Gas-chromatographic and Mass-spectrometric Properties of Perdeuterated Fatty Acid Methyl Esters

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PREVIOUS observations[1–3] that deuterated compounds exhibit shorter gas-chromatographic retention times than their protium analogues have led us to study gas-chromatographic properties of large molecules having a high percentage of hydrogen isotopes, namely long-chain fatty acid esters. In addition, the extensive studies[4] of mass spectra of long-chain esters make these compounds ideal models for a comparative study of mass-spectrometric behaviour of protium and deuterium analogues.

We have used direct combination gas chromatography–mass spectrometry[3] to identify a number* of perdeuterated ($\sim$ 98·1%) fatty acids isolated as

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* Preliminary studies have revealed perdeuterated C_{14}—C_{18} straight-chain, C_{16}—C_{18} monoenoic, C_{16} and C_{18} dienoic, and C_{18} trienoic acids; see also ref. 5.

TABLE 1

Gas-chromatographic data

Compound	Methylene unit values[a] (deuterium MU/protium MU) 1% OV-1	1% EGSS-X
[$^2H_{30}$]-Methyl tetradecanoate	17·00/17·16	19·08/19·28
[$^2H_{34}$]-Methyl hexadecanoate	18·88/19·14	20·98/21·27
[$^2H_{38}$]-Methyl octadecanoate	20·86/21·12	23·02/23·38
[$^2H_{32}$]-Methyl hexadecenoate	18·67/18·88	21·28/21·53
[$^2H_{36}$]-Methyl octadecenoate	20·52/20·80	23·21/23·52
[$^2H_{34}$]-Methyl octadecadienoate	20·48/20·70	23·74/24·04
[$^2H_{34}$]-Hexadecane[b]	15·78/16·00[c]	15·79/16·00[c]

[a] Temperature programmed from 70° at 3°/min. and 20 p.s.i. in glass columns 4 mm. I.D. × 4 m. length, using even-numbered straight-chain hydrocarbons as reference points.

[b] Prepared by reduction of [$^2H_{34}$]-methyl hexadecanoate.

[c] By definition.

their [$^2H_3$]methyl esters from a commercially available crude mixture of acids.[6]

Gas-chromatographic data (see Table 1) have been obtained using both nonpolar (OV-1) and polar (EGGS-X) stationary phases[7] in packed columns. Data for pairs of isotopic analogues were obtained under identical conditions (same run) for direct comparison and are presented in terms of methylene units (MU).[8] The influence of deuterium is so great that in every case the protium and deuterium forms are completely resolved. Replicate determinations of the number of theoretical plates for the methyl hexadecanoate analogues on OV-1 did not reveal peak broadening due to deuterium, as reported for carbohydrate derivatives.[2] The separations of analogues are greater on the more polar liquid phase, and generally increase on both phases as the ratio of hydrogen isotopes to carbon increases, reaching a limiting value with hexadecane and its [$^2H_{34}$]-analogue. In the latter case, although the increased mass of perdeuterohexadecane would normally lengthen the retention time by about 2·4 MU, the observed value ($-0·2$ MU) indicates a net inverse isotope effect equivalent to 2·6 MU. Although the reason for the greater mobility of the deuterium analogues is not clear,[9] it would appear to be inter-related to the lower boiling points of deuterated compounds[1,10] and the concept of deuterium as effectively a smaller atom than hydrogen[11] (hence, smaller molecular volume).

Mass spectra of [$^2H_{34}$] methyl hexadecanoate[12] and its protium analogue were recorded under identical conditions using a reservoir-type inlet system. Percent Σ-values[13] for each analogue were calculated in terms of the oxygen-containing ions represented in Table 2. Direct comparison of the relative abundance of the same type of ion in each analogue was obtained by taking the ratio of % Σ for ions of appropriate mass.

TABLE 2

Mass spectrum of [$^2H_{34}$]-methyl palmitate[a]

m/e[b]	Relative intensity[c]	$\%\Sigma_D/\%\Sigma_H$
80 (74)	90·9	0·99
82 (75)	6·2	0·39
94 (87)	57·9	0·90
110 (101)	3·9	0·88
126 (115)	1·6	0·85
142 (129)	4·1	0·66
158 (143)	10·4	0·89
174 (157)	0·48	0·54
190 (171)	1·2	0·63
206 (185)	1·8	0·70
222 (199)	1·3	0·77
238 (213)	0·61	0·64
254 (227)	8·4	0·86
270 ($M-C_2D_5$) (241)	2·5	0·89
270 ($M-OCD_3$) (239)	10·5	0·92
304 (270)	100·0	1·36

[a] CEC 21–110B mass spectrometer; 8 Kv, 70 ev., ion source 130°. Recorded by magnetic scanning at $R =$ 1300. High-resolution measurements made when required. Only the species listed were used to calculate % Σ-values.

[b] Values in brackets indicate corresponding masses for methyl palmitate. For a detailed discussion of the structural identities and origins of ions in the mass spectra of long chain methyl esters see ref. 4.

[c] Sum of all isotopic species (^{13}C, D) for each ion.

The most striking effect of deuterium substitution is the increased stability of the molecular ion, in general agreement with data reported for smaller molecules.[14] Processes which principally involve C–C or C–O bond breakage (*m/e* 87, 94,$M-OCD_3$) are least affected by deuterium substitution. While formation of the "protonated" McLafferty ion[4] seems to be strongly suppressed in the deuterated analogue (*m/e* 82), ions of the type $CD_3O{\cdot}CO{\cdot}[CD_2]^+_{3-11}$ appear to be less strongly discriminated against.† In the latter case, interpretation of the data may be somewhat hindered by relative errors associated with low intensities, and by the possibility of hydrocarbon ion formation (not included in % Σ calculations) as competitive processes.

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† The mode of formation of these ions is known to be complex (ref. 4), and may involve extensive hydrogen rearrangements.

[1] W. E. Falconer and R. J. Cvetanović, *Analyt. Chem.*, 1962, **34**, 1064.

[2] R. Bentley, N. C. Saha, and C. C. Sweeley, *Analyt. Chem.*, 1965, **37**, 1118, and references therein.

[3] F. A. J. M. Leemans and J. A. McCloskey, *J. Amer. Oil Chemists' Soc.*, 1967, **44**, 11.

[4] R. Ryhage and E. Stenhagen in "Mass Spectrometry of Organic Ions," F. W. McLafferty, ed., Academic Press, New York, 1963, ch. 9.

[5] W. Klenk and W. Knipprath, *Z. physiol. Chem.*, 1959, **317**, 243.

[6] Merck, Sharp, and Dohme of Canada, Ltd., Montreal 6, Quebec; acids had been isolated from *Scenedesmus obliquus* (algae) grown in 99·7% D_2O.

[7] Supelco, Inc., Bellefonte, Pennsylvania, and Applied Science Laboratories, State College, Pennsylvania, respectively.

[8] W. J. A. Vanden Heuvel, W. L. Gardiner, and E. C. Horning, *Analyt. Chem.*, 1964, **36**, 1550.

[9] For more detailed discussion, see ref. 2.

[10] D. Craig, F. A. Regenass, and R. B. Fowler, *J. Org. Chem.*, 1959, **24**, 240.

[11] L. S. Bartell, *J. Amer. Chem. Soc.*, 1961, **83**, 3567.

[12] A plotted spectrum of methyl perdeuterodocosanoate has been published in a recent note (Ng. Dinh-Nguyen and E. Stenhagen, *Acta Chem. Scand.*, 1966, **20**, 1423); no experimental conditions or mechanistic conclusions were reported.

[13] Defined as the percentage of the total ion current (Σ) carried by ions of given mass or type; see K. Biemann, "Mass Spectrometry," McGraw-Hill, New York, 1962, p. 43.

[14] For a detailed discussion of isotope effects, see F. H. Field and J. L. Franklin, "Electron Impact Phenomena," Academic Press, New York, 1957, p. 204—217.