HYDROXY ACIDS

TARTARIC MALIC

PENTARIC HEXARIC

STRUCTURE ANALYSIS

DEUTERIUM LABELING

Article included in PhD thesis

Organic Mass Spectrometry 6 (1972) 565-576

Link to publisher: doi: 10.1002/oms.1210060513

Fragments erroneously marked as radical ions on printing are correctly denoted below.

Mass spectrometry of hydroxy dicarboxylic acids as trimethylsilyl derivatives Rearrangement fragmentations

Göran Petersson

Department of Engineering Chemistry, Chalmers University of Technology, Göteborg, Sweden

Related articles:

Mass spectrometry of aldonic acids as trimethylsilyl derivatives

A McLafferty-type rearrangement of a trimethylsilyl group

Front pages 2011 - Göran Petersson

Abstract

Gas chromatography combined with mass spectrometry offers a convenient method for the separation and identification of hydroxy dicarboxylic acids as open-chain trimethylsilyl (Me_3Si) derivatives. Mass spectra were studied of aldaric (tartronic, tartaric, pentaric and hexaric) acids and deoxyaldaric (malic, 2-deoxypentaric, 2-deoxyhexaric, 3-deoxypentaric and 3-deoxyhexaric) acids. The structural types can be readily identified from their characteristic spectra.

The most prominent fragmentations involving the rupture of one bond are the loss of a silicon-linked methyl group and the formation of α-cleavage ions by carbon chain cleavage. Further decay is characterized by a number of significant rearrangements specific of Me_3Si derivatives. Several of these can be classified as involving migration of a Me_3Si group to an oxygen atom or the migration of an ester Me_3SiO group to a silicon atom. Concomitant loss of a stable molecule often provides a driving force.

Prominent odd-electron ions are formed by a McLafferty-type rearrangement of a Me_3Si group. The decomposition of several even-electron ions can be regarded as analogous to that rearrangement.

Sammanfattning

Gaskromatografi kombinerad med masspektrometri är en fördelaktig metod vid separation och identifiering av hydroxidikarboxylsyror som trimetylsilylderivat av icke-cykliska former av syrorna. Masspektra studerades för både aldarsyror (tartronsyra, vinsyror, pentarsyror och hexarsyror) och vanliga deoxialdarsyror (äppelsyra, 2- och 3-deoxipentarsyror samt 2- och 3-deoxihexarsyror). De olika typerna av hydroxisyror kan klart identifieras från strukturspecifika spektra.

De dominerande primära klyvningarna av en enda bindning är avspaltning av en kiselbunden metylgrupp samt bildning av joner från α-klyvningar av kolkedjan. Fortsatt sönderfall karakteriseras av framträdande omlagringar som är specifika för Me_3Si-derivat. Flera av dessa innefattar migrering av en Me_3Si-grupp till en syreatom eller av en Me_3SiO-estergrupp till en kiselatom. Samtidig avspaltning av en liten stabil molekyl ger ofta drivkraft.

Specifika radikaljoner bildas via en McLafferty-omlagring av en Me_3Si-grupp. Fragmenteringar av flera joner med jämnt antal elektroner kan ses som analoga omlagringar.

Mass spectrometry of hydroxy dicarboxylic acids as trimethylsilyl derivatives Rearrangement fragmentations

Göran Petersson

Department of Engineering Chemistry,
Chalmers University of Technology,
Göteborg, Sweden

IN A RECENT investigation[1] mass spectrometry was shown to be well suited for structural analysis of aldonic and deoxyaldonic acids as open-chain trimethylsilyl (TMS) derivatives. For aldaric and deoxyaldaric acids the application of gas chromatography–mass spectrometry offers an additional advantage since the gas chromatographic separation characteristics are more favourable for these acids.[2] The acyclic derivatives of the lactone-forming acids in the present study were prepared from sodium salts of the acids.[3] The alternative analysis of the acids as lactone derivatives is less advantageous since one acid may form several different lactones.

The analytical use of the spectra is discussed as well as fragmentations which may be of general interest for TMS derivatives. Special attention is given to the nature of the prominent rearrangement fragmentations.

Comprehensive fragmentation studies emphasizing rearrangements have been published earlier for TMS esters[4,5] and ethers[4,6] of compounds with one additional functional group. Applications of gas chromatography–mass spectrometry for structural analysis of non-cyclic TMS derivatives include the well established use for determination of the positions of hydroxyl groups in hydroxy fatty acids.[7 to 10] Acids of the Krebs cycle have also been studied.[11]

Aldaric acids

Mass spectra were recorded for derivatives of all diastereomeric aldaric acids with six C atoms or less; tartronic acid, the two tartaric (tetraric) acids, the three pentaric acids and the six hexaric acids. Normalized spectra of a series of these acids with 3, 4, 5 and 6 C atoms are reproduced in Fig. 1.

At the standard electron energy (70 eV) the well-known trimethylsilyl ion of mass 73 gave rise to the base peak for all aldaric acids. A peak at the *m/e*-value [M] of the molecular ion was obtained only for the tartaric acids but an intense peak at [M − 15]

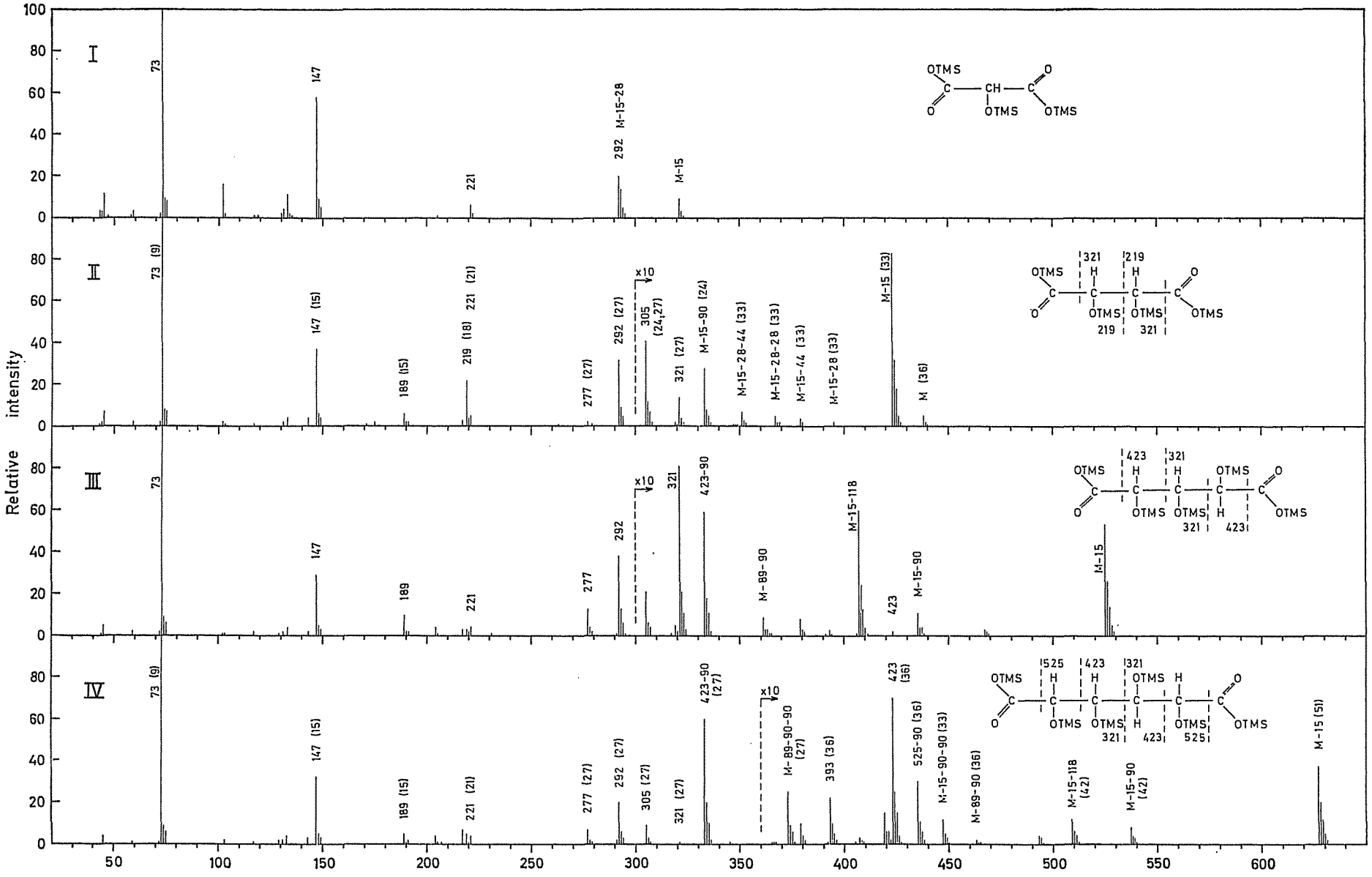


FIG. 1. Mass spectra at 70 eV of the trimethylsilyl (TMS) derivatives of tartronic (I), erythraric (II), arabinaric (III) and glucaric (IV) acids. Mass shifts for d_9-TMS derivatives are given in parentheses.

was found in all investigated spectra. The corresponding ions are formed by loss of an Me group linked to silicon and are characteristic of most TMS derivatives. This peak at an odd mass number in the uppermost part of the spectrum permits a reliable determination of the molecular weight for aldaric and deoxyaldaric acids.

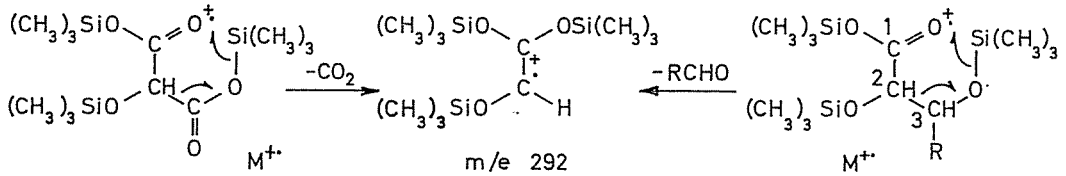
The characteristic spectra in Fig. 1 can be used for an unambiguous recognition of the four types of aldaric acids. Yet reproducible differences in relative intensities were found between most of the diastereomers permitting a mass spectrometric differentiation by comparisons with adequate reference spectra.

The structural formulae in Fig. 1 indicate the masses of ions from carbon chain cleavages with charge stabilization from adjacent ether oxygen atoms. The carbonyl dipole destabilizes ions with the charge site near to it to some extent. This results in less prominent ions from chain cleavages and more prominent ions from rearrangements for aldaric acids compared to aldonic acids.[1] There is also an increase in the intensities of the [M — 15] peaks for any series of an alditol,[12] an aldonic acid and an aldaric acid with the same number of C atoms.

As an aid in the interpretation of the fragmentation tri(trideuteromethyl)silyl (d_9-TMS) derivatives of erythraric and glucaric acids were prepared according to the method introduced by McCloskey *et al.*[13] The mass shifts towards higher masses for prominent ions in the spectra of the d_9-TMS derivatives are indicated in Fig. 1. The mass shift is 9 atomic mass units (amu) for each intact TMS group and 3 amu for each fractional Me group. Thus the base peak is shifted 9 amu from *m/e* 73 to *m/e* 82 and the [M — 15] peaks are shifted to [M — 18]. The relative intensities are not noticeably changed and the corresponding peaks are easily found.

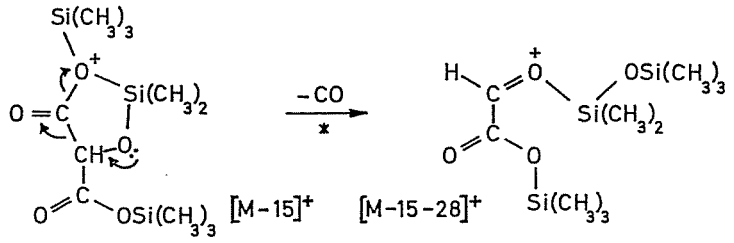
The most important rearrangement fragmentations are described below. The individual acid structures are discussed separately because their main fragmentations are different.

Tartronic acid (M = 336). Among the structure-related peaks in the upper part of the spectrum of tartronic acid (I) the *m/e* 292 peak is the most prominent. This peak is intense in the spectra of all aldaric acids. Its relative intensity increases at decreased electron energy and it was found to be the base peak in 20 eV spectra of the acids with 3, 4 and 5 C atoms. The odd-electron *m/e* 292 ion is obtained for aldonic and deoxyaldonic acids as well, and its character was described in the previous investigation of these acids.[1] Its formation in high abundance requires the structure of a 2,3-dihydroxy acid and occurs through a McLafferty-type rearrangement of a TMS group (to the right in the Scheme). The formation of the ion for tartronic acid (to the left in the Scheme) demonstrates that the rearrangement also occurs in the special case of migration of an ester TMS group.



SCHEME 1

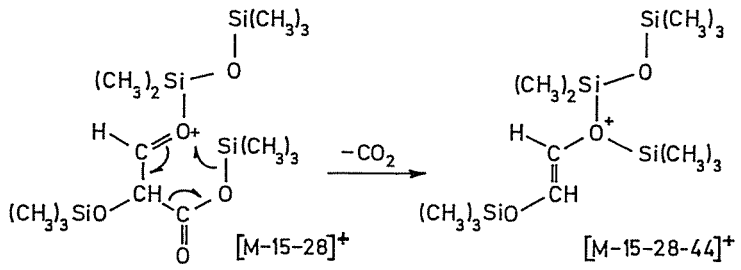
Subtraction of the contribution from the first isotope peak of the *m/e* 292 ion leaves a prominent peak at *m/e* 293 unaccounted. A very large metastable peak (m^* = 266·5 to 269; calc. 267·4) proves that the *m/e* 293 ions are formed from



SCHEME 2

$[M - 15]^+$ ions. Expulsion of CO from cyclic $[M - 15]^+$ ions is a likely fragmentation path. The $[M - 15]^+$ ions are formed preferably by loss of an Me group from an ether TMS group.[5] Many of the other acids investigated exhibit peaks from analogous $[M - 15 - 28]^+$ ions or from ions corresponding in mass to further elimination of trimethylsilanol (90 amu). An alternative to a five-membered ring for the $[M - 15]^+$ ion is a six-membered ring or a non-cyclic structure. Irrespective of the detailed formulation of the fragmentation the net result is a rearrangement of an ester OTMS group to a siliconium ion centre. The same rearrangement was observed for a number of α-monohydroxy acids[1] and the $[M - 15 - 28]$ peaks are also a characteristic feature of the spectra of amino acid TMS derivatives.[14] The formation of $[M - 15 - 28]$ ions for methyl esters of α-hydroxy acid TMS ethers involving migration of the OMe group[15] gives further evidence for the universal character of the rearrangement.

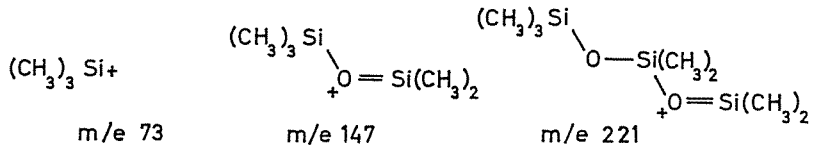
Tartaric acids (M = 438). The peaks at $[M - 15]$ and $[M - 15 - 28]$ in the spectrum of erythraric acid (II) are both shifted 33 amu for the d_9-TMS derivative. This shift confirms that the neutral molecule lost in the fragmentation is CO. Similarly the identical mass shifts of 33 amu and the mass differences of 28 and 44 amu for the next three peaks of lower mass indicate that the ions are formed by losses of CO and CO_2 from $[M - 15]^+$ ions. The ion of mass 351 ($[M - 15 - 28 - 44]$) is the most abundant of these. Its formation from the $[M - 15 - 28]^+$ ion requires a rearrangement of the ester TMS group. With one of the two ether oxygen atoms as acceptor site a reasonable oxonium ion structure is obtained.



SCHEME 3

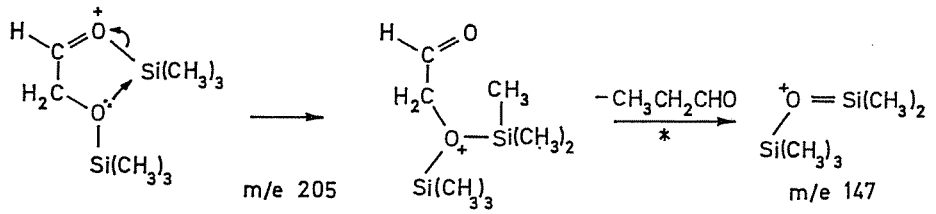
The structure of the $[M - 15 - 28]^+$ ion is probably a good example of intermediate structures involved in the formation of the *m/e* 147 ion. Heterolysis of the bond between silicon and the charge-bearing oxygen atom leads to its formation from the $[M - 15 - 28]^+$ ion. It gives rise to an intense peak for all the investigated hydroxy dicarboxylic acids. The trimethylsilyl ion of mass 73 is probably formed mainly by similar secondary decompositions of intermediate rearrangement ions. Its formation from *m/e* 147 ions has been proved earlier.[6] Formation of the next higher analogue of the *m/e* 73 and *m/e* 147 ions can explain the small peaks at *m/e* 221.

The structure is supported by the mass shifts for the d_9-TMS derivatives. The related structure of the $[M - 15 - 28 - 44]^+$ ion indicates the nature of the extensive rearrangements needed for the formation of the *m/e* 221 ion. At low electron energies the relative abundances of the ions of masses 73, 147 and 221 were found to be much lower. For the derivatives of high molecular weight in particular they are almost negligible in 20 eV spectra. This seems to be in accordance with their multistep formation and the high bond strength of the Si—O bonds to be cleaved.



SCHEME 4

The observations made for the *m/e* 73 and *m/e* 147 ions agree well with those made for aldonic and deoxyaldonic acids.[1] For many of these acids the *m/e* 205 ions from vicinal diol end groups were found to decompose to *m/e* 147 ions. This reaction must again be ascribed to a migration of a TMS group and contributes to a more complete picture of the pathways leading to the *m/e* 147 ion. The formation of the



SCHEME 5

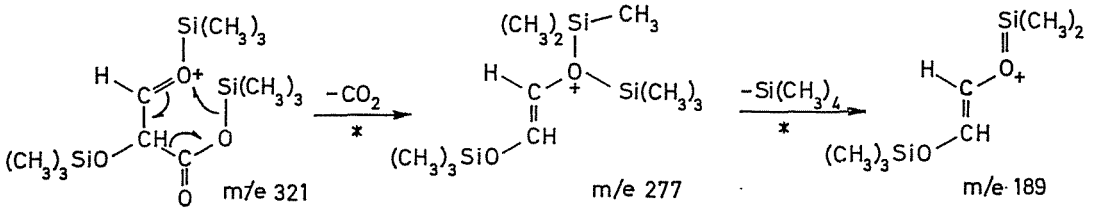
m/e 221 ion from the $[M - 15 - 28]^+$ ion for tartronic acid (m^* = 166 to 168·5; calc. 166·7) can be viewed in a similar way. The partial positive charge at the migrating silicon atom might give a clue to the frequent migrations of TMS groups to oxygen atoms in even-electron ions. It should facilitate such rearrangements by making the silicon *d* orbitals more available to the non-bonding electrons at oxygen.

The mass shifts for the d_9-TMS derivative support the ion structures discussed and those suggested in Fig. 1 for the *m/e* 219, *m/e* 321 and *m/e* 333 ions. The *m/e* 305 peak is resolved into two peaks at *m/e* 329 (40%) and *m/e* 332 (60%). Thus the labelling technique demonstrates the existence of two contributing ions of the same integral mass but of a different elemental composition. The first ion might be due to the loss of TMSOH and CO from $[M - 15]^+$ ions and the second to the loss of CO_2 and an OTMS radical from the molecular ion.

Pentaric acids (M = 540). For the pentaric acids the counterpart to the *m/e* 305 peak for the tetraric acids is the peak at *m/e* 407. This peak and the [M — 15] peak are the most intense in the upper region of the spectra of arabinaric (III), ribaric and xylaric acids. The mass shift for the d_9-TMS derivative of glucaric acid of the analogous ions (*m/e* 509) indicates a relationship to the $[M - 15]^+$ ions. The formation of these $[M - 15 - 118]^+$ ions may proceed in two steps involving elimination of TMSOH and expulsion of CO. An alternative decomposition path is the one-step loss from $[M - 15]^+$ ions of HCOOTMS, the TMS ester of formic acid. The latter

route is supported by metastable peaks for the pentaric acids (m^* = 315 to 317·5; calc. 315·5). Cyclic $[M - 15]^+$ ions may be involved in this fragmentation as well as in several other decomposition sequences.

The most prominent chain cleavage ion for the pentaric acids is that of mass 321. The decomposition of this ion to *m/e* 277 ions is demonstrated by metastable peaks (m^* = 238·5 to 240·5; calc. 239·0) for the C_5 acids as well as for the C_4 and C_6 acids. A further decomposition (*m/e* 277 → *m/e* 189) is also indicated by metastable peaks (m^* = 128·5 to 130; calc. 129·0) which are most apparent in low electron energy spectra of the pentaric acids. The identical mass shifts for the *m/e* 277 and



SCHEME 6

m/e 321 ions in the spectra of the d_9-TMS derivatives strongly indicate that the neutral moiety lost in the first fragmentation step is CO_2. The d_9-TMS derivatives can also be used to confirm the identity of the precursor ion and the daughter ion involved in a metastable transition. Thus a metastable peak (m^* = 265 to 267; calc. 265·6) for the d_9-TMS derivatives indicates the decomposition *m/e* 348 → *m/e* 304 and confirms the metastable transition *m/e* 321 → *m/e* 277 for the unlabelled derivatives.

The indicated process of formation for the *m/e* 277 ions is analogous to that of the $[M - 15 - 28 - 44]^+$ ions for the tartaric acids. This fragmentation type can be regarded as the analogue in even-electron systems to the McLafferty-type rearrangement described in the paragraph on tartronic acid.

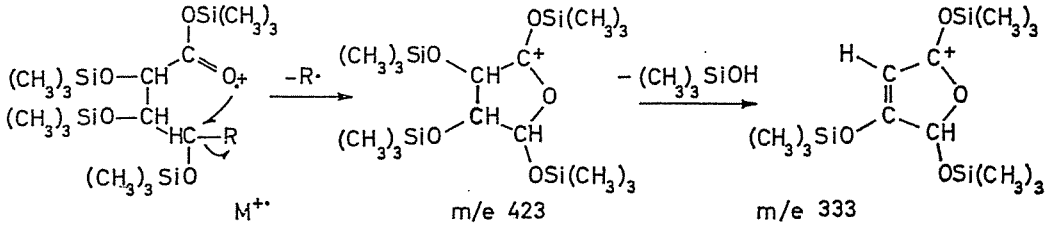
The decomposition of the *m/e* 277 ions is probably due to loss of tetramethylsilane and leads to a well resonance stabilized ion of mass 189. The structural formulae imply loss of a silicon-linked Me group together with one of the three groups linked to the adjacent oxonium ion centre. The fragmentation path given above for the decomposition *m/e* 205 → *m/e* 147 is of the same type and several minor peaks in the spectra investigated indicate that this fragmentation mode may be widely operative. A similar fragmentation of the immonium ion $CH_2{=}N^+(TMS)_2$ (*m/e* 174 → *m/e* 86) has been demonstrated earlier.[16]

Hexaric acids (M = 642). For the hexaric acids, e.g. glucaric acid (IV), the C_4 chain cleavage ions of mass 423 are more abundant than the *m/e* 321 ions. Moreover, strong metastable peaks (m^* = 261·5 to 264; calc. 262·1) indicate that the very abundant *m/e* 333 ions are formed from *m/e* 423 ions, obviously by elimination of TMSOH. This decomposition is confirmed by a metastable peak (m^* = 281·5 to 284·5; calc. 282·4) for the d_9-TMS derivative corresponding to the decay *m/e* 459 → *m/e* 360. The *m/e* 333 peak was found to be the base peak in the 20 eV spectrum of glucaric acid.

The hexonic acids[1] exhibit similar very prominent peaks from the same ions. Because of the great importance of the *m/e* 333 ions an effort was made to clarify which OTMS group is lost in the elimination. For this purpose the spectra of the TMS derivatives of a 2-O-methylhexonic and a 3-O-methylhexonic acid were studied.

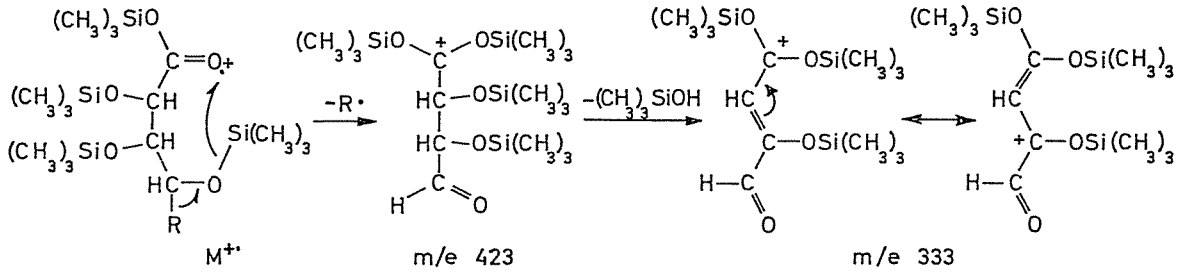
Investigations of Methyl and TMS[12] derivatives of alditols demonstrate that α-cleavages as well as eliminations of MeOH and TMSOH are analogous for Me and TMS derivatives. Therefore the position in the spectra of the methylated acids of the analogues to the *m/e* 333 peak should reveal the position of the substituent lost. For 2-O-methylgluconic acid such a peak was found only at the unchanged mass number 333 (365 — 32) from the elimination of MeOH. Evidently the C-2 substituent is lost in the elimination. Consistently the *m/e* 333 peak was small for 3-O-methylgulonic acid and a large peak was found at *m/e* 275 (365–90) from the elimination of TMSOH.

The *m/e* 423 and 333 ions as major ions from the TMS derivative of 6-phosphogluconic acid have been discussed by Zinbo and Sherman.[18] To account for the high intensity of these ions compared to the other chain cleavage ions the authors proposed a cyclic structure with the carbonyl oxygen atom in the ring. Elimination of the C-2 substituent apparently leads to an effective delocalization of the positive charge for the *m/e* 333 ion. The structure is rigid and probably strained, however.



SCHEME 7

The rearrangement modes of TMS groups described above permit alternative formulations to be made involving migrations of the TMS group at C-4. Migration of this TMS group to the carbonyl oxygen by analogy with the formation of the *m/e* 292 ions offers a plausible fragmentation path. Analogous rearranged structures for α-cleavage ions have been proposed for TMS derivatives of hydroxy fatty acid esters.[7,19] The structure of the *m/e* 333 ion appears to be very favourable and is compatible with loss of CO to form *m/e* 305 ions.



SCHEME 8

The peaks from the chain cleavage ions formed by loss of a terminal COOTMS radical from the molecular ion are small for all aldaric acids studied. The [M — 117 — 90] peaks from the further elimination of TMSOH are also comparatively small both for the pentaric (*m/e* 333) and the hexaric (*m/e* 435) acids.

The number of abundant ions from TMSOH eliminations increases with an increased chain length of the acid. Thus the C_5 acids exhibit peaks related to the $[M - 15]^+$ ions at [M — 15 — 90] and the C_6 acids at both [M — 15 — 90] and [M — 15 — 90 — 90]. The ions from loss of a OTMS group (89 amu) eliminate TMSOH to give rise to similar peaks. The mass shifts indicated in Fig. 1 for the

d_9-TMS derivative of glucaric acid are in accordance with all the ion structures proposed.

Deoxyaldaric acids

The following monodeoxyaldaric acids were studied: malic acid, the *erythro* and *threo* isomers of the 2-deoxy- and 3-deoxypentaric acids, 2-deoxy-*arabino*-hexaric and 2-deoxy-*lyxo*-hexaric acids and the four diastereomeric 3-deoxyhexaric acids. The spectrum of malic acid is reproduced in Fig. 2 and spectra of the four different structural types existing for the C_5 and C_6 acids in Fig. 3.

The [M — 15] peaks are intense. Due to the presence of the methylene group (14 amu) there is a characteristic shift in their position compared to the aldaric acids.

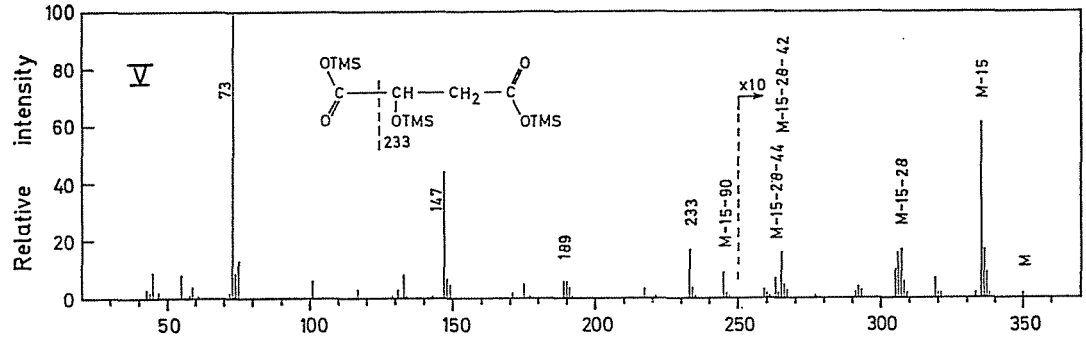


FIG. 2. Mass spectrum at 70 eV of the TMS derivative of malic acid (V).

Distinct peaks from the molecular ion were found in the spectra of malic acid and the 3-deoxypentaric acids. The base peak is at *m/e* 73 in all the 70 eV spectra. The abundant *m/e* 73 and *m/e* 147 ions are formed in a similar manner as with the aldaric acids.

All 5- and 6-carbon deoxyaldaric acids can be recognized as such acids from a comparison with the spectra in Fig. 3. The spectra of the diastereomeric acids differ less than those of the aldaric acids.

The spectra of the 2-deoxyaldaric and 3-deoxyaldaric acids differ by a number of characteristic features. As with the deoxyaldonic acids[1] the position of the 'deoxy group' predictably influences the formation of chain cleavage ions. Only α-cleavage ions with an OTMS group near to the positive charge can be formed in high abundance. The most obvious consequence is a characteristic peak at *m/e* 233 which distinguishes the spectra of the 2-deoxyaldaric acids from those of the 3-deoxyaldaric acids. Some of the further differences are pointed out in the fragmentation discussion below.

Malic acid (M = 350). Many of the features of the spectrum of malic acid (V) are explained by fragmentations analogous to those of the tartaric acids. Thus $[M - 15]^+$ ions expel CO to form $[M - 15 - 28]^{+\cdot}$ ions (m^* = 280·5 to 283; calc. 281·3) and the $[M - 15 - 28]^+$ ions in turn lose CO_2 to give $[M - 15 - 28 - 44]^+$ ions (m^* = 225 to 226·5; calc. 225·3). The *m/e* 265 ions are likely to be formed from $[M - 15]^{+\cdot}$ ions by the two-step loss of CO and CH_2CO. The second step is best represented as a migration of the ester OTMS group to the charge-bearing carbon atom. Similar migrations in chain cleavage ions of ester or ether OTMS groups to the positively charged C atom explain small *m/e* 191 peaks for most of the acids studied. Chain cleavage ions from methyl esters fragment to lose CH_2CO in an

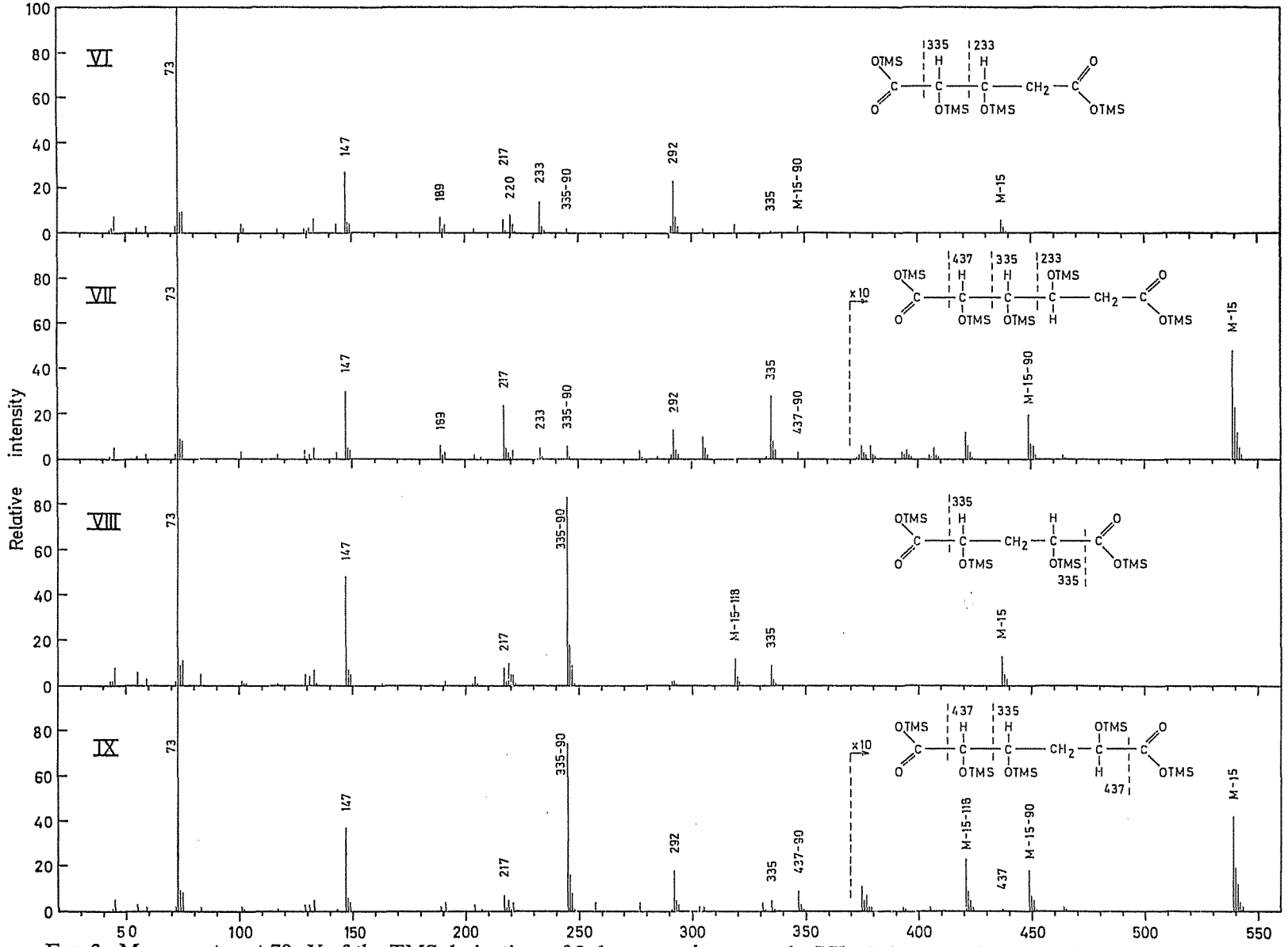
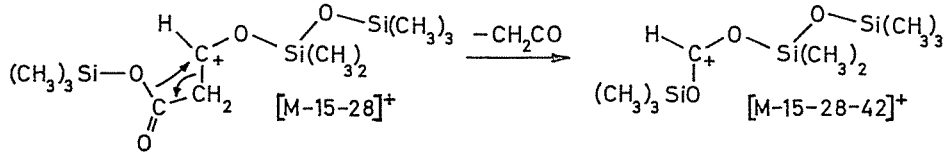


FIG. 3. Mass spectra at 70 eV of the TMS derivatives of 2-deoxy-*erythro*-pentaric (VI), 2-deoxy-*arabino*-hexaric (VII), 3-deoxy-*erythro*-pentaric (VIII) and 3-deoxy-*arabino*-hexaric (IX) acids.



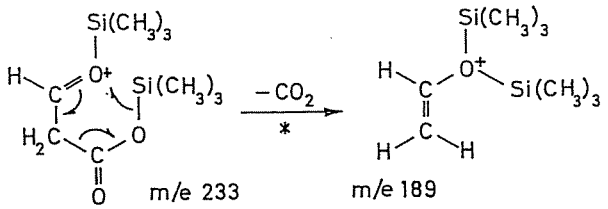
SCHEME 9

analogous manner by migration of the ester OMe group to the carbonium ion centre. In a comprehensive study of this general fragmentation type for dimethyl esters of aliphatic dicarboxylic acids[20] the fully methylated derivative of malic acid was included. Few fragmentation analogies with the TMS derivative exist because of the favourable fragmentations related to the specific [M — 15] ions for the TMS derivatives.

2-*Deoxypentaric* (M = 452) and 2-*deoxyhexaric* (M = 554) *acids*. The spectra of 2-deoxy-*erythro*-pentaric (VI) and 2-deoxy-*arabino*-hexaric (VII) acids are reproduced in Fig. 3.

These acids exhibit prominent *m/e* 292 peaks due to the McLafferty-type rearrangement of a TMS group. Malic acid and the 3-deoxypentaric acids are the only acids in this study lacking the 2,3-dihydroxy acid structure required for this rearrangement. The insignificance of the *m/e* 292 peak for these acids demonstrates the utility of the rearrangement for structural conclusions. The only additional prominent peak from odd-electron ions for the C_5 and C_6 deoxyaldaric acids is that at *m/e* 220 for the 2-deoxypentaric acids. It is probably due to ions from the conventional McLafferty rearrangement.

The *m/e* 233 ions are characteristic of the 2-deoxyaldaric acids and even give rise to the base peak in the 20 eV spectrum of malic acid. For the Methyl derivative of malic acid[20] the analogue of the *m/e* 233 ion expels CH_2CO by analogy with the fragmentation scheme above to give the most abundant ion in the spectrum. One reason for the insignificance of CH_2CO loss from the *m/e* 233 ions is the competing loss of CO_2 to give *m/e* 189 ions. This decay is supported by metastable peaks (m^* = 153 to 154·5; calc. 153·3) for all investigated 2-deoxyaldaric acids. The fragmentation is analogous to that (*m/e* 321 → *m/e* 277) described for the C_3 chain cleavage ions of the aldaric



SCHEME 10

acids. Its occurrence for the *m/e* 233 ions indicates a six-membered transition state emphasizing the similarity to the McLafferty rearrangement. The same fragmentation was observed for the *m/e* 233 ions from 2-deoxyaldonic acids.[1]

The *m/e* 233 ions are more abundant than the corresponding *m/e* 321 ions for the aldaric acids. The difference can be ascribed to the destabilizing effect on the ion from an electron-withdrawing OTMS group at C-2. For the 2-deoxyhexaric acids the C_4 chain cleavage ions of mass 335 are also more abundant but the *m/e* 245 ions

are less abundant than for the other 6-carbon acids. This is consistent with the preferred elimination of a C-2 substituent for the C_4 ions as discussed for the hexaric acids. Possibly the lacking C-2 substituent for the *m/e* 335 ions leads to an elimination of HCOOTMS, explaining the high intensity of the *m/e* 217 peaks. Similar relationships between the ions of masses 335, 245 and 217 were observed for 2-deoxyaldonic acids.[1]

3-*Deoxypentaric* (M = 452) and 3-*deoxyhexaric* (M = 554) *acids*. The spectra of 3-deoxy-*erythro*-pentaric (VIII) and 3-deoxy-*arabino*-hexaric (IX) acids are given in Fig. 3 below those of the 2-deoxyaldaric acids to permit a direct comparison between the structural isomers.

A characteristic feature of the 3-deoxyaldaric as well as the 3-deoxyaldonic[1] acids is the high intensity of the *m/e* 245 peaks. The formation of the corresponding ions from the *m/e* 335 chain cleavage ions by elimination of TMSOH is supported by metastable peaks (m^* = 178·5 to 180·5; calc. 179·2). The mechanism is likely to be similar to that for the C_4 chain cleavage ions from hexaric acids. The *m/e* 245 ions fragment further to give *m/e* 217 ions (m^* = 191·5 to 194; calc. 192·2).

Abundant ions of mass [M − 15 − 118] are obtained for the 3-deoxyaldaric acids. A distinct metastable peak (m^* = 232·5 to 234·5; calc. 232·9) for the 3-deoxypentaric acids indicates that these ions are formed from [M − 15] ions by loss of HCOOTMS as with the pentaric acids. Elimination of TMSOH from [M − 15] ions gives rise to prominent peaks at [M − 15 − 90] for the 3-deoxyhexaric acids as well as for the 2-deoxypentaric and 2-deoxyhexaric acids.

EXPERIMENTAL

Tartronic, erythraric, threaric and malic acids were commercial samples. The other hydroxy dicarboxylic acids were prepared by nitric acid oxidation of appropriate hydroxy monocarboxylic acids or neutral monosaccharides in the recent work[2] by Jansén and Samuelson. 3-O-Methylgulonic acid was obtained by borohydride reduction of the Na salt of 4-O-methylglucuronic acid.[21] 2-O-Methylgluconic acid was prepared by bromine oxidation of 2-O-methylglucose. Fifteen mg $BaCO_3$ and 3 μl Br_2 were added to 3 mg of the sugar in 1 ml H_2O. The stirred mixture was kept at room temperature for 40 hrs. After evaporation $BaCO_3$ and the Ba salt of 2-O-methylgluconic acid were left to be silylated.

The TMS derivatives were prepared from the sodium salts of the acids[2] in dry pyridine. Reagent consumption from remaining H_2O in the Na salt samples after vacuum drying over P_2O_5 was taken into account. Bis(trimethylsilyl)acetamide (BSA) in about 2- or 3-fold excess was used as the reagent together with half the volume trimethylchlorosilane (TMCS). Derivatives of the non-lactone-forming tartaric and malic acids were also conveniently prepared from the free acids without the addition of TMCS. Tartronic acid was found to give predominantly decomposition products if only BSA or bis(trimethylsilyl)trifluoroacetamide (BSTFA) was used whereas the pure derivative was obtained after initial addition of TMCS. The reaction mixtures were stirred for at least 2 hrs. at room temperature, the pyridine was evaporated at 40° and the derivatives were dissolved in diethyl ether. The d_9-TMS derivatives were prepared in pyridine with d_{18}-BSA (Merck, Sharp and Dohme of Canada Ltd) as the only reagent.

The mass spectrometric measurements were performed on a LKB 9000 gas chromatograph—mass spectrometer. Samples of the TMS derivatives in the μg range were introduced through a 300 × 0·2 cm i.d. steel column filled with 3% DC QF-1 on 100 to 120 mesh Gas Chrom Q. The oven temperature range was 120° to 190°. The temperature of the molecule separator was kept at 210° and the temperature of the ion source was 270°. The trap current was 60 μA and the accelerating voltage 3·5 kV. The exit slit was set to 0·05 mm and the collector slit to 0·10 mm. Background peaks were subtracted from the spectra.

Acknowledgements—The author thanks professor Erik von Sydow for the permission to use the mass spectrometer at the Swedish Institute for Food Preservation Research.

The financial support of the Swedish Board for Technical Development is gratefully acknowledged.

REFERENCES

1. G. Petersson, *Tetrahedron* **26,** 3413 (1970).
2. L. Jansén and O. Samuelson, *J. Chromatog.* **57,** 353 (1971).
3. O. Raunhardt, H. W. H. Schmidt and H. Neukom, *Helv. Chim. Acta* **50,** 1267 (1967).
4. G. H. Draffan, R. N. Stillwell and J. A. McCloskey, *Org. Mass Spectrom.* **1,** 669 (1968).
5. J. Diekman, J. B. Thomson and C. Djerassi, *J. Org. Chem.* **34,** 3147 (1969).
6. J. Diekman, J. B. Thomson and C. Djerassi, *J. Org. Chem.* **33,** 2271 (1968).
7. P. Capella and C. M. Zorzut, *Anal. Chem.* **40,** 1458 (1968).
8. W. J. Esselman and C. O. Clagett, *J. Lipid Res.* **10,** 234 (1969).
9. E. G. Perkins and C. J. Argoudelis, *Lipids* **4,** 619 (1969).
10. G. Eglinton, D. H. Hunneman and A. McCormick, *Org. Mass Spectrom.* **1,** 593 (1968).
11. M. G. Horning, E. A. Boucher, A. M. Moss and E. C. Horning, *Anal. Letters* **1,** 713 (1968).
12. G. Petersson, *Tetrahedron* **25,** 4437 (1969).
13. J. A. McCloskey, R. N. Stillwell and A. M. Lawson, *Anal. Chem.* **40,** 233 (1968).
14. W. J. A. VandenHeuvel, J. L. Smith, I. Putter and J. S. Cohen, *J. Chromatog.* **50,** 405 (1970).
15. P. Capella, C. Galli and R. Fumagalli, *Lipids* **3,** 431 (1968).
16. K. Bergström, J. Gürtler and R. Blomstrand, *Anal. Biochem.* **34,** 74 (1970).
17. L. S. Golovkina, N. S. Vul'fson and O. S. Chizhov, *J. Org. Chem. USSR* (English Transl.) **4,** 718 (1968).
18. M. Zinbo and W. R. Sherman, *J. Am. Chem. Soc.* **92,** 2105 (1970).
19. W. J. Richter and A. L. Burlingame, *Chem. Commun.* 1158 (1968).
20. I. Howe and D. H. Williams, *J. Chem. Soc.* (*C*) 202 (1968).
21. E. R. Nelson, P. F. Nelson and O. Samuelson, *Acta Chem. Scand.* **22,** 691 (1968).