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

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(Received July 18th, 1966)

INTRODUCTION

A relationship utilizing separation factors for the estimation of retention behavior of the methyl esters of fatty acids has recently been reported[1]. It has been shown that separation factors analogous to the methylene-unit separation factors reported by JAMES AND MARTIN[2] with the methyl esters of *n*-saturated fatty esters are applicable to certain unsaturated fatty esters. The methyl esters of even-numbered mono-unsaturated and methylene interrupted fatty acids of different chain length, constant numbers of double bonds and constant end carbon chain exhibit the same separation factors as the *n*-saturated esters.

Considering the fatty esters in terms of x and y (the total chain length and the number of methylene interrupted double bonds present respectively) a relationship which is similar to that developed by CLAYTON[3] with sterol derivatives may be shown by the equations:

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

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

Where $V_{R(x+2,\,y)}$ and $V_{R(x,\,y+1)}$ are separation factors, the former based on increasing chain length[1,2] and the latter is that described by ACKMAN[4] as Type I where pairs of esters are considered with the same chain length, the same end carbon chain, but with a varying number of double bonds.

Branched chain and normal odd numbered fatty acids have been found as minor constituents of fatty materials derived from a wide range of natural sources[5], and the separation factors previously reported are shown to allow inclusion of iso (terminal isopropyl group), ante-iso (terminal isobutyl group), neo (terminal isopentyl group) esters and the odd numbered saturated fatty esters.

While the earlier report substantially utilised the data of FARQUHAR and his coworkers[6] as modified by ACKMAN[5] by graphical examination of a variety of reports and suitable interpolation and extrapolation, the data is now largely available as experimentally determined. ACKMAN *et al.*[7,8] has reported retention data of the methyl esters of fatty acids of marine origin using ethylene glyc stearate[7], diethylene glycol succinate, neopentyl glycol succinate and organosilicone polyesters EGSS-X, EGSS-Y (Applied Science Laboratories) columns and separation factors as a means of tentative identification. Similarly retention data is available for several esters

previously reported as provisionally identified but included where compatible with the correlations of ACKMAN[4,5] and of HAKEN AND SOUTER[9].

The availability of the data determined on several polyester stationary phases further indicates the validity of the use of separation factors in the type of relationship presented as an aid to the tentative identification of the methyl esters of fatty acids. The linear relationship shown by ACKMAN[5] with homologous unsaturated fatty esters with the same number of double bonds and the same carbon end chain length has been examined by HOFFSTETTER and his coworkers[10]. This work represents the largest single series of esters reported and Equivalent Chain Length Values (ECL) when plotted against carbon chain length produced straight lines for the homologous series examined. The use of separation factors has been reported for cyclopentenyl[11] and several acetylenic fatty esters[12] but the range of compounds available is insufficient to allow a general comparison.

DATA

The retention data of the saturated esters shown in Table I are those of HAWKES[13], FARQUHAR[6] and GERSON[14] and their coworkers and were determined using polydiethylene glycol succinate at 200°, ethylene glycol adipate at 197° and APL at 207°, respectively. The data of homologous cyclopentenyl esters determined by ZEMEN[11] using polyethylene glycol stearate and apiezon M columns operated at 190° and 240° respectively is shown in Table II.

TABLE I

RETENTION DATA OF METHYL ESTERS OF SATURATED NORMAL AND BRANCHED CHAIN FATTY ACIDS

Chain length	*Saturated acids*			*Iso acids*			*Ante-iso acids*			*Neo acids*
	DEGS at 200°[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*DEGS at 200°*[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*DEGS at 200°*[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*EGA at 197°*[6]
12	0.57	0.165	0.088	0.66						
13	0.73	0.222	0.140	0.88						
14	1.00	0.302	0.203	1.14	0.252	0.164	1.22			
15	1.31	0.401	0.308	1.56		0.263		0.367	0.277	
16	1.77	0.550	0.456	2.08	0.472	0.390	2.16			0.402
17	1.29	0.733	0.681	2.78		0.579		0.676	0.603	
18	3.09	1.00	1.00		0.858	0.860				0.723
19	4.02	1.35	1.48					1.24		
20	5.50	1.82	2.17		1.59					1.35
21	7.30	2.46	3.20					2.29		
22	9.83	3.27	4.58		2.91					2.45*
23	13.71		6.87							
24	17.51	6.14	10.2		5.32					4.52
26	31.83		21.5							

* Estimated.

DISCUSSION

Separation factors reflecting an increase in chain length of an ethylene unit are shown in Table III for the methyl esters of *n*-saturated, iso and ante-iso fatty acids

TABLE II

RETENTION DATA OF METHYL ESTERS OF HOMOLOGOUS CYCLOPENTENYL AND *n*-SATURATED FATTY ESTERS

Ester	*Stationary phase*	
	Apiezon M at 240°[11]	*Polyethylene glycol stearate at 190°*[11]
10:0	0.15	0.20
12:0	0.28	0.34
14:0	0.52	0.58
16:0	1.00	1.00
18:0	1.88	1.69
20:0	3.51	2.92
22:0	6.60	5.50
5-Cyclopent-2-enyl pentanoate	0.19	0.45
7-Cyclopent-2-enyl heptanoate	0.37	0.73
9-Cyclopent-2-enyl nonanoate	0.70	1.21
11-Cyclopent-2-enyl henadecanoate	1.31	2.04
13-Cyclopent-2-enyl tridecanoate	2.51	3.38

TABLE III

SEPARATION FACTORS OF METHYL ESTERS OF FATTY ACIDS WITH VARYING CHAIN LENGTH, SAME SIDE CHAIN AND SAME TERMINAL CARBON CHAIN LENGTH

$\frac{C_{n+2}}{C_n}$	*Saturated acids*			*Iso acids*			*Ante-iso acids*			*Neo acids*
	DEGS at 200°[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*DEGS at 200°*[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*DEGS at 200°*[13]	*EGA at 197°*[6]	*APL at 207°*[14]	*EGA at 197°*[6]
14/12	1.75	1.82	2.30	1.73						
15/13	1.79	1.85	2.20	1.77						
16/14	1.77	1.82	2.24	1.82	1.87	2.37	1.77			
17/15	1.75	1.82	2.21	1.72		2.20		1.84	2.18	
18/16	1.74	1.82	2.19		1.81	2.20				1.80
19/17	1.75	1.84	2.17					1.83		
20/18	1.77	1.81	2.17		1.85					1.86
21/19	1.81	1.82	2.17					1.84		
22/20	1.80	1.81	2.11		1.83					1.81*
23/21	1.87		2.16		1.82					
24/22	1.78	1.86	2.22							1.84*
26/24	1.81		2.10							

* Estimated.

and it is apparent with the three sets of data used that a constancy exists in each case. The ethylene unit has been used as an increment as previously, the separation factors obtained being larger and differences in systems where they are experienced are more readily observed. GERSON[14] derived simple empirical equations relating retention data and chain length for *n*-saturated, (+)-ante-iso and iso fatty esters and inspection of these supports the constancy of separation factors reported here; however, in the same work a relationship is derived for *cis*-monoenoic acid esters which, it is now known, is not of general application[5]. The values obtained with the

three sets of data obviously differ as the stationary phase–fatty acid interactions and the mechanical parameters are widely variant.

The work of FARQUHAR and his coworkers[6] includes several branched chain acids with terminal isopentyl groups (neo acids). While one of the five retention values used was measured from a plot of log retention time and chain length such that two of the separation factors shown are estimated, examination of the plotted data of the four series of esters on both polyester (shown in Table I and III) and apiezon M stationary phases supports the conclusions reported.

The linear relationship shown by ACKMAN[5] with homologous fatty esters of the same end chain length has been further demonstrated by HOFFSTETTER and his coworkers[10] using a larger series of unsaturated esters. Eight series of esters produced linear and parallel plots of ECL values against chain length, three other series produced straight lines which were not parallel to the other lines nor to themselves. As one of these series had been previously examined by ACKMAN[5] a discrepancy obviously exists in one of the reports. The data presented as ECL values do not permit direct comparison or calculation of the relative slopes which is of importance. The series at variance are ones with 3 and 4 double bonds, as the number of esters of interest decreases with increased unsaturation, minor differences in slope need not exclude the use of separation factors with the particular series concerned.

Examination of retention data of seal oil esters[7,8] shows that parallel plots are not obtained with all homologous ester series. This, however, does not affect the validity of the relationship proposed by ACKMAN[4,5] or, as the deviations are slight, the application of separation factors as proposed.

Table IV shows separation factors of *n*-saturated and cyclopentenyl fatty esters and again a marked similarity exists. Separation factors based on a methylene unit as reported by ZEMEN[11] are shown in Table IV and the differences experienced on the polyester phase are considered to be significant. The data do not allow calculation of these individual values, the values shown probably being measured from a data plot. While retention data of the homologous cyclopentenyl fatty esters would be of interest the separation factors available allow estimation of retention behaviour with reasonable accuracy and suitable for tentative identification.

A preliminary study involving two homologous methyl esters of fatty acids containing a triple bond[12] indicates very little difference in separation factors for the methylene group for the three series of acids (saturated, containing one double bond and containing one triple bond, the end carbon chain lengths being constant).

TABLE IV

SEPARATION FACTORS OF METHYL ESTERS OF *n*-SATURATED AND CYCLOPENTENYL FATTY ESTERS

	Apiezon M		*Polyethylene glycol stearate*	
C_{n+2}/C_n	*n-Saturated*	*Cyclopentenyl*	*n-Saturated*	*Cyclopentenyl*
12/10	1.86	1.94	1.70	1.62
14/12	1.85	1.89	1.70	1.66
16/14	1.92	1.87	1.72	1.68
18/16	1.88	1.91	1.69	1.65
20/18	1.86		1.72	
C_{n+1}/C_n[11]	1.37	1.37	1.31	1.29

A suggestion of a slight decrease in separation factors due to increased polarity is likely, but the examination of more homologs is necessary. If further data of acetylenic esters support these findings, it would seem likely that the esters would be included in the concept of separation factors reported here.

In all sixteen series of unsaturated, and five of saturated fatty esters, have been examined; and produce linear relationships with the various homologous series providing that the end chain length remains constant. The end carbon chain length being measured from the middle of the terminal double bond or by considering the position of the branched chain with the saturated esters. The data indicate that the relationship previously reported for the calculation of retention behaviour as an aid to tentative identification and extended here to include branched chain esters may be more generally applicable.

ACKNOWLEDGEMENTS

The author is indebted to the University of Southern Mississippi for a visiting professorship during the period that this study was undertaken and to Dr. R. T. HOLMAN of the Hormel Institute, University of Minnesota, for a loan of the chromatograms reported in ref. 10.

SUMMARY

A recent study of the retention time behaviour of methyl esters of the even-numbered *n*-saturated, mono-unsaturated and methylene-interrupted unsaturated fatty acids in gas chromatography has been further investigated.

The availability of further data demonstrates the validity of the relationship proposed where retention times of fatty esters of different chain length and degree of unsaturation may be calculated, the end carbon chain in a particular case being constant.

The relationship based on separation factors is shown to allow inclusion of the methyl esters of iso, ante-iso, neo and cyclopentenyl fatty acids. The data of several homologous acetylenic fatty esters has been examined, and if further homologs, when available, substantiate the data examined, it would seem that the relationship considering end carbon chain with unsaturated esters is applicable as would be the use of separation factors.

REFERENCES

1 J. K. HAKEN, *J. Chromatog.*, 23 (1966) 375.
2 A. T. JAMES AND A. J. MARTIN, *Biochem. J.*, 63 (1956) 144.
3 R. B. CLAYTON, *Biochemistry*, 1 (1962) 357.
4 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 564.
5 R. G. ACKMAN, *J. Am. Oil Chemists' Soc.*, 40 (1963) 558.
6 J. W. FARQUHAR, W. INSULL, Jr., P. ROSEN, W. STAFFEL AND E. H. AHRENS, Jr., *Nutr. Rev.*, Suppl., 17 (1959) 1.
7 R. G. ACKMAN, R. D. BURGHER AND P. JANGAARD, *Can. J. Biochem. Physiol.*, 41 (1963) 1627.
8 R. G. ACKMAN AND R. D. BURGHER, *Can. J. Biochem. Physiol.*, 41 (1963) 2501.
9 J. K. HAKEN AND P. SOUTER, *J. Gas Chromatog.*, 4 (1966) 295.
10 H. H. HOFFSTETTER, N. SEN AND R. T. HOLMAN, *J. Am. Oil Chemists' Soc.*, 42 (1965) 537.
11 J. ZEMEN AND J. POKORNY, *J. Chromatog.*, 10 (1963) 15.
12 J. ZEMEN, *J. Gas Chromatog.*, 3 (1965) 18.
13 J. C. HAWKE, R. P. HANSEN AND F. B. SHORTLAND, *J. Chromatog.*, 2 (1959) 547.
14 T. GERSON, *J. Chromatog.*, 6 (1961) 178.