MASS SPECTROMETRY AND GAS CHROMATOGRAPHY OF METHYL ESTERS OF HIGHER ALIPHATIC BRANCHED ACIDS AND THEIR α-HYDROXY DERIVATIVES

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Mass spectrometer–gas chromatograph was used to measure the mass spectra and retention data of methyl esters of *n*-heptadecanoic acid (*I*), 15-methyl- (iso, *II*), 14-methyl- (anteiso, *III*), 6-methyl- (*IV*), and 2-methylhexadecanoic acid (*V*), their α-hydroxy derivatives (*VI*—*X*) and trimethylsilyl ethers. The mass spectra of methyl esters of 2-hydroxyheptadecanoic acid (*VI*) and 2-hydroxy-15-methylhexadecanoic acid (*VII*) are very similar and these substances may be identified with the use of gas chromatographic retention data. In spectrum of the methyl ester of 2-hydroxy-14-methylhexadecanoic acid (*VIII*) there is a characteristic peak of $[M-(CH_3OH + C_4H_9)]^+$ ions and the spectrum of 2-hydroxy-6-methylhexadecanoic acid (*IX*) methyl ester exhibits significant peaks of ions formed by cleavage of the aliphatic chain at the branching point. In the case of 2-hydroxy-2-methylhexadecanoic acid (*X*) methyl ester, typical fragments are the α-cleavage product and ions arisen by the β-cleavage under the simultaneous elimination of the methoxycarbonyl group. The spectra of trimethylsilyl derivatives of α-hydroxy acid methyl esters are as similar that the localisation of the carbon chain branching is hardly possible except for the derivative of 2-hydroxy-2-methylhexadecanoic acid (*X*).

The higher aliphatic α-hydroxy acids occur in Nature relatively often in lipidic fractions of microorganisms, plants, and animals (*e.g.*[1–5]) as well as in geological sediments[6]. The mass spectra of methyl esters of unbranched α-hydroxy acids have been investigated in detail[6–10] while those of branched α-hydroxy acid methyl esters have been paid little attention[11] in spite of the occurrence of these branched acids in Nature[11–14]. In connection with our investigations on naturally occurring lipidic substances[15,16] it appeared of interest to measure the mass spectra of methyl esters of branched α-hydroxy acids and compare them with spectra of analogous nonhydroxylated acid methyl esters. We wanted to examine in this manner to what extent is the fragmentation affected by the presence of a hydroxylic function in the molecule and whether it is possible to localize on the basis of mass spectra the branching point of the carbon chain in higher α-hydroxy acids. For this purpose, we have synthetically prepared some isomeric aliphatic monocarboxylic acids containing 17 carbon atoms, namely, n-heptadecanoic acid (*I*) and monomethylhexadecanoic acids branched at positions 15 (iso, *II*), 14 (anteiso, *III*), 6 (*IV*) and 2 (*V*). Furthermore,

analogous acids (*VI–X*) have been prepared carrying an additional hydroxylic function at the α-position. In the mass spectral measurements, the C_{17}-acids were used in the form of methyl esters and the corresponding α-hydroxy acids as trimethylsilyl derivatives. Contrary to acids isolated from naturally occurring materials, our synthetic products are optically inactive. This difference does not affect the results of mass spectrometry[17,18] or of the conventional gas chromatography.

Methyl Esters of C_{17}*-Acids*

In mass spectra of methyl esters of the aliphatic acids *I – V* (Fig. 1) there are dominant peaks of ions obtained by the McLafferty rearrangement and the γ-cleavage[19–21], *i.e.*, the ions of *m/e* 74, 87 or of *m/e* 88, 101 with the 2-methylhexadecanoic acid (*V*) methyl ester which simultaneously allow to localize the branching point of the hydrocarbon chain in this substance. The resolution of similar mass spectra of methyl esters of n-, iso-, and anteiso-acids has been successfully performed[22] on the basis of peak intensity ratios of the $[M\text{-}29]^+$, $[M\text{-}31]^+$, and $[M\text{-}43]^+$ ions. Values of these ratios are tabulated for methyl esters of C_{14}—C_{24}-acids (the value of iso-C_{17} is lacking), *e.g.*, 22 : 46 : 100 (n-C_{17}), 19 : 28 : 100 (iso-C_{16}), 15 : 19 : 100 (iso-C_{18}), and 50 : 25 : 100 (anteiso-C_{17}) (measured at 17 eV). Although these data are somewhat different from the present values recorded at 70 eV (17 : 31 : 100 with n-C_{17}, 19 : 21 : 100 with iso-C_{17}, and 50 : 20 : 100 with anteiso-C_{17}), there is a principal agreement in the $[M\text{-}29]^+ > [M\text{-}31]^+$ relation in the case of the anteiso-compounds and in the observation that the difference in peak intensities of these ions is greater with n-compounds than with iso-compounds. The identification of iso-compound may be facilitated[20] by the poorly intensive peak of $[M\text{-}(CH_3 + CH_3OH + H_2O)]^+$ ions (at *m/e* 219 in the present case). Also in the present case, the peak of $[M\text{-}15]^+$ ions is completely absent in the mass spectrum of the n-compound in contrast to those of the branched compounds[20,23,24]. Peaks of the $[M\text{-}(C_3H_7 + CH_3OH)]^+$ and $[M\text{-}(C_3H_7 + CH_3OH + H_2O)]^+$ ions claimed as characteristic of the iso-compounds[24], have not been recorded in the mass spectrum of the methyl ester of 15-methylhexadecanoic acid (*II*). The methyl esters of iso-acids might be expected to be characterised by an intensive peak of $[M\text{-}C_3H_7]^+$ ions but this peak is intensive in spectra of methyl esters of all aliphatic acids because of its formation with loss of carbon atoms $C_{(2)}$, $C_{(3)}$, and $C_{(4)}$ under the hydrogen transfer[25,26]. In addition to the characteristic peak ratio of $[M\text{-}29]^+$ and $[M\text{-}31]^+$ ions in the mass spectrum of the anteiso-compound *III*, there also are significant peaks of the $[M\text{-}(C_2H_5 + CH_3OH)]^+$ (*m/e* 223) and $[M\text{-}(C_2H_5 + CH_3OH + H_2O)]^+$ (*m/e* 205) ions and the peak of the $[M\text{-}57]^+$ ions which is not however higher[23,24] than that of the $[M\text{-}43]^+$ ions.

Position of the methyl group in the methyl ester of 6-methylhexadecanoic acid (*IV*) is particularly determined by the peak of the *m/e* 208 $[M\text{-}(CH_3OH + CH_2=$

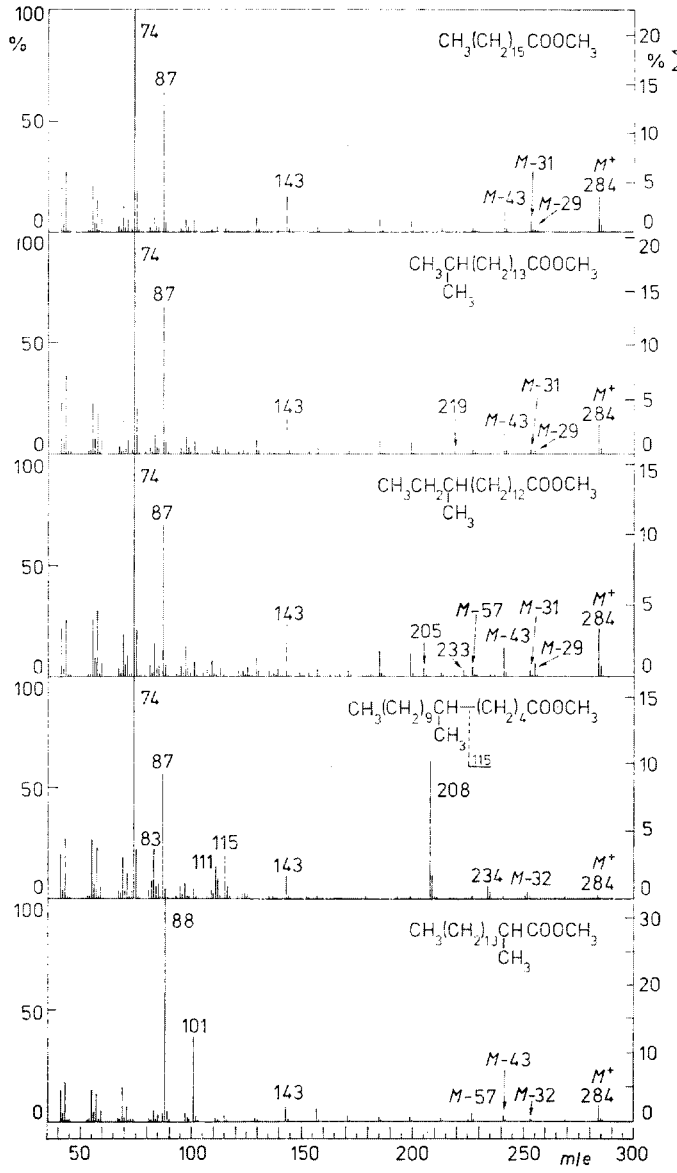


FIG. 1

Mass Spectra of Methyl Esters of n-Heptadecanoic Acid, 15-, 14-, 6-, and 2-Methylhexadecanoic Acids (*I*—*V*)

%Σ, % of total ionisation encompassing the mass range from *m/e* 41 to the molecular ion.

$=CHOH)]^{+\cdot}$ (m^* 284 → 208; calculated 152·3, observed 152·3) ions formed by a rearrangement characteristic of 6-substituted methyl esters[27,28]. Further characteristic fragments are formed by cleavage before and beyond the branching point of the aliphatic chain, *i.e.*, of *m/e* 115 and 143; from these fragments, both $CH_3O\cdot$ and CH_3OH is eliminated with the formation of the *m/e* 83, 84, 111, and 112 ions. From the poorly intensive molecular ion of this compound there is eliminated (in addition to the usual neutral fragments $CH_3\cdot$, $C_2H_5\cdot$, and $C_4H_9\cdot$) CH_3OH (m^* 284 → 252; calculated 223·6, observed 223·6) and (from the ions formed) H_2O (m^* 252 → 234; calculated 217·3, observed 217·2). This elimination also occurs simultaneously as confirmed by the metastable transition (m^* 284 → 234; calculated 192·8, observed 192·9).

In addition to the mentioned *m/e* 88 and 101 peaks, the mass spectrum of 2-methylhexadecanoic acid (*V*) methyl ester exhibits a significant peak of $[M-57]^+$ (> > $[M-43]^+$) ions corresponding to elimination of $C_{(2)}$ to $C_{(4)}$ carbon atoms under the hydrogen transfer.

Methyl Esters of α-Hydroxy Acids

Characteristic feature of mass spectra of α-hydroxy acids *VI*–*X* methyl esters (Fig. 2) is elimination of the methoxycarbonyl group from the molecular ion ($[M-59]^+$) and the presence of a significant peak of *m/e* 90 ions formed by the α-cleavage accompanied by hydrogen transfer[8–10]. In the case of 2-hydroxy-2-methylhexadecanoic acid (*X*) methyl ester, an analogous peak of *m/e* 104 ions may be observed. Methyl esters of all α-hydroxy acids eliminate to a small extent from the molecular ion H_2O and CH_3OH and the *m/e* 103 and 145 ions are formed by a simple 3, 4 and 6, 7 cleavage[8]. The earlier reported[8] $[M-(60 + 18)]^{+\cdot}$ ions are accompanied by a peak of $[M-(61 + 18)]^+$ ions; the most significant peaks are observed in the case of 2-hydroxy-6-methylhexadecanoic acid (*IX*) methyl ester (*m/e* 222 and 221). Elimination of the methoxycarbonyl group is partly accompanied by the loss of two hydrogen atoms ($[M-61]^+$). As shown by measurement of methyl esters of the α-hydroxy acids *VIII* and *X* containing deuterium in the hydroxylic function, one of the eliminated hydrogen atoms is the hydroxylic hydrogen. The absent peak of $[M-61]^+$ ions in spectrum of 2-hydroxy-2-methylhexadecanoic acid (*X*) methyl ester and the present peak of $[M-(59 + 16)]^+$ *m/e* 225 ions indicate the simultaneous elimination of $COOCH_3\cdot$ and $CH_4\cdot$. It may be inferred from these obsevations that in eliminations of two hydrogen atoms from the remaining methyl esters *VI*–*IX* there are involved the hydroxylic hydrogen and the α-hydrogen atoms. $C_3H_7\cdot$ and $CH_4\cdot$ are eliminated in the case of 2-hydroxy-2-methylhexadecanoic acid (*X*) methyl ester.

Concerning the localisation of the branching point in the aliphatic chain it has been found that mass spectrometry cannot distinguish between the methyl esters of n- and iso-α-hydroxy acids despite the availability of the mass spectra of the

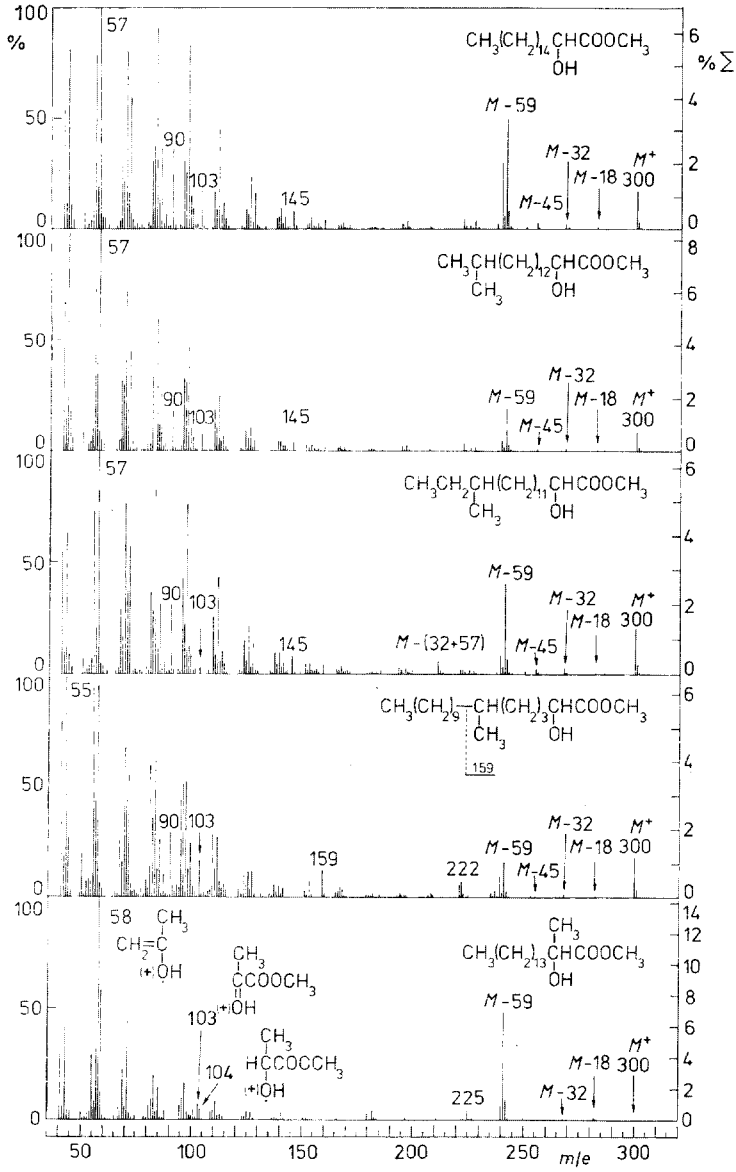


FIG. 2

Mass Spectra of the α-Hydroxy Derivatives of Methyl n-Heptadecanoate and Methyl 15-, 14-, 6-, and 2-Methylhexadecanoates (*VI*—*X*)

%Σ, % of total ionisation encompassing the mass range from *m/e* 41 to the molecular ion.

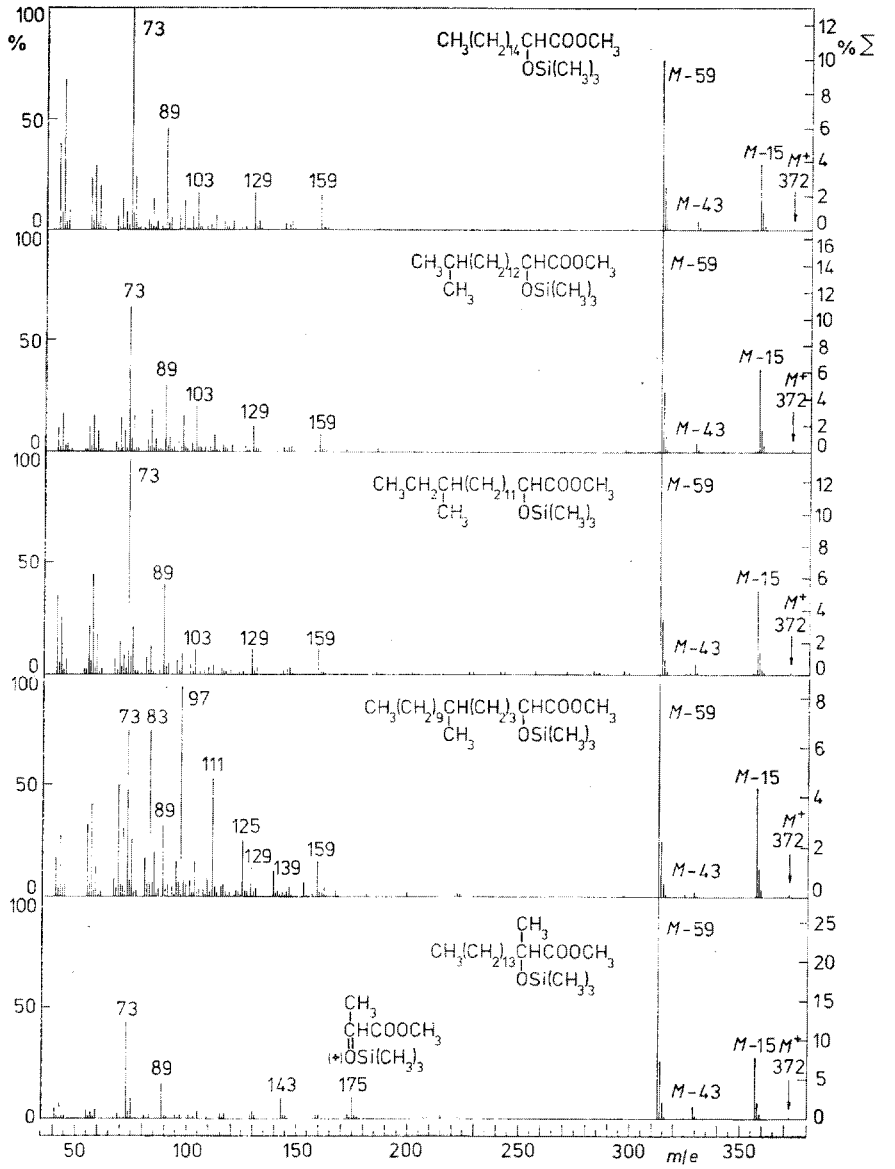


FIG. 3

Mass Spectra of Trimethylsilylated α-Hydroxy Derivatives of Methyl n-Heptadecanoate and Methyl 15-, 14-, 6- and 2-Methylhexadecanoates (*VI*—*X*)

%Σ, % of total ionisation encompassing the mass range from *m/e* 41 to the molecular ion.

iso-C_{15} and iso-C_{16} α-hydroxy acid methyl esters in addition to those of the present n-C_{17} (*I*) and iso-C_{17} (*II*) methyl esters. Introduction of the hydroxylic function into the molecule of the acids *I* and *II* leads to such a decrease in spectral differences that the α-hydroxy compounds can not be identified by mass spectrometry. In the case of 2-hydroxy-14-methylhexadecanoic acid (*VIII*) methyl ester there was observed a characteristic fragment corresponding to elimination of CH_3OH and cleavage of the aliphatic chain before the branching point, namely, $[M-(32 + 57)]^+$. Another typical ion corresponding to elimination of CH_3OH and cleavage beyond the branching point, *i.e.*, $[M-(32 + 29)]^+$, can not be distinguished by the low resolution spectrum because of the same mass with $[M-(COOCH_3 + H_2)]^+$.

In the case of 2-hydroxy-6-methylhexadecanoic acid (*IX*) methyl ester, the branching point is determined by the existence of the peak of $[M-C_{10}H_{21}]^+$ *m/e* 159 ions corresponding to the cleavage beyond the branching point. From these ions, CH_3OH and H_2O (*m/e* 127, 109) are successively eliminated.

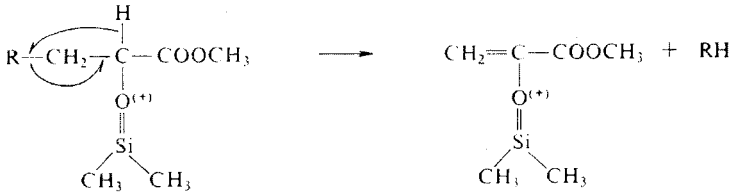
The methyl ester of 2-hydroxy-2-methylhexadecanoic acid (*X*) affords an insignificant molecular peak since the methyl group at the α-position prefers formation of a stable oxonium ion (*m/e* 241). Position of the methyl group is confirmed by peaks of *m/e* 103 and 104 ions ($C_4H_7O_3$ and $C_4H_8O_3$ according to the high resolution measurement) and by the basic spectral peak of *m/e* 58 (C_3H_6O). As shown by measurement of this compound deuterated in the hydroxylic function, the *m/e* 58 ion contains this function and may be thus ascribed the structure given in the spectrum on Fig. 2.

Trimethylsilyl Derivatives of α-Hydroxy Acid Methyl Esters

The mass spectra of all trimethylsilyl derivatives of the α-hydroxy acids *VI*–*X* methyl esters shown on Fig. 3 display a poorly intensive molecular peak and a more significant peak of $[M-15]^+$ ions as usual with silylated substances. The peak of $[M-59]^+$ ions formed by elimination of the methoxycarbonyl group represents the significant peak and, in most cases, also the basic spectral peak[6,9,10]. In addition to these fragments, the spectra exhibit structurally insignificant peaks of hydrocarbon ions and ions which are usually furnished by trimethylsilyl derivatives of α-hydroxy acid methyl esters[6,9,10] (*m/e* 73, 75, 89, 103, 129). Structure of the *m/e* 159 ion, also usual in spectra of the above type has been investigated by Capella and coworkers[10]. Thus, the *m/e* 159 has been observed to contain a methoxyl group and only two methyl groups on the silicon atom. It may be assumed on the basis of the earlier data and the present high resolution measurement that the *m/e* 159 ion is formed by β-cleavage under the hydrogen transfer from the $[M-15]^+$ ion.

As indicated by the spectrum of trimethylsilylated α-hydroxy acid methyl esters, the fragmentation centre has been transferred by the silylation process almost

exclusively into the neighbourhood of the trimethylsilyl group; any additional fragmentation is quite insignificant. Only the compound *X* with the methyl group at position 2 may be regarded as an exception. Owing to this substitution there is formed a stable oxonium ion (*m/e* 175) from which CH_3OH is split off with the formation of a *m/e* 143 ion. Furthermore, the methyl group at position 2 strongly favours elimination of the methoxycarbonyl group. Consequently, this fragment is farly the most significant and the formation of further ions is even more strongly suppressed than with other trimethylsilyl derivatives.



$[M-15]^+$ *m/e* 159

In the mass spectrum of the trimethylsilyl derivative of 2-hydroxy-6-methylhexadecanoic acid (*IX*) methyl ester there may be observed an abundant occurence of hydrocarbon fragments due to the branching inside the chain but is was not possible to localize the branching point on the basis of these peaks.

As suggested by the above data, the branching point of the aliphatic chain in methyl esters of α-hydroxy acids may be determined on the basis of mass spectra in the case of the free hydroxy compounds only and not with the use of the corresponding trimethylsilyl derivatives. Even under those conditions however, it can not be distinguished between the n- and iso-compounds. It is of interest in this connection that the methyl esters of α-hydroxylated iso- and anteiso-acids have been distinguished in the form of their acetates[11] on the basis of peak intensities of the *m/e* 43 and *m/e* 57 ions. In the present case, the methyl esters of α-hydroxy n- and iso-acids have been successfully resolved in combination with gas chromatography since the retention time of the iso-compounds is shorter than that of the corresponding n-compounds[29] (Table I).

EXPERIMENTAL

Methyl esters were prepared by reaction of free acids with diazomethane. Unless stated otherwise, the products were purified by column chromatography on silica gel in combination with monitoring by means of thin-layer chromatography. According to gas chromatography, the

purity of methyl esters was always higher than 90%. Since the thus-prepared substances were sufficiently characterised by mass spectra and retention data, the elemental analyses were not performed.

Trimethylsilyl derivatives. A mixture of the methyl ester of the appropriate α-hydroxy acid (4 mg), acetonitrile (0·2 ml), and bis(trimethylsilyl)trifluoroacetamide (0·2 ml) was heated in a sealed ampoule for one hour at 150°C. The reaction mixture was directly used in the gas chromatograph.

n-*Heptadecanoic acid* (I). The acid *I* was prepared from 1-hexadecanol (1 000 g) *via* the bromide and cyanide as described in the case of tridecanoic acid[30]. Rectification of the methyl ester on a 30 TP column afforded 530 g of the pure fraction.

15-*Methylhexadecanoic acid* (II). 14-Methylpentadecanoic acid (2·9 g) occurring as the main component of one distillation fraction of lanolin (73·1% of iso-C_{16}, 2·5% of iso-C_{15}, 19·7% of n-C_{16}, and 1·4% of n-C_{15}) was homologuenized as usual[31]. The resulting product contained 76% of the methyl ester of iso-C_{17} acid. This acid has been prepared earlier by other routes, *e.g.*[32,33].

14-*Methylhexadecanoic acid* (III). 6-Methyloctanoic acid (8·0 g) and monomethyl sebacate (21·6 g) were subjected to the anodic electrolysis[15,34] under conditions reported earlier by Milburn and Truler[35]. The required acid was isolated from the reaction mixture in the form of the methyl ester (5·8 g) by fractional distillation.

TABLE I

Kovats Retention Indexes of C_{17}-Acid Methyl Esters

Acid	Methyl ester	Methyl ester, 2-hydroxy-	Methyl ester, 2-trimethylsilyloxy-
n-Heptadecanoic	2 009	2 128	2 221
15-Methylhexadecanoic	1 974	2 089	2 185
14-Methylhexadecanoic	1 983	2 102	2 193
6-Methylhexadecanoic	1 953	2 067	2 154
2-Methylhexadecanoic	1 944	2 025	2 137

6-*Methylhexadecanoic acid* (IV). n-Decanoic acid (25·8 g) and monomethyl 3-methylglutarate (24·0 g) were subjected to anodic electrolysis[15,34]. The isolated (preparative gas chromatography) methyl 3-methyltridecanoate (10·7 g) was saponified. A portion of the resulting 3-methyltridecanoic acid (4·6 g) was lengthened by an additional anodic electrolysis[15,34] using monomethyl glutarate (3·3 g). Column chromatography on silica gel (light petroleum containing increasing amounts of ether as eluant) afforded 1·3 g of the methyl ester of acid *IV*. The free acid has been prepared earlier by another route[32].

2-*Methylhexadecanoic acid* (V). A mixture of methyl 2-hydroxy-2-methylhexadecanoate (60 mg), phosphorus oxychloride (37 mg), and pyridine (121 mg) was heated in a sealed ampoule for 90 min at 150°C. The resulting α,β-unsaturated methyl ester (47 mg) was hydrogenated over

palladium on active charcoal as catalyst in acetic acid as solvent at ordinary pressure and temperature for 10 h. After isolation and purification, there was obtained 40 mg of the pure substance. The IR absorption bands were situated at 1164, 1197, 1438, and 1742 cm^{-1} (—$CH(CH_3)$—$COOCH_3$). The free acid has been obtained earlier by other reactions[32].

2-*Hydroxyheptadecanoic acid* (VI). n-Heptadecanoic acid (*I*; 5·0 g) in the form of its chloride was brominated, the product hydrolysed (6·0 g of the 2-bromo acid), the bromo atom exchanged by the hydroxylic group (5·2 g of the α-hydroxy acid), and the carboxylic function esterified. Column chromatography on silica gel with the solvent system 15 : 4 : 1 light petroleum–chloroform–ether as eluant yielded 3·5 g of the methyl ester of compound *VI*. The IR absorption bands were situated at 1228, 1265, 1445, and 1741 cm^{-1} (—$COOCH_3$) and at 3610 cm^{-1} (—OH). The above reaction sequence has been used earlier by Sweet and Estes[36] in the preparation of 2-hydroxyhexadecanoic acid.

2-*Hydroxy*-15-*methylhexadecanoic acid* (VII). 15-Methylhexadecanoic acid (*II*; 0·95 g) was transformed into the 2-hydroxy derivative by an analogous sequence of reactions[36] as compound *I*; in the present case, the reaction with bromine was performed in a sealed ampoule (0·24 ml of bromine). Column chromatography on silica gel yielded a mixture of methyl esters (0·35 g) containing 64% of the required methyl ester of compound *VII* and 9·9% of the lower homologue, namely, methyl 2-hydroxy-14-methylpentadecanoate, the mass spectrum of which was also measured. IR spectrum: 1225, 1265, 1442, and 1741 cm (—$COOCH_3$), 1367 and 1384 cm^{-1} (—$CH(CH_3)_2$), and 3618 cm^{-1} (—OH).

2-*Hydroxy*-14-*methylhexadecanoic acid* (VIII). By an analogous process as in the preparation of compound *VII*, 14-methylhexadecanoic acid (*III*; 1·0 g) yielded 0·26 g of the methyl ester of compound *VIII*. IR spectrum: 1228, 1263, 1441, and 1740 cm^{-1} (—$COOCH_3$), and 3605 cm^{-1} (—OH).

2-*Hydroxy*-6-*methylhexadecanoic acid* (IX). From 6-methylhexadecanoic acid (*IV*; 0·79 g) there was obtained (analogously to the preparation of the methyl ester of compound *VII*) the methyl ester of compound *IX* (0·34 g, after the final purification). IR spectrum: 1230, 1263, 1442, and 1741 cm^{-1} (—$COOCH_3$), and 3615 cm^{-1} (—OH).

2-*Hydroxy*-2-*methylhexadecanoic acid* (X). By the addition of hydrogen cyanide[37], 2-hexadecanone (1·0 g) was converted to the corresponding cyanohydrin (0·75 g) which was subjected to acidic hydrolysis in methanol. Column chromatography on silica gel yielded 0·26 g of the methyl ester of compound *X*. IR spectrum: 1178, 1195, 1250, 1440, and 1739 cm^{-1} (—$COOCH_3$), and 3615 cm^{-1} (—OH).

Analytical methods. Mass spectrometry was performed on a combined Pye Series 104 Model 64 Chromatograph and AEI MS 902 apparatus with the use of the Watson-Biemann separator. The methyl esters of C_{17}-acids, methyl esters of α-hydroxy acids, and trimethylsilyl derivatives of α-hydroxy acid methyl esters were chromatographed on a 0·4 × 150 cm column packed with 3% SE-30 on GAS-CHROM Q (100—120 mesh), maintained at the temperature of 190°C, 200°C, and 210°C, resp. In all cases, the ion source temperature was 190°C and the electron energy 70 eV. Preparative gas chromatography was carrried out on an Automatic gas chromatograph (produced by Experimental Workshops, Czechoslovak Academy of Sciences, Prague) equipped with a catharometer. The metal column (1 × 240 cm) contained 15% of butanediol succinate on Chromosorb W (100—120 mesh). The column temperature was 200°C; the temperature of the inlet chamber, detector, and connecting lines was 250°C. Flow rate of helium, 100 ml per min; 0·3 ml samples. Analytical gas chromatography was performed on a Perkin-Elmer F 11 apparatus with flame ionisation detectors and a dual system of glass columns (0·3 × 180 cm)

packed with 3% SE-30 on silanised Chromosorb G (100—120 mesh); thermostat temperature, 190°C; inlet chamber temperature, 250°C; flow rate of nitrogen, 50 ml per min. The samples were used as a 4% solution in tetrachloromethane. To the methyl esters of C_{17}-acids, methyl esters of α-hydroxy acids, and trimethylsilyl derivatives of α-hydroxy acid methyl esters, there were simultaneously added the n-C_{19} and n-C_{21}, the n-C_{20} and n-C_{22}, and the n-C_{21} and n-C_{23} alkanes, resp. The Kovats retention indexes[38] values (Table I) are averaged from three measurements and are rounded to integer numbers. The IR spectra were taken on a UR-20 Carl Zeiss (Jena) apparatus in 0·01 cm cells with the use of tetrachloromethane as solvent; concentration of samples, 6%.

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