The Composition of Beeswax Alkyl Esters

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Abstract

The alkyl esters of beeswax, after isolation from the unhydrolyzed wax by preparative layer chromatography (PLC), have been analyzed directly by high temperature GLC using 1.5% OV1 as liquid phase. In two commercial wax samples examined the ester homologues are predominantly even carbon numbered ranging from C_{36} to C_{54}. The principal alkyl esters are C_{40}, C_{42}, C_{44}, C_{46} and C_{48}. The GLC analysis of the ester hydrolysis products revealed that the variations in ester chain length are produced by variations in the esterified primary alcohol chain lengths. The esterified fatty acid is chiefly hexadecanoic acid. The esterified fatty acids differ in composition from the free fatty acids which are also present in the wax.

Introduction

Our present knowledge of the constituents of beeswax has been derived principally from studies of its hydrolysis products. Hydrolyzed beeswax contains chiefly primary alcohols (homologue range C_{24}–C_{36}, mainly even between C_{24}–C_{32}) and fatty acids (range C_{12}–C_{34}, mainly even, chiefly C_{16} and C_{24}), with smaller amounts of alkanes (range C_{19}–C_{33}, mainly odd, between C_{27}–C_{33}) and hydroxy-fatty acids (range C_{12}–C_{32}, mainly even, chiefly 14-hydroxyhexadecanoic acid) (1,2). Minor constituents include alkenes (3), branched alkanes (4), diols (1), cholesteryl esters and pollen pigments (5). The unhydrolyzed wax comprises 70–80% esters (from the saponification value), up to 15% free fatty acids (from the acid value) and 10–16% alkanes which are unaffected by hydrolyses (5). However, TLC of the unhydrolyzed wax does reveal a much more complex composition (6–8).

The exact composition of the esters is still open to conjecture. Most reference books describe the esters as being mainly myricyl palmitate (triacontanyl hexadecanoate) $C_{15}H_{31}COOC_{30}H_{61}$. Such statements are misleading as triacontanol normally comprises only about 30% of the total primary alcohols obtained from the hydrolysis of the wax (9). Also the fatty acids obtained from the hydrolysis include both the free and esterified acids which may be different in composition. In addition, the statement ignores the fact that beeswax contains significant amounts of hydroxy fatty acids which are almost certainly esterified with the primary alcohols (10).

This paper describes further chromatographic work on beeswax alkyl esters and their constituent acids and alcohols performed without hydrolyzing the whole wax.

Experimental Procedures

Materials

White beeswax samples were supplied by Brohme and Schimmer Ltd., London. Sample A melted at 63.0–64.0 C, Sample B at 64.0–65.0 C.

Preparative Layer Chromatography

The alkyl ester content was determined after the chromatography of known amounts of beeswax on 1.5 mm thick layers of Kieselgel H as previously described (11). The isolated fraction was identified by IR spectroscopy, the ferric hydroxyamate color test (12) and TLC tests (8). Hydrolysis of the alkyl ester and the recovery of the resultant alcohols and acids were carried out using the method of Mazliak (13).

The free fatty acid fraction of the beeswax samples was separately determined by PLC on Kieselgel H using the solvent system, chloroform-ethyl acetate (1:1 v/v) (14). The fraction was identified by IR spectroscopy and TLC tests (8).

Gas-Liquid Chromatography

A Microtek GC 2000-R gas chromatograph fitted with dual columns and flame ionization detectors and a 0 to 1 mV recorder was used. The chromatographic columns were of stainless steel ⅛ in. internal diameter, 6 ft in length packed with 60–80 mesh Chromosorb G AW DMCS coated with 1.5% OV1 (dimethyl silicone, Applied Science Laboratories, Inc.). The carrier gas was nitrogen, flow rate 60 ml/min (60 psi) through each column and the flow rates for each detector were hydrogen 60 ml/min (20 psi) and air 280 ml/min (20 psi). Analyses were made both isothermally and using temperature programming in the temperature range 150–400 C. The detectors were maintained at 360 C and the injection inlet at 250 C. Before use the columns were conditioned overnight at 400 C using full carrier gas flow. All samples were injected as 2.0% solutions in hexane.

Beeswax alkyl ester, constituent primary alcohol and fatty acid, and free fatty acid homologues were first identified by isothermal GLC using methods previously described (11). Their quantitative composition was determined from the chart using peak areas obtained by triangulation. Mean values were calculated from five replicate chromatograms.

Results

The fractions isolated from the beeswax samples by PLC, with R_f values corresponding to an alkyl ester, gave a strong positive (violet) color with the ferric hydroxamate test. Their IR spectra (potassium chloride disc) showed no hydroxyl absorption, CH_3– and $-CH_2-$ absorption bands and strong bands at 1730 cm^{-1} and 1180 cm^{-1} typical of long chain alkyl esters (11). The ester content of Sample A was 65.0% (mp 64.5–65.0 C) and Sample B 68.4% (mp 62.0–63.0 C). These are mean values based on three separate PLC determinations.

The direct analysis of the alkyl ester fractions by high temperature isothermal and temperature programmed GLC revealed an homologous series of esters in both beeswax samples. Homologues with an even number of carbon atoms predominated (Table I). The principal esters in both samples were of chain lengths C_{40}, C_{42}, C_{44}, C_{46} and C_{48}.

The GLC analysis of the esters, however, give only their respective chain lengths but more information on the exact composition of the esters is obtained from the analysis of their hydrolysis products. GLC analysis of the liberated alcohols (as acetate derivatives) from both samples showed an homologous series of primary alcohols with a predominantly even number of carbon atoms between C_{24}–C_{34} (Table I). The quantitative data are in agreement with Australian

TABLE I

Composition (%) of the Alkyl Esters, the Primary Alcohols and Fatty Acids From Hydrolysis of the Esters and the Free Fatty Acids From Two Commercial Beeswax Samples (A and B). tr < 0.2%

Carbon number	Alkyl esters A	Alkyl esters B	Esterified primary alcohols A	Esterified primary alcohols B	Esterified fatty acids A	Esterified fatty acids B	Free fatty acids A	Free fatty acids B
<14	.....	.....	.....	.....	.....	.....	.....	.....
14	.....	.....	.....	.....	1.2	0.8	.....	.....
15	.....	.....	.....	.....	.....	.....	Tr	Tr
16	.....	.....	.....	.....	93.4	95.0	15.4	14.3
17	.....	.....	.....	.....	.....	.....	Tr	Tr
18	.....	.....	.....	.....	Tr	4.0	3.6	5.8
19	.....	.....	.....	.....	.....	.....	Tr	1.6
20	.....	.....	.....	1.5	.....	0.2	2.1	2.0
21	.....	.....	.....	.....	4.7	.....	1.9	Tr
22	.....	.....	Tr	Tr	Tr	Tr	5.6	3.7
23	.....	.....	.....	.....	Tr	.....	3.1	Tr
24	.....	.....	15.1	4.2	Tr	.....	31.3	35.2
25	.....	.....	.....	.....	Tr	.....	1.5	Tr
26	.....	.....	10.7	5.4	1.7	.....	16.4	19.6
27	.....	.....	Tr	Tr	.....	.....	Tr	Tr
28	.....	.....	13.9	14.8	Tr	.....	8.4	7.2
29	.....	.....	Tr	Tr	.....	.....	Tr	Tr
30	.....	.....	33.1	41.0	Tr	.....	5.3	8.1
31	.....	.....	Tr	Tr	.....	.....	Tr	Tr
32	.....	.....	20.9	27.7			3.1	2.5
33	.....	.....	Tr	Tr			Tr	.....
34	.....	.....	5.0	4.5			2.2	Tr
35	.....	.....	Tr	Tr			.....	.....
36	.....	1.0	1.3	0.9			Tr	.....
37	.....	.....	.....	.....			.....	.....
38	.....	.....	Tr	Tr				
39	.....	.....	.....	.....				
40	15.0	5.2						
41	.....	.....						
42	11.0	6.1						
43	.....	.....						
44	14.4	16.0						
45	.....	.....						
46	32.3	39.5						
47	.....	.....						
48	20.4	27.1						
49	.....	.....						
50	6.3	4.6						
51	.....	.....						
52	0.6	0.5						
53	.....	.....						
54	Tr	Tr						
>54	.....	.....						

work (1) on the hydrolysis products of the whole wax. The percentage composition of the esterified primary alcohol fraction also was in agreement with the composition of the corresponding alkyl esters suggesting that the alcohols were esterified with one acid only. Confirmation was obtained from the GLC analysis of the esterified acids (as methyl ester derivatives). Hexadecanoic acid (C_{16}) was the major fatty acid in both samples with much smaller amounts of other acid homologues (Table I). These results differ considerably from those obtained by Australian workers (1) after the hydrolysis of the whole wax. Beeswax alkyl esters must therefore be predominantly hexadecanoates and the principal esters accordingly tetracosanyl hexadecanoate (C_{40}), hexacosanyl hexadecanoate (C_{42}), octacosanyl hexadecanoate (C_{44}), triacontanyl hexadecanoate (C_{46}) and dotriacontanyl hexadecanoate (C_{48}).

The free fatty acid fraction in both beeswax samples was also isolated by PLC, analysed by GLC and compared with the esterified fatty acids. The IR spectra of the isolated fractions (potassium chloride disc) showed CH_3– and $-CH_2$– absorption bands, strong bands at 1710 cm^{-1}, 1300–1305 cm^{-1} and 940 cm^{-1} typical of long chain fatty acids and was identical with a reference spectrum of octacosanoic acid. The free fatty acid content of Sample A was 15.7% (mp 74.0–76.0 C) and Sample B 12.5% (mp 75.0–77.5 C). GLC analysis (as methyl ester derivatives) revealed a complex mixture of predominantly even carbon numbered homologues covering a wide range from C_{14}–C_{36} (Table I). Hexadecanoic (C_{16}) and tetracosanoic (C_{24}) acids were the principal fatty acid homologues in both samples. The free fatty acids differ markedly in composition from the esterified fatty acids present in the same wax.

Discussion

The direct analysis of alkyl esters obtained from natural waxes is a comparatively new field made possible by the development of silicone stationary phases which are stable at temperatures above 300 C, in conjunction with temperature programmed GLC. Homologous series of alkyl esters with a predominantly even number of carbon atoms have been reported in carnauba (C_{44}–C_{62}), ouricury (C_{44}–C_{66}), Montan (C_{44}–C_{68}) (15), grape (C_{32}–C_{40}) (16) and spermaceti (C_{26}–C_{38}) (11) waxes. In both spermaceti and grape wax the ester chain length variations are produced only by variations in chain lengths of the esterified fatty acids. Beeswax is the first natural wax in which this variation is produced by variations in the chain lengths of the esterified primary alcohols.

Although only two commercial samples were studied the results obtained are sufficient to show that descriptions of beeswax alkyl esters as mainly triacontanyl hexadecanoate are obviously inadequate. Such criticism have already been made by Callow (9) in a review of previously published work. A more accurate description is an homologous series of predominantly hexadecanoate alkyl esters between C_{36}–C_{54}, the principal esters being C_{40}, C_{42}, C_{44}, C_{46} and C_{48}.

In addition the constitution of alkyl esters cannot always be accurately ascertained from analyses of the hydrolysis products of the whole wax. In the case of beeswax such analyses are complicated by the presence of free fatty acids, the composition of which differs considerably from the esterified fatty acids. However, if combined the quantitative results of both fatty acid fractions of beeswax gave results very similar to the Australian work (1) on the acids obtained after the hydrolysis of the whole wax. A similar situation could arise in waxes which contain significant quantities of free alcohols together with alkyl esters.

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