

CHROM. 3842

LIMITATIONS OF SYSTEMATIC RELATIONSHIPS OF GAS CHROMATOGRAPHIC RETENTION BEHAVIOUR AND STRUCTURE OF FATTY ESTERS

J. K. HAKEN

Department of Polymer Science, University of New South Wales, P.O. Box 1, Kensington, N.S.W. 2033 (Australia)

(Received October 28th, 1968)

SUMMARY

The general applicability of systematic procedures that have been used in the tentative identification or calculation of retention data of certain fatty esters has been examined by considering the retention behaviour of several complete series of unsaturated isomeric fatty esters.

INTRODUCTION

Several systematic procedures based on the end carbon chain^{1,2,*} relationship have been applied with considerable success in the tentative identification of fatty esters using linear plots³, separation factors⁴ and their extensions⁵⁻⁷.

The esters mainly considered in these works have been those characteristic of marine products where, in common with other naturally occurring systems, the unsaturation tends to be located towards the centre of the fatty acid chain rather than near either end.

A further possible relationship⁸ between the position of unsaturation and gas chromatographic retention behaviour was recently shown for the methyl esters of certain monoenoic and methylene-interrupted trienoic acids present in cod liver oil triglycerides. The monoenoic esters are shown in Fig. 1, where a plot of the difference between chain length and end carbon chain and retention data produced a series of substantially parallel straight lines through the points of esters with constant end carbon chain. A second series of substantially parallel lines could be drawn through the points representing esters of constant chain length. Extrapolation of the plots

* The abbreviated notation used in this work essentially follows the ω -notation, which has its primary use in showing metabolic relationships with the double bonds counted from the terminal methyl group. As the polyunsaturated fatty esters considered are methylene interrupted the formula may be simply shown by the position of the first double bond. Thus 9,12,15-octadecatrienoic acid becomes 18:3 ω 3,6,9 or more conveniently 18:3 ω 3. The general case may be shown as $x:y\omega z$ where x , y , and z are respectively the total chain lengths, the number of methylene-interrupted double bonds and the 'end carbon chain', which is defined as the number of carbon atoms in the chain after the centre of the double bond furthest from the carboxylic group. The various nomenclatures applicable to polyunsaturated fatty esters have been recently described by HOLMAN¹⁰.

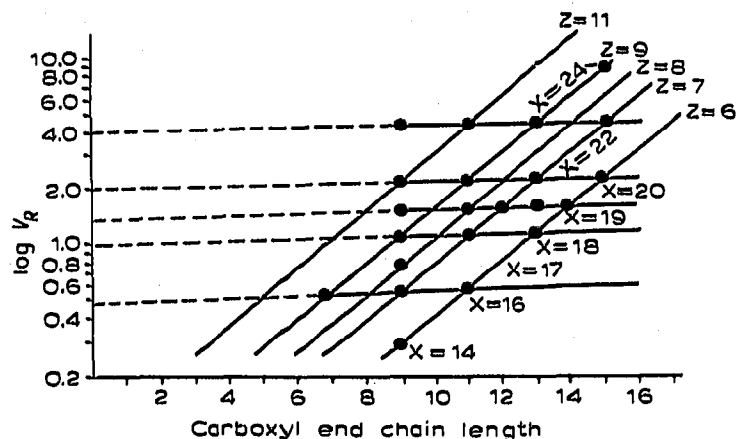


Fig. 1. Relationship between relative retention time and the carboxyl end chain of monoenoic fatty esters.

such that the end carbon chain approximated the total chain length produced retention values approximating those of the saturated esters. The total chain length and the end carbon chain of the monoenoic and trienoic acids were 14:1 ω 5; 16:1 ω 7,8,9; 17:1 ω 8; 18:1 ω 5,7,9; 19:1 ω 5,6,7,8,10; 20:1 ω 5,7,9,11; 22:1 ω 7,9,11,13; 24:1 ω 9 and 16:3 ω 3,4,6; 18:3 ω 3,6; 20:3 ω 3,6,9, respectively.

While it was apparent that interactions could cause deviations from a systematic relationship when the unsaturation was at or near the extremities of the chain the available data were both insufficient and in part inconsistent.

Retention data of the *cis*- and *trans*-octadecenoates and of the *cis,cis*-methylene-interrupted octadecadienoates have been reported by GUNSTONE and his co-workers⁹ and by CHRISTIE¹⁰. Other fatty ester series have been reported and examination of these data with regard to a systematic relationship between the position of unsaturation or of a substituent group is reported. It is also possible to discuss the effect of these data on the end chain relationship, separation factors and indications of additivity that have been observed in the gas chromatography of fatty esters.

The available series of retention data for esters of saturated, monoenoic, dienoic and acetylenic acids have been assembled and include:

saturated esters	hydroxy and acetoxy hexadecanoates	(ref. 11)
	hydroxy and acetoxy octadecanoates	(ref. 12)
	methyloctadecanoic esters	(ref. 13)
monoenoic esters	<i>cis</i> - and <i>trans</i> -octadecenoates	(ref. 9)
dienoic esters	<i>cis,cis</i> -methylene-interrupted octadecadienoates	(ref. 10)
acetylenic esters	nonynoates	(ref. 14)
	dodecyanoates	(ref. 9)

Retention data for the unsaturated series are shown in Table I together with the stationary phases and temperatures at which the data were obtained. Conversion

TABLE I

RETENTION DATA OF HOMOLOGOUS UNSATURATED ESTERS ON DIETHYLENE GLYCOL SUCCINATE

End carbon chain	18:1 (ECL.)	18:2 (ECL.)	9:1 (Rel. ret.) ^a	12:1 (ECL.)
0	18.91	18.64	3.74	15.74
1	19.38	19.36	4.13	16.00
2	19.01	19.05	2.99	15.13
3	18.86	19.06	2.59	14.88
4	18.76	19.19	2.52	14.78
5	18.62	19.23	3.31	14.68
6	18.58	19.28	3.51	14.47
7	18.53	19.38		b
8	18.49	19.46		b
9	18.45	19.62		b
10	18.45	19.75		b
11	18.45	20.25		
12	18.34	19.96		
13	18.42			
14	18.74			
15	18.32			

^a Relative to methyl nonoate.^b Not reported.

or manipulation of the data into a mutually compatible form has not been attempted as the series are in themselves consistent and suitable for study.

The effect of interactions that are experienced with the octadecenoates when the end chain constituent is either very small or where it approaches the total chain length is shown in Fig. 2. As could be expected a more marked effect occurs with a polar stationary phase than with a non-polar one.

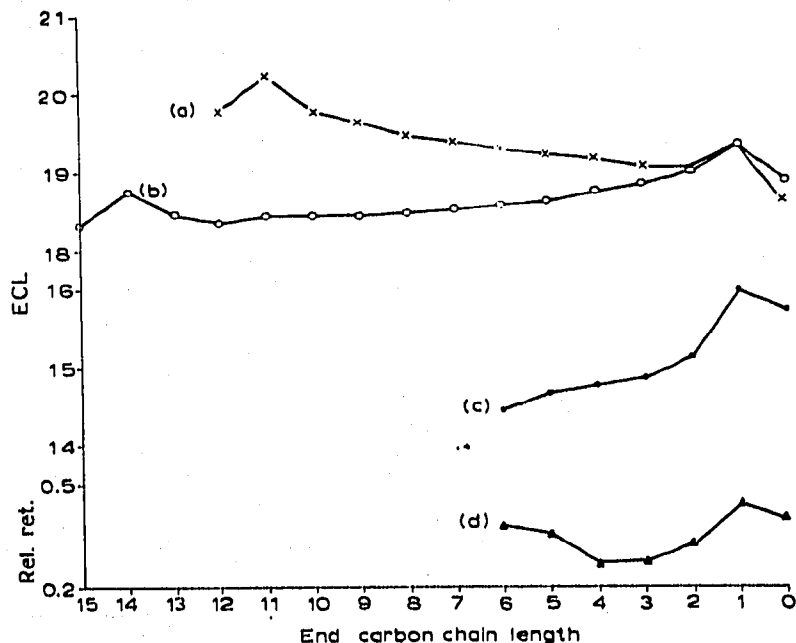


Fig. 2. Retention data of unsaturated fatty ester series. Curves: (a) *cis,cis*-octadecadienoates; (b) *cis*-octadecenoates; (c) dodecynoates; (d) nonynoates.

The behaviour observed is markedly different from that shown in Fig. 1, where for a limited range of homologous monoenoic esters a network plot was produced where both total chain length and end carbon chain could be linked⁹. A third series of lines may be drawn through esters of increasing chain length and increasing end carbon chain length. The data used were obtained on a butanediol succinate capillary column¹⁵ and are shown in Table II.

While a variety of minor plots relating fatty esters and their retention behaviour have been proposed at various times^{9,8} these, with limited numbers of esters of doubtful structure were often based on three compounds and were discarded as further homologues, identification or improved resolution became available.

TABLE II

RETENTION DATA OF MONOENOIC ESTERS RELATIVE TO METHYL STEARATE

End carbon chain	Chain length							
	14	16	17	18	19	20	22	24
13							4.38	
11						2.18	4.38	
10					1.53			
9		0.538		1.08		2.21	4.49	9.05
8			0.770		1.57			
7		0.555		1.13	1.60	2.28	4.67	
6					1.65			
5	0.275	0.583		1.19	1.69	2.41		

At least two types of relationships are currently proposed, the end carbon chain plots having found some general acceptance while with the monooctadecenoates, PANOS¹⁷ has proposed the reverse procedure where the position of the double bond from the carboxyl remains constant. Three or possibly four parallel lines were drawn using limited data (two, three, two, and one esters respectively). Investigation of this relationship using retention data of two series of five and six comparable esters shows that the plots obtained are neither parallel or straight.

While the relationship shown in Fig. 1 considered seventeen esters and seemed attractive as extrapolation to $x - z = 0$ (*i.e.* the end carbon chain approximates the total chain length) assumed values approximating those of the saturated ester it is now clear that interactions cause very significant deviations and operation with the range of available data is necessary. As plots of the octadecenoates and the octadecadienoates are similar it is likely that this pattern of behaviour is common to other unsaturated fatty ester series and on this basis examination of the probable effects on the end carbon chain relationship and its various extensions is possible.

Calculation of equivalent chain length (ECL) values for multiple branched-chain fatty esters has been reported by ACKMAN¹⁸. The significant effect of interaction experienced with fatty esters has been overcome by the determination of fractional chain length contributions due to unsaturation or a substituent group rather than use of the concept of a summation of contributions due to the skeleton and of non-interacting substituents. The estimation of values for the multiple-branched chain esters by a study of the methyl-substituted esters is considered valid as the resulting

system is one where interaction between the methyl groups is not experienced due to the isoprene skeleton of the esters concerned.

In common with the unsaturated esters the methyl group in the 2-5 and 12-17 positions showed fractional chain length (FCL) values significantly different from the values of the 6-11 positions. The respective groups were designated as carboxyl end C_x , terminal end w_y and the central positions m_x .

End carbon chain relationship

An extensive examination of the end carbon chain relationship has been carried out by HOFSTETTER, SEN AND HOLMAN¹⁹, who studied the behaviour of approximately eighty fatty esters on several stationary phases. When ECL values of the esters were plotted against the carbon number of the acids, parallel straight lines were obtained for the homologous series $x:1\omega 7,8,9$, $x:2\omega 5,6$, $x:3\omega 5,6$ and $x:5\omega 3$. The lines for the series $x:3\omega 9$ and $x:4\omega 5,6$ were not parallel to the others nor to themselves, but they were straight lines. The homologous series used in the development of the relationship were $x:1\omega 9$, $x:2\omega 4,6,7,9$, $x:3\omega 3,6$, $x:4\omega 3,6$ and $x:5\omega 3$.

A knowledge of the retention behaviour of all of the isomers of several fatty series allows examination of the effect of the significant interactions experienced at the chain extremities on the end chain carbon relationship. At the terminal end of the fatty chain the effects of interactions are noticeably decreased retention with the ester of end chain 1 and markedly increased retention with end chain 2, and possibly increased retention with end chains 3 and 4. If these effects as exhibited with the octadecenoates and methylene-interrupted octadecadienoates are generally occurring, it would be expected that straight lines would result from series with low end chain lengths. At the carbonyl end of the chain the same compensating effect does not occur.

Where the double bond of a monoenoic ester or the final double bond of a polyunsaturated ester is in the close proximity of the double bond of the carbonyl group particularly at the position $x - w = 2$, where a conjugated system exists, a deviation from linearity of the end chain plot of the series will occur with the lowest chain length homologue.

Separation factors

Four types of separation factors described as Types I to IV have been proposed for use with fatty esters and are described below:

Type I. Relationship between relative retention times or volumes of pairs of esters of fatty acids of the same chain length and with the same end carbon chain, but with varying number of methylene-separated double bonds.

Type II. Relationship between relative retention times or volumes of esters of fatty acids of the same chain length, with varying number of double bonds and with different end carbon chains.

Type III. Relationship between relative retention times or volumes of esters of fatty acids of the same chain length, with the same number of double bonds and with different end carbon chains.

Type IV. Relationship between relative retention times or volumes of esters of different total chain length, with the same number of double bonds and with the same end carbon chain.

Separation factors

$$\text{Type I} \quad \frac{V_{R(x, y+1, z)}}{V_{R(x, y, z)}}$$

$$\text{Type II} \quad \frac{V_{R(x, y+1, z)}}{V_{R(x, y, z+3)}}$$

$$\text{Type III} \quad \frac{V_{R(x, y, z)}}{V_{R(x, y, z+3)}}$$

$$\text{Type IV} \quad \frac{V_{R(x+2, y, z)}}{V_{R(x, y, z)}}$$

The various types may be shown by the relationships where x , y and z indicate the total chain length, the number of double bonds and the end carbon chain respectively.

Separation factors of Types I, II and III were developed using essentially the same data as for the end carbon chain relationship^{3,4}.

Type I separation factors provide a valuable aid in the prediction of retention behaviour in tentative identification. A tendency has been observed⁴ for the separation factor to decrease with increased unsaturation, as the end chain length is decreased and with increasing chain length. The decrease is suggested to be due to the polyester stationary phase. It is apparent however that these effects all demonstrate the retention behaviour experienced near the chain ends.

Type II separation factors concern pairs of esters of the same chain length but with varying unsaturation and a difference of three carbon atoms in the end carbon chain such that the carboxyl end chains are equal.

Relatively few data are available but the systems studied with chain lengths 18, 20 and 22 show extraordinary uniformity for all of the 3/6, 6/9 and 3/9 series studied with the exception of the docosahexaenoates, docosapentaenoates and docosatrienoates where the 6/9 and 3/9 series exhibit marked deviations.

With these esters the position of unsaturation is closer to the carbonyl than of the other systems listed while with the 3/9 series the positions of unsaturation at both ends of the chain of the docosahexaenoate are in regions where some discontinuity may be expected.

Type III separation factors consider pairs of esters of the same chain length and the same number of double bonds but with differences of three carbon atoms in the end chain.

With the data originally presented the variations recorded were considerably greater than experienced with Types I and II separation factors and with the availability of retention data of the series of octadecenoates, octadecadienoates and the nonynoates it is now apparent that a constancy even within a single homologous series does not occur.

The Type IV separation factors are with the saturated acids identical to the methylene separation factors reported by JAMES AND MARTIN²⁰ but a constancy is apparent with a series of esters independent of unsaturation providing the end carbon chain remains constant in any series⁷. This relationship has more recently been re-

ported by JAMIESON AND REID²¹ without comparison to the saturated acids as "retention time ratios of similarly unsaturated derivatives".

The effect of interactions near both ends of the fatty chain will cause deviations with fatty ester pairs where unsaturation tends to occur in these regions. Like the Type I separation factors if a constancy of interactions occurs, systems with small end carbon chains will be little effected due to a compensating effect. At the other end of the chain however significant deviations will be experienced when the double bond or final double of a polyunsaturated ester of the lower chain ester is in the close proximity of the carbonyl group.

Calculation of retention data

While the retention behaviour of certain fatty esters has been successfully calculated by a consideration of a basic chain contribution plus additive contributions from non-interacting substituents (*i.e.* a reasonably centrally located double bond or a methylene-interrupted polyunsaturated system) it is now apparent that the whole of a homologous series cannot be estimated in this way. As the calculation is based on the product of separation factors of Types I and IV, the interactions of series of esters of low end carbon chain tend to compensate one another but in the vicinity of the carboxyl group significant and non-systematic interactions occur and greater variations in calculated retention data are experienced in this region. For the general calculation of retention behaviour a procedure similar to the fractional chain length (FCL) contribution reported for the calculation of ECL values of multiple-branched¹⁸ chain esters is necessary.

Nomographic representation

The retention data of the fatty esters represent a four-dimensional system where relative retention is a function of three structural parameters. The system has been conveniently represented in two dimensions by a nomograph.

As the end carbon chain relationship was reported to be independent of the saturated esters a nomograph was prepared for a series of unsaturated esters directly from the experimental data²². It was subsequently found however that data for the saturated esters could be included using suitable index points^{5,6}.

With the nomograph constructed from the experimental data, retention times of esters with low end carbon chain may be read off by the use of a suitable end chain co-ordinate line. The nomographs previously reported allow for minor discrepancies with data of end carbon chain 3 and extension to shorter values is simple. Retention of esters where the end carbon chain or the final double bond approaches the total chain length could also be determined by the use of a suitably constructed nomograph.

CONCLUSIONS

The systematic procedures based on the end carbon chain relationship are not generally applicable to all of the isomers of a fatty acid series. The interactions which occur both with esters of very short end chain length and where the end chain length approaches the total chain length cause significant deviations from any simple systematic relationship.

With the Types I and IV separation factors variations with short end carbon

chains are partially compensating while with Type II separation factors the reverse effect occurs as the same carboxyl end chains are present.

Type III separation factors show only an approximate constancy and then only on the central area of the fatty acid chain.

Calculated retention data are only valid within the limits of the Types I and IV separation factors as errors present are compounded with the relationships used.

For application to fatty esters generally a scheme considering the retention behaviour near the chain extremities is necessary. A procedure analogous to the FCL values as applied to the branched-chain esters is feasible as is nomographic representation.

ACKNOWLEDGEMENT

The author is indebted to Dr. W. W. CHRISTIE of The Hannah Dairy Research Institute, Scotland for providing his paper (ref. 10) prior to its publication.

REFERENCES

- 1 R. G. ACKMAN, *Nature*, 194 (1962) 970.
- 2 R. G. ACKMAN, *Nature*, 195 (1962) 1198.
- 3 R. G. ACKMAN, *J. Am. Oil Chemists Soc.*, 40 (1963) 558, and references therein.
- 4 R. G. ACKMAN, *J. Am. Oil Chemists Soc.*, 40 (1963) 564.
- 5 J. K. HAKEN AND P. SOUTER, *J. Gas Chromatog.*, 4 (1966) 295.
- 6 J. K. HAKEN, in A. ZLATKIS AND L. S. ETTRE (Editors), *Proc. 3rd Intern. Symp. Advances Gas Chromatog.*, Houston, Texas, 1965; *J. Gas Chromatog.*, 4 (1966) 83.
- 7 J. K. HAKEN, *J. Chromatog.*, 23 (1966) 375.
- 8 J. K. HAKEN, *Rev. Pure Appl. Chem.*, 26 (1967) 17, and references therein.
- 9 F. D. GUNSTONE, A. ISMAIL AND M. LIE KEN JIE, *Chem. Phys. Lipids*, 1 (1967) 376.
- 10 W. W. CHRISTIE, *J. Chromatog.*, 37 (1968) 27.
- 11 J. S. O'BRIEN, AND G. ROUSER, *Anal. Biochem.*, 7 (1964) 288.
- 12 A. P. TULLOCK, *J. Am. Oil Chemists' Soc.*, 41 (1964) 833.
- 13 S. ABRAHAMSSON, S. STALLBERG-STENHAGEN AND E. STENHAGEN, *Prog. Chem. Fats Lipids*, Vol. 7, Part I (1963).
- 14 R. E. ANDERSON AND H. RAKOFF, *J. Am. Oil Chemists' Soc.*, 42 (1965) 1102.
- 15 R. G. ACKMAN, *Lipids*, 2 (1967) 251.
- 16 R. T. HOLMAN, *Prog. Chem. Fats Lipids*, 9 (1966) 3.
- 17 C. J. PANOS, *J. Gas Chromatog.*, 3 (1965) 278.
- 18 R. G. ACKMAN, *J. Chromatog.*, 28 (1967) 225.
- 19 H. H. HOFSTETTER, N. SEN AND R. T. HOLMAN, *J. Am. Oil Chemists' Soc.*, 42 (1965) 537.
- 20 A. T. JAMES AND A. J. P. MARTIN, *Biochem. J.*, 63 (1956) 144.
- 21 G. R. JAMIESON AND E. H. REID, *J. Chromatog.*, 26 (1967) 8.
- 22 J. K. HAKEN, AND P. SOUTER, *Tetrahedron Letters*, 52 (1965) 4752.

J. Chromatog., 39 (1969) 245-252