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Note

Gas–liquid chromatography characteristics of some long-chain acetylenic methyl esters

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Systematic separation factors have been used to help in the identification of long-chain methylene-interrupted polyolefinic methyl esters and also to predict their chromatographic behaviour[1–6]. Although the use of these separation factors was developed with methyl esters it has been shown[7] that the same separation factors can be used when the $-CO_2CH_3$ group is replaced by $-CH_2OH$, $-CH_2O_2C \cdot CH_3$ and $-CH_3$. It was also found[8] that the presence of a common non-methylene-interrupted olefinic centre in polyolefinic methyl esters does not alter the Type II separation factors; the same Type II factors were obtained from the normal methylene-interrupted esters and from the Δ^5-polyolefinic esters from conifer lipids[9].

Recently it has been shown[6,10–12] that lipids from certain moss species contain unusual C_{18} and C_{20} polyunsaturated acids in which an acetylenic group and olefinic groups are present in a methylene-interrupted pattern. An investigation has been carried out to determine how far the systematic relationships found for polyolefinic esters could be applied to these polyolefinic-acetylenic esters.

EXPERIMENTAL AND RESULTS

Methyl esters from the lipids of the moss, *Fontinalis antipyretica*, were separated by preparative thin-layer chromatography on silica gel[12] and the separated acetylenic methyl esters were examined by gas–liquid chromatography using columns of different liquid phase polarity. The major peaks in the chromatograms (76.3, 4.5 and 16.7%, respectively) were due to 18:3 6a,9,12, 18:4 6a,9,12,15 and 20:3 8a,11,14. A minor component (about 1%) was tentatively identified by the use of separation factors as 18:2 6a,9.

Retention data for these acetylenic acids and for standard olefinic acids on three different columns are given in Table I. On each of these columns the C_{18} acetylenic acids have a greater retention than 18:4 6,9,12,15, and 20:3 8a,11,14 has a greater retention than 20:5 5,8,11,14,17.

Type I and Type II separation factors are given in Table II. Type I 6/9 and 8/11 factors show the effect of introducing an additional olefinic centre into the carboxyl end-chain of polyolefinic systems and Type I 6a/9 and 8a/11 factors show the effect of introducing an acetylenic group into the same system. Since the 6a/9 and 8a/11 factors are higher than the normal 6/9 and 8/11 factors this indicates that the introduc-

TABLE I

RETENTION DATA OF C_{18} AND C_{20} POLYOLEFINIC ACIDS

Columns, polyester open tubular. Polarity $ECL_{18:3\ \omega-3}$; $a = 19.79$, $b = 19.50$, $c = 19.00$. t_R = Retention time relative to 18:0.

Methyl ester		Column					
		a		*b*		*c*	
		t_R	*ECL*	t_R	*ECL*	t_R	*ECL*
18:1	9	1.13	18.35	1.11	18.31	1.06	18.16
18:2	9, 12	1.39	18.94	1.30	18.79	1.19	18.44
18:3	6, 9, 12	1.64	19.42	1.45	19.12	1.32	18.74
18:3	9, 12, 15	1.85	19.79	1.62	19.50	1.45	19.00
18:4	6, 9, 12, 15	2.20	20.26	1.83	19.81	1.60	19.27
20:2	11, 14	2.70	20.91	2.48	20.73	2.47	20.41
20:3	8, 11, 14	3.18	21.31	2.78	21.03	2.64	20.64
18:2	6a, 9	2.60	20.73	2.26	20.42	1.95	19.80
18:3	6a, 9, 12	3.18	21.31	2.63	20.87	2.18	20.09
18:4	6a, 9, 12, 15	4.30	22.17	3.34	21.68	2.65	20.65
20:3	8a, 11, 14	6.14	23.21	5.05	22.81	4.49	22.02

TABLE II

TYPES I AND II SEPARATION FACTORS

Separation factor	Methyl ester		Column		
			a	*b*	*c*
Type I					
6/9	18:4 18:3	6, 9, 12, 15 9, 12, 15	1.19	1.13	1.10
6/9	18:3 18:2	6, 9, 12 9, 12	1.19	1.12	1.11
6a/9	18:4 18:3	6a, 9, 12, 15 9, 12, 15	2.32	2.06	1.83
6a/9	18:3 18:2	6a, 9, 12 9, 12	2.29	2.02	1.83
6a/9	18:2 18:1	6a, 9 9	2.30	2.04	1.84
8/11	20:3 20:2	8, 11, 14 11, 14	1.18	1.12	1.07
8a/11	20:3 20:2	8a, 11, 14 11, 14	2.27	2.04	1.82
Type II					
3/6	18:4 18:3	6, 9, 12, 15 6, 9, 12	1.34	1.26	1.21
3/6	18:3 18:2	9, 12, 15 9, 12	1.33	1.25	1.22
3/6	18:4 18:3	6a, 9, 12, 15 6a, 9, 12	1.35	1.27	1.22
3/9	18:3 18:1	9, 12, 15 9	1.64	1.46	1.37
3/9	18:4 18:2	6a, 9, 12, 15 6a, 9	1.65	1.48	1.36
6/9	18:2 18:1	9, 12 9	1.23	1.17	1.12
6/9	18:3 18:2	6a, 9, 12 6a, 9	1.22	1.16	1.12

tion of the acetylenic bond has a greater effect on retention than the introduction of an olefinic bond into the same positions and the effect is greater the more polar the liquid phase. There is good agreement between the Type II factors for olefinic esters and olefinic-acetylenic esters. These separation factors show the effect of introducing an additional olefinic bond between the existing unsaturation and the end-methyl group. The replacement of an acetylenic group for an olefinic group at the carboxyl end of the chain does not significantly alter the Type II separation factors.

An approach has been made to the problem of correlating retention data with liquid phase polarity[13]. Linear relationships were found between the equivalent chain-lengths (ECL values) for a number of C_{16}–C_{22} polyolefinic methyl esters and the ECL values for methyl linolenate (18:3 $n-3$). The data were computer processed with the formula

$$\mathrm{ECL}_X = a_X \cdot \mathrm{ECL}_{(18:3\ n-3)} + b_X$$

where a_X and b_X are the computer-derived constants for the fatty acid methyl ester (X). Various applications of this approach have been discussed recently[6]. The variations of the ECL values of the acetylenic-olefinic methyl esters with liquid phase polarity could also be represented by the above equation where

X = 18:3 6a,9,12, $a = 1.51$, $b = -8.58$;
X = 20:3 8a,11,14, $a = 1.50$, $b = -6.53$;
X = 16:4 6a,9,12,15, $a = 1.75$, $b = -12.49$.

The mean deviations of calculated ECL values and experimental values (20 determinations) were 0.04, 0.05 and 0.05 ECL units, respectively. The a values for 18:3 6a,9,12 and 20:3 8a,11,14 are similar to the mean value of 1.50 for pentaenoic methyl esters, and the value for 18:4 6a,9,12,15 similar to the value of 1.78 for hexaenoic methyl esters. The value for the component tentatively identified as 18:2 6a,9 is identical to the mean value for tetraenoic methyl esters.

It may be deduced from a consideration of the a values that, for acetylenic-polyolefinic esters, the contribution of the acetylenic group to the variation of retention with polarity of liquid phase is similar to that of three olefinic groups. A similar conclusion was made by Hofstetter, Sen and Holman[14] from ECL values on polar and non-polar liquid phases.

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