Comparison of Some Commercial Waxes by Gas Liquid Chromatography[1]

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ABSTRACT

Volatile components (hydrocarbons, monoesters, free acids as methyl esters and free alcohols as acetates) of seven unhydrolyzed commercial waxes—ouricury, carnauba, Chinese insect, lac, esparto, candelilla and Japan wax—have been analyzed and compared by gas liquid chromatography. Though appreciable portions of the waxes were nonvolatile, the results were sufficient to distinguish the seven waxes completely. Methanolysis products were analyzed directly by gas liquid chromatography, and the results agreed with those previously obtained for hydrolysis products of these waxes. Ouricury wax gave 18% C_{24}-C_{34} $\alpha\omega$-diols and 4% C_{24}-C_{32} ω-hydroxy acids, in addition to 28% C_{20}-C_{32} acids and 17% C_{22}-C_{34} alcohols, on methanolysis.

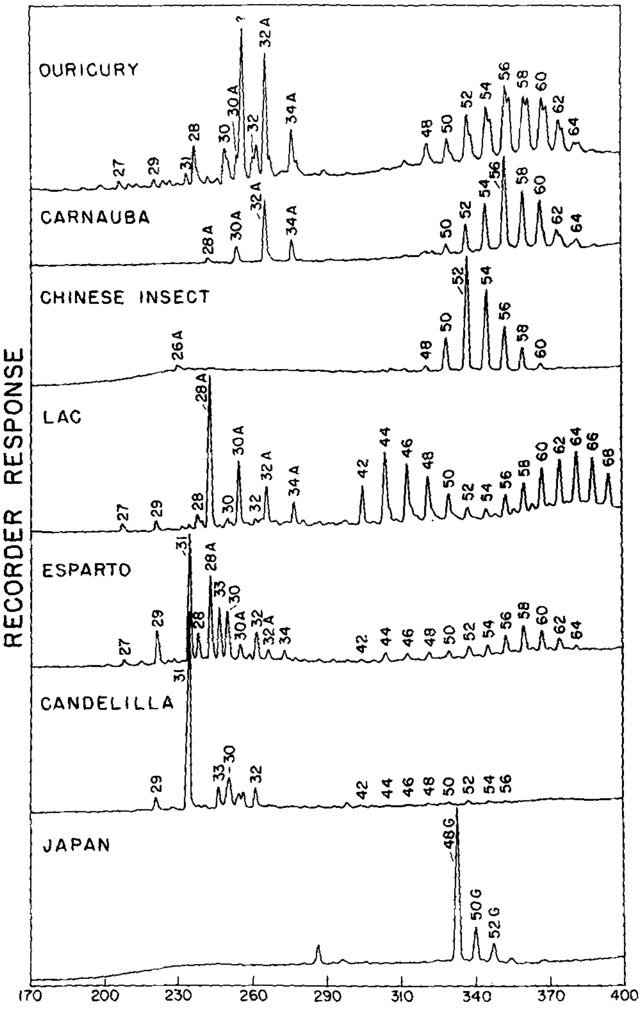

FIG. 1. Gas liquid chromatographic separations of commercial waxes after treatment with diazomethane and acetylation. Peaks with odd numbers (peak numbers also indicate chain lengths) from 27-33 are hydrocarbons; peaks with even numbers from 28-34 are fatty acid methyl esters; peaks with numbers 26A-34A are alcohol acetates; peaks with numbers 42-68 are monoesters; and peaks with numbers 48G-52G are triglycerides. Temperature was programed from 125 to 400 C at 3 C/min, but only results from 170 to 400 C are shown. Only Japan wax showed C_{16} and C_{18} methyl ester peaks between 125 and 170 C.

INTRODUCTION

Beeswax was previously analyzed by gas liquid chromatography (GLC) without preliminary fractionation or hydrolysis, and the amounts and compositions of the hydrocarbons, free fatty acids (as methyl esters) and monoesters (in all 65% of the wax) were determined (1). It then appeared useful to compare a number of other commercial waxes in the same way. Some hydrolysis products of carnauba (2-4), ouricury (5), Chinese insect and lac (6), esparto (7), candelilla (5,8) and Japan waxes (9) have been analyzed by GLC, after fractionation by conventional methods, but the whole waxes have not been so examined. Thin layer chromatographic (TLC) analysis has been used to compare unhydrolyzed waxes (10-12), but the indicated components were not completely identified. Hydrocarbons and free fatty acids of candelilla wax and hydrocarbons of esparto wax have been isolated by preparative TLC and analyzed by GLC (13). Ouricury wax has been fractionated by column chromatography on alumina and the fractions identified by functional group analysis (14).

In this paper the above mentioned seven waxes have been compared by GLC, and amounts and compositions of the volatile components determined. Since there is probably considerable variation in composition (indicated by the range of acid and ester values) between commercial samples of natural waxes, methanolysis products of these waxes have also been examined for comparison with results obtained in the investigations of hydrolysis products referred to above. The mixtures of methyl esters, alcohols, ω-hydroxy acids and diols produced by methanolysis have been analyzed by GLC without preliminary separation. The compositions of some of the waxes, hitherto inadequately

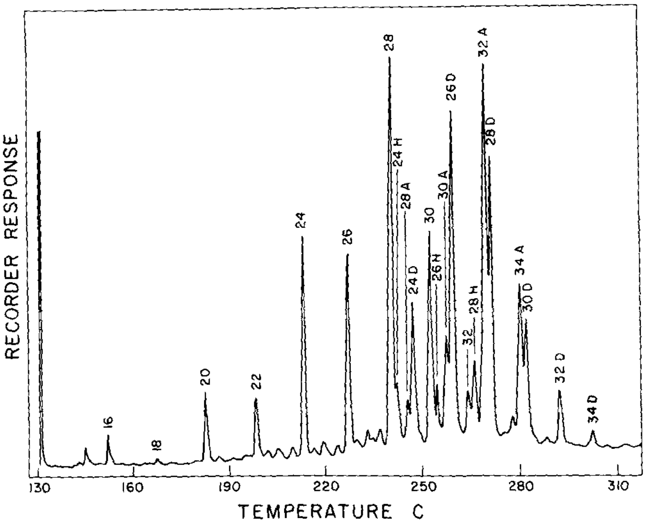

FIG. 2. Gas liquid chromatographic separation of acetylated methanolysis products from ouricury wax. Peak numbers (peak numbers also indicate chain lengths) 16-32 are methyl esters; peak numbers 28A-34A are alcohol acetates; peak numbers 24H-28H are ω-acetoxy methyl esters; and 24D-34D are α,ω-diol diacetates. Temperature was programed from 125 to 300 C at 3 C/min.

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TABLE I

Composition of Commercial Waxes Obtained by Gas Liquid Chromatography (%)

Components	Wax Carnauba	Ouricury	Chinese insect	Lac	Esparto	Candelilla	Japan
Hydrocarbons	—	1	—	1	19	41	—
Free acids	—	4	—	2	9	8	3
Free alcohols	11	5	2	11	8	4	—
Monoesters	36	11	81	24	9	6	36[a]
Hydroxy esters	—	7	—	—	—	—	—
Unidentified[b]	—	4(1)	—	—	—	6(2)	5(3)
Total volatile	47	32	83	38	45	65	46

[a] Triglycerides.

[b] Number of components in parentheses.

investigated, have been clarified considerably.

EXPERIMENTAL PROCEDURES

Wax sample acid and ester values were: carnauba, 4.5, 77.4; ouricury, 14.5, 88.1; Chinese insect, 6.5, 83.5; lac, 13.1, 79.9; esparto, 22.5, 41.2; candelilla, 13.3, 42.6; and Japan, 32.5, 195.3.

Solvent for TLC analysis of waxes was chloroform containing 1% ethanol (v/v). Methanolysis products were examined in the same solvent and R_f values were: methyl triacontanoate, 0.49; triacontyl acetate, 0.43; triacontanol, 0.14; methyl 23-acetoxyoctacosanoate, 0.09; 1,26-hexacosanediol diacetate, 0.07; methyl 28-hydroxyoctacosanoate, 0.02 and 1,26-hexacosanediol 0.00. Chloroform containing 5% ethyl acetate (v/v) was also used and R_f values were: methyl triacontanoate, 0.70; triacontanol, 0.32; methyl 28-hydroxyoctacosanoate, 0.20 and 1,26-hexacosanediol, 0.03.

GLC analyses were carried out using a Hewlett Packard Model 402 gas chromatograph with flame ionization detectors. Columns were 3 ft x 1/8 in. stainless steel packed with 80-100 mesh, acid-washed and silanized Chromosorb W coated with 1.5% Dexsil 300. Wax (0.050 g) was weighed out and 5 ml of chloroform solution containing ca. 0.001 g each of *p*-dioctylbenzene and octadecyl octadecanoate added, followed by a solution of diazomethane in methylene chloride. Solvents were taken off, and the mixture was acetylated with acetic anhydride and pyridine (0.5 ml each) at 100 C. Reagents were removed and the sample analyzed by GLC as a chloroform solution; temperature was programed from 125 to 400 C at 3 C/min; helium flow rate was 60 ml/min. Peaks were integrated with an Infotronics Model CRS-104 digital integrator. Components were provisionally identified by reanalysis after addition of appropriate synthetic compounds: triacontane, methyl octacosanoate and triacontanoate, octacosyl and triacontyl acetates and triacontyl hexadecanoate and triacontanoate. The percentage of hydrocarbons was calculated from the response relative to *p*-dioctylbenzene and the percentage of methyl esters, and alcohol acetates and monoesters from the response relative to octadecyl octadecanoate. Investigations with internal standards and the above synthetic compounds showed that correction factors were not required with the column used in the present investigation.

TABLE II

Composition of Hydrocarbons of Waxes (%)

Chain length	Wax Ouricury	Lac	Esparto	Candelilla
25	9	—	1	—
26	—	—	1	—
27	23	39	3	—
28	—	—	2	—
29	22	51	16	6
30	—	—	2	2
31	36	10	54	80
32	—	—	—	1
33	10	—	20	11
35	—	—	1	—

Methanolysis of Waxes

Wax (0.050 g) was refluxed 18 hr with 10 ml methanol containing 5% HCl and 10 ml benzene. The solution was poured into water and the products extracted with chloroform; solvent was taken off and the residue acetylated as before. During GLC analysis the temperature was programed from 125 to 300 C at 3 C/min. To identify components, synthetic methyl 28-hydroxyoctacosanoate (as acetate), diacetates of α,ω-hexacosane and triacontane diols, and dimethyl octadecanedioate and docosanedioate were used in addition to the above mentioned methyl esters and alcohol acetates. Again correction factors were not required.

Ouricury wax (0.5 g) was subjected to acid methanolysis, and the products were chromatographed on silicic acid (100 g, Biosil A). Methyl esters, alcohols and a mixture of alcohols, hydroxy esters and unidentified components were eluted with hexane-chloroform 4:1. Diols were eluted with chloroform. The mixture containing alcohols and hydroxy esters was acetylated and rechromatographed. Alcohol acetates were eluted with hexane-ether 99:1 and ω-acetoxy esters with hexane-ether 95:5. The composition found was: 28% methyl esters, 17% alcohols, 4% hydroxy esters, 18% diols, and 33% unidentified components (noncrystalline material eluted at different stages of the separation and material not recovered from the column).

Synthetic Compounds Used in Identification of Wax Components

Preparations of the following compounds have been preivously described: C_{28} acid and alcohol (15); C_{30} acid and alcohol, and triacontyl hexadecanoate and triacontanoate (16); 1,26-hexacosane diol and its diacetate (17). Electrolytic coupling of hexadecanoic acid gave triacontane and of methyl hydrogen decanedioate and dodecanedioate gave dimethyl octadecanedioate and docosanedioate, respectively. Electrolytic coupling of a mixture of methyl hydrogen tetradecanedioate (17) and 16-acetoxyhexadecanoic acid gave methyl 28-acetoxyoctacosanoate. α,ω-triacontanediol diacetate and dimethyl hexacosanedioate. The products were separated by column chromatography on silicic acid (A.P. Tulloch, unpublished work).

RESULTS AND DISCUSSION

Use of Dexsil 300 as GLC liquid phase gave good separations of hydrocarbons, even carbon methyl esters,

TABLE III

Composition of Free Acids and Alcohols of Waxes (%)

Chain length	Carnauba Acid	Carnauba Alcohol	Ouricury Acid	Ouricury Alcohol	Chinese insect Acid	Chinese insect Alcohol	Lac Acid	Lac Alcohol	Esparto Acid	Esparto Alcohol	Candelilla Acid	Candelilla Alcohol	Japan Acid	Japan Alcohol
16	—	—	—	—	—	—	—	—	—	—	—	—	84	—
18	—	—	—	—	—	—	—	—	—	—	—	—	16	—
22	—	—	3	—	—	—	—	—	—	—	—	—	—	—
24	—	—	3	—	—	—	—	—	1	—	—	—	—	—
26	—	—	4	2	—	75	5	3	3	5	—	—	—	—
28	—	7	29	4	—	21	52	55	25	70	9	—	—	—
30	—	16	32	12	—	4	34	25	37	15	48	77	—	—
32	—	60	29	60	—	—	9	11	26	8	43	20	—	—
34	—	17	—	22	—	—	—	6	8	2	—	3	—	—

and alcohol acetates and monoesters as shown in Figure 1. Alcohol acetates emerge at approximately the position of methyl esters with one more carbon, but for the present analyses it is reasonable to assume that odd carbon acids and alcohols are absent. The same column also partly resolved acetylated ω-hydroxy methyl esters from methyl esters and diol diacetates from alcohol acetates, the difunctional component emerging just after the monofunctional component with four more carbons. Figure 2 shows the separation of methyl esters, ω-acetoxy methyl esters, alcohol acetates and diol diacetates derived from methanolysis of ouricury wax. Though separation was not complete, tests with mixtures of synthetic compounds showed that electronic integration gave satisfactory quantitative results.

Compositions of the waxes, according to GLC analysis, are shown in Table I; Table II shows the composition of hydrocarbons, Table III that of free acids and free alcohols, and Table IV that of monoesters. These four tables together with Figure 1 show that there are obvious and useful differences between the seven waxes. In the case of five of the waxes, the per cent volatile according to GLC is less than 50%. For commercial waxes this figure might vary appreciably, depending on the extent of refining processes. Composition would also be expected to vary with origin of the wax, time of year at which it was collected, exact species of plant or insect used, etc. The present results will therefore be compared with those obtained previously. Acid and ester values of the waxes were all close to or within accepted ranges of values (18-21). Also, results of TLC analysis were very similar to those of Holloway and Challen (11) except for the carnauba wax sample, which showed spots for monoesters, alcohols, hydroxy esters and more polar components only.

Carnauba Wax

Results in Table I agree fairly well with those obtained by Vandenberg and Wilder by column chromatography (12% free alcohols, 40% monoesters, [4]). The hydroxy esters, *p*-hydroxy- and *p*-methoxycinnamic acid diesters found by these authors did not appear on GLC. Methanolysis products found by GLC were: 27% acids, 57% alcohols, 13% ω-hydroxy esters and 3% α,ω-diols. Downing et al. (2) found, by conventional separation of alkali hydrolysis products: 28% acids, 47% alcohols, 15% hydroxy acids, 6% diols and 6% unidentified. The composition of the methanolysis products was very similar to that given by Downing et al. (2). Cinnamic acid derivatives (4) were not detected by the present method.

Ouricury Wax

Figure 1 and Table I show appreciable differences from carnauba wax. Free acids are detectable in agreement with the higher acid value. An unidentified component (4%) appears at the position of C_{26} α,ω-diol diacetate; but it was not this diol, because the emergence position was not affected by acetylation and column chromatography of methanolysis products indicated it was eluted with the alcohols. Peaks in the monoester region are all double peaks, the later portion being due to acetylated hydroxy esters. This part of the peak did not appear if the sample was not acetylated and if the wax was treated with

TABLE IV

Composition of Monoesters of Waxes (%)

Chain length	Carnauba	Ouricury	Chinese insect	Lac	Esparto	Candelilla
40	—	—	—	1	1	—
42	—	—	—	6	1	4
44	—	2	—	14	7	4
46	1	3	1	12	7	7
48	2	5	2	9	7	7
50	3	6	11	6	8	11
52	9	12	37	2	8	12
54	13	13	25	1	8	9
56	27	18	14	3	13	3
58	16	16	8	5	17	3
60	17	15	2	7	12	4
62	8	8	—	8	7	4
64	3	2	—	9	2	2
66	—	—	—	8	—	—
68	—	—	—	6	—	—
Unidentified[a]	—	—	—	3(4)	2(2)	30(10)

[a] Number of components in parentheses.

TABLE V

Methanolysis Products of Ouricury Wax Found by Gas Liquid Chromatography of Total Methanolysis Products

Chain length	Composition, %			
	Acids	Alcohols	ω-Hydroxy acids	α,ω-Diols
20	6	—	—	—
22	5	1	—	1
24	17	2	24	15
26	16	5	26	40
28	34	6	38	25
30	19	15	9	11
32	3	53	—	6
34	—	22	—	2
Total volatile, %	37	26	5	31[a]

[a] An unidentified component has been included as part of the C_{26} diol.

N-trimethylsilylimidazole; TMS ethers of the hydroxy esters were completely separated from monoesters. Assuming, by analogy with relative emergence temperatures of ω-acetoxy methyl esters and methyl esters (see above), that acetoxy ester has four carbons less than preceding monoester, per cent composition is: C_{40}, 2; C_{42}, 3; C_{44}, 10; C_{46}, 15; C_{48}, 23; C_{50}, 20; C_{52}, 17; C_{54}, 7; C_{56}, 3.

The composition of methanolysis products of ouricury wax found by GLC is shown in Table V. Since nonvolatile material has not been taken into account, the percentages of the classes of breakdown products are too high; also, the unidentified component detected in the original wax makes the C_{26} diol and the total diols too high. Column chromatography of methanolysis products gave: 28% methyl esters, 17% alcohols, 4% hydroxy esters, 18% diols, 33% unidentified components. Diols isolated in this way had the percentage composition: C_{22}, 1; C_{24}, 17; C_{26}, 22; C_{28}, 35; C_{30}, 16; C_{32}, 7; C_{34}, 2; C_{28} diol is thus the major component, rather than C_{26}.

Miltenberger (5), who seems to have made the only other investigation of hydrolysis products of ouricury wax, found a fatty acid composition similar to that in Table V and reported C_{32} as the major alcohol but did not mention hydroxy acids or diols.

Since diols are the major difunctional components, hydroxy esters must be mainly monoesters of diols. Ouricury wax differs in this respect from carnauba wax, which has hydroxy acids as principal difunctional components. Free acids were mainly C_{28} to C_{32} so that combined acids are shorter (C_{20}-C_{30}). Principal components of acids, hydroxy acids and diols all have the same chain length (C_{24}-C_{30}), but alcohols are longer (C_{30}-C_{34}). In carnauba wax, acids and hydroxy acids were also C_{24}-C_{30} and alcohols C_{30}-C_{34}, but diols had a greater range (C_{24}-C_{34}).

Cole and Brown (14) found fairly similar amounts of free acids and free alcohol and also 23.5% esters and 22.4% hydroxy esters, though the esters may not have been entirely volatile material.

Chinese Insect Wax

Chinese insect wax was the least complex of the waxes, consisting mainly of monoesters; hydrocarbons and free acids were less than 1%. The composition of methyl esters obtained on methanolysis is almost the same as that found by Faurot-Bouchet and Michel by alkaline hydrolysis (6). These authors also found alcohol percentages: C_{26}, 63; C_{28}, 28; C_{30}, 2; but results obtained here were: C_{26}, 43; C_{28}, 43; C_{30}, 8. The major C_{52} and C_{54} monoesters (Table IV) must be mainly hexacosyl and octacosyl hexacosanoates.

Lac Wax

Lac wax differed from Chinese insect wax in having an appreciable free alcohol content and a large chain length range of monoesters with an unusual minimum at C_{52}-C_{54}. Methanolysis products were as shown in Table VI. The figures are fairly close to those obtained by alkaline hydrolysis (6), but C_{14} and C_{16} acids are larger and C_{28} alcohol is smaller. No hydroxy acids or diols were found, so that the composition of more than half of the wax is unexplained. Since C_{28} alcohol is a major component of free alcohols (Table III), the major combined alcohols must be C_{30}-C_{34}. The unusual chain length distribution of the monoesters reflects the chain length range of the combined acids. The C_{42}-C_{48} esters must be C_{14} and C_{16} acid esters of C_{28}-C_{34} alcohols, and the C_{56}-C_{68} esters must be C_{28} to C_{34} esters of these alcohols.

Esparto Wax

GLC showed that this wax contained 19% hydrocarbons (Table I), which agrees with the figure of 23% reported by Savidan (22) some time ago. C_{31} was the major hydrocarbon. Miet et al. (7) reported 70% hydrocarbons; however the saponification value of their sample was much lower than usual and the percentage of even carbon hydrocarbons was unusually high (38%), suggesting considerable natural variation in esparto wax or even the possibility of adulteration by petroleum paraffin. Another possible explanation is that only part of the paraffin found by column chromatography was volatile during GLC analysis; this has also been observed during investigations of other grass waxes (A.P. Tulloch, unpublished work). Holloway (13) found 60% hydrocarbons by preparative TLC with a composition similar to that reported here. The considerable variation in the acid and ester values reported for esparto wax (18,21,22) suggests that it is a rather variable product.

The free acids (Table III) were similar in content and composition to those reported by Miet et al. (7), and

TABLE VI

Methanolysis Products—Lac Wax

Products	Chain length												
	12	14	16	18	20	22	24	26	28	30	32	34	36
Acids	1	8	14	2	1	1	1	1	10	11	23	26	1
Alcohols	—	—	—	—	—	—	—	2	40	23	21	13	1

TABLE VII

Methanolysis Products–Candelilla Wax

Products	Chain length 16	18	20	22	24	26	28	30	32	34
Acids	2	1	12	4	1	1	7	32	33	7
Alcohols	—	—	—	5	—	3	9	65	15	3

octacosanol was the major free alcohol as has been found for leaf waxes of other members of the grass family (23,24). Acids obtained on methanolysis ranged from C_{16} to C_{34}, making allowance for the C_{28}-C_{34} acids contributed by the free acids; the combined acids would be similar to those reported before (7). The large chain length range of the acids again accounts for the C_{40}-C_{64} range of monoesters (Table IV). Comparison of response of nonhydrocarbon constituents with that of hydrocarbons after and before methanolysis showed an increase of ca. 80%, indicating an appreciable percentage of acids and alcohols combined as nonvolatile components. Since hydroxy acids and diols were not detected, the components are presumably not polyesters.

Candelilla Wax

Hydrocarbon content of this wax was reported to be ca. 50-55% with C_{31} the principal component (13,25,26), and the present sample contained 41% of volatile hydrocarbons (80% C_{31}) (Tables I and II). Holloway (13) found free acids similar to those reported here (Table III). The methanolysis products were as shown in Table VII. Comparison with free acids (Table III) shows that the C_{16}-C_{22} acids are all in the combined acids. The large range of chain lengths of acids give rise to the large range in the esters (Table IV). Alkali hydrolysis gave acids of a similar range (5), and Chibnall et al. (27) reported major C_{30} and C_{32} acids and alcohols. There was a 50% increase in nonhydrocarbon content on methanolysis, due to acids and alcohols combined in nonvolatile components.

Japan Wax

That this wax is a glyceride wax and gives, on hydrolysis, mainly palmitic acid and also 6% of long chain dicarboxylic acids has been known for some time (28). Methanolysis gave methyl esters with a composition very similar to that reported (28), together with esters of eicosanedioic (2%) and docosanedioic (2.6%) acids. Tazaki (9) reported almost the same percentages of dicarboxylic acids. Figure 1 shows that tripalmitin is the major volatile component; the small peak with an emergence temperature of ca. 285 C was not trilaurin. The relatively low total volatile material (46%, Table I) is due presumably to the presence of molecules consisting of two diglyceride units linked by dicarboxylic acids (29).

Results obtained in this investigation agree sufficiently well with previous studies of these waxes to use this GLC method to identify waxes and probably, in some cases, to estimate the approximate amounts of different waxes in mixtures.

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