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The analysis of oils and fats by gas chromatography

Part IX. Separation factors of non-methylene-interrupted polyolefinic esters

Systematic relationships have been shown to exist between the gas chromatographic retention times of methylene-interrupted polyolefinic fatty acid esters of the same chain length[1] and these relationships have been used in terms of separation factors to help in the identification of methyl esters and also to predict their chromatographic behaviour[2–5]. Although the use of these separation factors was developed with methylene-interrupted polyolefinic methyl esters it has been shown[6] that the same separation factors can be used with methylene-interrupted polyolefinic compounds in which the carbomethoxy group has been replaced by other functional groups.

Recently it has been shown[7] that the leaf lipids of various conifer species contain a series of C_{18} and C_{20} non-methylene-interrupted polyolefinic fatty acids with an olefinic bond in the $\Delta 5$ position. An investigation has been carried out to determine how far the systematic relationships found for the methylene-interrupted polyolefinic esters could be applied to these non-methylene-interrupted esters.

Retention data for various C_{18} and C_{20} olefinic acids on two high-polarity and two low-polarity stationary phases are given in Table I. The differences in equivalent chain length (ECL) values between the phase of highest polarity and that of lowest polarity are approximately proportional to the number of olefinic bonds present in the fatty acid methyl ester. This is analagous to the findings of HOFSTETTER *et al.*[8] who showed that the differences in ECL values from a polar and a non-polar phase were approximately proportional to the number of olefinic bonds present in a non-conju-

TABLE I

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

Columns: a = EGSS-X open tubular (new); b = EGSS-X open tubular (1 year); c = EGSS-X SCOT (new); d = EGSS-X SCOT (6 months).

Methyl ester		*Column*								*ECL(a)–ECL(d)*
		a		*b*		*c*		*d*		
		t_r[a]	*ECL*	t_r	*ECL*	t_r	*ECL*	t_r	*ECL*	
18:1	9	1.16	18.49	1.15	18.44	1.08	18.24	1.08	18.22	0.27
18:2	5,9	1.34	18.93	1.29	18.79	1.17	18.48	1.14	18.32	0.61
18:2	9,12	1.48	19.25	1.45	19.16	1.28	18.79	1.25	18.63	0.62
18:3	5,9,12	1.72	19.74	1.66	19.57	1.37	19.02	1.32	18.83	0.91
18:3	6,9,12	1.84	19.96	1.75	19.74	1.48	19.18	1.40	18.95	1.01
18:3	9,12,15	2.07	20.33	1.97	20.12	1.60	19.51	1.57	19.26	1.07
20:3	5,11,14	3.10	21.66	3.01	21.45	2.62	20.89	2.51	20.56	1.10
20:3	8,11,14	3.22	21.77	3.14	21.57	2.76	21.04	2.68	20.77	1.00
18:4	5,9,12,15	2.37	20.78	2.20	20.46	1.71	19.71	1.65	19.40	1.38
18:4	6,9,12,15	2.53	20.99	2.35	20.69	1.88	19.89	1.75	19.56	1.43
20:4	5,11,14,17	4.21	22.59	4.02	22.30	3.33	21.60	3.11	21.18	1.41
20:4	8,11,14,17	4.53	22.87	4.30	22.55	3.45	21.71	3.33	21.37	1.50

[a] t_r = retention time relative to 18:0 = 1.00.

TABLE II

TYPE I AND TYPE II SEPARATION FACTORS

Carboxyl end chain ratio	*Methyl ester*		*Column*			
			a	*b*	*c*	*d*
Type I						
6/9	18:4	6,9,12,15	1.22	1.19	1.17	1.11
	18:3	9,12,15				
6/9	18:3	6,9,12	1.24	1.21	1.16	1.12
	18:2	9,12				
5/9	18:4	5,9,12,15	1.14	1.12	1.06	1.05
	18:3	9,12,15				
5/9	18:3	5,9,12	1.16	1.14	1.06	1.05
	18:2	9,12				
Type II						
3/6	18:4	6,9,12,15	1.38	1.34	1.27	1.25
	18:3	6,9,12				
3/6	18:3	9,12,15	1.40	1.36	1.25	1.25
	18:2	9,12				
3/6	18:4	5,9,12,15	1.38	1.33	1.25	1.25
	18:3	5,9,12				
3/6	20:4	8,11,14,17	1.41	1.37	1.25	1.24
	20:3	8,11,14				
3/6	20:4	5,11,14,17	1.36	1.34	1.27	1.24
	20:3	5,11,14				
3/9	18:3	9,12,15	1.78	1.71	1.47	1.45
	18:1	9				
3/9	18:4	5,9,12,15	1.77	1.71	1.46	1.45
	18:2	5,9				
6/9	18:2	9,12	1.28	1.26	1.18	1.16
	18:1	9				
6/9	18:3	5,9,12	1.28	1.28	1.17	1.16
	18:2	5,9				

gated unsaturated fatty acid ester, regardless of the positions of the olefinic bonds in the molecule.

Type I and II separation factors are given in Table II. It is found that Type I 5/9 factors are lower than Type I 6/9 factors. This indicates that the introduction of an olefinic bond into the carbon chain nearer to the carbomethoxy group has a greater effect on retention values at position 6 than at position 5. This is in agreement with the results of GUNSTONE *et al.*[9] for isomeric methyl octadecenoates, the $\Delta 5$ isomer having lower retention values on polar phases than the $\Delta 6$ isomer. However, the 5/9 separation factors are lower than would be expected on considering the retention values of the $\Delta 5$ and $\Delta 6$ isomers. When retention data for a methylene-interrupted polyolefinic ester are calculated by a summation of increments of retention values of the corresponding mono-olefinic compounds, the results are lower than the values found experimentally. There is an "exaltation" of retention values for methylene-interrupted polyunsaturated compounds. In the calculation of Type I 6/9 factors each of the pair of compounds has methylene-interrupted unsaturation but for Type I 5/9 factors only one of the pair has this type of unsaturation.

There is a good agreement between Type II separation factors for methylene-interrupted olefinic esters and those for non-methylene-interrupted esters. Type II

factors show the effect of introducing an additional olefinic bond between the existing unsaturation and the end-methyl group. Each of the pair of compounds used to find the Type II factor has either unsaturation of the methylene-interrupted type or of the non-methylene-interrupted type.

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