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# CHROMATOGRAPHY OF THE ISOMERIC METHYLENE-INTERRUPTED METHYL *CIS*, *CIS*-OCTADECADIENOATES

## 2. GAS–LIQUID CHROMATOGRAPHY*

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SUMMARY

The equivalent chain lengths on gas–liquid chromatography of the isomeric methylene-interrupted methyl *cis*, *cis*-octadecadienoates (2,5- to 14,17-) were determined on eight different liquid phases, two of which were in capillary columns. The equivalent chain length values increased with distance of the double bonds from the carboxyl group but the values for the 3,6- and 13,16-isomers on polar columns or the 13,16-isomer alone on the non-polar column were much greater than those of the adjacent isomers. Isomers differing by 0.04 in equivalent chain length could be separated on the Apiezon L capillary column and those differing by 0.08 in equivalent chain length on the neopentyl glycol succinate capillary column.

## INTRODUCTION

Preliminary or tentative identification of unknown unsaturated fatty acids in lipid samples by their relative retention times on various gas–liquid chromatography (GLC) columns is common practice in many laboratories. This has been considerably facilitated by the introduction of the concept of carbon numbers (WOODFORD AND VAN GENT)[1] or equivalent chain lengths (ECLs) (MIWA, MIKOLAJCZAK, EARLE AND WOLFF)[2] for presenting retention data. Recent reviews[3,4] describe the merits of the systems in fatty acid analysis. ECLs for large numbers of fatty acids on many GLC liquid phases are available in the literature and there have been many attempts, particularly in the laboratories of ACKMAN[5,6] and HOLMAN[4,7], to correlate these values with the structures of the acids. The number of different fatty acids available from natural sources is limited, however, and studies with complete series of model compounds are necessary for more meaningful conclusions to be drawn. GUNSTONE, ISMAIL AND LIE KEN JIE[8] have recently recorded retention data for the complete series of isomeric methyl *cis* and *trans* octadecenoates. A similar study of the retention data and separations achieved of the isomeric methylene-interrupted methyl *cis-cis-*

* Part 1. see ref. 19.

octadecadienoates (*i.e.* methyl *cis*-2-, *cis*-5- to *cis*-14,17-octadecadienoate[9]) is now described.

## EXPERIMENTAL

The columns used with the appropriate operating conditions are detailed in Table I. Capillary columns were used in an F11 gas chromatograph (Perkin-Elmer Ltd, Beaconsfield) and packed columns in a Pye 104 (W.G. Pye Ltd, Cambridge). Both instruments were equipped with flame ionization detectors and nitrogen was the carrier gas. Solid supports were acid-washed and silanised. $C_{14}$, $C_{16}$ and $C_{17}$ or $C_{18}$

TABLE I

CONDITIONS OF GAS–LIQUID CHROMATOGRAPHY WITH EACH OF THE LIQUID PHASES

| *Liquid phase* | *Content (%)* | *Support* | *Mesh* | *Column dimensions* | *Flow rate (ml/min)* | *Temp.* |
|---|---|---|---|---|---|---|
| ApL (1)* | | | | 50 m × ¼ mm | *ca.* 2 | 220 |
| NPGS* | | | | 50 m × ¼ mm | *ca.* 2 | 190 |
| ApL (2) | 5 | Gas Chrom Z | 70–80 | 5 ft. × ¼ in. | 50 | 200 |
| Carbowax 20 M-terephthalic acid | 5 | Chromosorb G | 80–100 | 5 ft. × ¼ in. | 50 | 200 |
| PEGA | 15 | Gas Chrom Z | 70–80 | 5 ft. × ¼ in. | 50 | 190 |
| DEGS | 20 | Gas Chrom Z | 70–80 | 5 ft. × ¼ in. | 50 | 180 |
| EGS | 20 | Chromosorb W | 100–120 | 7 ft. × ¼ in. | 50 | 180 |
| EGS–2% $H_3PO_4$ | 20 | Gas Chrom P | 80–100 | 7 ft. × ¼ in. | 50 | 180 |

* Capillary columns.

saturated methyl esters were used as internal standards. The relative retention times for each of the esters were determined on the eight different liquid phases and are presented as ECLs. All columns were freshly packed since it is well documented that ECL values can alter as columns age. The capillary columns were purchased precoated (Perkin-Elmer Ltd, Beaconsfield).

## RESULTS AND DISCUSSION

The liquid phases chosen were those that are most commonly in use in lipid laboratories. Apiezon L (ApL) is non-polar and unsaturated esters are eluted before the corresponding saturated esters. Polyethylene glycol adipate (PEGA), diethylene glycol succinate (DEGS) and ethylene glycol succinate (EGS) are the commonest polar polyester liquid phases in use. EGS with added phosphoric acid is reported to be more stable and to give better separations than EGS alone[10]. Neopentyl glycol succinate (NPGS) is commercially available in capillary (open-tubular or Golay) columns. Carbowax 20 M terephthalic acid is commonly used for the chromatography of free acids. The ECL values obtained for each isomer on each of the eight columns are listed in Table II. Those from three representative columns are illustrated graphically in Fig. 1.

On all the polar liquid phases, the ECL values of the isomers increase with

TABLE II

EQUIVALENT CHAIN LENGTHS OF THE ISOMERS ON POLAR AND NON-POLAR COLUMNS

| Isomer | Liquid phase | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *ApL (1)*[a] | *NPGS* | *ApL (2)*[b] | *Carbowax* | *PEGA* | *DEGS* | *EGS* | *EGS–$H_3PO_4$* |
| Methyl 2,5-octadecadienoate | 17.68[c] | 18.14 | 17.64 | 18.25 | 18.37 | 18.64 | 18.65 | 18.72 |
| Methyl 3,6-octadecadienoate | 17.62 | 18.65 | 17.57 | 18.67 | 18.94 | 19.36 | 19.54 | 19.68 |
| Methyl 4,7-octadecadienoate | 17.47 | 18.36 | 17.42 | 18.45 | 18.69 | 19.05 | 19.28 | 19.42 |
| Methyl 5,8-octadecadienoate | 17.43 | 18.38 | 17.38 | 18.38 | 18.71 | 19.06 | 19.27 | 19.40 |
| Methyl 6,9-octadecadienoate | 17.46 | 18.44 | 17.40 | 18.46 | 18.81 | 19.19 | 19.37 | 19.57 |
| Methyl 7,10-octadecadienoate | 17.44 | 18.46 | 17.38 | 18.46 | 18.80 | 19.23 | 19.42 | 19.63 |
| Methyl 8,11-octadecadienoate | 17.48 | 18.53 | 17.42 | 18.51 | 18.87 | 19.28 | 19.47 | 19.65 |
| Methyl 9,12-octadecadienoate | 17.50 | 18.60 | 17.47 | 18.57 | 18.95 | 19.38 | 19.55 | 19.75 |
| Methyl 10,13-octadecadienoate | 17.60 | 18.70 | 17.56 | 18.60 | 19.03 | 19.46 | 19.69 | 19.81 |
| Methyl 11,14-octadecadienoate | 17.68 | 18.82 | 17.63 | 18.75 | 19.15 | 19.62 | 19.83 | 20.00 |
| Methyl 12,15-octadecadienoate | 17.78 | 18.90 | 17.72 | 18.88 | 19.28 | 19.75 | 19.97 | 20.16 |
| Methyl 13,16-octadecadienoate | 18.00 | 19.27 | 17.95 | 19.20 | 19.68 | 20.25 | 20.37 | 20.60 |
| Methyl 14,17-octadecadienoate | 17.80 | 19.04 | 17.75 | 18.95 | 19.33 | 19.78 | 19.96 | 20.18 |

[a] Capillary column.
[b] Packed column.
[c] Some sample decomposition also occurs.

distance of the double bonds from the carboxyl group, though there are sharp discontinuities for the 3,6- and 13,16- isomers where the ECLs are considerably higher than those of the adjacent isomers. A similar feature was noted with the methyl octadecenoates[8] where the ECLs of the 3- and 16- isomers were also much higher than those of nearby isomers. Such discrepancies are not seen in the ECLs of the isomeric methyl hydroxy and acetoxy palmitates[11], hydroxy, acetoxy and oxo-stearates[12] and methylene octadecanoates[13]. With the isomeric methyl-branched octadecanoates[14,15], similarly elevated ECL values are found for the 4- and 16-isomers. No explanation of this

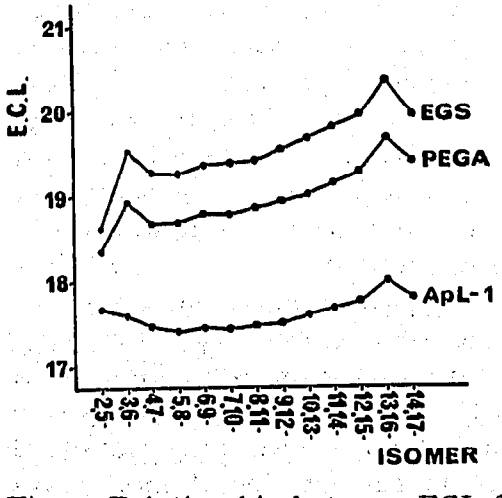


Fig. 1. Relationship between ECL for each isomer and double position on EGS, PEGA and ApL columns.

phenomenon can at present be offered. With the ApL columns, the ECL values conform to a gentle curve with a minimum at the 5,8-isomer, though the 13,16-isomer again has a greater ECL value than those of the isomers on either side. ECLs on the capillary ApL column are uniformly 0.05 higher than those on the packed column. The use of capillary columns is reported to result in losses of polyunsaturated fatty acids[16]. This was certainly so with methyl 2,5-octadecadienoate, which is the most labile of the isomers chemically. When this was chromatographed on the ApL capillary column, a number of late running spurious peaks were observed.

If the data of GUNSTONE, ISMAIL AND LIE KEN JIE[8] are used to calculate the increment in chain lengths for double bonds in each position (*i.e.* ECL—18) and thence, by adding these, to predict ECL values for the dienoic esters, values of the correct order of magnitude are obtained. However, in each instance, the calculated value was somewhat lower than that actually found. If the value for the 2,5-isomer is omitted, a mean difference of 0.13 is obtained for the ApL column, 0.16 for the NPGS column and 0.18 for the DEGS column. This may mean that there is some interaction between the double bonds which increases the dipole moment of the unsaturated system. Alternatively, the two double bonds may polarise the diallyl methylene group, again increasing the dipole moment of the whole and hence lengthening the retention time of the dienoic esters.

HOFSTETTER, SEN AND HOLMAN[7] have noted that for many unsaturated esters the difference between $ECL_{EGS}$ and $ECL_{ApL}$ is approximately constant per double bond in the molecule. This can only be so, however, if the graphs of double bond position against ECL of the isomers for the two liquid phases are parallel. As can be seen from Fig. 1, this is not so, but the relationship does have some validity when the double bonds are near the middle of the molecule. If such values are again calculated from the monoene data[8] and summed to predict similar values for the octadecadienoates, values are obtained that agree closely with those actually found, particularly with $ECL_{NPGS} - ECL_{ApL}$ where the agreement is within 0.05 for the 2,5- to 11,14-isomers. If this relationship can be shown to hold also for more unsaturated esters, it may be of value in the tentative identification of unknown fatty acids.

ECL values of the 8,11- to 12,15- isomers have been reported elsewhere[7] for ApL, DEGS and EGS columns. The ApL values agree within 0.06 and this probably reflects the particular stability of this liquid phase. Quite large discrepancies exist, however, between the values reported here and those of the previous report for polyester columns. This may be partly due to the fact that nitrogen was the carrier gas in the study now reported and helium was used by HOFSTETTER, SEN AND HOLMAN[7] or to differences in the ages or conditioning of the column packings. GUNSTONE *et al.*[8] similarly find good agreement between their values and those previously recorded by other workers for certain of the methyl octadecenoates on ApL columns. The use of common standards for correlations between laboratories is advised[8], however, and it would appear that ECLs on polar polyester phases may have less absolute value than has been supposed.

Both capillary columns gave excellent separations of isomers, representative examples of which are illustrated in Fig. 2. The ApL column (*ca.* 40,000 theoretical plates) separated isomers differing in ECL by 0.04 and the NPGS column (*ca.* 15,000 theoretical plates) those differing in ECL by about 0.08. LANDOWNE AND LIPSKY[17] have also described the separation of the methyl 8,11- to 11,14- octadecadienoates on polar and non-polar capillary columns. In theory, a capillary column could be con-

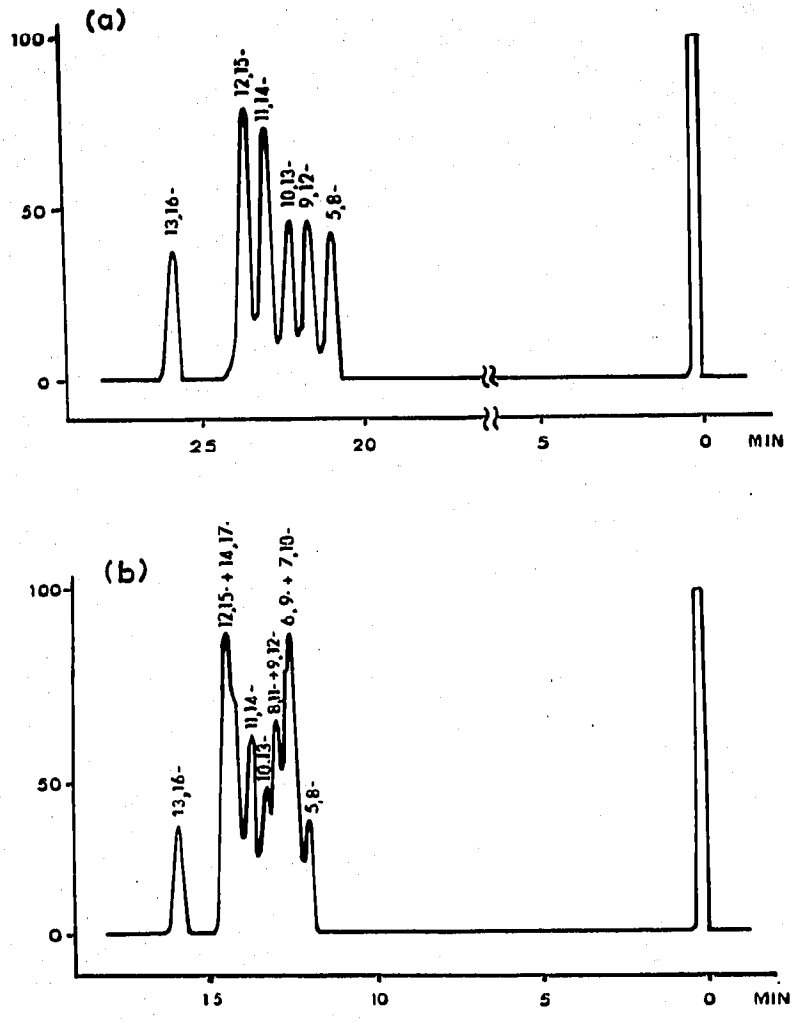


Fig. 2. GLC recorder tracing of mixtures of isomers on the capillary columns. (a) = ApL. (b) = NPGS.

structed from one or other of the liquid phases investigated to separate any two of the isomers with adjacent double bond systems; only the 4,7- to 7,10-isomers should cause any difficulty. For example, DEGS or EGS capillary columns should easily separate the 5,8-, 6,9-, 8,11- and 9,12- octadecadienoates which co-occur in the tissues of fat-deficient rats[18].

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