GAS CHROMATOGRAPHY–MASS SPECTROMETRY DETERMINATION OF HIGHER ALIPHATIC SECONDARY ALCOHOLS

K.Ubik, K.Stránský and M.Streibl

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

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On a combined gas chromatograph–mass spectrometer (GC-MS), a series of isomeric tricosanols and their trimethylsilyl derivatives has been measured and the dependence of fragmentation on the position of the hydroxylic group has been examined. In addition to the known *a* and *b* ions resulting by the α- and β-cleavage, there have been identified *a*-1, *b*-1, *a*-2, *c*-2, and *d*-2 ions corresponding to the elimination of one or two hydrogens from products of the α-, β-, γ-, and δ-cleavage. Dependence of the intensity of products from the α- and β-cleavage on the number of carbons has been determined. The poorly intensive but characteristic *a*-(18 + 29) ions have also been found.

Higher secondary aliphatic alcohols as biosynthesis products are frequent components of naturally occurring plant[1–4] or animal[5–7] cuticular waxes both in the free or ester form. Analogously to alkanes and ketones, the odd members always predominate in homologous series as an indication of their biogenetic relationship[2,8]. Relatively little attention has been paid to mass spectra of alcohols of this type. From the literature, there are known the spectra of 2-eicosanol[8,9], the combined spectra of two to four higher secondary alcohols[5], and spectra of trimethylsilyl derivatives of nonanols and dodecanols[10]. Kraft and Spiteller[11] have studied by means of deuterated compounds elimination of water from both the molecular ion and products of the α-cleavage, and also have examined the formation of $C_nH_{2n-1}^{(+)}$ and $C_nH_{2n+1}^{(+)}$ ions.

In the present paper, we wish to report dependence of fragmentation of secondary alcohols on the position of the hydroxylic group. In this connection, we have prepared[12] a series of tricosanols (C_{23}) with hydroxylic groups at positions 2, 3, 4, 5, 6, 7, 8, 9, 10, and 12. For purposes of comparison, the primary alcohol 1-docosanol (C_{22}) was also measured. All the mentioned alcohols were measured free as well as in the form of the corresponding trimethylsilyl derivatives. The gas chromatography–mass spectrometry technique was adopted in our investigations since secondary alcohols (as components of lipids) frequently come to analysis as mixtures and the preparative separation would be extremely difficult.

EXPERIMENTAL

Preparation of alcohols. 1-Docosanol was prepared by the lithium aluminium hydride reduction of methyl behenate (docosanoate). Secondary tricosanols were synthetized by the lithium aluminium hydride reduction of the appropriate tricosanones as reported earlier[12] and purified (final purity, 90 – 99%) by thin-layer chromatography.

Mass spectra were measured on combined PYE Series 104 Chromatograph Model 64 and A.E.I. MS 902 apparatus with the use of the Watson–Biemann separator. Glass chromatographic column (0·4 × 150 cm) containing 3% SE-30 on Gas-Chrom Q (100 – 120 mesh) was maintained at 220°C (free alcohols) or at 230°C (trimethylsilyl derivatives of alcohols). The interface temperature was 200°C in each case. The ion source temperature was 150°C (measurement of free alcohols) or 200°C (trimethylsilyl derivatives). In both cases, the electron energy was 70 eV and the trap current was 500 μA.

In order to determine composition of ions at particular masses, the alcohols were measured in addition to the usual fast scan technique also under conditions of the high resolution (resolving power 10000). Prior to the high resolution measurement of compounds eluted from the chromatographic column, only the analyzer slit was narrowed; as soon as the substance began to enter the source (indication by the total ion current at 20 eV), also the source slit was narrowed and the measurement was carried out at 70 eV by the slow scan technique. The scan time was considerably longer with the use of this technique. Consequently, concentration of the substance markedly changed in the course of the measurement (depending on elution of the chromatographic peak) and the thus-obtained spectra were distorted[13]. Nevertheless, intensity ratios at near masses and particularly at the same mass remained intact. As an example see the significant part of the 5-tricosanol spectrum on Fig. 1. It is obvious from this record that the α-cleavage product at mass *m/e* 87 is not accompanied by hydrocarbon fragments while at mass *m/e* 86 the $C_5H_{10}O$ (*a*-1) and $C_5 . . {}^{13}CH_{13}$ ions show the intensity ratio of 16 : 1 and mass *m/e* 85 is composed from C_5H_9O (*a*-2) and C_6H_{13} ions in the ratio of 1 : 2. All the remaining alcohols were measured and interpreted in this manner.

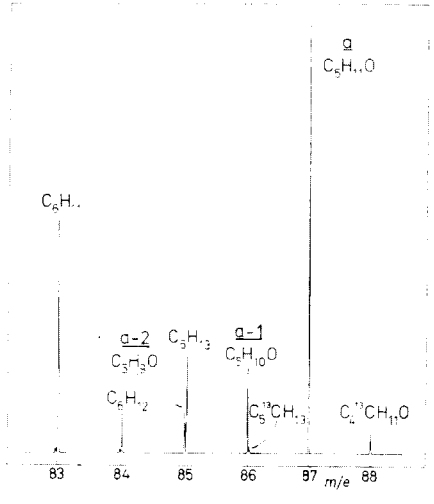
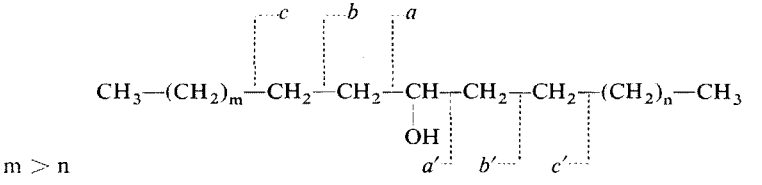


FIG. 1
Part of the High Resolution Mass Spectrum of 5-Tricosanol

RESULTS AND DISCUSSION

Free Alcohols

In descriptions of fragmentations, the significant ions are designated by symbols *a*, *b*, *c*, ..., *a'*, *b'*, *c'* ... corresponding to the cleavage position in their formation, *i.e.*, to the α-, β-, γ-, ... cleavage (Scheme 1).



SCHEME 1

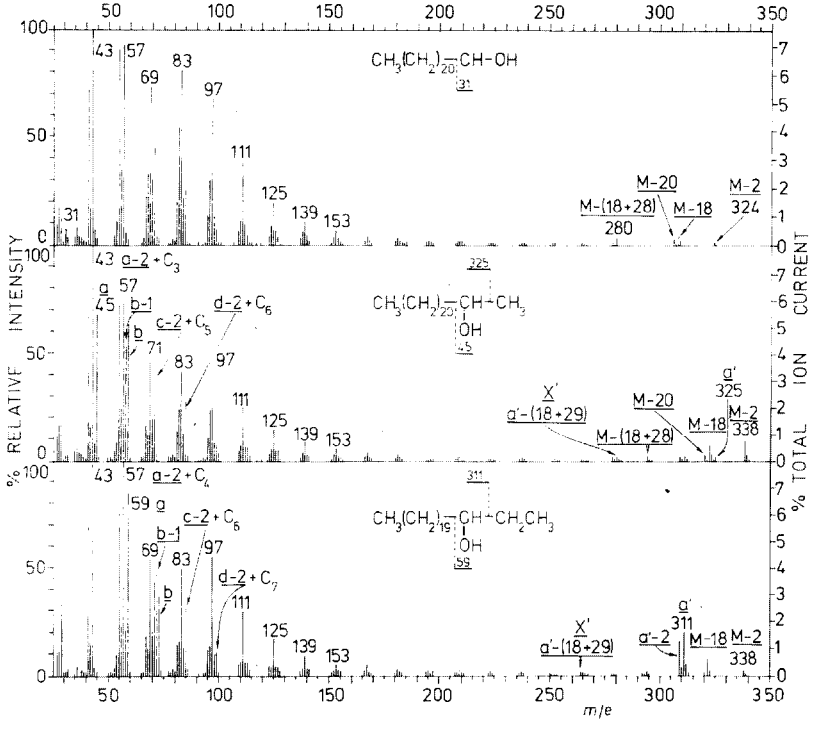


FIG. 2

Mass Spectra of 1-Docosanol, and 2- and 3-Tricosanol

Mass spectra of free alcohols are recorded in Figs 2–5 and intensity ratio of oxygen-containing hydrocarbon fragments to hydrocarbon fragments occurring at the same mass are listed for particular alcohols in Table I.

The following conclusions were drawn from the spectral analysis. 1-Docosanol did not afford any molecular peak; only a very small peak of $[M-1]^+$ ions and a greater $[M-2]^{+\bullet}$ peak were observed. In addition to the peak of $[M-18]^{+\bullet}$ ions, the spectrum exhibits a relatively intensive $[M-20]^{+\bullet}$ peak. Further characteristic fragments *m/e* 31 and $[M-(18+28)]^{+\bullet}$ were also recorded. Our measurements of 1-docosanol correspond in principle to those data obtained earlier by the direct inlet technique[8,14].

Secondary alcohols did not afford in any case molecular peaks but there were observed peaks of $[M-2]^{+\bullet}$ ions which were most distinct in the molecular group. This peak is intensive in the case of 2-tricosanol while with other compounds the intensity is very low. With 2-tricosanol, the $[M-2]^{+\bullet}$ ions are as stable as that their peak is more intensive than that of $[M-18]^{+\bullet}$ ions. Similarly to spectra of primary alcohols[8,15], the spectra of all secondary tricosanols exhibit a peak of $[M-(18+28)]^{+\bullet}$

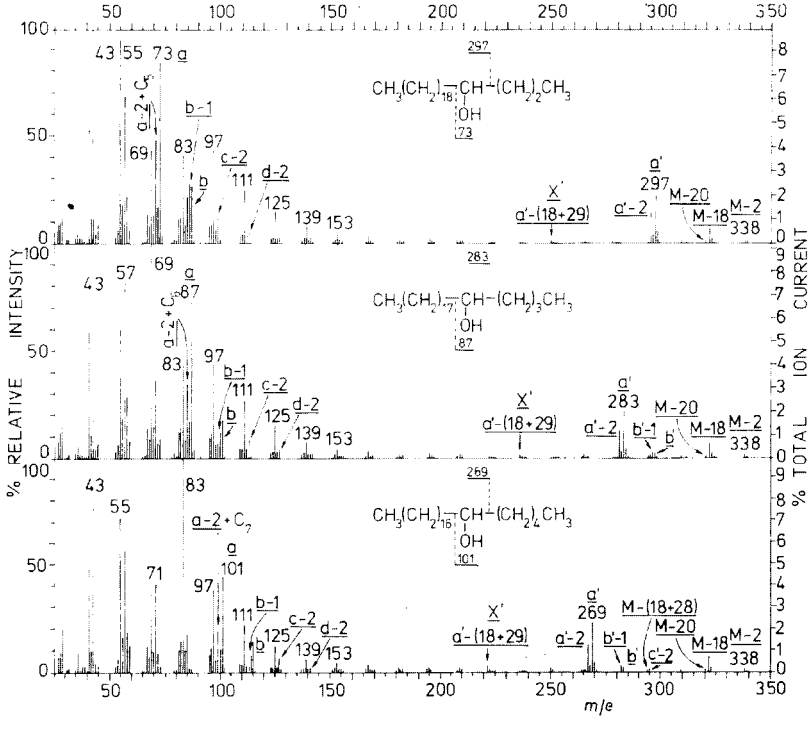


FIG. 3

Mass Spectra of 4-, 5-, and 6-Tricosanol

TABLE I

Ratios of Intensities of Oxygen Containing to Those of Plain Hydrocarbon Fragments Occurring at the Same Mass in the Mass Spectra of Secondary Tricosanols

m/e	Composition	Position of hydroxyl group									
		2	3	4	5	6	7	8	9	10	12
43	$C_2H_3O : C_3H_7$	1 : 1·9	1 : 25	1 : 84	1 : 1·7	1 : 27	1 : 40	1 : 32	1 : 26	1 : 19	1 : 22
44	$C_2H_4O : C_2\,^{13}CH_7$	1 : 1									
57	$C_3H_5O : C_4H_9$	1 : 22	1 : 4·3	1 : 8·1	1 : 4·9	1 : 4·7	1 : 6·1	1 : 7	1 : 7·5	1 : 6·6	1 : 6·8
58	$C_3H_6O : C_3\,^{13}CH_9$	99 : 1	7 : 1	4 : 1	6·3 : 1	2·1 : 1	4·3 : 1	9 : 1	4·2 : 1	3·7 : 1	6 : 1
71	$C_4H_7O : C_5H_{11}$	1 : 1·5	1 : 12	1 : 2·8	1 : 5·3	1 : 4·6	1 : 4·3	1 : 3·9	1 : 3	1 : 4	1 : 3·6
72	$C_4H_8O : C_4\,^{13}CH_{11}$	1 : 1	9 : 1	5 : 1	2·7 : 1	1·2 : 1	1·3 : 1	1·5 : 1	1 : 1	1·5 : 1	1 : 1
85	$C_5H_9O : C_6H_{13}$	1 : 3	1 : 2·8	1 : 22	1 : 2	1 : 4·4	1 : 5	1 : 8	1 : 3·4	1 : 4·8	1 : 14
86	$C_5H_{10}O : C_5\,^{13}CH_{13}$			6·5 : 1	16 : 1	1·1 : 1	1·5 : 1	1 : 1	1 : 1	1 : 1·2	1 : 1·2
99	$C_6H_{11}O : C_7H_{15}$		1 : 3·1	1 : 1·4	1 : 6	1 : 1	1 : 4·7	1 : 2	1 : 2·5	1 : 2·3	
100	$C_6H_{12}O : C_6\,^{13}CH_{15}$				10 : 1	15 : 1	3·3 : 1	3·5 : 1	30 : 1		
113	$C_7H_{13}O : C_8H_{17}$			1 : 4·5	1 : 1·2	1 : 7	3·5 : 1	1 : 2·1	1 : 1·2	1 : 3·5	1 : 1·3
114	$C_7H_{14}O : C_7\,^{13}CH_{17}$					9 : 1	32 : 1				
127	$C_8H_{15}O : C_9H_{19}$				1·3 : 1	1·7 : 1	1 : 1·7	3·9 : 1	1·2 : 1	2 : 1	
128	$C_8H_{16}O : C_8\,^{13}CH_{19}$							80 : 1			
141	$C_9H_{17}O : C_{10}H_{21}$					1 : 1·8	1·4 : 1	1 : 2·8	9 : 1	3 : 1	1 : 1
142	$C_9H_{18}O : C_9\,^{13}CH_{21}$								32 : 1		
155	$C_{10}H_{19}O : C_{11}H_{23}$						1 : 2	3·8 : 1	1 : 1	16 : 1	4·3 : 1
156	$C_{10}H_{20}O : C_{10}\,^{13}CH_{23}$								70 : 1		
169	$C_{11}H_{21}O : C_{12}H_{25}$							3·4 : 1	2·3 : 1	1 : 1	2·1 : 1
183	$C_{12}H_{23}O : C_{13}H_{27}$								2·1 : 1		55 : 1

ions (m/e 294) but its intensity is lower. The peak of $[M-18]^{+\bullet}$ ions arisen by elimination of water from molecular ions, is always accompanied by a lower peak of $[M-20]^{+\bullet}$ ions.

For the determination of the position of the hydroxylic group, fragments a and a' are characteristic[11]. With alcohols, the hydroxylic function of which is placed near the ends of the carbon chain, it can be written that $a \gg a'$. The more is the OH group shifted to the centre, the more decreases the intensity of fragments a and increases the intensity of fragments a'; consequently with 9- and 10-tricosanol, it can be written that $a \approx a'$. It may be seen on curve *1* (Fig. 6) expressing dependence of the peak intensity of a (a') ions on the number of carbon atoms in the resulting oxonium ion that the formation of fragments a is highest when the oxonium ion contains four carbon atoms. Curve *1* then falls up to oxonium ions containing nine carbon atoms. A further increase of carbon atoms does not affect the amount of detected a (a') ions any more. The rapid decrease of the formation of a' ions as

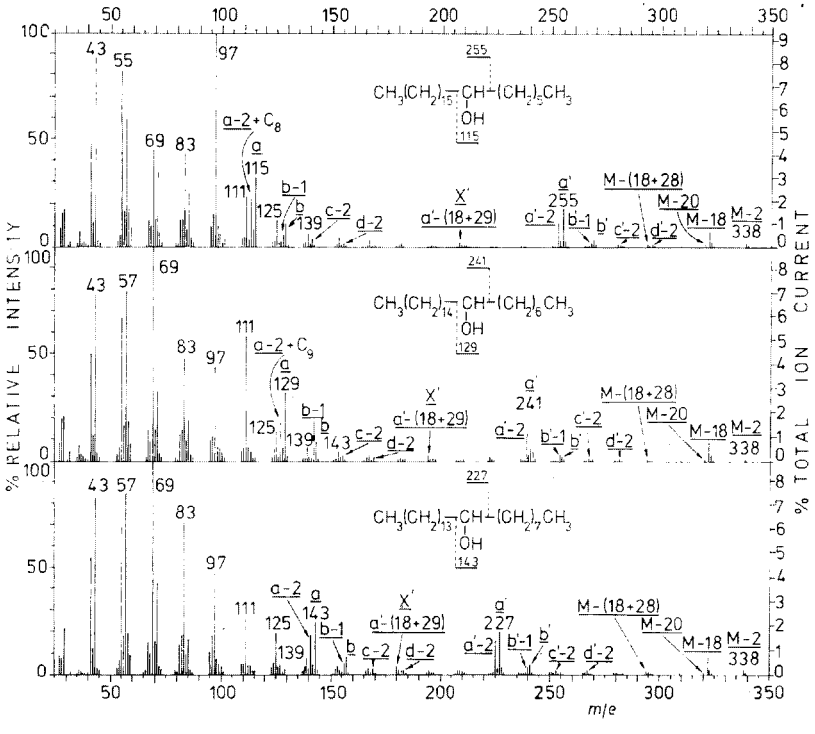


FIG. 4

Mass Spectra of 7-, 8-, and 9-Tricosanol

observed at the end of curve *1* is obviously due to the fact that the hydrocarbon chain of the alcohol practically terminates in this region. It may be inferred from the course of the curve that a further elongation of the hydrocarbon chain would not affect the amount of *a'* fragments as expressed in units of the total ion current.

The *a* (*a'*) fragments are accompanied by *a*-1, *a*-2 and *a'*-1, *a'*-2 fragments, resp., with the following intensity ratios: $a > a\text{-}2 > a\text{-}1$ and $a' > a'\text{-}2 > a'\text{-}1$. With 2-

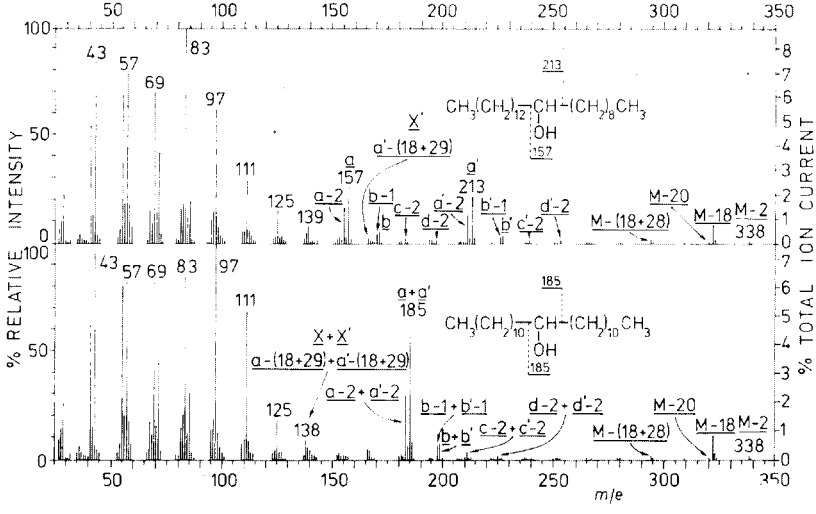


FIG. 5

Mass Spectra of 10- and 12-Tricosanol

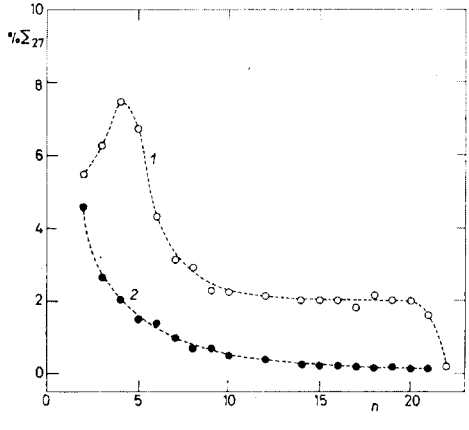


FIG. 6

Dependence of the Peak Intensity of the α-Cleavage (*1*) and β-Cleavage (*2*) Products of Free Secondary Tricosanols on the Position of the Hydroxylic Group

$\%\sum 27$, % of total ionisation encompassing the mass range from *m/e* 27 to the molecular ion; *n*, number of carbon atoms in products of α- and β-cleavage.

and 3-tricosanol, the *a*-2 ions, when summed up with hydrocarbon fragments, cooperate in the formation of the base peaks *m/e* 43 and *m/e* 57. In spectra, there were also identified the *b*, *b*-1, *c*-2, *d*-2, and *b'*, *b'*-1, *c'*-2, *d'*-2 ions, resp., of intensities (except for 3- and 4-tricosanol) decreasing in the order $b > b\text{-}1 > c\text{-}2 > d\text{-}2$ and $b' > b'\text{-}1 > c'\text{-}2 > d'\text{-}2$, resp. With 3- and 4-tricosanol, *b*-1 is somewhat higher than *b*. Similarly to the *a* (*a'*) ions, the intensity of *b* (*b'*) ions can be plotted *versus* the number of carbon atoms (Fig. 6, curve 2). It may be seen from this plot that the quantity of ions formed by the β-cleavage hyperbolically decreases with the growing number of carbon atoms.

From the *a* (*a'*) ions, there is eliminated water (as confirmed by metastable ions) and the resulting $C_nH_{2n-1}^{(+)}$ hydrocarbon ions add to ions of the same composition arisen by another type of the aliphatic chain fragmentation, and thus cooperate in the formation of marked spectral peaks. In the case of 5-, 6-, and 7-tricosanol, base peaks are formed in this manner. We did not observe any elimination of water from the *b* (*b'*), *b*-1 (*b'*-1), *c*-2 (*c'*-2), and *d*-2 (*d'*-2) ions.

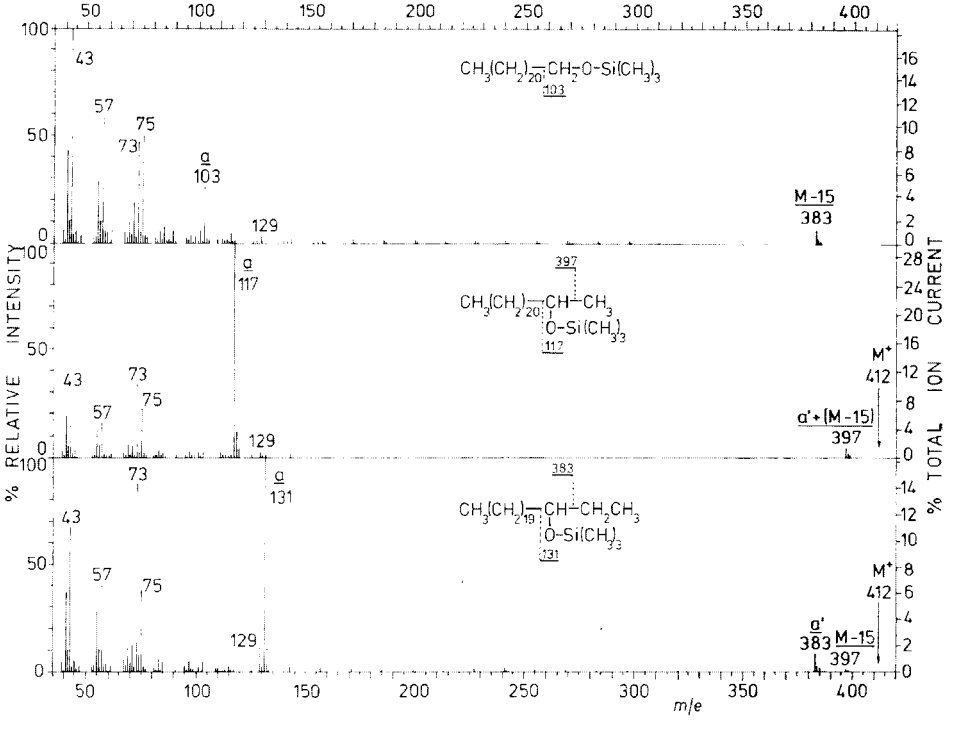


FIG. 7

Mass Spectra of Trimethylsilyl Derivatives of 1-Docosanol and 2- and 3-Tricosanol

In spectra of all tricosanols, there were observed poorly intensive but obviously very characteristic X and X′ ions corresponding to the composition $a\text{-}(18 + 29)^+$ and $a' - (18 + 29)^+$, resp. We did not succeed in explaining satisfactorily the process of their formation.

The problem of base peaks with 8-, 9-, 10-, and 12-tricosanols is also of interest. In these tricosanols, the base peaks are formed by the $C_nH_{2n-1}^{(+)}$ ions (m/e 69, 69, 83, 97) which also are dominant in their groups with other tricosanols; it is however difficult to explain why these ions should be present in such a quantity in the case of the above mentioned 8-, 9-, 10-, and 12-tricosanols.

Trimethylsilyl Derivatives of Alcohols

Mass spectra of the trimethylsilyl derivatives of alcohols are shown in Figs 7 – 10. The molecular peak was absent in spectrum of the trimethylsilyl derivative of 1-docosanol; only the peak of $[M-15]^+$ ions was present. Product of the α-cleavage,

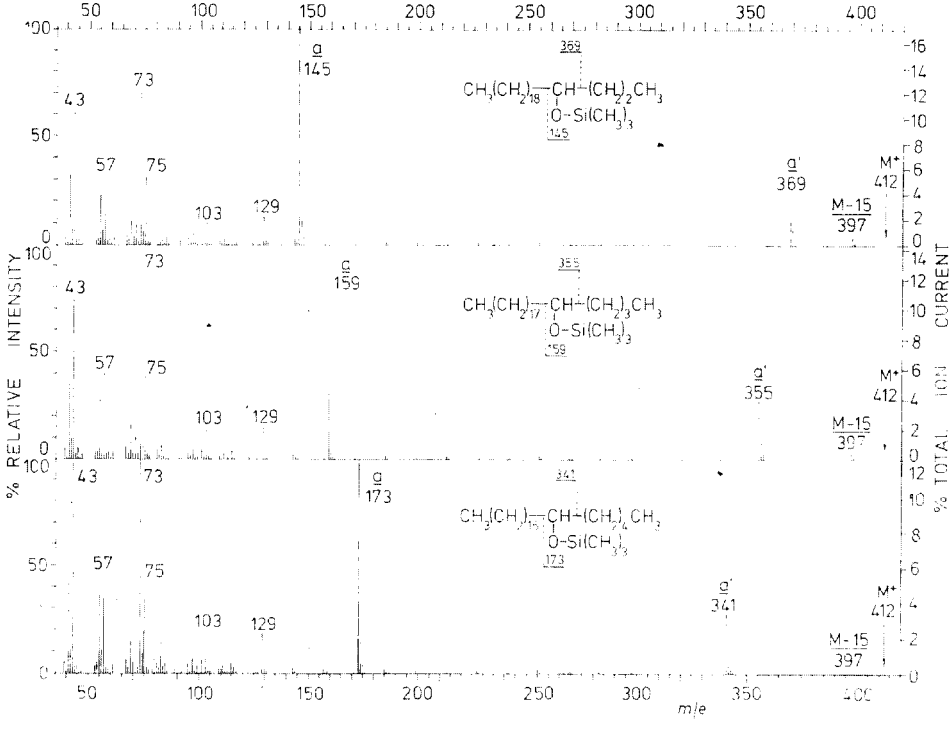


FIG. 8

Mass Spectra of Trimethylsilyl Derivatives of 4-, 5-, and 6-Tricosanol

the *m/e* 103 ion, is relatively little intensive. The hydrocarbon fragments *m/e* 41, 43, 55, 57, 71 and so on assert themselves in the spectrum to a greater extent.

Mass spectra of the trimethylsilyl derivatives of tricosanols are very simple. Introduction of the trimethylsilyl group into the molecule resulted in concentration of the fragmentation center in the neighbourhood of this group. For the determination of the position of the —O—Si$(CH_3)_3$ group and, simultaneously, of the oxygen atom, there are authoritative the marked peaks due to the α-cleavage. With the trimethylsilyl derivatives of 2-, 3-, 4-, 5- and 6-tricosanols, these ions form the base spectral peaks. The more is the —O—Si$(CH_3)_3$ group shifted to the center of the hydrocarbon chain, the more asserts itself fragmentation of this chain at positions more distant from the substituent; with further tricosanols, the base peak is thus formed by $C_3H_7^+$ ions. In addition to further *m/e* 41, 55, 57, 69, 71 etc. hydrocarbon fragments, the spectra exhibit only the *m/e* 73, 75, 103, 129 ions commonly occurring in spectra of silylated substances[10,16–18]. In spectra of all trimethylsilyl derivatives of tricosanols, there was observed a molecular peak, the intensity of which, however, did not exceed 0·1%

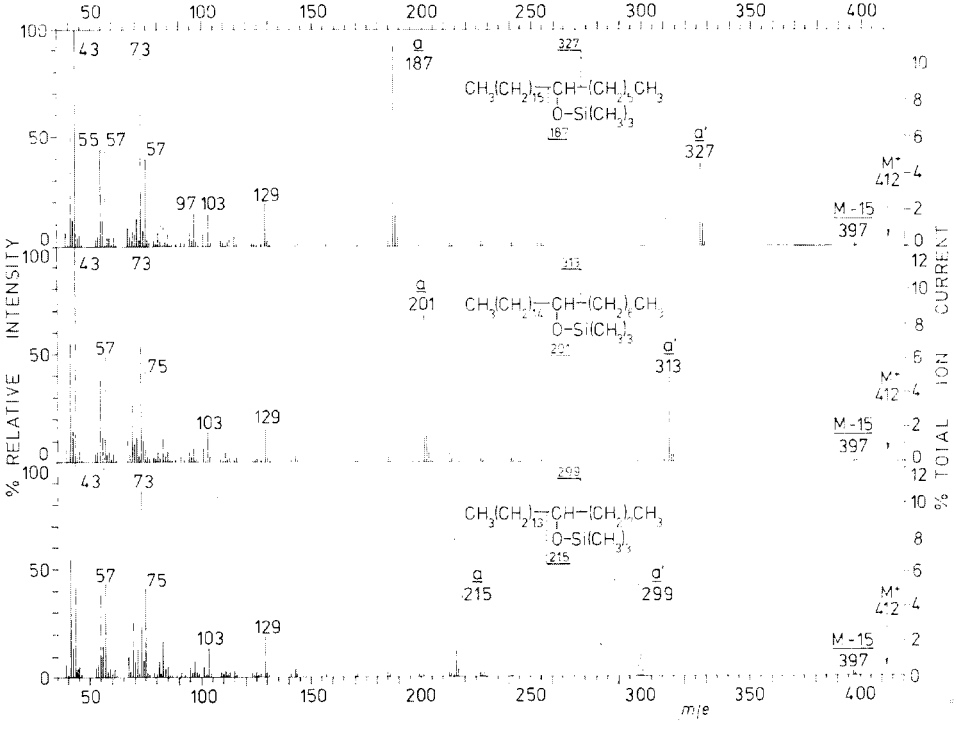


FIG. 9

Mass Spectra of Trimethylsilyl Derivatives of 7-, 8-, and 9-Tricosanol

of the relative intensity of the base peak. Considerably more intensive was in all spectra the peak of $[M-15]^+$ ions resulting by elimination of one methyl group attached to the silicon atom[16-18]. Peak intensities of the α-cleavage products resulting from the trimethylsilyl derivatives of secondary tricosanols can be plotted (similarly to those of free alcohols) *versus* the number of carbon atoms in these fragments (Fig. 11). Though the experimental values are more scattered than in the case of free alcohols, the intensity of the α-cleavage products evidently decreases with the increasing number of carbon atoms.

Comparison of Results

The existing reports on mass spectra of higher aliphatic alcohols contain some differences relating particularly to the occurence and intensity of ions in the molecular group as well as in the group of the α-cleavage products, and ions arisen by elimination of water from the molecular group ions. For the secondary alcohols, there are given[8,9,11] in the molecular group either poorly intensive peaks of $M^{+\cdot}$ ions and somewhat more intensive $[M-1]^+$ ions, or peaks of $[M-2]^{+\cdot}$ ions. With primary alcohols, peaks are absent[15] in the molecular group, or peaks of $[M-1]^+$ and $[M-2]^{+\cdot}$ ions are presented[8,9,14,15,19,20] as principal peaks. These results are substantially in accordance with our findings on the dominating peaks of $[M-2]^{+\cdot}$ ions in the case of secondary tricosanols and the relation $[M-1]^+ < [M-2]^{+\cdot}$ with 1-docosanol. The results of Blomquist and coworkers[5] are markedly different especially with respect to

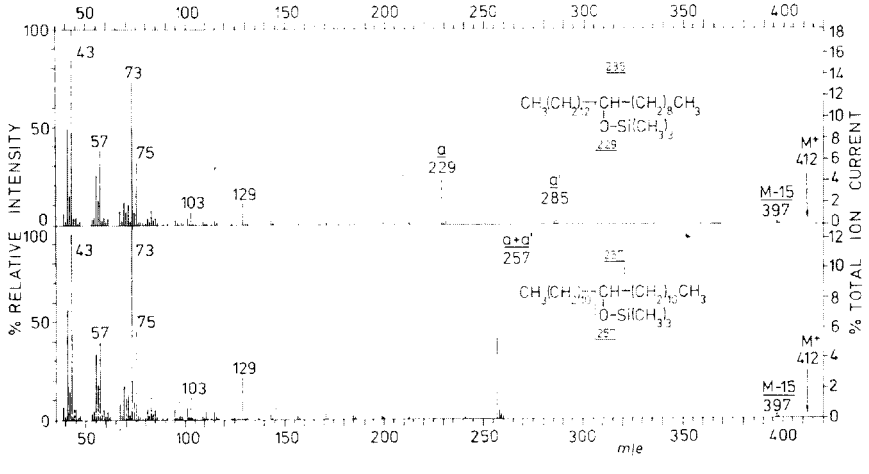


FIG. 10

Mass Spectra of Trimethylsilyl Derivatives of 10- and 12-Tricosanol

the occurence of the relatively intense peaks of $M^{+\cdot}$ ions in mixtures of secondary alcohols. In accordance with some reports[9,11,15], Blomquist and coworkers[5] mention peaks of $[M-18]^{+\cdot}$ ions which are not accompanied by peaks of $[M-20]^{+\cdot}$ ions. According to our and some other measurements[8,9,20], peaks of $[M-20]^{+}$ ions do

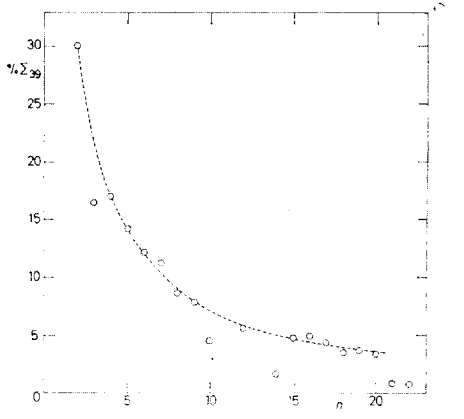


FIG. 11

Dependence of the Peak Intensity of the α-Cleavage Products of Trimethylsilyl Derivatives of Secondary Tricosanols on the Position of the Hydroxylic Group

% Σ 39, % of total ionisation encompassing the mass range from *m/e* 39 to the molecular ion; *n*, number of carbon atoms in products of α-cleavage.

occur in spectra and sometimes[14] are even higher than those of $[M-18]^{+\cdot}$ ions. None of the earlier papers mentions the *a*-1, *a*-2, *b*-1, *c*-2, *d*-2 or *a'*-1, *a'*-2, *b'*-1, *c'*-2, *d'*-2 ions, only in the paper of Blomquist and coworkers[5], the *a*-1, *a*-2 or *a'*-1, *a'*-2 ions are mentioned without any comment in some spectra only. We incline to the idea[8,14,21] that the origin of $[M-1]^{+}$, $[M-2]^{+\cdot}$, $[M-20]^{+\cdot}$ and also *a*-1, *a*-2, *b*-1, *c*-2, *d*-2 or *a'*-1, *a'*-2, *b'*-1, *c'*-2, *d'*-2 ions is due to thermal degradation. It remains however to determine where this degradation takes place, whether in the interface or in the source.

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