ON NATURAL WAXES. XX.*

FREE ACIDS OF THE WAX OF THE HONEYBEE (*Apis mellifera* L.)

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The chemical composition of free acids from virgin beeswax was estimated using chromatography on silica gel, gas chromatography and IR and mass spectrometry. The principal portion (63%) is formed by a homologous series of normal fatty acids (C_{14}—C_{40}) with predominant C_{24}. Further, a group of epoxy acids, keto acids and hydroxy acids was detected together with a considerable amount of more complex compounds containing greater numbers of carbon atoms (C_{35}—C_{54}). Their chemical nature is discussed on the basis of the results of alkaline hydrolysis and of reduction with $LiAlH_4$.

In connection with a systematic analysis of beeswax we analyzed in detail the hydrocarbons[1-3], simple wax esters[4] and bifunctional esters[5]. In the present communication an analysis of the chemical composition of the chromatographic fraction of free acids is discussed.

The presence of free carboxylic acids in beeswax has been recognized for some time[6-8]. Mattissohn[9,10] observed that their main component is tetracosanoic acid (C_{24}). Later on, the content of these free acids has been determined several times[11-16] as 8—14%. Carlier and coworkers[17] used mass spectrometry to establish that the homologous series of normal acids is present from C_6 to C_{38} with predominating homologues C_{24}, C_{26}, C_{30} and C_{32}. The odd-numbered members of the homologous series have not been identified in the mass spectrum. Holloway[18] carried out a detailed analysis of free acids using gas chromatography; in two samples of bleached beeswax he detected the presence of both even-numbered and odd-numbered saturated unbranched monocarboxylic acids C_{15}—C_{36} with predominating homologues C_{16}, C_{24}, C_{26}. On the other hand, the wax of *Apis indica* was found[19] to contain 4% free tetradecanoic acid (C_{14}).

Gradient elution chromatography of the wax on a column of silica gel[4] yielded as the last group IX (fractions 60—78) a mixture of compounds displaying in an IR spectrum absorption bands of a free carboxyl group. Their methyl esters were separated by further column chromatography into a total of 10 groups of compounds (A—J) (Table I) which were then studied separately with the exception of A and B.

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EXPERIMENTAL

Material. Silica gel, as well as the solvents were of the same degree of purity as described[4]. For the study we used the fraction of free acids (group IX)[4] of nonsaponified virgin beeswax (1·34 g) which were esterified for further separation with an ether solution of diazomethane.

Chromatographic Separation of Methyl Esters of the Acids. 1·58 g of methyl esters of the acids was divided into two halves, each of which was chromatographed on a column (2 × 86 cm) containing 125 g silica gel deactivated with 15% water. The elution was done with a mixture of chloroform and hexane (1 : 1). Fractions 1—14 were collected in 25 ml volumes, fractions 15—34 in 50 ml volumes and fractions 35—40 in 100 ml volumes. The composition of the fractions was monitored by chromatography on a thin layer of silica gel. Unseparated intermediate fractions were rechromatographed and the fractions obtained were combined with the corresponding fractions from the main chromatography. A total of 10 groups of substances was obtained (Table I).

Alkaline Hydrolysis and Reduction with Lithium Aluminium Hydride

Alkaline hydrolysis. 20 mg of compounds of groups F, G and J were refluxed with 4 ml ethanol with 0·04 ml water and 40 mg KOH. After 1 h of boiling the mixture was made acid with 0·4 ml H_2SO_4 (1 : 20) and extracted with ether. The concentrated residue was esterified with diazomethane for the purposes of gas chromatography.

Reduction with lithium aluminium hydride. Ten mg of compounds of groups D—J were dissolved in 10 ml ether and refluxed for 30 min with 40 mg lithium aluminium hydride. The reaction mixture was decomposed with 2·5 ml H_2SO_4 (1 : 20) and 5 ml water and extracted with ether. After distilling off the solvent the mixture was analyzed by thin-layer chromatography and by gas chromatography, or by means of mass spectra obtained directly from the individual gas-chromatography elution waves.

12-Oxotridecanoic Acid (C_{13})

17·4 g (0·15 mol) 4-oxopentanoic acid and 32·5 g (0·15 mol) monomethyl ester of 1,10-decandioic acid were electrolyzed in 90 ml methanol under the addition of 0·40 g sodium (Pt electrodes[20] 4 × 5·5 cm, 3·5—1 A, 45—90 V). A total of 35·4 g mixture was obtained which, according to gas chromatography, contained as main products 2,7-octanedione (10%), methyl ester of 12-oxotridecanoic acid (35%) and dimethyl ester of 1,18-octadecanedioic acid (42%) which were further separated by vacuum fractional distillation at 10 Torr. Fraction 2 contained 70% methyl ester of 12-oxotridecanoic acid. For analytical purposes, it was isolated in a pure state by preparative gas chromatography. The IR spectrum contained absorption bands at 1719 cm^{-1} (—CO—) and 1170, 1438 and 1740 cm^{-1} (—$COOCH_3$). For $C_{14}H_{26}O_3$ (242·4) calculated: 69·38% C, 10·81% H; found: 69·15% C, 10·79% H.

Methyl Ester of 20-Oxoheneicosanoic Acid (C_{21})

3·28 g (0·01 mol) 12-oxotridecanoic acid (obtained by saponification of 70% ester) and 3·24 g (0·015 mol) monomethyl ester of 1,10-decanedioic acid were electrolyzed in 25 ml methanol with an addition of 0·20 g sodium (Pt electrodes 2 × 4 cm), 0·9—0·4 A, 70 V). A total of 5·10 g reaction mixture was obtained which, according to gas chromatography, contained as principal components the dimethyl ester of 1,18-octadecanedioic acid (19%) and the methyl ester of 20-oxoheneicosanoic acid (22%). The mixture was subjected to vacuum distillation (10 Torr and 0·005 Torr) and divided into ten fractions. Gas chromatography established that the methyl ester

of 20-oxoheneicosanoic acid is most represented in fraction 7 (63·8%). Preparative gas chromatography was used to isolate about 60 mg pure substance. The IR spectrum displayed absorption bands at 1719 cm^{-1} (—CO—) and at 1170, 1438 and 1740 cm^{-1} (—$COOCH_3$). For $C_{22}H_{42}O_3$ (354·6) calculated: 74·52% C, 11·94% H; found: 74·23% C, 11·88% H. The mass spectrum is shown in Fig. 1*b*.

Analytical Methods

All *IR spectra* were determined in a UR-10 spectrometer in 0·01 cm cuvettes. The sample concentration in tetrachloromethane was 6—7%.

Analytical gas chromatography was carried out in a Pye Argon Chromatograph with a radioactivity ionization detector (glass column 0·4 × 120 cm) and Pye Series 104 Chromatograph, Model 24 with flame ionization detectors and a dual system of glass columns (0·4 × 150 cm). As the liquid phase we used 3% SE-30 on Gas-Chrom Z (100—120 mesh). For high temperatures we used 2·5% SE-30 G.C. Grade (General Electric, U.S.A.) on Gas-Chrom P (100—120 mesh). All the acids were chromatographed as methyl esters, alcohols as such[4,5], or acetates[22] or trimethylsilyl derivatives[21]. The individual samples were applied as 1—4% solution in tetrachloromethane. Qualitative evaluation and identification was done by mass spectrometry and by comparing the retention volumes with synthetic derivatives. At the same time, a linear relationship between the number of carbon atoms and the logarithm of the retention volume was used. The quantitative evaluation of the chromatograms was done in such a way as to compare the products of the retention times and of the corresponding wave heights (without using correction factors).

Preparative gas chromatography was done in an apparatus of original construction[5] provided with a catharometer. The individual fractions were condensed in glass traps with silica gel. Elution from silica gel was done with 4 ml tetrachloromethane, eluting always practically all the methyl ester while the stationary phase partly volatilized from the column was not eluted. The purity of the compounds was checked in an analytical apparatus.

Gas chromatography-mass spectrometry was done in a single focus mass spectrometer LKB 9000 with magnetic registration of the mass scale. The chromatographic glass column (0·25 × 250 cm) contained 1% SE-30 on Chromosorb W (80—100 mesh). The thermostat temperature was at 180—280°C, rate 4°C/min. The samples were applied as 4% solutions in 2,2,4-trimethylpentane or tetrachloromethane. The mass spectra of the individual components were registered at the peak of the chromatographic wave. The ionization energy during chromatographic record was 20 eV, during mass spectra recording 70 eV, the source temperature was 290°C. Synthetic methyl ester of 20-oxoheneicosanoic acid was introduced into the ion source by a direct inlet system. The temperature of the direct inlet system was 20°C, that of the ion source 250°C, the rate of spectrum recording was about 10 times less than with spectra recorded directly from the chromatographic waves. In the course of 15 min evaporation of the compound several mass spectra were obtained, the temperature of the inlet system being raised gradually up to 50°C.

RESULTS AND DISCUSSION

Group C formed the greatest portion of the original free acids (Table II). The IR spectrum showed absorption bands at 1175 and 1737 cm^{-1} which are characteristic for the —$COOCH_3$ group. The predominant part is formed by an extensive homologous series of normal saturated monocarboxylic acids. Even-numbered homologues with a maximum at C_{24} predominated. Using chromatography on a thin layer

of silica gel impregnated with silver nitrate and by means of gas chromatography trace amounts of unsaturated fatty acids could be demonstrated, oleic acid (C_{18}) predominating. The composition of the free monocarboxylic saturated acid fraction from bleached beeswax had been published by Holloway[18]. Our results differ from his particularly in the amount of C_{16}, C_{32} and C_{34} acids.

Groups D and E absorb in the IR spectrum at 1175 and 1737 cm^{-1} (—$COOCH_3$). The frequency of the free hydroxyl group was not detected. According to gas chromatography, the groups D and E differ only in the per cent participation of the individual homologues; in agreement with the elution sequence during column chromatography on silica gel the D group contains primarily higher members designated as C_{37} and C_{39} (Table III) while the E group contains rather the lower ones (Table IV). In gas chromatograms of compounds of the D and E groups ob-

TABLE I

Chromatographic Separation of Free Acids (as methyl esters) from Beeswax

Group	Yield mg	Acids %	Beeswax[a] %	Group	Yield mg	Acids %	Beeswax[a] %
A	0·5	0·03	+[b]	F	67·6	4·67	0·63
B	1·3	0·09	0·01	G	42·9	2·96	0·40
C	913·6	63·1	8·46	H	102·5	7·07	0·95
D	98·6	6·81	0·91	I	65·0	4·49	0·60
E	23·1	1·59	0·21	J	133·7	9·23	1·24
				Total	*1 448·8*	*100·0*	*13·4*

[a] Expressed per free acid; [b] traces.

TABLE II

Composition of Normal Monocarboxylic Acids (Group C)

No of C at.	14	15	16	18[a]	18	19	20	21
%	+[b]	+	0·5	0·1	0·1	+	0·1	+
No of C at.	22	23	24	25[a]	25	26	28	30
%	1·8	0·1	41·1	+	+	11·6	11·3	10·3
No of C at.	32	34	36	38	40			
%	10·4	10·8	1·7	0·1	+	Total 100%		

[a] Unsaturated; [b] traces.

TABLE III
Composition of Epoxy Acids (Group D)

No of C at.	%[a]	No of C at.	%[a]
13	0·3(×)	31	0·5(A_D), 0·6(B_D), 0·2(C_D)
14	0·1(×)	32	0·1(A_D), 0·5(B_D), 0·1(C_D)
15	0·1(×)	33	1·8(×)
16	+(×), +(×)	34	0·7(×)
17	0·1(×), +(×)	35	4·9(×)
18	+(×), 0·1(×)	36	1·5(×)
19	0·2(×), 0·1(×)	37	49·4(×)
20	+(×), 0·1(×)	38	3·8(×)
21	1·2(A_D), 0·1(×)	39	19·5(×)
22	+(×), +(×)	40	0·7(×)
23	0·3(A_D), +(B_D), +(C_D)	41	3·5(×)
24	0·1(C_D)	42	0·7(×)
25	0·2(A_D), +(B_D), +(C_D)	43	1·1(×)
26	0·1(B_D)	44	0·7(×)
27	0·1(B_D), 0·1(C_D)	45	0·7(×)
28	0·1(B_D), 0·1(C_D)	46	0·8(×)
29	1·6(A_D), 2·4(B_D), 0·2(C_D)	Total	3·9(A_D), 4·1(B_D), 1·0(C_D), 91·0(×)
30	0·3(B_D), 0·2(C_D)		

[a] The symbol in parentheses shows the classification into homologous series A_D, B_D, C_D; × stands for acids where the classification could not be effected; + stands for traces.

tained at the constant temperature of 250°C one may, on the basis of the linear relationship between the logarithm of the retention volume and the number of carbon atoms, detect always three homologous series A_D, B_D, C_D and A_E, B_E, C_E. The mass spectrum of the dominant peak C_{29} of the B_E series obtained directly during gas chromatography is somewhat contaminated by ions of the volatile liquid phase. In addition to ions typical of the methyl esters of fatty acids[23,24] (m/e 74, 87, 101, 115, 129, 143 *etc.*), clear fragments of m/e 239 and 269 are present. On the basis of studies[25–27] of the mass-spectrometric behaviour of epoxy acids we assume that the C_{29} peak of the homologous B_E series is the methyl ester of 14,15-epoxynonacosanoic acid. In addition to the ions of this compound, the mass spectrum contains other ions, apparently belonging to an admixture of the same elution volume.

To obtain more accurate information on the composition of groups D and E, we reduced then with lithium aluminium hydride and the reaction mixture (original acetylated and trimethylsilylated), was analyzed by thin-layer and gas chromatography. Similarly to the analysis of groups G and H, we obtained here as reaction products a homologous series of even-numbered 1, (ω-1)-diols (C_{20}—C_{30}), a homo-

TABLE IV
Composition of Epoxy Acids (Group E)

No of C at.	%[a]	No of C at.	%[a]
10	0·6(×)	29	9·2(A_E), 23·2(B_E)
11	+(×)	30	2·8(B_E), 0·3(C_E)
12	0·1(×)	31	1·2(A_E), 1·8(B_E), +(C_E)
13	+(×)	32	+(A_E), 2·2(B_E), 1·2(C_E)
14	+(×)	33	0·3(A_E), 3·7(B_E), +(C_E)
15	+(×)	34	2·9(×)
16	+(×)	35	4·6(×)
17	+(×)	36	1·4(×)
18	0·4(×)	37	19·1(×)
19	+(×)	38	3·1(×)
20	+(C_E)	39	7·9(×)
21	0·1(A_E)	40	2·0(×)
22	1·6(C_E)	41	3·4(×)
23	+(B_E)	42	2·2(×)
24	0·1(A_E), 1·0(C_E)	43	+(×)
25	+(A_E), 0·4(B_E)	45	+(×)
26	1·2(C_E)	47	+(×)
27	0·3(B_E), 0·3(C_E)	Total	10·9(A_E) 34·4(B_E), 7·0(C_E), 47·7(×)
28	1·4(C_E)		

[a] In parentheses, the classification into homologous series A_E, B_E, C_E is shown; × stands for acids where the classification could not bee effected; + stands for traces.

logous series of primary monoalcohols (C_{16}—C_{34}) with dominant 1-hexadecanol (C_{16}) and 1,15-hexadecanediol (C_{16}). The last-named diol was missing in the D group. On the basis of these experimental results it does not appear probable that the higher members of the D groups designated* as C_{35}—C_{46} are simply higher homologues of the epoxy acids. In any case we seem to be dealing here with a mixture of compounds since during gas chromatography under higher temperatures the resolving power of the columns considerably decreases.

Groups F and G. The IR spectra contain absorption bands at 1175 and 1735 cm^{-1} ($-COOCH_3$) with an inflexion at 1718 cm^{-1} indicating the presence of a keto group. The absorption bands of the free hydroxyl group are again missing. The gas chromatograms of compounds of groups F and G are similar and display the presence always of four homologous series A_F, B_F, C_F, D_F and A_G, B_G, C_G, D_G, of compounds

* It has not been definitely established whether the numerical designation is identical with the actual number of carbon atoms.

(Tables V and VI). The dominant series in group G is the C_G. The mass spectra of the more highly represented peaks C_{24} (Fig. 1*a*) and C_{26} obtained during gas chromatography show a fragmentation typical of methyl esters of (ω–1)-oxomonocarboxylic acids[23,25] and fully analogous with the fragmentation of the synthetically prepared methyl ester of 20-oxoheneicosanoic acid (C_{21}) (Fig. 1*b*). Its molecular ion has a value of *m/e* 354, the most intense ion (the base peak) being that with *m/e* 43. The ion sequence *m/e* 74, 87, 101, 115, 129, 143 *etc.* is typical of methyl esters of fatty acids[23,24]. In the upper range of *m/e* values of the spectrum there appear ions $[M-15]^+$, $[M-31]^+$, $[M-32]^+$ and $[M-57]^+$ (confirmed by metastable ions). The ion at *m/e* 297 $[M-57]^+$ is further split, giving rise to an ion with *m/e* 265 $[M-57-32]^+$ which is dehydrated to an ion with *m/e* 247 $[M-57-32-18]^+$.

A synthetic methyl ester of 20-oxoheneicosanoic acid shows at gas chromatography retention values confirming its belonging to the C_G as well as the C_F homologous series. Also the R_F values of thin-layer chromatography show an agreement. On the basis of all these facts we assume that the whole C_G series present in the G group and the C_F series present in the F group are methyl esters of (ω-1)-oxomonocarboxylic acids.

The compounds of the remaining homologous series A_F, B_F, D_F and A_G, B_G, D_G could not be identified by means of mass spectra, either for the lack of clarity of the

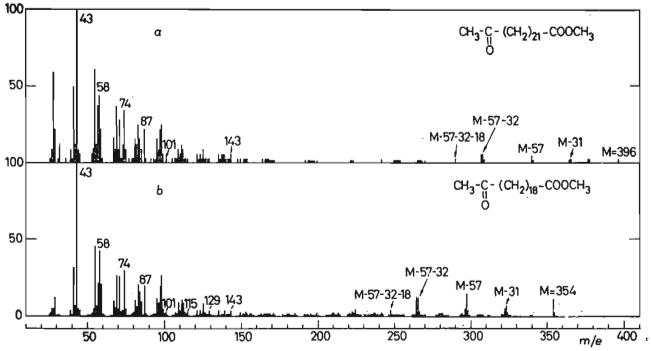

FIG. 1

Mass Spectra

a Homologue C_{24} of series C_G (group G). LKB 9000, glass column 0·25 × 250 cm, 1% SE-30 on Chromosorb W (80—100 mesh), thermostat temperature 180—280°C, 4°C/min, ionization energy 70 eV, ion source temperature 290°C. *b* The synthetic methyl ester of 20-oxoheneicosanoic (C_{21}). LKB 9000, direct inlet system, 50°C, ion source temperature 250°C.

TABLE V
Composition of Keto Acids (Group F)

No of C at.	%[a]	No of C at.	%[a]
10	0·2(×)	31	0·8(×), 1·1(×)
11	0·2(×)	32	2·9(B_F), 1·3(C_F)
12	0·4(×)	33	1·8(×)
13	0·2(×)	34	3·0(B_F), 2·8(C_F)
14	0·1(×), 1·0(×)	36	3·1(×)
15	0·3(×)	38	4·0(×)
16	0·1(×), 0·5(×)	39	4·1(×)
17	0·1(×), 0·4(×)	40	3·4(×)
18	0·1(×), 0·2(×), 0·5(×)	41	4·2(×)
19	0·4(×), 0·5(×), 0·1(×)	42	2·9(×)
20	0·7(C_F), 0·4(×)	43	4·5(×)
21	0·4(×), 0·4(×)	44	3·0(×)
22	+(A_F), 0·5(B_F), 0·9(C_F)	45	6·5(×)
23	0·2(A_F), 0·2(B_F)	46	6·7(×)
24	3·8(A_F), 4·1(C_F)	47	3·2(×)
25	1·1(B_F), 1·4(D_F)	49	+(×)
26	3·3(A_F), 3·1(C_F)		
27	0·7(B_F), 1·0(D_F)	Total	7·3(A_F), 16·3(B_F), 17·9(C_F),
28	+(A_F), 4·1(B_F), 3·0(C_F)		2·7(D_F), 55·8(×)
29	+(A_F), 0·3(B_F), 0·3(D_F)		
30	3·5(B_F), 2·0(C_F)		

[a] In parentheses, the classification into homologous series A_F, B_F, C_F, D_F is shown; × stands for acids where the classification could not be effected; + stands for traces.

spectrum obtained (A_F and A_G) or for extremely low concentrations of the eluted compounds (series B_F, D_F and B_G, D_G). We carried out then an alkaline hydrolysis and a reduction with lithium aluminium hydride in both groups. The reaction mixtures were further analyzed by thin-layer and gas chromatography and mass spectrometry, using model compounds. The F group after saponification contains a homologous series of the even-numbered members of monohydroxy acids* between C_{16} and C_{32}, a homologous series of original keto acids (C_F), a minor homologous series of primary monoalcohols between C_{24} and C_{36} and, as the dominating components, hexadecanoic acid (C_{16}) and 15-hydroxyhexadecanoic acid (C_{16}). The composition of the reaction mixture after hydrolysis of the G group compounds was qualitatively practically the same as with the F group. The results of the reductive cleavage with lithium aluminium hydride are in agreement with the results of hydrolysis. Gas chromatography and mass spectrometry[5,28] detected in both groups a homo-

* See note added in proof.

logous series of primary monoalcohols in the range of C_{16}—C_{34}, a homologous series of 1,(ω-1)-diols from C_{18} to C_{32} and a smaller amount of a homologous series of diols of an unknown type (C_{24}—C_{32}). The dominating components are again 16-hexadecanol formed from palmitic acid, and 1,15-hexanediol formed from the C_{16} hydroxy acid.

Group H absorbs in the IR region at 1175 and 1736 cm^{-1} (—$COOCH_3$). There are low intensity absorptions at 3620 cm^{-1} (—OH) and diffusion bands at 1045 and 1080 cm^{-1} (C—O). A gas chromatogram permits to distinguish between 4 homologous series A_H, B_H, C_H and D_H of compounds which are of a different type than in the F and G groups. The mass spectra of the dominating peaks C_{24}, C_{25}, C_{26}, C_{27} of the D_H series (Table VII) could not be interpreted satisfactorily. With greatest likelihood, the individual chromatographic peaks may contain mixture of compounds. The gas chromatogram after reduction cleavage of compounds of the H group with lithium aluminium hydride is analogous to the other groups. It contains again a considerable amount of 1,15-hexadecanediol (C_{16}), a homologous series of even-

TABLE VI
Composition of Keto Acids (Group G)

No of C at.	%[a]	No of C at.	%[a]
12	0·2(×)	29	0·6(B_G), 0·7(D_G)
13	0·1(×)	30	4·6(B_G), 7·3(C_G)
14	0·4(×)	31	0·5(B_G), +(D_G)
15	0·1(×)	32	2·5(B_G), 6·0(C_G)
16	0·1(×), 0·4(×)	33	+(B_G)
17	0·3(×)	34	3·0(B_G), 6·5(C_G)
18	0·3(×), 0·4(×)	36	2·0(×)
19	0·3(×)	38	0·5(×)
20	0·6(C_G), 0·2(×)	40	0·7(×)
21	0·2(×)	41	+(×)
22	0·2(A_G), 0·6(C_G)	42	0·9(×)
23	0·2(×)	43	+(×)
24	1·5(A_G), 13·4(C_G)	44	0·8(×)
25	2·0(B_G), 4·2(D_G)	45	+(×)
26	1·4(A_G), 12·0(C_G)	46	0·9(×)
27	1·1(B_G), 2·0(D_G)		
28	7·0(A_G), 13·3(C_G)	Total	10·1(A_G), 14·3(B_G), 59·7(C_G), 6·9(D_G), 9·0(×)

[a] In parentheses, the classification into homologous series A_G, B_G, C_G, D_G is shown; × stands for acids where the classification could not be effected; + stands for traces.

numbered primary monoalcohols between C_{16} and C_{36} (with a maximum at C_{24}), a homologous series of even-numbered 1,(ω-1)-diols from C_{20} to C_{32} (with a maximum at C_{24}) and a lower amount of a homologous series of diols (C_{24}—C_{32}) of an unknown type. It is assumed that similarly to the preceding two groups F and D the mother compounds of the diols are carbocylic acids with another oxygen function.

Group I shows in the IR spectrum bands of the ester group and intense bands of a free hydroxyl group (1080 and 3620 cm^{-1}). According to gas chromatography two homologous series of compounds, A_I and B_I, are present, the B_I series predominating (Table VIII). From the magnitude of change of the retention volumes of compounds of both series in the original state and in the trimethylsilylated form it could be concluded that the compounds possess one free hydroxyl group. The mass spectrum of the trimethylsilyl derivative of the major homologue C_{25} of the B_I series contains important ionic species at *m/e* 43, 73, 57, 55, 71, 75 *etc.* The molecular ion *m/e* 484 is accompanied by an ion of equal intensity at *m/e* 483 $[M-1]^+$ and *m/e* 441 $[M-43]^+$ and by ions, more intense by two orders of magnitude, at *m/e* 469 $[M-15]^+$, 425

TABLE VII

Composition of Unidentified Acids (Group H)

No of C at.	%[a]	No of C at.	%[a]
11	0·4(×)	29	0·5(A_H), 3·6(D_H)
12	0·3(×)	30	7·9(B_H)
13	0·2(×), 0·6(×)	31	0·8(A_H), 2·7(D_H)
14	0·2(×)	32	1·0(A_H), 3·1(B_H)
15	0·1(×), 0·4(×)	33	0·6(A_H), 2·7(C_H)
16	0·2(×), 0·2(×)	34	2·5(A_H)
17	0·1(×), 0·3(×)	35	2·2(×)
18	+(×)	36	0·7(×)
19	0·2(×), 0·7(×)	37	0·2(×)
20	0·6(×)	38	0·6(×)
21	0·2(×), 0·2(×)	40	2·1(×)
22	0·7(×)	42	1·9(×)
23	0·6(B_H), 1·0(D_H)	44	2·2(×)
24	+(B_H), 13·9(D_H)	46	0·8(×)
25	1·1(B_H), 20·0(D_H)	47	0·9(×)
26	+(A_H), 9·7(D_H)		
27	0·9(B_H), 6·3(D_H)	Total	5·5(A_H), 13·6(B_H), 6·6(C_H), 63·2(D_H), 17·2(×)
28	3·9(C_H)		

[a] In parentheses, the classification into homologous series A_H, B_H, C_H, D_H is shown; × stands for acids where the classification could not be effected; + stands for traces.

$[M-59]^+$ and 351 $[M-43-90]^+$. Eglinton and coworkers[29,30] and Capella and coworkers[31] had shown before that these fragments with approximately equal intensity as in the present case are characteristic for trimethylsilyl derivatives of methyl esters of α-hydroxy acids. On this basis, one may assume that the compound in question is a trimethylsilyl derivative of the methyl ester of α-hydroxypentacosanoic acid

TABLE VIII
Composition of Hydroxy Acids (Group I)

No of C at.	%[a]	No of C at.	%[a]
16	+(×)	31	4·5(B_I)
17	2·0(×)	32	1·3(A_I)
18	+(×)	33	2·7(B_I)
19	0·2(A_I), 0·5(B_I)	34	2·5(A_I)
20	0·2(×)	35	2·4(B_I)
21	0·2(A_I), 0·3(B_I)	36	+(×)
22	0·2(×)	37	0·2(×)
23	0·6(A_I), 3·1(B_I)	38	0·4(×)
24	1·2(A_I)	39	+(×)
25	+(A_I), 47·2(B_I)	40	3·4(×)
26	1·2(A_I)	41	0·4(×)
27	0·6(A_I), 9·8(B_I)	42	2·6(×)
28	0·9(A_I)	44	1·9(×)
29	7·1(B_I)	46	1·2(×)
30	0·8(A_I)	48	0·4(×)
		Total	9·5(A_I), 77·6(B_I), 12·9(×)

[a] In parentheses, the classification into homologous series A_I, B_I is shown; × stands for acids where the classification could not be effected; + stands for traces.

TABLE IX
Composition of Unidentified Acids (Group J)

No of C at.	24	25	26	27	28	29	30	31	32	33
%	+[a]	+	+	1·2	0·6	0·4	+	0·9	+	0·9
No of C at.	34	35	36	37	38	39	40	41	42	43
%	+	0·5	0·6	1·3	1·1	2·1	2·0	2·0	4·0	6·8
No of C at.	44	45	46	48	50	52	54			
%	20·6	2·1	17·2	17·9	13·0	4·8	+	Total 100%		

[a] + Stands for traces.

(C_{25}) and that the original homologous series B_I is thus a series of α-hydroxy acids. In addition to the above ions the spectrum contains ions of a different compound, [the most intense in the upper region being the ion m/e 176 (12%)], leaving the chromatographic column together with the α-hydroxy acid. However, we are not dealing here with the β-hydroxy acid as described by Eglinton and coworkers[29]. The gas chromatogram of the reaction mixture after reduction of this group of compounds with lithium aluminium hydride was practically identical with the analogous record of group H.

Group J. The IR spectrum contains absorption bands similar to those of the I group, with the difference that the intensity of the band of the free hydroxy group (3 620 cm^{-1}) is lower. Gas chromatography provided suitable chromatographic waves only at a relatively high temperature (300°C) (Table IX). The records reveal at least two homologous series, containing homologues with a relatively high number* of carbon atoms (C_{40}—C_{54}). In spite of this, in a chromatogram obtained after alkaline hydrolysis even after reduction with lithium aluminium hydride of compounds of this group, one could detect compounds with a smaller molecule, at most about C_{36}. The hydrolyzate contained again hexadecanoic acid (C_{16}), 15-hydroxyhexadecanoic acid (C_{16}), a homologous series of even-numbered primary alcohols (C_{24}—C_{36}) with a maximum at C_{30} and C_{32} and additionally also a shorter homologous series of even-numbered 1,(ω-1)-diols (C_{24}—C_{30}). The gas-chromatographic record of this group of compounds after reduction with lithium aluminium hydride again agrees with the results of hydrolysis.

CONCLUSIONS

The fraction of free acids which amounted to 13·4% of beeswax is very complex. The major part of it is represented by the homologous series of normal saturated monocarboxylic acids (group C) which are the first to be eluted during adsorption column chromatography. It was also possible to detect the presence of homologous series of epoxy acids (groups D and E), keto acids (groups F and G), and hydroxy acids (group I). In addition to the above bifunctional acids (up to C_{34}—C_{36}) the individual chromatographic groups (D—I) contain also more complex acids** with higher numbers of carbon atoms (up to C_{54}). Their polarity apparently corresponds to that of lower acids which they accompany on leaving the column. The complex character of these compounds is supported by the fact that during alkaline hydrolysis or during reduction with lithium aluminium hydride they are decomposed into smaller building blocks (C_{16}—C_{36}). It may be assumed that ester groups are probably split off during the process. The limiting case is represented by the J group which is the last to the eluted from the column and which contains only these more complex compounds

* See the footnote on p. 2456.

** See note added in proof.

(roughly from C_{35} to C_{54}). It should be mentioned here that after hydrolysis, significant amounts of hexadecanoic and 15- and 14-hydroxyhexadecanoic acids (C_{16}) were found, with corresponding hexadecanol and 1,15- and 14-hexadecanediol (C_{16}) after reduction. The existing results, however, do not permit to obtain any definitive information on the actual composition of the higher compounds present.

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Note added in proof: Recently a paper has appeared by A. P. Tulloch [Chem. Phys. Lipids *6*, 235 (1971)], where the isolation of acid mono- and polyesters is also described. Their building blocks obtained after hydrolysis are similar with hydrolysis products, quoted here.