ON NATURAL WAXES. XVIII.*

SIMPLE ALKYL ESTERS OF THE WAX OF THE HONEYBEE (*Apis mellifera* L.).

K.STRÁNSKÝ and M.STREIBL

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague 6

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Unsaponified beeswax was divided by gradient elution chromatography on a column of silica gel into 9 principal groups of compounds: hydrocarbons, two groups of esters, five groups of hydroxy esters and free acids. The first group of simpler alkyl esters (esters 1) was further separated into the saturated and the unsaturated fraction and the individual fractions were analyzed in detail by GLC, TLC and by IR and MS spectrometry. The saturated esters are formed chiefly by a homologous series of palmitates in the range of $C_{34}-C_{52}$ with maxima at C_{40} and C_{46}. The unsaturated esters are of two types. Most of them are members of a homologous series of oleates from C_{40} to C_{52} with a maximum at C_{48}; the rest are formed by palmitates of unsaturated alcohols C_{32} and C_{34}. The chemical composition of the minor components is also discussed.

In three of our previous publications[1-3] we studied in detail the chemical composition of beeswax hydrocarbons. In addition to the partly described homologous series of n-paraffins we identified homologous series of saturated branched alkanes and several homologous series of *cis*- and *trans*-monoalkenes. The present communication describes the separation of unsaponified beeswax with the aid of gradient column chromatography and the results of a detailed analysis of the first group of esters.

In contrast with most existing approaches to the analysis of the individual components of beeswax where the starting material was first hydrolyzed, we divided the wax here chromatographically without previous hydrolysis. By gradient elution chromatography on a column of silica gel the beeswax was then divided into 9 principal groups of compounds. Another difference as compared with our previous work was in the fact that in place of commercial or even bleached beeswax, fresh virgin wax was used here; this made it possible to isolate and to identify several groups of unsaturated oxygen-containing compounds not known previously, just as the hydrocarbon fraction had been previously shown to contain olefins[1].

* Part XVII: Fette, Seifen, Anstrichmittel *73*, 301 (1971).

Chromatographic Separation of Beeswax

The first group separation of unsaponified beeswax on a column of silica gel was carried out by Fuchs and de Jong[4]. They obtained 11—13% paraffins, 41—43% esters *I*, 10% esters *II*, 5—8% hydroxyesters and 13% free acids. The authors did not undertake any further analysis of the individual groups of substances. Column chromatography on silica gel was also used by Scholz[5,6] for a partial separation of saponified beeswax. For separating the acids of saponified beeswax some authors used even paper chromatography[7,8]. Much greater attention has been paid to thin-layer chromatography on silica gel[9—16]. Most of these investigations were done with the aim of comparing different types of wax. Only Holloway and Challen[11] made use of various colour reactions directly on the thin layer of adsorbent for identifying some groups of compounds. Carlier and coworkers[13], Kalique and Kader[14], Holloway[15] and Tulloch[16] have analyzed directly some components separated by thin-layer chromatography on silica gel from unsaponified beeswax. A completely different technique, molecular distillation, was used by Findley and Brown[17] for separating the components of unsaponified beeswax.

In the present work we decided to separate beeswax components on a preparative scale using a column of silica gel. We used 10 g of wax which was sufficient for a possible analysis of minor fractions: the entire chromatographic procedure was done without interruption. Since beeswax contains a number of groups of substances with different polarity, we chose the gradient type of elution. On the basis of preliminary tests using thin-layer chromatography we selected the solvent system of hexane–chloroform–ether. Chloroform, the concentration of which was kept at 10% throughout the chromatography, was used for increasing the solubility of the compound tested. The concentration of ether, on the other hand, was gradually increased in the course of separation. Fig. 1 shows a diagram indicating the composition of the elution mixture

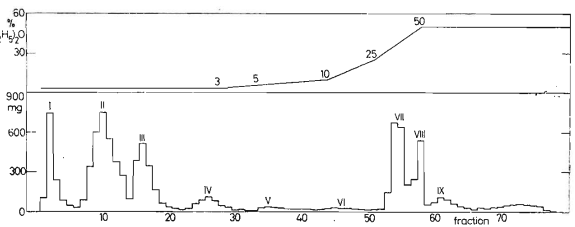

FIG. 1

Course of Gradient Elution Chromatography of Beeswax on a Silica Gel Column

The composition of the elution mixture (ether–hexane) brought on the column is shown in the top part of the figure. *I* Hydrocarbons 12·7%, *II* esters 1 31·2%, *III* esters 2 12·8%, *IV* hydroxy esters 1 (?) 5·1%, *V* hydroxy esters 2 (?) 2·8%, *VI* hydroxy esters 3 (?) 2·0%, *VII* hydroxy esters 4 6·0%, *VIII* hydroxy esters 5 9·5%, *IX* free acids 13·4%.

placed on the column and the course of the chromatographic separation. Using this gradient elution chromatography, beeswax was separated into 9 principal groups of compounds ($I-IX$). Each of the fractions was checked by thin-layer chromatography on silica gel and for further work, only fractions with identical R_F values were combined. Thin-layer chromatography also revealed that, together with the chief components ($I-IX$) representing almost 100% of the entire wax, some 30 other groups of compounds are present, some of them only in trace amounts. It may be assumed that some of them are not known from nature. The intermediate fractions from the main chromatography were rechromatographed and the fractions obtained were combined with the principal ones.

Beeswax Esters

Oxygenous compounds forming the major part of beeswax have been studied chemically for more than 150 years. As early as 1814 a compound called myricin was isolated[18] which was identified 30 years later as the myricyl ester of palmitic acid ($C_{15}H_{31}COOC_{30}H_{61}$)*. Later on, a number of saturated acids and alcohols have been isolated and, using the existing techniques, identified in saponified beeswax[23-38]. The presence of unsaturated acids was pointed out by Leys[39], Lejeune[40] and by Tischer and Illner[37]. Ikuta[33] and Hata[36] mention oleic acid as one of the acids without supplying direct evidence. The presence of hydroxypalmitic and 7-hexadecenoic acid in the wax of *Apis indica* was reported by Lipp and coworkers[24,25] and by Ikuta[34]. Toyama and Hirai[41-43] identified it later as 14-hydroxypalmitic acid. The saponified beeswax was analyzed by Mehta and Murthy[44,45]. In addition to straight-chain alcohols and acids they applied fractionation with urea to isolate small amounts of cyclic and branched alcohols, unsaturated acids and hydroxy acids which were not identified further.

The heretofore most detailed analysis of oxygenous compounds was carried out by Downing and coworkers[46]. Saponified beeswax was separated chromatographically into hydrocarbons, monoalcohols, diols, monocarboxylic acids and hydroxy acids. Each of the groups was analyzed by gas chromatography. The structure of diols, hydroxy acids and a small amount of unsaturated C_{18} acid was not further studied. The report thus could not give a picture on the representation of esters but it provides a review of the individual chief components of these esters or directly in beeswax. Unsaponified beeswax was partly separated and then further analyzed by Carlier and coworkers[13]. The first group of esters was isolated by thin-layer chromatography, saponified and the individual components were identified by mass spectrometry directly in the mixture by using molecular or other characteristic ions. They found that these esters contain a homologous series of saturated monocarboxylic acids in the region C_6-C_{59}, while up to C_{38} the even-numbered homologous predominated (particularly C_{16}, C_{18} and C_{20}), higher up the odd-numbered homologues. They found also the admixture of unsaturated monocarboxylic acids C_{14}, C_{16} and C_{18} and of unsaturated dicarboxylic acids from C_4 to C_{11}. The homologous series of alcohols contained only even-numbered homologues between C_8 and C_{36} with predominant homologues $C_{26}-C_{34}$. The analysis, although only qualitative and orientation in character, pointed to the complexity of participation of esters in beeswax.

Further work was done by Khalique and Kader[14]. The wax of *Apis indica* was first bleached with a solution of permanganate and then separated preparatively on a thin layer of silica gel.

* The compound is frequently described[19-22] as a basis of beeswax. According to our analysis it represents only 7·28%.

TABLE I

Composition (%) of Saturated and Unsaturated Esters 1

Number of C atoms	Saturated esters 1	Unsaturated esters 1
34	0·1	—
36	0·2	—
38	0·4	—
40	20·7	0·1
42	13·4	3·3
44	13·3	4·9
46	27·8	8·6
48	20·6	44·7
50	2·3	35·7
52	1·2	2·5
Total	*100·0*	*99·9*

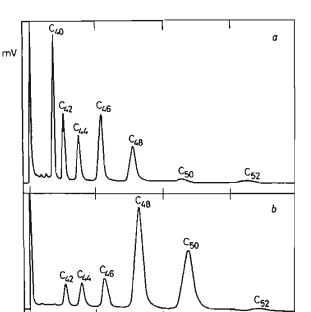

FIG. 2

Chromatogram of Saturated Esters 1 (*a*) and Unsaturated Esters 1 (*b*) of Beeswax

Perkin-Elmer F 11 apparatus with a dual system, flame ionization detectors, glass columns 0·4 × 150 cm, 2·5% SE-30 on silanized Chromosorb G (100–120 mesh), temperature of the thermostat 300°C, temperature of the injection chamber 340°C, flow of nitrogen 50 ml/min.

Classical identification methods did not suffice for carrying out a sufficiently detailed analysis of the complex mixture.

While our study of beeswax esters was in progress a paper by Holloway[15] appeared. He used preparative thin-layer chromatography on silica gel to isolate the first group of esters and he was the first to analyze by gas chromatography both the other esters present and the components obtained by their saponification. He does not mention the presence of unsaturated esters. It is likely that bleached commercial wax which was used for the analysis did not contain them any more. A similar account was recently published by Tulloch[16]. Also this author makes no comment on the presence of the unsaturated ester fraction.

The group of esters which we designated as esters 1 represents the second largest group of compounds of beeswax, amounting to 31·2% of the total weight (*II*, Fig. 1). Thin-layer chromatography on silica gel impregnated with silver nitrate revealed together with the saturated fraction also the presence of a considerable amount of the unsaturated fraction. In order to concentrate on them separately we separated them by column chromatography on impregnated silica gel just as we did with hydrocarbons before[1].

IR spectroscopy of both fractions showed that we are dealing here with esters containing long aliphatic chains. High-temperature gas chromatography[47] on a silicone elastomer SE-30 at 300°C showed that both the saturated and the unsaturated fraction are formed by homologous series of esters with even numbers of carbon atoms (Fig. 2, Table I).

In spite of the fact that mass spectra of higher aliphatic esters and of their mixtures make it possible to identify relatively exactly the individual ester components[48–50] this is not possible with high esters of most natural waxes. Experimental difficulties,

TABLE II
Overall Composition (%) of Esters 1 after Saponification

Compound	Saturated esters 1			Unsaturated esters 1		
	saturated esters 1	esters 1	beeswax	unsaturated esters 1	esters 1	beeswax
Monocarboxylic acids	39·0	32·8	10·2	35·4	5·63	1·75
Monocarboxylic acids with another oxygen function	0·9	0·7	0·2	3·0	0·48	0·15
Alcohols	60·2	50·6	15·8	61·6	9·8	3·03
Total	*100·0*	*84·1*	*26·2*	*100·0*	*15·9*	*4·93*

due to unsatisfactory temperature stability of gas-chromatographic stationary phases, do not guarantee reproducible and fully accurate results when using gas chromatography coupled with mass spectrometry. Hence the composition of esters *I* was studied after the usual hydrolytic decomposition.

Using a very gentle alkaline hydrolysis, the course of which was monitored by thin-layer chromatography, both the saturated and the unsaturated esters *I* were saponified separately. After acidification and quantitative extraction with ether,

TABLE III
Composition (%) of Acids and Alcohols from Saturated Esters I

Number of C atoms	Acids		Alcohols	
	series A	series B	series A	series B
10	–	+	–	–
11	–	–	–	–
12	–	0·2	–	–
13	+[a]	–	–	–
14	–	0·6	–	+
15	+	+	–	–
16	–	93·6	–	+
17	2·5	–	–	–
18	+	2·8	–	+
19	+	–	–	–
20	–	0·2	–	0·1
21	–	–	–	–
22	–	0·1	–	0·2
23	–	+	–	–
24	–	+	0·2	19·7
25	–	+	–	–
26	–	–	0·2	12·9
27	–	–	–	+
28	–	–	0·3	13·9
29	–	–	–	–
30	–	–	0·8	28·8
31	–	–	–	–
32	–	–	0·8	19·9
33	–	–	–	–
34	–	–	–	2·0
Total	*2·5*	*97·4*	*2·3*	*97·5*

[a] + stands for traces.

thin-layer chromatography actually exhibited the presence of two groups of compounds, *i.e.* acids and alcohols. Esterification of the whole mixture with diazomethane converted the acids to methyl esters while the alcohols remained unchanged. On the

TABLE IV
Composition (%) of Acids and Alcohols from Unsaturated Esters I

Number of C atoms	Monocarboxylic acids		Monocarboxylic acids with another oxygen function			Alcohols		
	saturated	unsaturated	series A	series B	series C	saturated	unsaturated series A	unsaturated series B
8	—	+	—	—	—	—	—	—
9	—	—	—	—	—	—	—	—
10	—	+	—	—	—	—	—	—
11	—	—	—	—	—	—	—	—
12	+[a]	—	—	—	—	—	—	—
13	—	+	—	—	—	—	—	—
14	+	—	—	—	0·3	—	—	—
15	+	—	0·1	—	—	—	—	—
16	10·3	0·5	—	2·3	—	—	—	—
17	—	+	—	—	0·1	—	—	—
18	0·4	84·8	—	93·9	—	+	—	—
19	0·5[b]	—	0·4	0·1	—	+	—	—
20	+	3·2	—	1·2	—	0·1	—	—
21	+[b]	—	—	—	+	0·1	—	—
22	+	0·2	—	—	—	0·1	0·8	—
23	—	+	+	—	0·1	+	0·7	—
24	+	+	—	—	0·1	3·8	0·7	—
25	—	—	+	—	0·1	+	1·9	—
26	—	—	—	—	+	5·3	0·8	—
27	—	—	0·1	—	0·1	+	1·6	—
28	—	—	—	—	—	11·4	0·5	2·7
29	—	—	0·2	—	0·3	+	—	—
30	—	—	—	—	—	38·5	—	5·9
31	—	—	—	—	0·3	+	—	0·5
32	—	—	—	—	—	36·8	—	60·9
33	—	—	—	—	—	—	—	2·1
34	—	—	—	—	—	4·1	—	20·5
35	—	—	—	—	—	—	—	—
36	—	—	—	—	—	—	—	0·5
Total	*11·2*	*88·7*	*0·8*	*97·5*	*1·4*	*100·2*	*7·0*	*93·1*

[a] + stands for traces; [b] probably branched homologues.

basis of a considerable difference between the R_F values of the methyl esters of acids and free alcohols, the individual groups could be separated by chromatography on a column of silica gel. Fractions 1–3 contained the methyl esters of monocarboxylic acids, fractions 4–7 the methyl esters of unidentified acids (detectable in appreciable amounts only in the fraction of unsaturated esters *I*), fractions 8–17 contained free alcohols. A review of the amounts of acids and alcohols obtained by this procedure may be found in Table II, their composition in the individual esters is shown in Tables III and IV.

EXPERIMENTAL

Materials

The virgin wax of the honeybee *Apis mellifera* L. not produced on the partition was used. The wax was slightly yellowish and contained no honey or pollen. It originated from a swarm near Brno.

Silica gel according to Pitra[51] (made at the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Service Laboratories, Praha-Lysolaje) for column chromatography (granulated between 0·1 and 0·25 mm), was extracted while cold in a column with a mixture of chloroform–methanol (1 : 1) as long as the eluate yielded a residue. Then it was activated for 24 h at 120°C and deactivated with 15% water.

Silica gel according to Pitra[51] for thin-layer chromatography was granulated between 10 and 40 μm. Extraction and activation were done as above. Before forming the thin layer it was mixed with 12·5% extracted alabaster gypsum.

Hexane and chloroform were twice redistilled. The ether was first dried with solid KOH and twice redistilled.

Chromatographic Separation of Beeswax

10·00 g wax dissolved at 40°C in 40 ml chloroform, the solution mixed with 30 g inactivated silica gel (0·10–0·25 mm) and, with gentle heating by an infra-red lamp, the solvent was evaporated. The adsorbed sample was immediately chromatographed on a column (6·4 × 62 cm) which contained 1 000 g silica gel (0·10–0·25 mm). Throughout the chromatography, the concentration of chloroform in the solvent system was maintained at 10% but the content of ether in hexane varied from 3 to 50%. Fractions 1–25 were 100 ml, fractions 26–33 125 ml, fractions 34–74 250 ml and fractions 75–78 500 ml; their composition was monitored by thin-layer chromatography on silica gel. The entire course of chromatography, as well as the composition of the solvent mixture applied to the column are shown in Fig. 1.

Beeswax Esters

Separation of saturated and unsaturated esters 1. 3·12 g esters 1 obtained from gradient elution chromatography (*II*, Fig. 1) were first adsorbed on 14 g inactive silica gel and then chromatographed on a column (3·5 × 49 cm) containing 250 g silica gel (0·10–0·25 mm) impregnated first with 20% silver nitrate and deactivated with 15% water. The elution was carried out with a mixture of hexane (refined with sulfuric acid) and chloroform (7 : 3), 50 ml fractions being collected. Fractions 1–4 contained saturated esters, fractions 11–20 unsaturated esters. The intermediate fractions 5–10 were combined and chromatographed in the same way, the individual

fractions being added to the fractions of the principal chromatography. A total of 2·62 g saturated esters (84·1%) and 496 mg unsaturated esters (15·9%) were obtained.

IR *spectra* of both groups of esters were measured on a UR-10 apparatus in 0·01 cm cuvettes (6% solution in $CHCl_3$). Peaks at 1179 (1181) and 1722 (1723) cm^{-1} correspond to the ester group and the band at about 720 cm^{-1} is typical of long aliphatic chains.

Saponification of esters 1 and separation of the individual components. 200 mg esters (both saturated and unsaturated) were refluxed with 40 ml ethanol containing 0·4 ml water and 400 mg KOH. The course of saponification was monitored by chromatography on a thin layer of silica gel. After 1 h of boiling, the mixture was cooled and made acid with 3 ml H_2SO_4 (1 : 20), 55 ml water was added and the mixture was extracted with 70 ml ether. The extraction was repeated with 5 × 20 ml ether. The combined extracts were extracted with 3 × 20 ml water and dried with magnesium sulfate. The excess ether was distilled away and the remaining solution of acids and alcohols combined with a redistilled ether solution of diazomethane and the mixture left to stand for 1 h. After removing the solvent by distillation, the mixture of methyl esters of acids and free alcohols was adsorbed on 3 g silica gel and the sample thus prepared was chromatographed on a column (2 × 21 cm) containing 32 g silica gel (0·07—0·10 mm) deactivated with 15% water. The elution was done with a mixture of hexane and ether (9 : 1). Fractions 1—10 were 25 ml, fractions 11—17 were 50 ml. The individual components of saturated as well as unsaturated esters 1 are summarized in Table II.

Separation and identification of monocarboxylic acids from unsaturated esters 1. Preparative chromatography on a thin-layer of silica gel impregnated with 20% silver nitrate was applied to separate the methyl esters of acids (fractions 1—3 from the chromatography of saponified unsaturated esters 1) into a saturated and an unsaturated fraction [chloroform–hexane (refined with H_2SO_4) 7 : 3]. After detection with rhodamine B in UV light[11] the individual zones were extracted with absolute ether on a small column of silica gel, the dye being trapped on the column.

IR *spectroscopy* of the methyl esters of unsaturated acids revealed the presence of a *cis*-double bond (absorption bands at 1655 and 3005 cm^{-1}) and, at the same time, excluded the presence of a *trans*-double bond (determined on a UR-10 apparatus with a 0·02 cm cuvette in substance).

Consumption of hydrogen determined by *microhydrogenation*[52] of the methyl esters of unsaturated acids corresponded to 1·02 double bond per molecule.

Hydroxylation was carried out according to Wolff and coworkers[53]. 54 mg OsO_4 dissolved in 5 ml of a mixture of ether and pyridine (8 : 1) was added to 25 mg methyl esters of unsaturated acids. After 2 h of standing, 150 ml of freshly prepared suspension of Na_2SO_3 in methanol were added (the suspension was prepared by mixing 40 ml 16% aqueous solution of sodium sulfite with 130 ml methanol). After 1 h of standing, the precipitate was filtered through a S 3 glass filter and the filtrate evaporated at reduced pressure practically to dryness. The residue was extracted three times with 5 ml of a mixture of hexane and ether (1 : 1). After evaporation, 25 mg methyl esters of dihydroxy acids was obtained. Five mg of these were converted to bis-trimethylsilyl derivatives (see the preparation of trimethylsilyl derivatives of alcohols).

Mass spectrometry. Spectra were obtained on a mass spectrometer MCH 1303 with a sector magnetic analyzer and magnetic detector of the mass scale and with an equipment for direct placing of samples. The ionization chamber was maintained at a constant temperature of 200°C.

Attempt at identifying the unknown-type acids in unsaturated esters 1. Combined fractions 4—7 from a chromatographic separation of saponified unsaturated esters 1, when chromatographed on a thin layer of silica gel, showed an R_F value (0·54) similar to that of the dimethyl ester of α,ω-dicarboxylic acid C_{22} (R_F 0·59) in hexane-ether (4 : 1).

The IR spectrum showed absorption peaks at 1174, 1438 and 1742 cm^{-1} which are typical of the $-COOCH_3$ group. The bands corresponding to the free hydroxyl group were completely absent (determined on a UR-10 apparatus in a 0·01 cm cuvette, 5% solution in CCl_4).

Conversion to the structurally equivalent hydrocarbon was achieved according to Downing and coworkers[54]. 1·5 mg of the compound was reduced with $LiAlH_4$ in ether. The compound formed by reduction had the same R_F value in thin-layer chromatography on silica gel as synthetic 1·18-octadecanediol (C_{18}) in chloroform–methanol (9 : 1). It was heated in a sealed ampoule with 3 mg red phosphorus and 5 mg iodine for 1·5 h at 100°C. The iodide formed was converted with $LiAlH_4$ to the hydrocarbon which was purified on a small column of silica gel for the purposes of gas chromatography and mass spectrometry.

Separation and identification of alcohols from unsaturated esters 1. 80 mg alcohols was heated[55] with 0·5 ml acetyl chloride for 20 min at 60°C. The excess acetyl chloride was distilled off *in vacuo*. The alcohol acetates formed were divided into a saturated and an unsaturated fraction with the aid of preparative chromatography on a thin layer of silica gel (20 × 20 cm) impregnated with 20% silver nitrate in chloroform–hexane (refined with H_2SO_4) (7 : 3). After detection with Rhodamine B in UV light[11] the individual zones were extracted with absolute ether in a small column of silica gel, the dye being trapped on the column. In this way, a total of 55·3 mg (90·7%) saturated ($R_F = 0·87$) and 5·7 mg (9·3%) unsaturated fraction ($R_F = 0·44$) were obtained.

Preparation of the trimethylsilyl derivatives[56]. To 5 mg of a mixture of alcohols (or of methyl esters of dihydroxy acids) 0·2—0·3 ml silylation mixture [trimethylchlorosilane–hexamethyldisilazane–pyridine (1 : 3 : 9)] was added. The reaction mixture was heated several times to about 60°C and well shaken. After 30 min, it was combined with 5 ml of water and the derivatives formed were extracted with 4 × 5 ml hexane. The combined extract was shaken with 3 × 7 ml water dried, and evaporated to dryness. The trimethylsilyl derivatives being rather unstable, the gas chromatography or mass spectrometry were done on the same or on the following day.

Gas Chromatography

Gas chromatography was done on a PYE Argon Chromatograph and a Perkin-Elmer F 11 (with a dual system) apparatus. The acid methyl esters were chromatographed on 10% silicone elastomer SE-30 placed on Chromosorb W (100—120 mesh), on 3% SE-30 placed on Gas-Chrom P (100 to 120 mesh) and on 10% butanediol succinate placed on Chromosorb W (100—120 mesh). The trimethylsilyl derivatives of alcohols were also chromatographed on 3% SE-30. The glass columns were 0·4 × 120 cm in size. The wax esters, alcohols, their acetates and trimethylsilyl derivatives were chromatographed on 2·5% SE-30 using silanized Chromosorb G (100—120 mesh). The glass columns were 0·4 × 150 cm in size. The quantitative evaluation of the chromatograms was done by comparing the products of the retention times and of the corresponding wave heights (not using any correction factors).

CONCLUSIONS

The gas chromatography of the individual groups of compounds makes it possible to draw the following conclusions.

Saturated Esters 1

Monocarboxylic acids (fractions 1—3) are represented by a prevalently homologous series of saturated acids with a straight chain between C_{10} and C_{25} (series B), palmitic acid (C_{16}) being clearly predominant (93·6%). It was determined by mass spectrometry and by comparing the retention times on different phases with a standard. Another homologous series (series A) is present in a small amount (2·5%)

and it was not studied in detail. In view of the shorter retention times we are dealing here probably with branched monocarboxylic acids.

The alcohols form a mostly homologous series of saturated straight-chain monoalcohols (series B) as was demonstrated by comparing the retention times of free alcohols, of their acetates and trimethylsilyl derivatives with those of synthetic standards. The series reaches from C_{14} to C_{34} with maxima at C_{24} and C_{30}. In a small amount, the homologous series A is also represented but it was not studied further. In view of the shorter retention times we are probably dealing here with branched alcohols.

Saturated esters 1 are thus formed predominantly by a homologous series of palmitates from C_{34} to C_{52} with maxima at C_{40} and C_{46} which is in agreement with the work of Holloway[15] and Tulloch[16].

Unsaturated Esters 1

Monocarboxylic acids obtained by their hydrolysis belong to two homologous series. The first series represented by 11·2% of the total is formed by saturated acids. Palmitic acid (C_{16}) is clearly predominant. The second, more abundant series is formed by unsaturated acids from C_8 to C_{24} with a maximum at C_{18} which predominates in this group.

Both types of acids were separated as methyl esters by thin-layer chromatography using silica gel impregnated with silver nitrate. Mass spectrometry bore out the conclusion of gas chromatography that the saturated acid involved here is palmitic acid (C_{16}).

Since almost 96% of all the unsaturated acid is formed by the C_{18} acid, the whole mixture could be considered for further identification as practically a chemical individual. Microhydrogenation[52] (consumption of 1 mol hydrogen) supported the presence of one double bond. IR spectroscopy revealed further that we are dealing here with a *cis*-double bond (absorption bands at 1655 and 3005 cm^{-1}), bands corresponding to a *trans*-double bond being completely absent. It thus remained to determine the position of this *cis*-double bond. For this purpose, the mixture was hydroxylated with osmium tetroxide and the methyl ester of the formed dihydroxy acid was silylated. Mass spectrometry[57,58] of the formed bis-trimethylsilyl derivative then determined unequivocally that the double bond is in position 9. The unsaturated acid was thus identified as *cis*-9-octadecenoic acid (oleic acid). This identification was further confirmed in gas chromatography by comparing the retention times with those of standard oleic acid on a polar phase. The other unsaturated acids may be considered as their homologues.

The presence of 11% saturated acids in the saponified acid fraction of the unsaturated esters 1 was somewhat surprising. We assumed that the carriers of this unsaturation in the original unsaturated esters 1 should be the alcohol components bound to saturated palmitic acid. This assumption was actually confirmed (see Alcohols).

The chromatographic fractions 4–7 contain three homologous series (A, B, C) of compounds where the C_{18} homologue of series B represents 93·9% of the whole mixture. The R_F value of this mixture during thin-layer chromatography on silica gel corresponds to the R_F value of the dimethyl esters of dicarboxylic acids. The IR spectrum showed absorption peaks which are typical of the —$COOCH_3$ group. The absorption bands corresponding to the free hydroxyl group were completely lacking. Results obtained with nuclear magnetic resonance indicate the presence of a —$COOCH_3$ group.

The retention data of the individual homologues of the mixture during gas–liquid chromatography were not identical with the retention data of methyl esters of monocarboxylic acids or the dimethyl esters of α,ω-dicarboxylic acids as assumed by Carlier and coworkers[13]. To verify the structure of the basic skeleton of the unknown acid we converted it to the structurally equivalent hydrocarbon which was identified by means of gas–liquid chromatography and of mass spectrometry as n-octadecane (C_{18}). Since reduction with $LiAlH_4$ gave rise first of all to diols we assumed that we are most probably dealing here with an unbranched monocarboxylic acid with a further oxygen function (see Note added in proof).

Alcohols. Using thin-layer chromatography on silica gel impregnated with silver nitrate it was found that, in addition to a great majority of saturated alcohols, there is a certain amount of unsaturated alcohols present. The mixture of both types of alcohols was converted to acetates which were separated by the above chromatography. In this way, a total of 90·7% acetates of saturated alcohols and 9·3% acetates of unsaturated alcohols was obtained. The amount of unsaturated alcohols from the unsaturated esters 1 corresponds satisfactorily to the amount of saturated acids in these esters.

The saturated fraction is formed by a homologous series of unbranched primary monoalcohols as was demonstrated by comparing the retention times of free alcohols, their acetates and the trimethylsilyl derivatives with those of synthetic standards. The series extends from C_{18} to C_{34} with a maximum at C_{30}.

The unsaturated fraction is formed by two homologous series (series A and B) of alcohols. Microhydrogenation[52] of this fraction consumed 1 mol hydrogen whence it follows that the individual homologues possess one double bond. The presence of one double bond is also in agreement with the R_F value from thin-layer chromatography on impregnated silica gel. Since hydrogenation gave rise only to saturated unbranched primary alcohols the homologues of both series A and B are thought to possess an unbranched skeleton. Series B, represented by 93%, extends from C_{28} to C_{36} with predominating homologues C_{32} and C_{34}, In view of the shorter retention times of the individual homologues during gas–liquid chromatography as compared with the retention times of the corresponding saturated alcohols, the position of the double bond will be farther from the chain end[59–61]. Because of scarcity of material we did not determine the exact position.

Homologous series A represented by 7%, extends from C_{22} to C_{28}. With great probability, the position of the double bond in the individual homologues will lie nearer the centre of the unbranched chain than the case is with homologues of series B.

The unsaturated esters 1 are thus of two types: 85% of them are formed by a homologous series of oleates from C_{40} to C_{52} with a maximum at C_{48}. Thus the unsaturated nature of the esters 1 originates from the acid. The second type, represented roughly by 10%, are fundamentally palmitates of unsaturated alcohols C_{32} and C_{34}, with a double bond placed farther from the chain end.

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Translated by A. Kotyk.

Note added in proof (see p. 2280 and 2284): Identification of the C_{18} homologue from series B (fraction 4—7 of unsaturated esters 1) as the methyl ester of 9,10-epoxy-octadecanoic acid resulted from an additional analysis of the mass spectrum. An identical mass spectrum has been quoted already earlier by Ryhage R. and Srenhagen E. [Arkiv Kemi *15*, 545 (1960)].