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FATTY ACIDS

VIII*. GAS-LIQUID CHROMATOGRAPHIC PROPERTIES OF ALL DIMETHYLENE INTERRUPTED METHYL *trans,trans*-OCTADECADIENOATES

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SUMMARY

All the dimethylene interrupted *trans,trans*-octadecadienoic acids were synthesised by lithium metal-ammonia reduction of the corresponding octadecadiynoic acids, except for the $\Delta^{2r,6r}$ isomer, which was obtained by the isomerisation of the $\Delta^{2c,6c}$ isomer. The gas-liquid chromatographic behaviour of the methyl esters was studied on polar [Carbowax 20M, FFAP, DEGA, DEGS, Silar 10C (recently renamed as Apolar 10) and SP 2330], semi-polar (XE-60), and non-polar (SE-30, OV-101 and Apiezon L) stationary phases. The equivalent chain length of each isomer is recorded (including the $\Delta^{2r,6c}$ isomer) and the possibility of separation of these isomers is discussed.

INTRODUCTION

Further to our study on the gas-liquid chromatographic (GLC) properties of the dimethylene interrupted C_{18} esters, we have now prepared all (except the $\Delta^{2r,6r}$ isomer) *trans,trans* isomers by lithium metal-ammonia reduction² of the corresponding diynoic acids. The $\Delta^{2r,6r}$ isomer was obtained from the $\Delta^{2c,6c}$ isomer by isomerisation of the *cis*-ethylenic bond located between C-2 and C-3 by mercuric acetate³ to give initially the $\Delta^{2r,6c}$ intermediate, which was further isomerised in the presence of nitrous acid⁴ to the required $\Delta^{2r,6r}$ isomer. Purification of the *trans,trans* compounds was readily achieved by silver ion thin-layer chromatography (TLC).

Hoffmann and Meijboom⁵ have isolated the $\Delta^{11r,15r}$ isomer from tallow, while Kuemmel and Chapman⁶ obtained on hydrogenation of corn oil small amounts of the $\Delta^{8r,12r}$ and $\Delta^{9r,13r}$ isomers. When rats were fed on trielaidin, the $\Delta^{5r,9r}$ isomer was detected in the tissues of the liver⁷. Of all the twelve possible *trans,trans* isomers only the $\Delta^{6r,10r}$ and $\Delta^{8r,12r}$ isomers were previously synthesised⁸.

* For Part VII, see ref. 1.

EXPERIMENTAL

General method of reduction of all isomers, except $\Delta^{2r,6r}$

Lithium metal (800 mg) was added piecewise into a mixture of octadecadiynoic acid (500 mg) and tetrahydrofuran (20 ml) in distilled liquid ammonia (300 ml) under reflux for 3 h. Ethanol (2 ml) was added to destroy the excess lithium and the ammonia was allowed to evaporate. Extraction with diethyl ether of the acidified reaction mixture gave *trans,trans*-octadecadienoic acid (80–90% yield). GLC analysis of the methyl ester derivative showed the presence of 2–10% methyl C_{18} -enynoate.

In the preparation of the $\Delta^{12r,16r}$ isomer, the product contained 10% enynoate, 57% dienolate and 23% enolate with ECL_{Silar 10C} of 20.30, 19.22, 18.35, respectively. And in the case of the $\Delta^{13r,17c}$ isomer, ammonium sulphate (500 mg) was added to the ammoniacal reaction mixture as a proton source for the reduction of the terminal acetylene. The product contained 68% dienolate and 32% enynoate with ECL_{Silar 10C} of 19.21 and 21.27. In both instances, the *trans* isomers were isolated by silver ion TLC.

Preparation of $\Delta^{2r,6r}$

A mixture of methyl *cis,cis*-2,6-octadecadienoate (100 mg), mercuric acetate (300 mg) and methanol (25 ml) was left at room temperature for four days. The reaction mixture was then refluxed for 1 h, diluted with hydrochloric acid (2 M, 20 ml) and extracted with diethyl ether after 2 h. GLC analysis of the extract showed the presence of methyl *trans*-2,*cis*-6-octadecadienoate (96%, ECL_{Silar 10C} 19.84) and methyl *cis,cis*-2,6-octadecadienoate (4%, ECL_{Silar 10C} 18.49). The *trans*-2,*cis*-6 isomer was isolated by silver ion TLC (90 mg, 90% yield).

The *trans*-2,*cis*-6 isomer (90 mg) in diethyl ether (20 ml) was vigorously shaken with a mixture of nitric acid (6 M, 20 ml) and sodium nitrite (2 M, 20 ml) for 3 h. The GLC analysis of the isolated product gave a shoulder peak with ECL_{Silar 10C} of 19.72 and 19.84. Methyl *trans,trans*-2,6-octadecadienoate (30 mg, 33% yield) was

TABLE I
CONDITIONS FOR GLC
Column length, 2 m.

Stationary phase	Temperature (°C)	Carrier gas (nitrogen) flow-rate (ml/min)	Internal diameter (mm)
5% Apiezon L	200	100	3.1
3% SE-30	190	40	3.1
1.5% OV-101	190	40	3.1
20% XE-60	190	95	6.2
10% Carbowax 20M	200	40	3.1
10% FFAP	195	40	3.1
10% DEGA	195	80	6.2
10% Silar 10C	165	70	6.2
20% DEGS	140	40	3.1
10% SP 2330	170	50	6.2

isolated by silver ion TLC. Infrared analysis (cm^{-1}): 3000(s) (C-H), 1720(s) (C=O), 1660(m) (C=C) and 970(s) (C-H bending at the *trans* ethylenic bonds). The following nuclear magnetic resonance signals (τ) were observed:

	CH_3-	$(\text{CH}_2)_9-$	CH_2-	$\text{CH}^{\delta}\text{=CH}-$	$(\text{CH}_2)_2-$	$\text{CH}^{\delta}\text{=CH}-$	COOCH_3
	9.1	8.76	7.8	4.62	7.8	4.09 3.1	6.3
Number of protons:	(3)	(18)	(2)	(2)	(4)	(1) (1)	(3)

Oxidative cleavage of the methyl *trans,trans*-octadecadienoates gave the corresponding mono- and/or dicarboxylic acid moieties only.

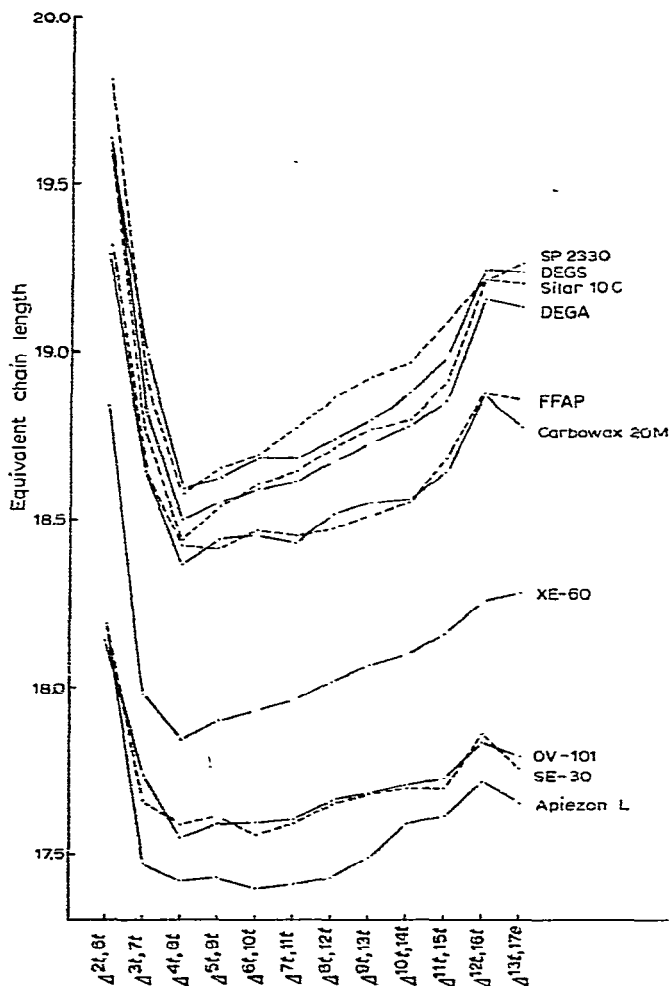


Fig. 1. Equivalent chain lengths of *trans,trans*-diolefinic C_{18} methyl esters.

TABLE II

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON APIEZON L

Isomer	ECL	A1*	A2**	A3**	A4***	A5‡
Δ ^{2z,6z}	18.19	18.17	—	—	—	—
Δ ^{3z,7z}	17.47	—	—	—	—	—
Δ ^{4z,8z}	17.42	—	—	—	—	—
Δ ^{5z,9z}	17.43	—	—	—	17.43	—
Δ ^{6z,10z}	17.40	—	—	—	—	17.43
Δ ^{7z,11z}	17.41	—	17.41	—	—	—
Δ ^{8z,12z}	17.43	—	—	17.46	—	—
Δ ^{9z,13z}	17.49	—	—	—	—	—
Δ ^{10z,14z}	17.59	—	—	—	—	17.43
Δ ^{11z,15z}	17.61	—	—	—	—	—
Δ ^{12z,16z}	17.72	17.72	17.69	17.72	—	—
Δ ^{13z,17z}	17.65	—	—	—	17.59	—
Δ ^{2z,6c}	18.14	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

RESULTS

The GLC results were obtained under the conditions given in Table I on a Pye 104 or Varian 940 chromatograph equipped with a flame ionisation detector. Equivalent chain length (ECL) values were calculated from distances between the solvent front and the peak of the eluted components with saturated esters (C₁₅, C₁₆, C₁₈, C₁₉ and C₂₀) as internal standards.

TABLE III

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON SE-30

Isomer	ECL	B1*	B2**	B3**	B4***	B5‡
Δ ^{2z,6z}	18.17	18.15	18.13	—	—	—
Δ ^{3z,7z}	17.66	17.68	—	—	—	—
Δ ^{4z,8z}	17.59	—	—	—	—	—
Δ ^{5z,9z}	17.61	—	—	—	—	—
Δ ^{6z,10z}	17.56	—	—	—	17.58	17.57
Δ ^{7z,11z}	17.59	—	—	17.62	—	—
Δ ^{8z,12z}	17.65	—	—	—	—	—
Δ ^{9z,13z}	17.68	—	—	—	—	—
Δ ^{10z,14z}	17.70	—	—	—	—	—
Δ ^{11z,15z}	17.70	—	—	—	—	17.57
Δ ^{12z,16z}	17.86	—	—	17.86	—	—
Δ ^{13z,17z}	17.76	—	17.80	—	17.76	—
Δ ^{2z,6c}	18.13	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

TABLE IV

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON OV-101

Isomer	ECL	C1*	C2**	C3**	C4***	C5‡
$\Delta^{2r,6r}$	18.14	18.14	18.18	—	—	—
$\Delta^{3r,7r}$	17.74	17.74	—	—	—	—
$\Delta^{4r,8r}$	17.55	—	—	—	—	—
$\Delta^{5r,9r}$	17.59	—	—	—	—	—
$\Delta^{6r,10r}$	17.59	—	—	—	17.60	—
$\Delta^{7r,11r}$	17.60	—	—	17.62	—	—
$\Delta^{8r,12r}$	17.66	—	—	—	—	—
$\Delta^{9r,13r}$	17.68	—	—	—	—	17.77
$\Delta^{10r,14r}$	17.71	—	—	—	—	—
$\Delta^{11r,15r}$	17.73	—	—	—	—	—
$\Delta^{12r,16r}$	17.84	—	—	17.84	—	17.77
$\Delta^{13r,17r}$	17.79	—	17.79	—	17.75	—
$\Delta^{2r,6c}$	18.12	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

The ECL values of all the dimethylene interrupted methyl *trans,trans*-octadecadienoates are compared in Fig. 1 and the actual values are recorded in Tables II–XI.

Methyl trans,trans-octadecadienoates

On all the ten stationary phases studied, the $\Delta^{2r,6r}$ isomer gave the highest

TABLE V

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON XE-60

Isomer	ECL	D1*	D2**	D3***	D4***	D5‡
$\Delta^{2r,6r}$	18.84	—	—	—	—	—
$\Delta^{3r,7r}$	17.98	—	—	—	—	—
$\Delta^{4r,8r}$	17.84	17.84	—	—	—	—
$\Delta^{5r,9r}$	17.90	—	—	17.93	17.94	—
$\Delta^{6r,10r}$	17.93	—	—	—	—	18.00
$\Delta^{7r,11r}$	17.96	—	17.96	—	—	—
$\Delta^{8r,12r}$	18.02	—	—	—	—	—
$\Delta^{9r,13r}$	18.07	—	—	—	—	18.00
$\Delta^{10r,14r}$	18.10	—	—	—	18.08	—
$\Delta^{11r,15r}$	18.16	—	—	18.12	—	—
$\Delta^{12r,16r}$	18.26	18.28	—	—	—	—
$\Delta^{13r,17r}$	18.28	—	18.32	—	—	—
$\Delta^{2r,6c}$	18.99	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

TABLE VI

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON CARBOWAX 20M

Isomer	ECL	E1*	E2**	E3***	E4***	E5 [†]
$\Delta^{2t,6t}$	19.28	19.28	—	—	—	—
$\Delta^{3t,7t}$	18.64	—	—	18.63	—	—
$\Delta^{4t,8t}$	18.36	—	—	—	—	18.40
$\Delta^{5t,9t}$	18.44	—	—	—	—	—
$\Delta^{6t,10t}$	18.45	—	—	—	18.46	—
$\Delta^{7t,11t}$	18.43	—	—	—	—	—
$\Delta^{8t,12t}$	18.52	—	—	—	—	18.40
$\Delta^{9t,13t}$	18.55	—	—	—	—	—
$\Delta^{10t,14t}$	18.56	—	18.56	—	—	—
$\Delta^{11t,15t}$	18.64	—	—	—	18.64	—
$\Delta^{12t,16t}$	18.88	—	18.89	18.83	—	—
$\Delta^{13t,17c}$	18.78	18.81	—	—	—	—
$\Delta^{2t,6c}$	19.26	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

[†] No separation.

ECL value of the series. On the three non-polar stationary phases (Apiezon L, SE-30 and OV-101) these isomers gave ECL values below 18.00, except in the case of the $\Delta^{2t,6t}$ isomer. The lowest values were recorded on Apiezon L, ranging from 17.40 to 18.19, while SE-30 and OV-101 gave an almost identical retention pattern, with ECL values ranging from 17.56 to 18.17 and 17.55 to 18.14, respectively.

On the semi-polar XE-60 phase, the ECL values ranged from 17.84 to 18.84,

TABLE VII

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON FFAP

Isomer	ECL	F1*	F2**	F3**	F4***	F5 [†]
$\Delta^{2t,6t}$	19.32	—	—	—	—	—
$\Delta^{3t,7t}$	18.63	—	—	18.64	—	—
$\Delta^{4t,8t}$	18.42	—	—	18.40	—	—
$\Delta^{5t,9t}$	18.41	—	—	—	—	18.54
$\Delta^{6t,10t}$	18.46	—	—	—	—	—
$\Delta^{7t,11t}$	18.45	18.45	—	—	—	—
$\Delta^{8t,12t}$	18.47	—	—	—	—	—
$\Delta^{9t,13t}$	18.51	—	18.54	—	—	—
$\Delta^{10t,14t}$	18.55	—	—	—	—	18.54
$\Delta^{11t,15t}$	18.68	—	—	—	18.76	—
$\Delta^{12t,16t}$	18.88	18.90	—	—	—	—
$\Delta^{13t,17c}$	18.86	—	18.85	—	18.87	—
$\Delta^{2t,6c}$	19.31	—	—	—	—	—

* Base line separation.

** Twin peak separation.

*** Shoulder separation.

[†] No separation.

TABLE VIII

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON DEGA

Isomer	ECL	G1*	G2**	G3***	G4***	G5†
$\Delta^{2t,6t}$	19.63	—	—	—	—	—
$\Delta^{3t,7t}$	18.82	—	—	18.80	—	18.72
$\Delta^{4t,8t}$	18.50	—	—	—	—	—
$\Delta^{5t,9t}$	18.55	—	—	—	18.56	—
$\Delta^{6t,10t}$	18.59	—	—	18.60	—	—
$\Delta^{7t,11t}$	18.61	—	18.61	—	—	—
$\Delta^{8t,12t}$	18.67	—	—	—	—	18.72
$\Delta^{9t,13t}$	18.73	—	—	—	18.59	—
$\Delta^{10t,14t}$	18.78	18.76	—	—	—	—
$\Delta^{11t,15t}$	18.86	—	18.86	—	—	—
$\Delta^{12t,16t}$	19.16	—	—	—	—	—
$\Delta^{13t,17t}$	19.13	19.15	—	—	—	—
$\Delta^{2t,6c}$	19.74	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

† No separation.

with the $\Delta^{4t,8t}$ isomer exhibiting the lowest value in the series. Unlike the behaviour of the $\Delta^{13t,17t}$ isomer¹, the $\Delta^{13t,17t}$ isomer gave an ECL value which was slightly higher than that observed for the $\Delta^{12t,16t}$ isomer. There was once more a significant and gradual increase in the ECL value as the double bonds moved closer to the ω -end of the molecule.

Of the polar phases, the newly employed SP 2330 phase furnished the highest

TABLE IX

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON SILAR 10C

Isomer	ECL	H1*	H2**	H3**	H4***	H5†
$\Delta^{2t,6t}$	19.64	—	—	—	—	—
$\Delta^{3t,7t}$	18.75	18.76	—	—	—	18.66
$\Delta^{4t,8t}$	18.44	—	—	18.44	—	—
$\Delta^{5t,9t}$	18.54	—	—	—	18.58	—
$\Delta^{6t,10t}$	18.60	—	—	—	—	18.66
$\Delta^{7t,11t}$	18.64	—	—	—	—	—
$\Delta^{8t,12t}$	18.71	—	—	—	18.68	—
$\Delta^{9t,13t}$	18.77	—	—	18.77	—	—
$\Delta^{10t,14t}$	18.80	—	18.81	—	—	—
$\Delta^{11t,15t}$	18.92	—	—	—	—	—
$\Delta^{12t,16t}$	19.22	19.21	—	—	—	—
$\Delta^{13t,17t}$	19.21	—	19.22	—	—	—
$\Delta^{2t,6c}$	19.84	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

† No separation.

TABLE X

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC C₁₈ METHYL ESTERS AND THEIR MIXTURES ON DEGS

Isomer	ECL	I1*	I2**	I3**	I4***	I5‡
$\Delta^{2t,6t}$	19.59	19.61	—	—	—	—
$\Delta^{3t,7t}$	18.99	18.98	—	—	—	—
$\Delta^{4t,8t}$	18.59	—	—	18.61	—	—
$\Delta^{5t,9t}$	18.62	—	—	—	18.73	—
$\Delta^{6t,10t}$	18.68	—	—	—	—	18.79
$\Delta^{7t,11t}$	18.68	—	—	—	—	—
$\Delta^{8t,12t}$	18.74	—	18.74	—	—	—
$\Delta^{9t,13t}$	18.80	—	—	—	—	—
$\Delta^{10t,14t}$	18.88	—	—	—	18.78	18.79
$\Delta^{11t,15t}$	18.99	—	—	18.98	—	—
$\Delta^{12t,16t}$	19.25	—	—	—	—	—
$\Delta^{13t,17c}$	19.24	—	19.23	—	—	—
$\Delta^{2r,6c}$	19.69	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

ECL values for most isomers. The $\Delta^{4t,8t}$ isomer gave the lowest ECL value in the series on almost every polar stationary phase. Carbowax 20M and FFAP produced quite similar ECL results with values ranging from 18.36 to 19.28 and 18.41 to 19.32, respectively. Silar 10C gave ECL values which were higher than those observed on DEGA, but were lower than those found on the DEGS phase; and the ECL values ranged from 18.44 to 19.64 (Silar 10C), 18.50 to 19.63 (DEGA) and 18.59 to 19.59

TABLE XI

EQUIVALENT CHAIN LENGTHS OF INDIVIDUAL *trans,trans*-DIOLEFINIC METHYL ESTERS AND THEIR MIXTURES ON SP 2330

Isomer	ECL	J1*	J2**	J3***	J4‡	J5‡
$\Delta^{2t,6t}$	19.81	—	—	—	—	—
$\Delta^{3t,7t}$	18.92	—	—	18.86	—	—
$\Delta^{4t,8t}$	18.58	—	18.59	18.58	—	—
$\Delta^{5t,9t}$	18.65	—	—	—	—	18.78
$\Delta^{6t,10t}$	18.69	18.69	—	—	—	—
$\Delta^{7t,11t}$	18.78	—	—	—	19.00	—
$\Delta^{8t,12t}$	18.87	—	—	—	—	18.78
$\Delta^{9t,13t}$	18.93	—	—	—	—	—
$\Delta^{10t,14t}$	18.97	—	18.91	—	—	—
$\Delta^{11t,15t}$	19.10	—	—	—	19.00	—
$\Delta^{12t,16t}$	19.22	—	—	—	—	—
$\Delta^{13t,17c}$	19.27	19.27	—	—	—	—
$\Delta^{2r,6c}$	19.93	—	—	—	—	—

* Baseline separation.

** Twin peak separation.

*** Shoulder separation.

‡ No separation.

TABLE XII

SEPARATION OF MIXTURES OF METHYL *trans,trans*-OCTADECADIENOATES

Stationary phase	Baseline	Degree of separation (difference in ECL)		
		Twin peak	Shoulder	No separation
APL	0.47 (A1)	≥0.29 (A2-3)	0.22 (A4)	0.19 (A5)
SE-30	0.51 (B1)	≥0.27 (B2-3)	0.20 (B4)	0.14 (B5)
OV-101	0.40 (C1)	≥0.24 (C2-3)	0.20 (C4)	0.16 (C5)
XE-60	0.42 (D1)	0.32 (D2)	>0.20 (D3-4)	0.14 (D5)
Carbowax 20M	0.50 (E1)	0.32 (E2)	>0.19 (E3-4)	0.16 (E5)
FFAP	0.43 (F1)	≥0.21 (F2-3)	0.18 (F4)	0.14 (F5)
DEGA	0.35 (G1)	0.25 (G2)	>0.18 (G3-4)	0.15 (G5)
Silar 10C	0.47 (H1)	≥0.33 (H2-3)	0.17 (H4)	0.15 (H5)
DEGS	0.60 (I1)	≥0.40 (I2-3)	0.26 (I4)	0.20 (I5)
SP 2330	0.58 (J1)	0.39 (J2)	0.34 (J3)	<0.32 (J4-5)

(DEGS). The SP 2330 stationary phase was claimed as a semi-polar phase⁹, but for these *trans,trans* isomers this phase gave the highest recorded ECL values for nine isomers in the series. The ECL values ranged from 18.58 to 19.81.

No sign of decomposition of the $\Delta^{22,6t}$ and $\Delta^{32,7t}$ isomers on the polar stationary phases was observed, as previously reported¹⁰ in the case of the corresponding diacetylenic esters.

Mixtures of these isomers were also examined (Tables II–XI) and the results are summarised in Table XII. The degree of separation is described as baseline, twin peak and shoulder. On the non-polar stationary phases the efficiency of separation of the isomers was best on OV-101, while SE-30 and APL gave almost identical performances. Amongst the polar phases, FFAP, DEGA and Silar 10C were superior in separating these isomers. The ECL values are highly reproducible on all stationary phases (including the newly studied SP 2330 phase), as demonstrated by the examination of the mixtures of these isomers.

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