RETENTION TIME RELATIONSHIPS IN THE GAS CHROMATOGRAPHY OF THE METHYL ESTERS OF FATTY ACIDS

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INTRODUCTION

The success of polyester substrates[1–4] in the separation of the methyl esters of fatty acids has introduced the problem of identifying the large number of chromatographic peaks obtained. The retention times of fatty esters have been examined on a polar and non-polar hydrocarbon column[5], on aged and new polyester columns[6], and on two different polyester phases[7]. The procedures produce a variation in retention times but are of limited value in a systematic evaluation.

ACKMAN has reported several correlations between the structure of unsaturated methylene-interrupted fatty esters and retention data. The correlations show that:

(1) Esters of monounsaturated fatty acids with the same end carbon chain length exhibit a linear relationship between the logarithm of their retention times, $\log V_R$, and the total chain length[8].

(2) Normal methylene-interrupted polyunsaturated acid esters with the same number of double bonds and the end carbon chain length constant also exhibit a linear relationship between $\log V_R$ and the total chain length. The slope of the plots is the same as that for the monounsaturated acids provided the end carbon chain length is in all cases constant[8].

(3) Separation factors can be derived by dividing retention data of one methyl fatty ester by the lesser value of another comparable ester. The three types of separation factors reported[9] consider esters of the same total chain length with varying unsaturation and (a) the same and (b) different carbon chain lengths, and (c) esters of the same total chain length, same number of double bonds and different end carbon chains.

These methods apart from being somewhat involved in their application do not give an overall picture of the relative effects of the various structural parameters on the retention behaviour.

A single correlation has been reported by HAKEN AND SOUTER[10,11] where all the data is represented nomographically using three structural parameters as the co-ordinate scales. The data of the saturated esters has been considered; these fall on the end carbon chain axis at a point corresponding to a large value and approxi-

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mating the total chain length. If this interpretation is valid, it implies that the saturated and unsaturated esters provide a mutually compatible set of data.

Retention data and structural features of sterols and their derivatives have been studied and the procedure of separation factors has been developed and extended by CLAYTON[12]. For each isolated double bond introduced into the sterol ether molecule the retention time of the saturated parent compound varies by a constant factor depending on the position of the unsaturation.

The relationship is shown by the equation:

$$V_{R(s+x)} = V_s \times k_x$$

where $V_{R(s+x)}$ is the retention time of the compound with a double bond in position x, V_s is the retention time of the parent compound and k_x is a constant characteristic of the position of the unsaturation.

With two non-interacting double bonds at positions x and y, the retention time $V_{R(s+x+y)}$ is given by the equation:

$$V_{R(s+x+y)} = V_R \times k_x \times k_y$$

where k_y is the retention factor characteristic of the second double bond.

The retention time of a polysubstituted steroid with non-interacting substituent groups a, b, c, ...etc., might be expressed by the general equation:

$$V_{R(n+a+b+c\cdots)} = V_R \times k_a \times k_b \times k_c \times \ldots \text{etc.}$$

where $V_{R(n+a+b+c)}$ is the retention time of the total structure, V_R is the retention time of the unsubstituted nucleus and k_a, k_b, k_c, ...etc. are constants characteristic of the substituent a, b, c, ...etc. and their individual structural position.

It is shown here that the methylene-unit separation factor reported by JAMES AND MARTIN[13] with the methyl esters of the n-saturated fatty acids is of more general application. Methyl esters of different chain length, constant number of double bonds and constant end carbon chain exhibit a constancy throughout the series of esters studied, while a relationship similar to that developed by CLAYTON[12] is reported where retention times of the fatty esters of different chain length and degree of unsaturation may be calculated, the end carbon chain length in a particular case being constant.

DATA

The retention data are shown in Table I and are those used by ACKMAN[8,9] and ACKMAN AND BURGHER[14]. The chromatography was conducted substantially by FARQUHAR and his coworkers[15] using an ionisation detector with a ethylene glycol adipate column (20% w/w on acid and alkali washed Celite 545; 100–120 mesh) operated at 197°.

A number of provisional identifications (noted in Table I) were made by FARQUHAR and this data where used are compatible with the correlations of ACKMAN[8,9] and of HAKEN AND SOUTER[11].

TABLE I

SEPARATION FACTORS OF ESTERS OF FATTY ACIDS WITH VARYING CHAIN LENGTH, SAME NUMBER OF DOUBLE BONDS AND SAME END CHAIN LENGTH WITH EACH SERIES

Fatty acid chain length and No. of double bonds	*Position of double bonds*	*End carbon chain length*	$\frac{V_{R\,(x+2,y)}}{V_{R\,(x,y)}}$	
14:0	—	—	0.302	1.82
12:0	—	—	0.165	
16:0	—	—	0.550	1.82
14:0	—	—	0.302	
18:0	—	—	1.00	1.82
16:0	—	—	0.550	
20:0	—	—	1.82	1.82
18:0	—	—	1.00	
22:0	—	—	3.30	1.81
20:0	—	—	1.82	
18:3	9, 12, 15	3	1.72	1.81
16:3	7, 10, 13	3	0.95	
20:3	11, 14, 17[a]	3	3.10	
18:3	9, 12, 15	3	1.72	1.80
22:3	13, 16, 19	3	5.65	
20:3	11, 14, 17[a]	3	3.10	1.82
20:4	8, 11, 14, 17	3	3.51	1.78
18:4	6, 9, 12, 15	3	1.97	
22:4	10, 13, 16, 19	3	6.40	1.82
20:4	8, 11, 14, 17	3	3.51	
22:5	7, 10, 13, 16, 19	3	7.00	1.81
20:5	5, 8, 11, 14, 17	3	3.85	
18:2	11, 14	4	1.45	1.80
16:2	9, 12	4	0.810	
18:2	9, 12	6	1.34	1.80
16:2	7, 10	6	0.745	
20:2	11, 14	6	2.45	1.82
18:2	9, 12	6	1.34	
22:2	13, 16	6	4.35	1.80
20:2	11, 14	6	2.45	
18:3	6, 9, 12	6	1.54	1.80
16:3	4, 7, 10	6	0.86	
20:3	8, 11, 14	6	2.76	1.80
18:3	6, 9, 12	6	1.54	
22:3	10, 13, 16[a]	6	5.00	1.81
20:3	8, 11, 14	6	2.76	
22:4	7, 10, 13, 16	6	5.50	1.80
20:4	5, 8, 11, 14	6	3.04	
18:2	8, 11	7	1.33	1.80
16:2	6, 9	7	0.74	
20:2	10, 13	7	2.39	1.80
18:2	8, 11	7	1.33	
18:1	9	9	1.12	1.80
16:1	7	9	0.625	
20:1	11	9	2.02	1.80
18:1	9	9	1.12	
22:1	13	9	3.68	1.80
20:1	11	9	2.02	
20:2	8, 11	9	2.32	1.80
18:2	6, 9	9	1.29	
22:2	10, 13[a]	9	4.20	1.81
20:2	8, 11	9	2.32	
			Average	1.81

[a] Provisional identification.

DISCUSSION AND RESULTS

Table I shows separation factors obtained by considering fatty esters of different total chain length but with the same number of double bonds, and with the same end carbon chain length. The first five separation factors (*i.e.* saturated esters) are analogous to the methylene separation factors reported by JAMES AND MARTIN[13] and occur with all homologous series that produce a linear relationship of log V_R with total chain length. It is apparent, however, that a constant value is obtained with all of the series of esters independent of unsaturation providing the end carbon chain length is the same in any series. The data available allow only a comparison of esters with the chain length increasing by an ethylene unit, but it would be expected that the same situation would occur if the chain length were increased by a methylene group throughout the whole series of esters.

Separation factors described by ACKMAN[9] as type I, where pairs of esters are considered with the same chain length and the same end carbon chain, but with different numbers of double bonds are shown in Table II. While it is apparent that a slightly lower value is obtained with increasing unsaturation or decreasing end carbon chain length a relatively constant value is obtained for all of the pairs of esters examined. For simplicity the esters considered are shown in all the tables in terms of x and y, the total carbon chain length and the number of methylene-interrupted double bonds present respectively.

In Table III retention data of esters of the type $(x + 2, y + 1)$ are shown calculated from data of esters which may be shown as (x, y) by multiplication of the average separation factors shown in Tables I and II according to the relationship:

$$V_{R\,(x+2,\,y+1)} = V_{R\,(x,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x,y+1)}$$
$$= V_{R\,(x,y)} \times 1.81 \times 1.12$$

Table IV similarly shows data calculated for esters of the type $(x + 4, y + 1)$ by the formula:

$$V_{R\,(x+4,\,y+1)} = V_{R\,(x,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x+2,\,y)} \times V_{R\,(x,\,y+1)}$$
$$= V_{R\,(x,y)} \times 1.81 \times 1.81 \times 1.12$$

The deviations from the experimentally determined values are shown in Table III and IV. CLAYTON[12] has shown that with bifunctional steroids agreement within $\pm$ 4.0 % is obtained and with certain sapogenins calculated from the work of VANDENHEUVEL AND HORNING[16] agreement of $\pm$2.0 % is possible. The variations experienced in this work are comparable with the earlier work; and as the values reported are in general lower than those determined experimentally, it would be possible to decrease the average variation by a slight modification of the separation factors.

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TABLE II

SEPARATION FACTORS OF ESTERS OF FATTY ACIDS WITH THE SAME CHAIN LENGTH, VARYING UNSATURATION, AND THE SAME END CARBON CHAIN LENGTH WITH EACH SERIES

Fatty acid chain length and No. of double bonds	*Position of double bonds*	*End carbon chain length*	$\frac{V_{R\,(x,y+1)}}{V_{R\,(x,y)}}$	
16:1	9	7	0.634	1.15
16:0	—	—	0.550	
16:1	7	9	0.625	1.13
16:0	—	—	0.550	
18:1	9	9	1.12	1.12
18:0	—	—	1.00	
20:1	11	9	2.02	1.11
20:0	—	—	1.82	
22:1	13	9	3.68	1.10
22:0	—	—	3.30	
16:3	6, 9, 12	4	0.904	1.11
16:2	9, 12	4	0.810	
18:4	6, 9, 12, 15	3	1.97	1.14
18:3	9, 12, 15	3	1.72	
20:5	5, 8, 11, 14, 17	3	3.85	1.10
20:4	8, 11, 14, 17	3	3.51	
22:4	10, 13, 16, 19	3	6.40	1.13
22:3	13, 16, 19	3	5.65	
20:4	8, 11, 14, 17	3	3.51	1.13
20:3	11, 14, 17	3	3.10	
22:5	4, 7, 10, 13, 16	3	7.00	1.09
22:4	7, 10, 13, 16	3	6.40	
16:3	4, 7, 10	6	0.86	1.15
16:2	7, 10	6	0.745	
18:3	6, 9, 12	6	1.54	1.13
18:2	9, 12	6	1.34	
20:3	8, 11, 14	6	2.76	1.12
20:2	11, 14	6	2.45	
20:4	5, 8, 11, 14	6	3.04	1.10
20:3	8, 11, 14	6	2.76	
22:3	10, 13, 16	6	5.00	1.13
22:2	13, 16	6	4.35	
22:5	4, 7, 10, 13, 16	6	6.09	1.11
22:4	7, 10, 13, 16	6	5.50	
22:4	7, 10, 13, 16	6	5.50	1.10
22:3	10, 13, 16	6	5.00	
16:2	9	7	0.74	1.16
16:1	9	7	0.634	
18:2	6, 9	9	1.29	1.14
18:1	9	9	1.120	
20:2	8, 11	9	2.32	1.14
20:1	11	9	2.02	
22:2	10, 13	9	4.20	1.14
22:1	13	9	3.68	
			Average	1.12

TABLE III

CALCULATION OF RETENTION DATA OF ESTERS OF THE TYPE $(x + 2, y + 1)$ FROM THAT OF ESTERS OF THE TYPE (x,y)

$V_{R(x,y)}$			$V_{R(x+2,\,y+1)}$					
Fatty acid	*End carbon chain length*	$V_{R(x,y)}$	*Fatty acid*	*End carbon chain length*	*Determined*	*Calculated*	*Error*	%
16:0	—	0.55	18 1	9	1.12	1.11	—0.01	—0.90
18:0	—	1.00	20:1	9	2.02	2.02	0	0
20:1	—	1.82	22:1	9	3.68	3.67	—0.01	—0.27
16:3	3	0.950	18:4	3	1.97	1.92	—0.05	—2.40
18:3	3	1.72	20:4	3	3.51	3.47	—0.04	—1.13
20:4	3	3.51	22:5	3	7.00	7.02	—0.02	—0.28
20:5	3	3.85	22:6	3	7.75	7.70	—0.05	—0.64
16:2	6	0.745	18:3	6	1.54	1.50	—0.04	—2.60
18:2	6	1.34	20:3	6	2.76	2.69	—0.07	—2.53
18:3	6	1.54	20:4	6	3.04	3.09	0.05	+1.64
20:3	6	2.76	22:4	6	5.50	5.52	0.02	+0.38
20:4	6	3.04	22:5	6	6.09	6.09	0	0
16:1	7	0.634	18:2	7	1.33	1.29	—0.04	—3.00
16:1	9	0.625	18:2	9	1.29	1.26	—0.03	—2.32
18:1	9	1.12	20:2	9	2.32	2.25	—0.07	—3.88
							Average	1.20

TABLE IV

CALCULATION OF RETENTION DATA OF ESTERS OF THE TYPE $(x + 4, y + 1)$ FROM THAT OF ESTERS OF THE TYPE (x,y)

$V_{R\,(x,y)}$			$V_{R\,(x+4,\,y+1)}$					
Fatty acid	*End carbon chain length*	$V_{R\,(x,y)}$	*Fatty acid*	*End carbon chain length*	*Determined*	*Calculated*	*Error*	%
16:3	3	0.950	20:4	3	3.51	3.41	—0.10	—2.85
16:4	3	1.08	20:5	3	3.85	3.89	+0.02	+0.52
18:3	3	1.72	22:4	3	6.40	6.31	—0.09	—1.40
18:4	3	1.97	22:5	3	7.00	7.09	+0.09	—1.28
16:2	6	0.745	20:3	6	2.76	2.69	—0.07	—2.53
16:3	6	0.86	20:4	6	3.04	3.10	+0.06	+1.89
18:2	6	1.34	22:3	6	5.00	4.85	—0.15	—3.00
18:3	6	1.54	22:4	6	5.50	5.54	+0.04	+0.73
16:0	—	0.55	20:1	9	2.02	1.98	—0.04	—1.98
16:1	9	0.625	20:2	9	2.32	2.26	—0.06	—2.58
18:0	—	1.00	22:1	9	3.68	3.61	—0.07	—1.90
18:1	9	1.12	22:2	9	4.20	4.12	—0.08	—1.90
							Average	1.35

SUMMARY

The influence of structural parameters on the retention behaviour of the methyl esters of methylene-interrupted fatty acids, their monounsaturated and saturated homologs may be shown by a simple mathematical relationship.

REFERENCES

1 C. H. ORR AND J. E. CALLEN, *J. Am. Chem. Soc.*, 80 (1958) 249.
2 C. H. ORR AND J. E. CALLEN, *Ann. N.Y. Acad. Sci.*, 72 (1959) 649.
3 S. R. LIPSKY AND R. A. LANDOWNE, *Ann. N.Y. Acad. Sci.*, 72 (1959) 666.
4 S. R. LIPSKY AND R. A. LANDOWNE, *Biochim. Biophys. Acta*, 27 (1958) 666.
5 A. T. JAMES, *J. Chromatog.*, 2 (1959) 552.
6 P. MAGEDMAN, S. F. HERB, R. A. BARFORD AND R. W. RIEMENSCHNEIDER, *J. Am. Oil Chemists' Soc.*, 39 (1962) 137.
7 R. G. ACKMAN, R. D. BURGHER AND P. M. JANGAARD, *Can. J. Biochem. Physiol.*, 41 (1963) 1627.
8 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 558.
9 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 564.
10 J. K. HAKEN, *Proc. Intern. Symp. Advan. Gas Chrom., 3rd., Houston, October, 1965.*
11 J. K. HAKEN AND P. SOUTER, *J. Gas Chromatog.*, 4 (1966).
12 R. B. CLAYTON, *Biochemistry*, 1 (1962) 357.
13 A. T. JAMES AND A. J. MARTIN, *Biochem. J.*, 63 (1956) 144.
14 R. G. ACKMAN AND R. D. BURGHER, *J. Am. Oil Chemists' Soc.*, 42 (1965) 38.
15 J. W. FARQUHAR, W. INSULL, P. ROSEN, W. STAFFEL AND E. H. AHRENS, *Nutr. Rev.*, 17 (1959) Suppl. 1.
16 W. J. A. VANDENHEUVEL AND E. C. HORNING, *J. Org. Chem.*, 26 (1961) 634.