Notes

CHROM. 3826

The analysis of oils and fats by gas chromatography

VI. Calculation of equivalent chain length and modified equivalent chain length values

The use of separation factors for the tentative identification of methylene-interrupted olefinic esters has been reviewed recently[1]. HAKEN[2,3] has applied the idea of separation factors to the correlation of GLC retention data and structural parameters by using an equation of the type:

$$V_{R(x+2,y+1)} = V_{R(x,y)} \times V_{R(x+2,y)} \times V_{R(x,y+1)} \quad (1)$$

where

x = total carbon chain length
y = number of methylene-interrupted double bonds
$V_{R(x+2,y)}$ = ethylene-unit separation factor[2,4]
$V_{R(x,y+1)}$ = Type I separation factor[5]

If eqn. (1) is put into a logarithmic form it becomes:

$$ECL_{(x+2,y+1)} = ECL_{(x,y)} + 2 + k_I \quad (2)$$

where k_I is the difference in equivalent chain length (ECL) values of pairs of esters used to calculate Type I separation factors. In the ECL system the ethylene-unit separation factor should be 2. However, it has been shown[1] that, for many liquid phases, the semi-log plot of saturated methyl esters is not parallel to those of unsaturated esters and the difference in ECL values of adjacent members of homologous series of unsaturated esters is less than 2. If modified equivalent chain length ($MECL$) values[6] are used the ethylene-unit separation factors are much closer to the expected value of 2. If eqn. (2) is used in the form

$$MECL_{(x+2,y+1)} = MECL_{(x,y)} + 2 + k_I \quad (3)$$

then agreement between calculated and determined $MECL$ values would be expected to be better than between the corresponding ECL values.

JAMIESON AND REID[7] have shown that the fatty acids of the leaf lipids of *Myosotis scorpioides* contain relatively large proportions of 18:2ω6, 18:3ω6, 18:3ω3, and 18:4ω3 and smaller proportions of 18:1ω9 and 18:0. Using the retention times of the methyl esters of these acids and of the mono-olefinic acids of rape seed oils as standards, $MECL$ values for a number of C_{20} and C_{22} esters occuring in natural lipids may be calculated using eqn. (3).

Experimental

Separations of methyl esters were carried out on a PE 800 gas chromatograph with the following columns:

TABLE I

AVERAGE ETHYLENE-UNIT SEPARATION FACTORS FOR HOMOLOGOUS METHYL ESTERS

Type of ester	*DEGS*			*EGSS-X*	*BDS*
	150°	*170°*	*190°*	*180°*	*200°*
Saturated	2.17	1.96	1.77	1.82	1.87
Monoene	2.06	1.90	1.75	1.78	1.81
Diene	2.06	1.90	1.75	1.78	1.81
Triene	2.06	1.90	1.77	1.79	1.79
Tetraene	2.06	1.91	1.76	1.78	1.79
Pentaene	2.04	1.90	1.73	1.78	1.81

(i) EGSS-X, 50 m × 0.5 mm stainless steel, open tubular; 180°
(ii) BDS, 8% on HMDS Chromosorb W; 6 ft. × $\frac{1}{8}$ in. stainless steel; 200°

ECL values were calculated from the retention data using saturated methyl esters as standards; *MECL* values were calculated using the retentions of mono-olefinic methyl esters from rape seed oil as standards[1]. *ECL* and *MECL* values on DEGS were calculated from the retention data of ACKMAN[8].

Results and discussion

Table I shows ethylene-unit separation factors obtained by considering fatty acid methyl esters of different homologous series; a homologous series being defined as one with varying chain length, the same number of double bonds and a constant carbon-end chain in each series. It is apparent that, with the stationary phases examined, there is a greater constancy between the different series of olefinic esters than between these esters and those of the saturated series. In a semi-log plot of retention *vs.* chain length the lines for the olefinic esters would be virtually parallel to each other but would converge with the saturate line.

TABLE II

k_I VALUES FOR PAIRS OF ESTERS USED TO CALCULATE TYPE I SEPARATION FACTORS

Carbon-end chain ratio	*DEGS*			*EGSS-X*	*BDS*
	150°	*170°*	*190°*	*180°*	*200°*
6/9	0.51	0.51	0.51	0.56	0.38
8/11	0.46	0.47	0.52	0.54	0.30
5/8	0.29	0.43	0.53	0.44	0.22
4/7	0.42	0.43	0.46	0.53	0.28

Separation factors obtained by difference of *ECL* values of pairs of esters used to calculate Type I separation factors are shown in Table II. As has been found previously[9] there are variations in these values depending on the respective carbon-end chains. The variations for these values on DEGS decrease as the column temperature increases.

TABLE III

CALCULATION OF *ECL* AND *MECL* VALUES OF ESTERS OF THE TYPES $(x + 2, y + 1)$ AND $(x + 4, y + 2)$ FROM THE VALUES OF ESTERS OF THE TYPE (x, y)

$Ester_{(x,y)}$			$Ester_{(x+2,y+1)}$							$Ester_{(x+4,y+2)}$						
				ECL			*MECL*				*ECL*			*MECL*		
	ECL	*MECL*		*Calc.*	*Det.*	*Diff.*	*Calc.*	*Det.*	*Diff.*		*Calc.*	*Det.*	*Diff.*	*Calc.*	*Det.*	*Diff.*
DEGS 150°																
18:2ω6	19.07	18.77	20:3ω6	20.58	21.44	+0.14	21.26	21.29	−0.03	22:4ω6	24.19	23.62	+0.57	23.77	23.59	+0.18
18:3ω6	19.59	19.32	20:4ω6	22.10	21.71	+0.39	21.83	21.58	+0.25	22:5ω6	24.61	24.04	+0.57	24.34	24.00	+0.34
18:3ω3	19.96	19.70	20:4ω3	22.47	22.30	+0.17	22.21	22.22	−0.01	22:5ω3	24.98	24.39	+0.59	24.72	24.48	+0.24
18:4ω3	20.45	20.23	20:5ω3	22.96	22.61	+0.35	22.74	22.50	+0.24	22:6ω3	25.47	24.81	+0.66	25.25	24.93	+0.32
					Mean	+0.26			+0.11				+0.60			+0.27
DEGS 170°																
18:2ω6	19.22	18.82	20:3ω6	21.73	21.62	+0.11	21.33	21.32	+0.01	22:4ω6	24.24	23.94	+0.30	23.84	23.74	+0.10
18:3ω6	19.73	19.32	20:4ω6	22.24	22.00	+0.24	21.83	21.72	+0.11	22:5ω6	24.75	24.40	+0.35	24.34	24.22	+0.12
18:3ω3	20.32	19.82	20:4ω3	22.83	22.58	+0.25	22.33	22.33	0	22:5ω3	25.34	24.87	+0.47	24.84	24.72	+0.12
18:4ω3	20.65	20.33	20:5ω3	23.16	22.92	+0.24	22.71	22.58	+0.13	22:6ω3	25.67	25.26	+0.41	25.35	25.20	+0.15
					Mean	+0.21			+0.06				+0.38			+0.12
DEGS 190°																
18:2ω6	19.34	18.83	20:3ω6	21.85	21.80	+0.05	21.34	21.38	−0.04	22:4ω6	24.36	24.32	+0.04	23.85	23.90	−0.05
18:3ω6	19.85	19.33	20:4ω6	22.36	22.34	+0.02	21.84	21.92	−0.08	22:5ω6	24.87	24.71	+0.16	24.35	24.43	−0.08
18:3ω3	20.34	19.85	20:4ω3	22.85	22.90	−0.05	22.36	22.46	−0.10	22:5ω3	25.36	25.20	+0.16	24.87	24.92	−0.05
18:4ω3	20.85	20.39	20:5ω3	23.31	23.27	+0.04	22.90	22.96	−0.06	22:6ω3	25.87	25.72	+0.15	25.41	25.45	−0.04
					Mean	+0.02			−0.07				+0.13			−0.05
EGSS-X 180°																
18:2ω6	19.35	18.75	20:3ω6	21.91	21.81	+0.10	21.31	21.34	−0.03	22:4ω6	22.47	24.17	+0.30	23.87	23.80	+0.07
18:3ω6	19.90	19.32	20:4ω6	22.46	22.25	+0.21	21.98	21.80	+0.18	22:5ω6	25.02	24.69	+0.33	24.54	24.36	+0.18
18:3ω3	20.31	19.76	20:4ω3	22.87	22.78	+0.09	22.32	22.36	−0.04	22:5ω3	25.43	25.14	+0.29	24.88	24.82	+0.06
18:4ω3	20.88	20.36	20:5ω3	23.44	23.23	+0.21	22.92	22.81	+0.11	22:6ω3	26.00	25.68	+0.32	25.48	25.38	+0.10
					Mean	+0.15			+0.06				+0.31			+0.10
BDS 200°																
18:2ω6	18.91	18.52	20:3ω6	21.29	21.12	+0.17	20.90	20.88	+0.02	22:4ω6	23.67	23.29	+0.36	23.28	23.19	+0.09
18:3ω6	19.29	18.91	20:4ω6	21.67	21.44	+0.23	21.29	21.17	+0.12	22:5ω6	24.05	23.63	+0.42	23.57	23.49	+0.08
18:3ω3	19.64	19.30	20:4ω3	22.02	21.84	+0.18	21.68	21.62	+0.06	22:5ω3	24.40	24.04	+0.32	24.06	23.98	+0.08
18:4ω3	20.05	19.66	20:5ω3	22.43	22.09	+0.34	22.04	21.91	+0.13	22:6ω3	24.81	24.38	+0.41	24.42	24.34	+0.08
					Mean	+0.23			+0.08				+0.38			+0.08

Using eqns. (2) and (3), *ECL* and *MECL* values of esters of the type $(x + 2, y + 1)$ and $(x + 4, y + 2)$ were calculated from the values for the C_{18} esters (x, y) and the results are shown in Table III. The deviations of calculated values from determined values are less when *MECL* is used rather than *ECL*, the only exception being those values from DEGS at 190°. With the C_{20} esters the largest deviations are in the results for the 20:4ω6 and 20:5ω3 esters. For these esters a Type I 5/8 separation factor would give closer agreement than the use of the larger 6/9 value calculated from the C_{18} esters.

The above method of calculation could be extended by the use of Type II separation factors, *e.g.* for the EGSS-X column:

$$MECL_{20:2\omega 9} = MECL_{20:4\omega 3} - \text{Type II}_{3/9} = 22.32 - 1.76 = 20.56 \text{ (determined } 20.63)$$

$$MECL_{20:3\omega 9} = MECL_{20:4\omega 6} - \text{Type II}_{3/6} = 21.98 - 0.75 = 21.23 \text{ (determined } 21.19)$$

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