Journal of Chromatography, 131 (1977) 239–243

CHROM. 9462

FATTY ACIDS

XIII*. THE GAS–LIQUID CHROMATOGRAPHIC BEHAVIOUR OF DIMETHYLENE-INTERRUPTED METHYL *CIS*-OCTADECENYNOATES

M. S. F. LIE KEN JIE
Department of Chemistry, University of Hong Kong, Pokfulam Road, Hong Kong (Hong Kong)
(Received June 21st, 1976)

SUMMARY

All of the dimethylene-interrupted methyl *cis*-octadecenynoate isomers, except the $\Delta^{13c,17a}$ isomer, have been prepared by partial hydrogenation of the corresponding methyl octadecadiynoate. Gas–liquid chromatographic analysis of the octadecenynoates has been carried out on Carbowax 20M, DEGA, DEGS, FFAP and Silar 10C stationary phases. The $\Delta^{3,7}$ isomers decompose on chromatography. The equivalent chain lengths of all of the remaining methyl *cis*-octadecenynoates are reported.

INTRODUCTION

Naturally occurring fatty acids containing conjugated enyne systems are not uncommon[2], but non-conjugated methyl octadecenynoate isomers are rare. Of the limited number of non-conjugated methyl octadecenynoates which have been isolated from seed oils[3–5], crepenynic acid (*cis*-9-octadecen-12-ynoic acid) is believed[6] to play a key role in the biogenesis of various oxygenated polyunsaturated fatty acids.

Several synthetic routes are available to octadecenynoates. Ames and Islip[7] have prepared methyl *cis*-12-octadecen-5-ynoate, and the synthesis of methyl *cis*-10-octadecen-5-ynoate has also been reported[8]. Bradshaw *et al.*[9] succeeded in producing crepenynic acid via the Wittig reaction, and Osbond[10] described the synthesis of methyl *cis*-12-octadecen-9-ynoate.

In this paper the gas–liquid chromatographic (GLC) behaviour is reported of mixtures of dimethylene-interrupted methyl *cis*-octadecenynoates on Carbowax 20M, DEGA, DEGS, FFAP and Silar 10C stationary phases.

EXPERIMENTAL

Gas–liquid chromatography

The GLC results were obtained under the conditions given in Table I on a

* For Part XII, see ref. 1.

Pye 104 or a Varian 940 chromatograph equipped with a flame ionization detector.

Equivalent chain length (ECL) values were calculated from the distances between the solvent front and the peaks of the other eluted components. Saturated methyl esters (C_{12}, C_{16}, C_{18} and C_{19}) were used as internal standards.

TABLE I

CONDITIONS USED FOR GLC

Column length, 2 m; temperature, 190°.

Stationary phase	*Carrier gas (nitrogen) flow-rate (ml/min)*	*Internal diameter (mm)*
10% Carbowax 20M	50	3.1
10% DEGA	70	6.2
20% DEGS	50	3.1
10% FFAP	50	3.1
10% Silar 10C	60	6.2

General procedure for hydrogenation of methyl octadecadiynoates

A mixture of methyl octadecadiynoate (100 mg), ethyl acetate (10 ml) and 5% palladium on charcoal (5 mg) was shaken in hydrogen at room temperature under atmospheric pressure. The reaction was stopped when 7.7 ml of hydrogen had been absorbed. The catalyst was filtered off and the solvent was evaporated under reduced pressure. The unwanted methyl *cis,cis*-octadecadienoate was removed by thin-layer chromatography (TLC) on silica impregnated with silver nitrate (solvent: 20% diethyl ether in light petroleum, b.p. 60–80°). The mixture of methyl octadecadiynoate and methyl *cis*-octadecenynoate was used without further purification.

RESULTS AND DISCUSSION

Preparation of methyl cis-octadecenynoates

The methyl *cis*-octadecenynoates were prepared by hydrogenation of the corresponding methyl octadecadiynoates[11] by allowing a 1:1 molar ratio of hydrogen to substrate to react in the presence of a palladium-on-charcoal catalyst. This method furnished a mixture of methyl *cis*-octadecenynoate isomers (10–15%), the corresponding methyl *cis,cis*-octadecadienoate (*ca.* 40%) and unreacted methyl octadecadiynoate (*ca.* 45%). The low yield of octadecenynoates after each hydrogenation reaction indicates that the conversion of the diacetylenic ester into the enynoate derivative is a slower process than hydrogenation of the intermediate octadecenynoate to the octadecadienoate.

Several attempts failed to isolate the methyl *cis*-octadecenynoate isomers from unreacted methyl octadecadiynoate and undesired *cis,cis*-octadecadienoate using TLC on silica impregnated with silver nitrate. Only methyl *cis,cis*-octadecadienoate could be removed by this technique. The mixtures of methyl *cis*-octadecenynoate and

octadecadiynoate were then used for GLC study. Each sample of methyl *cis*-octadecenynoates contained two positional isomers: thus $\Delta^{2a,6a}$ gave a mixture of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ isomers; $\Delta^{3a,7a}$ gave a mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$, etc.

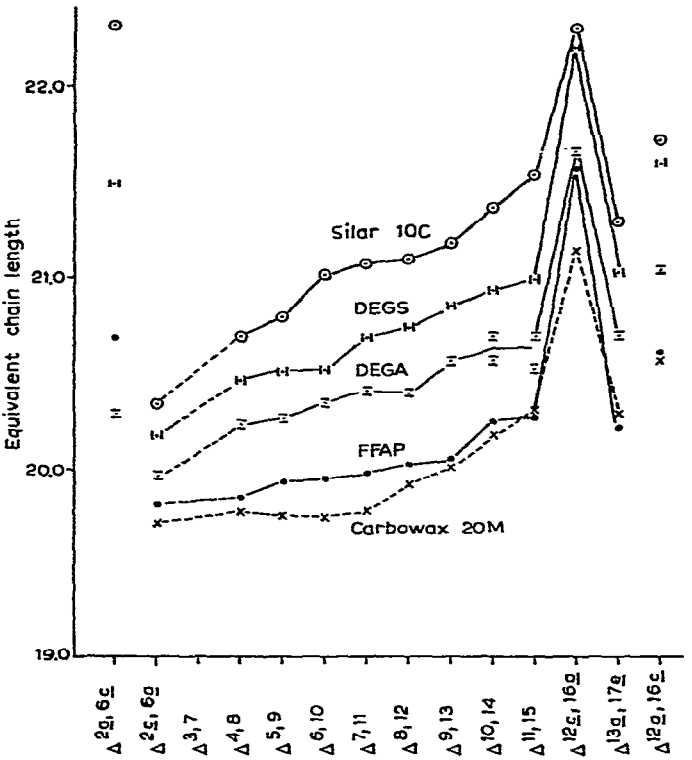

Fig. 1. Equivalent chain lengths of methyl *cis*-octadecenynoates.

TABLE II

EQUIVALENT CHAIN LENGTHS OF MIXTURES OF METHYL OCTADECENYNOATES

Isomers	*Stationary phase*				
	Silar 10C	*DEGS*	*FFAP*	*Carbowax 20M*	*DEGA*
$\Delta^{2a,6c}$, $\Delta^{2c,6a}$	22.31, 20.34	21.47, 20.18	20.68, 19.82	—, 19.72	20.29, 19.97
$\Delta^{3a,7c}$, $\Delta^{3c,7a}$	—	—	—	—	—
$\Delta^{4a,8c}$, $\Delta^{4c,8a}$	20.68	20.46	19.85	19.78	20.23
$\Delta^{5a,9c}$, $\Delta^{5c,9a}$	20.78	20.51	19.93	19.76	20.26
$\Delta^{6a,10c}$, $\Delta^{6c,10a}$	21.00	20.51	19.95	19.75	20.34
$\Delta^{7a,11c}$, $\Delta^{7c,11a}$	21.06	20.68	19.98	19.78	20.40
$\Delta^{8a,12c}$, $\Delta^{8c,12a}$	21.08	20.73	20.02	19.92	20.39
$\Delta^{9a,13c}$, $\Delta^{9c,13a}$	21.16	20.82	20.05	20.00	20.55
$\Delta^{10a,14c}$, $\Delta^{10c,14a}$	21.34	20.92	20.24	20.17	20.55, 20.68
$\Delta^{11a,15c}$, $\Delta^{11c,15a}$	21.51	20.98	20.26	20.28	20.51, 20.67
$\Delta^{12a,16c}$, $\Delta^{12c,16a}$	21.70, 22.28	21.58, 22.19	20.59, 21.55	20.56, 21.12	21.01, 21.64
$\Delta^{13a,17c}$	21.27	21.01	20.20	20.28	20.68

GLC behaviour of mixtures of methyl cis-octadecenynoates

The ECL values of the mixtures of methyl *cis*-octadecenynoate are compared in Fig. 1 and the actual values are recorded in Table II.

Behaviour on Silar 10C. Except for the isomeric mixtures of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ and $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$, all of the remaining sets of mixtures of positional isomers could not be separated on the Silar 10C stationary phase. The ECL values increased gradually as the enyne system became more distant from the ester group. In the case of the mixture of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$, the ECL values of 20.34 and 22.31 correspond to $\Delta^{2c,6a}$ and $\Delta^{2a,6c}$ respectively, as the triple bond at the Δ^2 position contributes a much higher fractional chain length than that at the Δ^6 position[11]. For the mixture of $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$, the ECL values of 21.70 and 22.28 are similarly attributed to $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ respectively as the triple bond at Δ^{16} in a C_{18} ester also contributes a higher fractional chain length than that at the Δ^{12} position. As only the $\Delta^{13a,17e}$ isomer is produced during hydrogenation of $\Delta^{13a,17a}$ (ref. 12), the ECL value of 21.27 in the case of $\Delta^{13,17}$ corresponds only to the $\Delta^{13a,17e}$ isomer. The mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$ decomposed on the column and could not be eluted as in the case of $\Delta^{3a,7a}$ (ref. 11).

Behaviour on DEGS. The ECL values of the mixtures of the positional isomers were generally lower than those obtained on Silar 10C. The mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$ decomposed on the column and could not be eluted. The mixtures of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ and of $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ gave two distinct peaks each at 21.47 and 20.18 and at 21.58 and 22.19 respectively. The remaining mixtures of isomers gave a single peak with ECL values ranging from 20.46 to 21.01.

Behaviour on FFAP. The mixtures of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ and of $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ were separated on FFAP with ECL values of 20.68 and 19.82 and of 20.59 and 21.55 respectively. The mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$ could not be eluted as these isomers decomposed on the column. The remaining mixtures of the series appeared as a single peak and the ECL values (19.85–20.26) increased gradually as the unsaturated centres approached the methyl end of the fatty ester chain. The mixture of $\Delta^{4a,8c}$ and $\Delta^{4c,8a}$ gave the lowest ECL value of the entire series.

Behaviour on Carbowax 20M. The mixture of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ gave only one peak with an ECL value of 19.72. Since $\Delta^{2a,6a}$ decomposed on Carbowax 20M (ref. 11) but the $\Delta^{2c,6c}$ isomer did not[13], it is believed that the peak at 19.72 is due to $\Delta^{2c,6a}$. The mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$ also decomposed on the column and was not eluted. The mixture of $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ gave two peaks with ECL values of 20.56 and 21.12 due to $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ respectively. The remaining mixtures gave ECL values ranging from 19.75 to 20.28.

Behaviour on DEGA. The retention times of the octadecenynoates on DEGA were exceptionally long compared to the four previously examined polar phases. The mixture of $\Delta^{2a,6c}$ and $\Delta^{2c,6a}$ gave two distinct peaks at 20.29 and 19.97 respectively. Although the compound $\Delta^{3a,7a}$ could be eluted on DEGA (ref. 11), the corresponding mixture of $\Delta^{3a,7c}$ and $\Delta^{3c,7a}$ could not be eluted on this stationary phase. The mixtures of $\Delta^{10a,14c}$ and $\Delta^{10c,14a}$ and of $\Delta^{11a,15c}$ and $\Delta^{11c,15a}$ gave twin peaks with ECL values of 20.55 and 20.68 and of 20.51 and 20.67 respectively. The mixture of $\Delta^{12a,16c}$ and $\Delta^{12c,16a}$ gave two distinct peaks with ECL values of 21.01 and 21.64 respectively. The remaining mixtures gave a single peak with ECL values ranging from 20.23 to 20.68.

CONCLUSIONS

This study reconfirms the order of polarity of the five stationary phases employed for the analysis of unsaturated fatty esters: *viz.* Silar 10C > DEGS > DEGA > FFAP > Carbowax 20M. The ECL values of the methyl *cis*-octadecenynoates correspond closely to the average of the ECL values of the corresponding methyl *cis,cis*-octadecadienoates and methyl octadecadiynoates. In the case of the $\Delta^{2,6}$ and $\Delta^{12,16}$ isomers, the separation of the two octadecenynoate isomers shows the possibility of identifying positional isomers which have different unsaturated centres located at the termini of the fatty ester chain. The $\Delta^{3a,7e}$ and $\Delta^{3e,7a}$ isomers are very labile compounds and decompose readily on contact with the stationary phases at high temperature.

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