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FATTY ACIDS

II. THE SYNTHESIS AND GAS–LIQUID CHROMATOGRAPHIC BEHAVIOUR OF FIVE TRIMETHYLENE-INTERRUPTED C_{18}-DIUNSATURATED FATTY ACIDS

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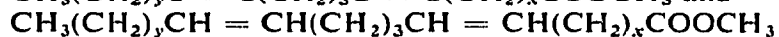
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SUMMARY

Five trimethylene-interrupted methyl octadecadiynoates, $C_{18}\Delta^{2a,7a}$; $\Delta^{3a,8a}$; $\Delta^{4a,9a}$; $\Delta^{5a,10a}$ and $\Delta^{6a,11a}$, and the corresponding *cis,cis*-octadecadienoates were synthesized, and their gas–liquid chromatographic properties were studied on Apiezon L, diethylene glycol succinate polyester and Silar 10C stationary phases. The equivalent chain lengths of these esters have been determined, and the separation of mixtures and the prediction of gas chromatographic behaviour of these isomers are discussed.

INTRODUCTION

Numerous values have been published for the gas chromatographic equivalent chain lengths[1] (ECL) of the methyl esters of C_{18}-unsaturated fatty acids. Gunstone and his co-workers published values for all methyl octadecenoate isomers[2,3] and some methyl octadecadienoates[4]; Christie[5] for all the methylene-interrupted *cis,cis*-octadecadienoates; Hofstetter *et al.*[6] for several positional isomers; Scholfield and Dutton[7] for some octadecadienoates and trienoates; and Ackman and Hooper[8] for some octadecatrienoate esters. In furthering this study and in order to examine the possibility of predicting the gas chromatographic behaviour of polyunsaturated C_{18} esters, we have prepared five trimethylene-interrupted methyl octadecadiynoates and the corresponding *cis,cis*-octadecadienoates. The general formulae of these compounds are:

$$CH_3(CH_2)_yC \equiv C(CH_2)_3C \equiv C(CH_2)_xCOOCH_3 \text{ and}$$
$$CH_3(CH_2)_yCH = CH(CH_2)_3CH = CH(CH_2)_xCOOCH_3$$

where $x = 0, 1, 2, 3$ or 4 and $y = 5, 6, 7, 8$ or 9.

None of these compounds seems to occur in nature, except the 4,9-isomer, which Lisina *et al.*[9] isolated as the acid from *Pinus sibirica*. Lemarchal and Munch[10] noted the conversion of elaidic acid to the 4,9- and 5,9-octadecadienoic acids in rats fed on a linoleic acid-free diet.
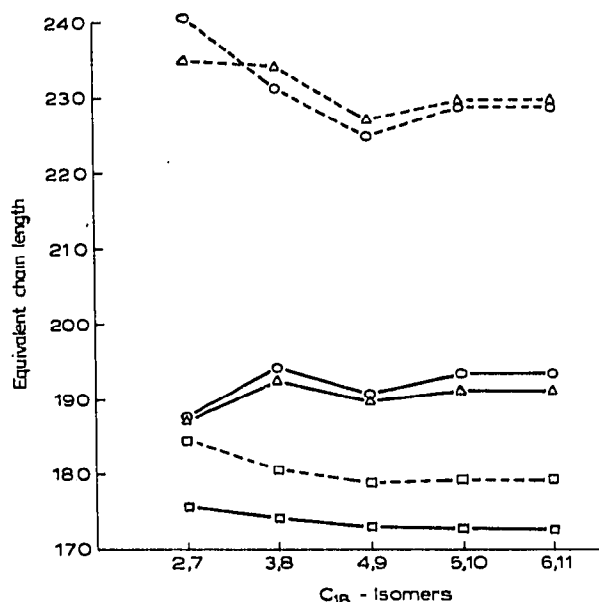
Fig. 1. Equivalent chain lengths of octadecadiynoates (— — —) and *cis,cis*-octadecadienoates (————) on different stationary phases. □, APL: △, DEGS: ○, Silar 10C.

GAS–LIQUID CHROMATOGRAPHY

The ECL values of both series of esters during gas–liquid chromatography (GLC) on Apiezon L (APL), diethylene glycol succinate polyester (DEGS) and Silar 10C are compared in Fig. 1 and actual values are recorded in Table I.

Methyl octadecadiynoates ($\varDelta^{2a,7a}$; $\varDelta^{3a,8a}$; $\varDelta^{4a,9a}$; $\varDelta^{5a,10a}$; $\varDelta^{6a,11a}$)

The ECL values of these isomers ranged from 17.89 to 18.44 on the non-polar APL column. The $\varDelta^{2a,7a}$ isomer exhibited the highest ECL value and was readily separated from the other isomers when mixtures of these isomers were examined.

TABLE I

EQUIVALENT CHAIN LENGTHS OF METHYL OCTADECADIYNOATES AND *cis,cis*-METHYL OCTADECADIENOATES

Isomer	A PL	DEGS	Silar 10C
$\varDelta^{2a,7a}$	18.44	23.48	24.05
$\varDelta^{3a,8a}$	18.07	23.40	23.12
$\varDelta^{4a,9a}$	17.89	22.68	22.49
$\varDelta^{5a,10a}$	17.92	22.89	22.87
$\varDelta^{6a,11a}$	17.93	22.90	22.86
$\varDelta^{2c,7c}$	17.57	18.74	18.73
$\varDelta^{3c,8c}$	17.43	19.25	19.40
$\varDelta^{4c,9c}$	17.32	18.98	19.04
$\varDelta^{5c,10c}$	17.30	19.11	19.34
$\varDelta^{6c,11c}$	17.29	19.12	19.34

On the polar stationary phases, the ECL values ranged from 22.68 to 23.48 on DEGS and from 22.49 to 24.05 on Silar 10C; on both these phases the $\Delta^{2a,7a}$ isomer gave the highest ECL value and the $\Delta^{4a,9a}$ isomer the lowest. The ECL behaviour of these isomers on the polar phases tended to be controlled by the effect of the triple bond nearer to the methoxycarbonyl group. Separation of mixtures on Silar 10C was more efficient than that on DEGS. Three distinct peaks were obtained when a mixture of the $\Delta^{2a,7a}$, $\Delta^{3a,8a}$ and $\Delta^{4a,9a}$ isomers was examined, and a mixture of the $\Delta^{4a,9a}$ and $\Delta^{5a,10a}$ isomers gave twin peaks on Silar 10C. On DEGS, a mixture of the $\Delta^{2a,7a}$ and $\Delta^{5a,10a}$ isomers gave twin peaks only.

cis,cis-Methyl octadecadienoates $(\Delta^{2c,7c};\ \Delta^{3c,8c};\ \Delta^{4c,9c};\ \Delta^{5c,10c};\ \Delta^{6c,11c})$

On the non-polar APL phase, the ECL values of the cis,cis-isomers ranged from 17.29 to 17.57, whereas on DEGS they ranged from 18.74 to 19.25 and on Silar 10C from 18.73 to 19.40.

Unlike the diynoate isomer, the $\Delta^{2c,7c}$ isomer gave the lowest (and almost identical) ECL values on both DEGS and Silar 10C; the $\Delta^{4c,9c}$ isomer also gave closely similar ECL values on DEGS and Silar 10C.

Mixtures of the isomers could not be separated on APL. On Silar 10C, base-line separation was achieved when the ECL values of the individual isomers differed by at least 0.35, but twin peaks were obtained when the ECL values differed by 0.25 or more. On DEGS, a near-to-base-line twin-peak separation was obtained when a mixture of the $\Delta^{2c,7c}$ and $\Delta^{3c,8c}$ isomers was analysed, and only shoulder separation for a mixture of $\Delta^{3c,8c}$ and $\Delta^{4c,9c}$ isomers.

PREDICTION OF CHROMATOGRAPHIC BEHAVIOUR

The concept of fractional chain length (FCL) to predict the ECL values of branched-chain[11,12] and unsaturated esters[4] has made it possible to recognise the pattern of behaviour of uncommon fatty acid esters. We have already studied the GC behaviour of two series of C_{11}-unsaturated methyl esters[13] on APL, DEGS and Silar 10C stationary phases in 2-m columns, and from this source the FCL values of the double and triple bonds were obtained by subtraction of 11.00 from the observed values. The FCL values for the triple and double bonds in the 2 to 6 positions are listed in Table II.

In calculating the ECL values of our C_{18} diunsaturated esters, we considered the FCL value of the unsaturated centres at the 6-position to be an acceptable average

TABLE II

FRACTIONAL CHAIN LENGTHS OF DOUBLE AND TRIPLE BONDS

Isomer	Stationary phase			Isomer	Stationary phase		
	APL	DEGS	Silar 10C		APL	DEGS	Silar 10C
Δ^{2c}	−0.02	+0.28	+0.03	Δ^{2a}	+0.61	+3.49	+3.39
Δ^{3c}	−0.13	+0.88	+0.70	Δ^{3a}	+0.17	+3.12	+2.65
Δ^{4c}	−0.24	+0.62	+0.44	Δ^{4a}	+0.02	+2.38	+1.99
Δ^{5c}	−0.25	+0.58	+0.48	Δ^{5a}	+0.02	+2.43	+2.12
Δ^{6c}	−0.21	+0.73	+0.73	Δ^{6a}	+0.05	+2.64	+2.38

TABLE III

CALCULATED AND OBSERVED EQUIVALENT CHAIN LENGTHS OF SOME OCTA-DECADIYNOATES AND cis,cis-OCTADECADIENOATES

Isomer	On APL (2 m)			On DEGS (2 m)			On Silar 10C (2 m)		
	Calc.	Obs.	Discrepancy	Calc.	Obs.	Discrepancy	Calc.	Obs.	Discrepancy
$\Delta^{2a,7a}$	18.66	18.44	−0.22	24.13	23.48	−0.65	23.77	24.05	+0.28
$\Delta^{3a,8a}$	18.22	18.07	−0.15	23.76	23.40	−0.36	23.03	23.12	+0.06
$\Delta^{4a,9a}$	18.07	17.89	−0.18	23.02	22.68	−0.34	22.37	22.49	+0.12
$\Delta^{5a,10a}$	18.07	17.92	−0.15	23.07	22.89	−0.18	22.50	22.87	+0.37
$\Delta^{6a,11a}$	18.10	17.93	−0.17	23.28	22.90	−0.38	22.76	22.86	+0.10
$\Delta^{2c,7c}$	17.77	17.57	−0.20	19.01	18.74	−0.27	18.76	18.73	−0.03
$\Delta^{3c,8c}$	17.66	17.43	−0.23	19.61	19.25	−0.36	19.43	19.40	−0.03
$\Delta^{4c,9c}$	17.55	17.32	−0.23	19.35	18.98	−0.37	19.17	19.04	−0.13
$\Delta^{5c,10c}$	17.54	17.30	−0.24	19.31	19.11	−0.20	19.21	19.34	+0.13
$\Delta^{6c,11c}$	17.58	17.29	−0.29	19.46	19.12	−0.34	19.46	19.34	−0.12

value for all unsaturation centres located between positions 6 and 11, as the FCL values of unsaturated centres in the middle of a chain have been found not to vary significantly[2,3]. Our calculated ECL values were obtained by addition of the FCL values to 18.00 (see Table III). The discrepancy between calculated and observed ECL values on the APL phase averages −0.206, and on DEGS −0.345; on Silar 10C, the averages for the diynoates and dienoates are +0.186 and −0.036, respectively. There is strong evidence from this study that this discrepancy between observed and calculated ECL values is a measure of some sort of interaction between the two centres of unsaturation. As these centres are three methylene units apart in each instance, it is possible to establish a constant of −0.206 for use in predicting the ECL values on APL phase and of −0.345 on DEGS. With Silar 10C, prediction of ECL values is more complex, and we are studying the effects on Silar 10C in greater detail.

SYNTHESIS AND PROPERTIES

Methyl 2,7-octadecadiynoate

1,6-Heptadecadiyne. 1,3-Dibromopropane (101 g, 0.5 mole) in tetrahydrofuran (THF) (200 ml) was added to a suspension of lithium acetylide obtained by the titration method[14] (from 7 g of lithium, 3 l of ammonia and acetylene). The mixture was stirred for 2 h, then lithamide (from 6 g of lithium, 1 l of ammonia and 2 g of ferric nitrate) was added and the mixture was stirred for 1.5 h. 1-Bromodecane (133 g, 0.6 mole) in THF (150 ml) was finally added, and the mixture was stirred overnight; distillation of the extract gave 1,6-heptadecadiyne (34 g, 30% yield, b.p. 113–116°/ 0.5 mm).

Methyl 2,7-octadecadiynoate. 1,6-Heptadecadiyne (23.2 g, 0.10 mole) in anhydrous diethyl ether (50 ml) was added to ethyl magnesium bromide (prepared from 0.22 g of magnesium, 21.8 g of bromoethane and 200 ml of diethyl ether), and the mixture was refluxed for 1 h. Excess of dry carbon dioxide gas was then passed through the cooled solution for 0.5 h, and the mixture was acidified and extracted with diethyl ether. The crude acid was esterified in boron trifluoride–methanol, and

pure methyl 2,7-octadecadiynoate (8.5 g, 28% yield) was obtained by column chromatography on silica.

Methyl 3,8-octadecadiynoate

1,6-Hexadecadiyne. The procedure used was analogous to that described above for 1,6-heptadecadiyne. Distillation of the extract gave 1,6-hexadecadiyne (29% yield, b.p. 94–100°/0.2 mm).

3,8-Octadecadiyn-1-ol. 1,6-Hexadecadiyne (30 g, 0.14 mole) in THF (50 ml) was added to a suspension of lithamide (from 2 g of lithium, 1 l of ammonia and 0.4 g of ferric nitrate), and the mixture was stirred for 2 h. Epoxyethane (10 g, 0.23 mole) in THF (20 ml) was then added to the mixture, which was stirred for a further 6 h. The product was isolated, and pure 3,8-octadecadiyn-1-ol (19.7 g, 53% yield, m.p. 59°) was obtained by column chromatography on silica.

Methyl 3,8-octadecadiynoate. Chromic acid solution (from 5 g of chromium trioxide, 1 ml of concentrated sulphuric acid and 15 ml of water) was added to a cooled solution (0–5°) of 3,8-octadecadiyn-1-ol (8 g, 0.03 mole) in pure acetone (150 ml), and the mixture was stirred at room temperature for 30 min, then diluted with water (300 ml) and extracted with diethyl ether. The acid fraction was isolated and esterified in boron trifluoride–methanol, and purification of the methyl 3,8-octadecadiynoate (1.9 g, 21% yield) was achieved by column chromatography on silica.

Methyl 4,9-octadecadiynoate

1-Chloro-4-tridecyne. 1-Decyne (68.0 g, 0.49 mole) in THF (100 ml) was added to a suspension of lithamide (from 4.2 g of lithium, 2 l of ammonia and 1 g of ferric nitrate) in anhydrous ammonia, and the mixture was stirred for 1.5 h; 1-bromo-3-chloropropane (94 g, 0.6 mole) in THF (50 ml) was then added, and the suspension was stirred overnight. Distillation of an ethereal extract gave 1-chloro-4-tridecyne (60 g, b.p. 112–120°/1.5 mm, 57% yield).

1-Iodo-4-tridecyne. 1-Chloro-4-tridecyne (55 g, 0.26 mole) was refluxed with sodium iodide (75 g, 0.5 mole) in acetone (500 ml) for 19 h; GLC of the crude product indicated 98% conversion of the chloro-compound into the iodo-derivative. The product (75 g, 94% yield) was not distilled, but used in the next reaction.

4,9-Octadecadiyn-1-ol. 4-Pentyn-1-ol (3.0g, 0.053 mole) in THF (20 ml) was added to a suspension of lithamide (from 0.8 g of lithium, 0.5 l of ammonia and 0.1 g of ferric nitrate) in ammonia, and the mixture was stirred for 1 h; 1-iodo-4-tridecyne (5.3 g, 0.017 mole) in THF (20 ml) was then added, and the mixture was stirred overnight. An ethereal extract of the acidified reaction product gave, after purification by column chromatography [150 g silica, diethyl ether–hexane (1:1) as eluent], pure 4,9-octadecadiyn-1-ol (8 g, 65% yield).

Methyl 4,9-octadecadiynoate. Oxidation of 4,9-octadecadiyn-1-ol by chromic acid gave 4,9-octadecadiynoic acid (21 % yield). Esterification of the acid with boron trifluoride–methanol gave methyl 4,9-octadecadiynoate, which was purified by column chromatography on silica.

Methyl 5,10-octadecadiynoate

1-Bromoheptane (89.5 g, 0.5 mole) in THF (100 ml) was added to a suspension

of lithium acetylide (from 4 g of lithium, 2 l of ammonia and acetylene) prepared by the titration method, and the mixture was stirred for 2 h. Lithamide (from 4 g of lithium, 1 l of ammonia and 1 g of ferric nitrate) was carefully added, and the mixture was stirred for a further 1.5 h. 1-Bromo-3-chloropropane (78.5 g, 0.5 mole) in THF (100 ml) was then added, and the mixture was stirred overnight; distillation of an ethereal extract gave 1-chloro-4-dodecyne (68 g, 68% yield, b.p. 70–73°/0.3 mm).

1-Iodo-4-dodecyne. 1-Chloro-4-dodecyne (65 g, 0.325 mole) was refluxed with sodium iodide (70 g, 0.46 mole) in acetone (300 ml) for 18 h, then the reaction mixture was diluted with water (500 ml) and extracted with light petroleum (b.p. 60–80°); GLC showed 96% conversion of the chlorododecyne into 1-iodo-4-dodecyne (89 g, 93% yield). The product was not distilled.

1-Chloro-4,9-heptadecadiyne. 1-Iodo-4-dodecyne (85 g, 0.29 mole) in THF (100 ml) was added to a suspension of lithium acetylide (from 2.5 g of lithium, 1 l of ammonia and acetylene) prepared by the titration method. The mixture was stirred for 1 h, then lithamide (from 2.5 g of lithium, 0.5 l of ammonia and 0.3 g of ferric nitrate) was carefully added, and the mixture was stirred for a further 1 h. 1-Bromo-3-chloropropane (60 g, 0.38 mole) in THF (100 ml) was then added and the mixture was stirred overnight. Solvent was removed from an ethereal extract of this mixture under vacuum, and the product was distilled to 120° (0.5 mm) to remove all volatile material. The residue was purified on a silica chromatographic column (hexane as eluent) to give 1-chloro-4,9-heptadecadiyne (29 g, 37% yield).

Methyl 5,10-octadecadiynoate. A mixture of 1-chloro-4,9-heptadecadiyne (19.0 g, 0.071 mole), dimethyl sulphoxide (150 ml) and sodium cyanide (8.5 g, 0.17 mole) was heated at 120° for 3 h. The reaction product was diluted with water (300 ml), and the C_{17} nitrile was isolated and kept in a solution of hydrogen chloride (25%, w/w) in methanol (300 ml) for 30 h. Extraction of the diluted reaction mixture gave methyl 5,10-octadecadiynoate (8.0 g, 37% yield) after purification by column chromatography on silica.

Methyl 6,11-octadecadiynoate

This isomer was prepared in a manner similar to that used for methyl 5,10-octadecadiynoate by appropriate chain-extension methods, with the following results: 1-chloro-4-undecyne (69% yield, b.p. 85–88°/25 mm; ref. 15: 80–82°/20 mm), 1-iodo-4-undecyne (95% yield), 1-chloro-5,10-heptadecadiyne (29% yield), methyl 6,11-octadecadiynoate (43% yield).

Semi-hydrogenation

A mixture of methyl octadecadiynoate (200 mg) in ethyl acetate (20 ml), Lindlar catalyst[16] (50 mg) and quinoline (30 mg) absorbed hydrogen (32 ml) in 3–12 min; GLC showed 98–99% conversion into the *cis,cis*-isomer.

Purity check

The *cis,cis*-methyl octadecadienoates on saturation with hydrogen over palladium on charcoal gave only methyl stearate. Cleavage oxidation[17] of each isomer gave only the corresponding mono- and dibasic acids.

TABLE IV
CONDITIONS FOR GLC

Stationary phase	Column length (m)	Temperature (°C)	Carrier gas and flow-rate (ml/min)
5% APL	2	190	N_2, 95
20% DEGS	2	190	N_2, 115
10% Silar 10C	2	190	N_2, 50

Gas–liquid chromatography

The GLC results were obtained under the conditions shown in Table IV on a Pye 104 chromatograph equipped with a flame ionization detector.

ECL values were calculated from the distances between the solvent front and the peak of the other eluted components. Saturated methyl esters were used as internal standards: C_{16}, C_{19} and C_{20} (on APL) and C_{18}, C_{20} and C_{22} (on DEGS and Silar 10C) for the diynoates, and C_{16}, C_{18} and C_{20} for the *cis,cis*-octadecadienoates.

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