.3	.....	22.89	.....	16.41	.....	11.32	.....
Ouricuri wax		.....	.....	1.50	22.50	1.08	16.13	0.40	6.00	.....

[a] Not detectable. [b] N.D.—not determined.

TABLE IV

Separation of F_1 (Ouricuri Wax) on Silica Gel

Subfraction	Eluant	Eluant volume, ml.	Wt.% of original wax	Ester		Alcohol		Mol.wt. (found)
				m.Eq./g.	Total m. Eq.	m.Eq./g.	Total m. Eq.	
F_1P_1	Heptane	700	1.3	.....	.....	.....	.....	336
F_1P_2	CH_2Cl_2: Heptane 50 50	1000	7.7	1.03	1.20	.....	.....	920
F_1P_3	CH_2Cl_2 45°	800	12.5	1.26	2.36	.....	.....	780
F_1P_4	C_3H_7OH: CH_2Cl_2 2 : 98	500	0.5	N.D.[a]	.....	3.02	0.20	N.D.[a]
Total recovered		.....	22.0	.....	3.56	.....	0.20	.....

[a] N.D.—not determined.

20.2% and also F_2P_1 (Table V) 3.3%. There is fair agreement between the M.M.W. calculated from the ester value and that found by experiment.

Free Alcohols and Hydroxy-Mono Esters (H.M.E.). Because of its position in the elution schedule, subfraction F_2P_2 was assumed to have a negligible amount of simple esters. The analytical data indicate the possible presence of H.M.E. and free alcohols. If the subfraction consisted entirely of H.M.E., then the ester value and hydroxyl value would coincide; actually the hydroxyl value exceeds the ester value by 0.49 m.Eq./g. The mixture present in F_2P_2 is then assumed to contain 0.49 mM of free alcohols and 1.24 mM of H.M.E. per gram. If the M.M.W. of the free alcohols in F_2P_2 is assumed to be the same as that found for F_2P_4, *i.e.* 331, then the estimated amount of free alcohols in F_2P_2 can be arrived at as follows: after multiplying 0.49 by the weight of F_2P_2 (2.347 g.) to obtain the milliequivalents of free alcohols present, the weight of free alcohols is then obtained by multiplying the number of milliequivalents by the M.M.W.; this gives 380 mg. The total estimate of free alcohols in the wax is the sum of F_1P_4 (0.5%) F_2P_2 (2.5%). F_2P_3 is considered to be entirely H.M.E. by virtue of the similarity of hydroxyl and ester values and the fact that the M.M.W., as calculated from the ester value, 691, agrees fairly well with the Rast determination 670.

The total percentage concentration of H.M.E. is obtained by adding the amounts present in F_2P_2 (13.1%), and F_2P_3 (9.3%).

Hydroxy-Di-Ester (H.D.E.). Since the M.M.W. of F_3 after chromatography on silica gel is 839, while the M.M.W. calculated from the ester value is 427, it is assumed that this material consists mostly of H.D.E. A similar assumption is made for F_4, in which the M.M.W. found, 725, is twice that predicted from the ester value, 373.

The probable constituents of F_6 (Table III) are more difficult to assess on the basis of the available data. The M.M.W. found for this fraction is 650, and the M.M.W. predicted from the ester value is 607. The similarity of these two figures would suggest that the fraction consists of a mixture of H.M.E. Since this fraction is eluted after acids, it is unlikely that H.M.E.'s are present. There is also a significant acid concentration present, which makes an assessment more difficult. It appears more plausible to assume a mixture of di-esters and acids. The M.M.W. calculated as di-esters is 1214; however the presence of sufficient amounts of acid of relatively low M.M.W. may explain the actual value found, 650. This fraction is calculated as H.D.E., and the sum of F_3 (12.1%), F_4 (2.0%), and F_6 (3.1%) gives the total H.D.E. present, *i.e.*, 17.2%.

Hydroxy-Acidic-Poly-Esters (H.A.P.E.). The M.M.W. of F_7, calculated from acid value, is 2140 (found by Rast method, 1950). At this level the discrepancy of 5% in the molecular weight determination permits the assumption that there is one free carboxyl group and 6 ester linkages per molecule (ester value/acid value).

Free Acids. F_5 (8.7%) is apparently mostly free acid, with perhaps a small contribution from hydroxy acids.

Resins. F_8 and F_9 (14.8%) consists entirely of very friable reddish-brown resins, which were partially soluble in the solvents tried only with great difficulty.

Discussion

At the time that this work was undertaken, there were no reports in the literature of a successful chromatographic separation of a plant wax. The original intention was to apply, to a plant wax, procedures which were proven successful with synthetic allegedly "wax-like" material and less complex waxes. At the outset however difficulty was experienced with the only alumina then available; the product obtained from Fisher was found to be too "harsh" for the synthetic material but was satisfactorily modified by washing with methanol and water as described. Significantly spermaceti wax required alumina of even lesser adsorptive activity for resolution. The result of this separation tends to support previous reports of the composition of spermaceti wax. We do find however that there is an indication that cetyl palmitate may not be present in the quantities generally believed (19).

Ouricuri wax, in turn, required alumina of lesser

TABLE V

Separation of F_2 (Ouricuri Wax) on Silica Gel

Subfraction	Eluant	Eluant volume, ml.	Wt.% of original wax	Ester		Alcohol		Acid		Mol. wt. (found)
				m.Eq./g.	Total m.Eq.	m.Eq./g.	Total m.Eq.	m.Eq./g.	Total m.Eq.	
F_2P_1	$CHCl_3$	300	3.3	1.81	0.90	.....	.....	0.06	0.03	528
F_2P_2	$CHCl_3$:EtOH 2 : 1	300	15.6	1.24	2.91	1.73	4.04	0.05	0.12	680
F_2P_3	$CHCl_3$:EtOH:ACOH: 50 : 50 : 2	1500	9.2	1.45	2.00	1.40	1.94	0.26	0.36	670
Total recovered		.....	28.1	.....	5.81	.....	5.98	.....	0.58	.....

activity. The alumina provided by M. Woelm, anionotrophic, pH4, was found to be entirely satisfactory and did not require prewashing. We obtained the highest grade and reduced it to the right adsorptive activity by increasing the moisture content. Since the more complex plant wax was more easily disrupted than either the synthetic mixture or spermaceti wax, the indication is therefore that more labile constituents are present. One main difference between ouricuri wax and the other groups of materials is the probable presence of estiolides in the former; in fact, Bertram (20) from theoretical considerations has suggested that estiolides predominate in wool wax, a very complex material. The suggestion has been made that these linkages are attacked by alumina, but although estiolides are probably present in significant amounts, it is unlikely that this linkage is disrupted because they are known to be quite stable even under conditions of mild saponification. Lactones, on the other hand, may be responsible for this evident lability. Even in the cases where disruption did not seem to occur, it was noted that acid value determinations of F_2, F_3, and F_4 were accompanied by fairly rapid reversion of the indicator color at end point, indicating that alkali labile material was present.

There are unfortunately no other reports of this kind with which our results may be compared. Ludecke (21) does report a composition for a refined ouricuri wax with 17% hydrocarbon present, but the original product was probably contaminated. Savidan (22), on the other hand, reports 1.3% hydrocarbon for a sample of authentic ouricuri. We have accounted for 98% of the wax, and, so far as we are aware, this is the only report which has attempted a resolution of this type. In most cases the groups into which the wax has been divided follow directly from the analytical data. There are however one or two cases where the assignment has been somewhat arbitrary because of incomplete evidence. For example, the initial analytical results show almost identical ester and hydroxyl values for F_3 (Table III). The inference would be that this fraction was H.M.E.'s. However the position of elution and the wide divergence between the calculated M.M.W., and that found by Rast determination indicate otherwise. On rechromatography, using one eluant, 96.4% of the charge was recovered. The results of ester, hydroxyl, and M.M.W. determination were in agreement and strongly indicated that this fraction was H.D.E.'s. More equivocal however is the identity of F_6 (Table III), where the ester, hydroxyl, and acid values would indicate components of high M.M.W., but the Rast determination gives a much lower value. There is a strong possibility that this fraction may contain lactones which have undergone ring opening during chromatography.

Two other fractions attract special attention, F_1P_3 (Table IV) because of its very wide melting-range, 82°–118.5°, and F_7 (Table III). The evidence for the existence of acidic-poly-esters in the latter is as yet incomplete but is strongly suggestive of the presence of this unique type of compound.

The acidic imbalance (Table III) does not reflect disruption of the main portion of the wax but is contributed to by the resinous fractions F_8 and F_9. These resins yielded to analytical techniques with great difficulty. They did not melt up to 200°, and Rast values could not be obtained. Resins are themselves a complex mixture, and their study is beyond the scope of this investigation; consequently no specific importance was attached to the imbalance.

Wiedenhof (23) has quite recently used this technique successfully in conjunction with x-ray analysis to identify some of the constituents of four plant waxes.

The approximate original composition of ouricuri wax is as follows: hydrocarbons 1.3%; simple esters 23.5%; H.M.E. 22.4%; H.D.E. 17.2%; H.A.P.E. 5.4%; free acids 8.7%; free alcohols 3.0%; resins 14.8%; moisture 1.4%; ash 0.4%. The presence of a small percentage of diols is also indicated. The suggested composition above may be subject to change with further investigation, but from the evidence it seems clear that the concept that plant waxes are entirely mixtures of simple wax esters should be modified.

Summary

A chromatographic method was developed which separated a known mixture of alleged wax constituents into four groups, *viz.*, dotriacontane, octadecyl stearate and stearone, octadecanol, and stearic acid on especially prepared alumina. This adsorbent, when prepared fresh, did not give a satisfactory separation of spermaceti wax. On re-use after regeneration by exhaustive washing with methanol and water, spermaceti wax was resolved into esters (95.4%), alcohols (2.6%), and acids (1.2%). A resolution of ouricuri wax into four major classes (nine fractions) was obtained on alumina (M. Woelm, aniontrophic, pH4) of lesser adsorptive activity than the two previous preparations. Two of the latter fractions were further resolved on silica gel. There is *a priori* evidence that the constituents of natural plant waxes do not conform rigidly to the pattern of mixtures of simple normal esters. An estimated composition of ouricuri wax (in per cent) is hydrocarbons 1.3, simple esters 23.5, hydroxy-mono-esters 22.4, hydroxy-di-esters 17.2, hydroxy-acidic-poly-esters 5.4, free acids 8.7, free alcohols 3.0, resins 14.8, moisture 1.4, and ash 0.4.

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Gas-Liquid Chromatography of Fatty Derivatives. III. Analysis of Fatty Amines[1]

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GAS-LIQUID chromatography has been shown to be an effective tool in the analysis of certain fatty derivatives (10,11). Its effectiveness in the analysis of fatty alcohols and amines has been hampered however by unsymmetrical peaks which interfere with area measurements and resolution. The asymmetry which occurs is evidenced by the presence of leading, tailing, or both and is generally encountered with the more highly polar molecules. These deviations from the desired Gaussian, or bell-shaped character, result primarily from some mode of interaction between the components being chromatographed and the liquid stationary phase and/or stationary support.

Knight (9) has shown that highly polar compounds can be chromatographed with a minimum of tailing if the carrier gas is first allowed to become either partially or completely saturated (depending on the analysis) with a volatile component. The polarity and partition isotherm of this component should be somewhat similar to the molecules being analyzed. Peak symmetry and resolution were thus improved. Johns (7) reduced the tailing of certain polar molecules on silicone (DC-550) columns by the addition of small amounts of polar solvents.

James and Martin (5) employed Celite 545, treated with methanolic sodium hydroxide, in the estimation of ammonia and methylamines, and James described the separation of amines containing 1 to 12 carbon atoms (4). Decora and Dineen (2) used a porous inorganic solid prepared from a commercial detergent and treated with potassium hydroxide for the separation of basic nitrogen compounds of pK_a range from 6 to 11 including n-hexylamine.

The procedures reported herein represent an application of the principles described in above techniques. These methods were independently developed in separate laboratories but because of similarity are being combined in one report. The described procedures permit the separation and quantitative determination of saturated fatty amine samples of carbon-chain length ranging from C_8 through C_{22}, using nonpolar liquid substrates on either Chromosorb W or Chromosorb solid supports previously treated to reduce their adsorptivity.

Experimental

Preparation of Solid Support. Procedure A[2]: Chromosorb W, 40–60 mesh, was initially deactivated by washing with concentrated hydrochloric acid and then with water to remove the residue acid, followed by heating at 200°C. for 2 hrs. After screening to 40–60 mesh 15.5 g. of the support were poured into a solution of 1.5 g. of potassium hydroxide in 80 ml. of methanol. After the methanol was removed, the support was again heated at 200°C. for 2 hrs., cooled, and poured into 3.2 g. of Apiezon L, which was dissolved in 60 ml. of methylene chloride. The solvent was removed by evaporation, packing material was screened, and the 40–60 mesh material was heated in a vacuum oven 1 hr. at 60°C. before being used to pack the column.

Procedure B[3]: Chromosorb, 30–60 mesh, was first acid-washed over-night with concentrated hydrochloric acid diluted 1:1 with water. The Chromosorb was then repeatedly washed with distilled water until free of acidity and finally oven-dried at 150°C. over-night. Forty grams of the support were treated with 200 ml. of a 5% solution of potassium hydroxide in methanol for a period of 2.5 hrs. The suspension was transferred to a sintered glass funnel, and the solvent was removed with suction. The support was then rinsed with 200 ml. of fresh methanol and finally with 100 ml. of chloroform. The support was then air-dried.

Twenty grams of Dow-Corning High Vacuum grease were washed, using the pretreatment procedure described by Cropper and Heywood (1). The washed grease was dissolved in sufficient ethyl acetate, and to the solution was added the previously deactivated support. The solvent was slowly removed with continual agitation, and the packing was finally allowed to air-dry. The packing was flushed with nitrogen at 200°C. for 3 hrs. before being used to pack a column.

Instrumental Conditions. Procedure A, using Apiezon L: the gas-liquid chromatographic apparatus used in this work was described previously (10,11). A 2-ft. column (stainless steel, ¼ in. O.D.) was packed with the Apiezon L substrate and equilibrated in the instrument for a few hours at 220°C.

Fatty amines were obtained by careful fractional distillation of commercially available materials.[4] The purified amines in Table I were run at 225°C. both individually and in mixtures using 250 ma. current, 0.002–.005-ml. samples, and a helium flow rate of 55 ml./min.

The symmetry of the peaks, as shown in Figure 1, is as good as that observed for the higher α-olefins

[1] The application of gas chromatography described in this paper was conceived and developed independently and simultaneously in the respective research laboratories of these two companies.

[2] Archer-Daniels-Midland Company procedure.
[3] Atlas Powder Company procedure.
[4] Adogens, Archer-Daniels-Midland Company.