Structural Correlation of Unsaturated Fatty Acid Esters Through Graphical Comparison of Gas-Liquid Chromatographic Retention Times on a Polyester Substrate

R. G. ACKMAN, Fisheries Research Board of Canada, Technological Station, Halifax, Nova Scotia

Abstract

The correlation of structures of esters of certain unsaturated fatty acids is possible through a linear relation when logarithms of retention time on polyester substrates are plotted against the number of carbon atoms in the fatty acid chain.

This correlation is independent of any relation to the retention times of the esters of the saturated fatty acids, and depends solely on the fatty acid chain length, the number of double bonds, and the length of the end carbon chain. It appears to depend on the influence of the latter on vapor pressure.

A sound basis is thus provided for advocating a modified equivalent chain length system, virtually independent of temperature effects, and based on certain commonly occurring monounsaturated acids rather than on the saturated acids.

Introduction

THE INTRODUCTION of polyester substrates (1,2,3,4) has facilitated the application of gas-liquid chromatography to the analysis of lipids. However there is a new problem: identification of the multiplicity of peaks corresponding to the methyl esters of fatty acids for which reference standards are not immediately available. Recovery with subsequent structural degradation (5,6) remains the final proof of identity, as the comparative application of retention times (or volumes) is difficult because variables cannot be fully reproduced by different workers. To assist in identification, the relation between retention times on two different substrates has been employed (7), or comparison, under the same operating conditions, between a fresh and aged polyester column (8), essentially the same concept. Differences in operating temperature are of limited application with esters of unsaturated fatty acids (9). In considering different isomers it has been pointed out (10) that as the double bond systems in polyethylenic fatty acids move away from the carboxyl group the retention time increases, while similar observations apparently apply to certain monoethylenic esters (11). For reasons discussed subsequently this rule must be considered subject to limitations.

The effect of polyester interaction with ethylenic double bonds, in esters of fatty acids with the same chain length, might be expected to be proportional to the number of double bonds, and on various small scale plots of log retention time against a number of double bonds may seem proportional (2,12,13,14). Other plots (6,15) have suggested an inflection in similar lines joining the monounsaturated and polyunsaturated acids. This is not unexpected as, in normal methylene-interrupted polyethylenic fatty acids, there may be some interaction between the double bonds themselves, and hence modification of their individual responses to polyester linkages. However, in a single analyses on any polyester, no substantial correlation has been obtained with various detailed plots of log retention time for fatty acid esters with the same chain length and differing numbers of double bonds. With esters of differing fatty acid chain lengths and the same numbers of double bonds, no adequate correlation has been shown except with certain monoethylenic fatty acids, which have been joined by apparently straight lines (14,16,17,18).

This report shows that the identification problem has appeared needlessly complex through the basic misapprehension that the unsaturated fatty acids can be structurally correlated through the corresponding saturated acids, the number of ethylenic double bonds, and their position relative to the carboxyl group. Actually it may be shown that there is a satisfactory relationship among the unsaturated fatty acids themselves, independent of the saturated fatty acids, and based on the chain length, the number of double bonds, and the position of the latter relative to the terminal methyl group.

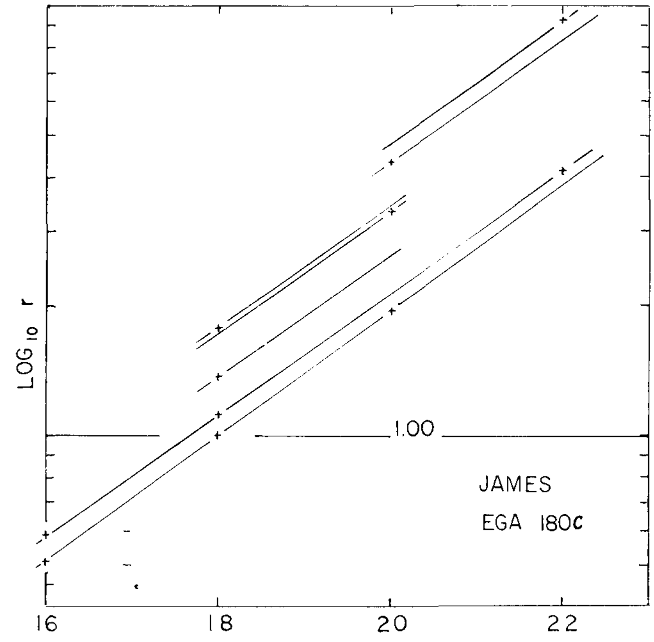

FIG. 1. Example of method of plotting log r against number of carbon atoms in fatty acid chains. Lines from bottom plot esters of: saturated fatty acids; monounsaturated fatty acids (see text); 9,12-octadecadienoic acid; 5,8,11,14-eicosatetraenoic acid; 9,12,15-octadecatrienoic acid; eicosapentaenoic acid; docosahexaenoic acid.

Procedure

Certain data were examined through plots of log methyl ester *r* against number of carbon atoms in the fatty acid chain (6,7,16,19,20,21,22,23,24), where the log *r* values for the unsaturated fatty acid esters were plotted on the same abscissa line as the saturated fatty acid (14,17,18). It was disclosed that for certain common fatty acids, a similar relationship existed among the various points independently of the polyester, worker, or operating conditions. Closer examination indicated that this relationship was improved if the points were considered in relation to the line joining those points (gas-liquid chromatography data should properly be presented in terms of corrected retention volume of carrier gas. The widespread use of *time* has led to the introduction of t_R for retention time, and t_R' for adjusted (not corrected) retention time. Relative retention time or separation factor is then t_{R_1}'/t_{R_2}' and denoted *r* (19). In the present study all data are based on retention time (adjusted) relative to methyl octadecanoate (18:0) and simply denoted *r* when abbreviated.) representing the monounsaturated esters, instead of the line joining the saturated ester points. This is illustrated in Figure 1 by drawing lines through the various points parallel to the line joining 9-octadecenoic acid and 13-docosenoic acid. This shows the typical relationship between the lines through the points representing 9,12,15-octadecatrienoate and 5,8,11,14-eicosatetraenoate, with the former line lying just above the latter. It will also be noted that the point for 9-hexadecenoate lies slightly above the extrapolation of the line joining 9-octadecenoate and 13-docosenoate.

In a number of these plots the points for 5,8,11,14,17-eicosapentaenoate and 7,10,13,16,19-docosapentaenoate appeared to fall on a common line. The sole correlation in the structure of these acids, other than the number of double bonds, is in the number of carbon atoms after the center of the double bond farthest removed from the carboxyl group; in each case three, including the terminal methyl group. The comprehensive data of Farquhar et al. (19) yielded another pair of linearly related points: 9,12-octadecadienoate and 11,14-eicosadienoate with, in each case, six carbon atoms after the center of the ultimate double bond.

End Carbon Chain. In a preliminary communication (12) this correlating number of carbon atoms, named the end carbon chain, was reckoned from, and including, the first methylene group after the double bond farthest removed from the carboxyl group. For purposes of biological comparisons, it is now considered better to conform to the more usual system of double bond identification and group the fatty acids by the number of carbon atoms after the center of the ultimate double bond. This system is therefore employed exclusively. A convenient system for comparing correlated fatty acids in the groups subsequently discussed, based on the carboxyl end of the acid rather than on the end carbon chain, is to consider that as two carbon atoms are added to the chain length, the first double bond moves two carbon atoms farther from the carboxyl group (e.g., 9-octadecenoate and 11-eicosenoate). The parallel probable first step in the biosynthesis of highly unsaturated fatty acids from essential fatty acids is an interesting coincidence. The end carbon chain is, however, the correlating constant, and of more importance in determining the relationship. As will be shown later, this must therefore be preferred as the means of comparison.

TABLE I

Modification of Relative Retention Times Through Interpolation

Fatty acid [a]		*r* values		
		Landowne	Farquhar	Interpolated
18:0	....	1.00	1.00	1.00
18:1	9	1.10	1.12	1.12
18:2	6,9	......	1.43	1.29
18:2	8,11	1.31	......	1.33
18:2	9,12	1.32	1.34	1.34
18:2	10,13	1.35	......	1.37
18:2	11,14	1.43	......	1.45
18:3	9,12,15	1.68	1.72	1.72

[a] Notation after Farquhar.

Transfer of Data. Unfortunately no one author has tabulated retention time data for all the potentially interesting methylene-interrupted polyethylenic fatty acids studied by GLC. The work of Farquhar is the most comprehensive, but the results of any one author do not always fit the presumptive pattern which it is desired to verify. However, in comparing retention times reported by various authors, note was taken of the parallel linearity established in relation to the line joining the monoethylenic fatty acid esters. A modified equivalent chain length system (MECL) was tested, based on the lines drawn through the points representing 9-octadecenoate, 11-eicosenoate and 13-docosenoate. It will be noted that these three points also fit the pattern of one line joining points representing the same number of double bonds (one) and the same end carbon chains (nine), after the double bond. The MECL system will be considered below, but only in those cases where one or more nearby points coincide moderately well, may the transfer of a point on a log *r* plot be considered reliable. In some cases it has been necessary to use such transfers even in cases of poor correlation in order to obtain a point not otherwise reported. In addition to errors inherent in plotting retention times, and reading intercepts from graph lines, some data include only one or two points representing suitable monoethylenic fatty acid esters, necessitating interpolation, extrapolation, or even the use of the otherwise unsuitable point for 9-hexadecenoate.

In the case of the important data of Landowne and Lipsky (10), simple interpolation has been used, since no overall plot may be drawn (Table I). The errors should be small since there is good agreement for the appropriate separation factors based on the mutual *r* values for 9-octadecenoate, 9,12-octadecadienoate and 9,12,15-octadecatrienoate (25). In one instance the ordinary ECL system (21) has been employed.

Results of Graphic Analysis

The available retention time data, drawn largely from Farquhar, are presented in Table II and in Figure 2. In most cases the structural relationships among the acids arranged in Groups I to X correlate well with the listed *r* values, although the different retention times tabulated from the literature for the sixteen-carbon unsaturated fatty acid esters are of doubtful utility. Thus Farquhar has indicated no separation of 6,9-hexadecadienoate from 9,12-hexadecadienoate, although this should be observed, and has in fact been reported (6,15). These difficulties may have arisen from the very short retention time in the analysis of a complex mixture with longer chain highly unsaturated materials in the first instance. But the general failure of this, and data from another source (6), to correlate well with either the predicted *r* values or Farquhar's values, is inexplicable. The

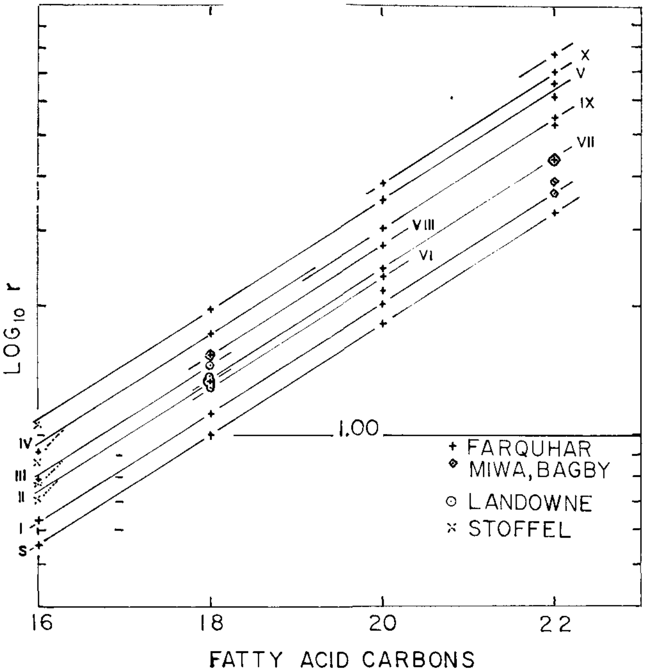

FIG. 2. Correlation of log *r* data through structural groups dependent on number of double bonds and the same end carbon chains. Data from Table II.

hexadecanoate point in the Farquhar data is satisfactory, presumably eliminating the possibility of effect of injection procedure, etc. Without other information, the *r* values listed in the "probable" column in Table II must be considered more accurate than those from the literature. In Group XI are listed a number of acids for which no point of correlation is available; also some tentative identifications of unknowns. The latter are discussed below, or considered elsewhere (25).

The importance attached to each plotted point may now be reviewed in detail, following the arrangement in groups. Since the basic data is that of Farquhar it is understood that points not discussed are from these data and are valid without comment.

Group I. The point for 13-docosenoate ester (*r* 3.68), obtained by extrapolation, coincides with that taken from the data of Miwa et al. (21) by application of the normal ECL system to the saturated ester line. This coincidence is encouraging when considering the transfer of certain other points from this group of workers.

Group II. Neither the data of Stoffel et al. (6), nor that of Farquhar, can be considered reliable in the case of the 6,9-hexadecadienoic acid, since in the one case none of the 16-carbon esters fit (*r* 0.702 by MECL), and in the other no separation is reported for the 6,9 and 9,12 isomers (common *r* 0.785). The probable value of 0.740 is based on the assumption that the *r* of 1.33 for 8,10-octadecadienoic ester, interpolated from the data of Landowne and Lipsky (Table I) is valid.

Group III. Arguments similar to the above apply in the case of the 9,12-hexadecadienoic ester, with the Stoffel *r* (6) (0.770 by MECL), and Farquhar *r* (0.784), values lying closer to the probable value of 0.810. The 11,14-octadecadienoic point is again modified from the data of Landowne and Lipsky (see Group VI discussion).

Group IV. The Farquhar value for 7,10-13-hexadecatrienoic ester (*r* 0.904) is suspect owing to the reported non-separation from the 6,9,12 isomer. A probable *r* of 0.950 is indicated by the linear plot. The reported *r*, 0.904, may be approximately correct for

TABLE II

Structures of Various Fatty Acids with Relative Retention Times Modified from the Literature, and as Employed in or Predicted by Figure 2

Group	Fatty acid [a]		End carbon chain	*r* values [b]					
				Probable	Plotted	Farq. (19)	Stof. (6)	Land. (10)	Bagby (24) Miwa (21)
S	16:0	....	....	0.550	0.550	0.550	....	......	......
	18:0	....	....	1.00	1.00	1.00	1.00	1.00	1.00
	20:0	....	....	1.82	1.82	1.82	....	......	......
	22:0	....	....	3.30	3.30	3.27	....	......	......
I	18:1	9	9	1.12	1.12	1.12	....	......	......
	20:1	11	9	2.02	2.02	2.02	....	......	......
	22:1	13	9	3.68	3.68	....	....	......	3.68
II	16:2	6,9	7	0.740	......	0.785	0.702	......	......
	18:2	8,11	7	1.33	1.33	....	....	1.33	......
III	16:2	9,12	4	0.810	......	0.785	0.770	......	......
	18:2	11,14	4	1.45	1.45	1.43	....	1.45	......
IV	16:3	7,10,13	3	0.950	......	0.904	....	......	......
	18:3	9,12,15	3	1.72	1.72	1.72	1.58	1.72	1.72
V	18:4	6,9,12,15	3	1.97	1.97	1.97	....	......	......
	20:4	8,11,14,17 [c]	3	3.51	3.51	3.51	2.76	......	......
VI	18:2	6,9	9	1.29	1.29	1.43	1.41	......	......
	20:2	8,11	9	2.32	2.32	2.32	....	......	......
VII	18:2	9,12	6	1.34	1.34	1.34	....	......	1.34
	20:2	11,14	6	2.45	2.45	2.45	....	......	......
	22:2	13,16 [c]	6	4.38	4.38	4.38	....	......	4.40
VIII	18:3	6,9,12	6	1.54	1.54	....	....	......	1.53
	20:3	8,11,14	6	2.76	2.76	....	....	......	......
IX	20:4	5,8,11,14	6	3.04	3.04	3.04	....	......	......
	22:4	7,10,13,16 [c]	6	5.50	5.50	5.50	....	......	......
X	20:5	5,8,11,14,17	3	3.85	3.85	3.85	....	......	......
	22:5	7,10,13,16,19	3	7.00	7.00	7.00	....	......	......
XI	16:1	7 [c]	9	0.625	......	....	....	......	......
	16:1	9	7	0.634	0.634	0.634	....	......	......
	16:2	7,10 [c]	6	0.745	......	0.67	....	......	......
	16:3	4,7,10 [c]	6	0.86	......	0.825	....	......	......
	16:3	6,9,12	4	0.904	0.904	0.904	0.860	......	......
	16:4	4,7,10,13 [c]	3	1.08	......	....	....	......	......
	16:4	6,9,12,15	1	1.11	......	....	1.06	......	......
	18:2	10,13	5	1.37	1.37	....	....	1.37	......
	18:?	?	?	2.18	2.18	2.18	....	......	......
	22:2	5,13	7	3.90	3.90	....	....	......	3.90
	22:5	4,7,10,13,16 [c]	6	6.09	6.09	6.09	....	......	......
	22:5	?	4–5 [c]	6.60	6.60	6.60	....	......	......
	22:6	4,7,10,13,16,19	3	7.75	7.75	7.75	....	......	......

[a] Notation after Farquhar (19).

[b] Excepting the data of Farquhar et al. all other values are modified from original author's data as specified in text.

[c] Identification postulated or reported by other workers as occurring in natural lipids (12,27).

the 6,9,12 isomer, present in menhaden oil in a much higher proportion.

Group V. The plotted line through the 6,9,12,15-octadecatetraenoate point (*r* 1.97) suggests that the *r* for 8,11,14,17-eicosatetraenoate will be 3.54, correlating with the *r* of 3.51 by Farquhar for an unknown acid with 20 carbon atoms. The value from the data of Stoffel et al. (6), *r* 2.76 by MECL, coincides with 8,11,14-eicosatrienoate (Group VIII). The similar structures suggest an error in structural determination by the latter authors.

Group VI. Another error seems to have been reproduced in the case of the 6,9-octadecadienoic acid, reported by Farquhar to have *r* 1.43 and by Stoffel 1.41 (by MECL from original 1.35). The data of Landowne and Lipsky strongly suggest an interpolated *r* of 1.29, correlating very well by plot with the 8,11-eicosadienoic ester, and coinciding with an unknown listed at approximately *r* 1.29 (not specified at 197C).

The identity of the component hitherto considered 6,9-octadecadienoate, *r* 1.43, remains to be considered. Tentatively it is suggested by biological comparison (29) that this is derived from 9,12-hexadecadienoic acid, and is therefore 11,14-octadecadienoate. The proposed *r* of 1.45 (see Group III) is then very satisfactory. In the degradative study of these octadecadienoic acids (29) only oxidative ozonolysis is reported. Since this is a minor component, the traces of butyric acid produced in ozonolysis may have been lost, and the dicarboxylic acid product would have a long retention time and an inconspicuous flat peak. The more obvious products of the 6,9 isomer would, in contrast, be readily recognized, and lead to the belief that only two isomers were present.

Group VII. Good correlation is obtained for the 9,12-octadecadienoic and 11,14-eicosadienoic esters. Extrapolation of this line gives a probable *r* of 4.38 for the 13,16-docosadienoic ester. This coincides with an unknown, *r* 4.38, reported by Farquhar, and also with a 22:2 diene listed by Bagby et al. (23) (*r* 4.40 by MECL from original listed ECL of 23.0).

Group VIII. Although a 6,9,12-octadecatrienoic acid is listed by Farquhar, the *r* is not specified. The linear plot suggests a probable value of 1.54, coinciding with an unknown listed by this author with this *r*, and with a value of 1.53 obtained from the data of Miwa et al. (21) by MECL. Correlation with the 8,11,14-eicosatrienoic ester is then satisfactory.

In considering the *r* data tabulated by Lindgren et al. (20), obtained on a DEGS type substrate (LAC-2R-728), the log *r* plot (although not satisfactory, as no straight line can be drawn joining the appropriate monoethylenic fatty acids) suggests that these authors have interchanged the retention values, relative to methyl stearate, for 6,9,12-octadecatrienoate and 9,12,15-octadecatrienoate. The listed comparative *r* values (20) of Hawke et al. (16) support this suggestion.

Group IX. 5,8,11,14-eicosatetraenoic ester (*r* 3.04) cannot be obviously correlated with any other known acid, but the linear plot extrapolation falls closely between a 22:4 ester (*r* 5.30) and an unknown (*r* approximately 5.50 at 197C by interpolation), suggesting that one of the latter is 7,10,13,16-docosatetraenoate. Tentatively this is assigned to the point with *r* of 5.50, since the separation factor (22:4/20:4) of 1.81 is then similar to that for all pairs in any group (see below), whereas the other would give 1.74.

Group X. Excellent correlation is obtained, as in many plots from various authors, between 5,8,11,14,17-eicosapentaenoate and 7,10,13,16,19-docosapentaenoate.

Group XI. The plotted point for 9-hexadecenoate (*r* 0.634) lies slightly above the Group I line, while the predicted value for 7-hexadecenoic ester is 0.625. Since, in general, the 16-carbon *r* values listed by Farquhar are too low, the unknown with interpolated *r* at 197C of approximately 0.67, may be 7,10-hexadecadienoate, reported in herring oil (27). The probable *r* of 0.745 lies very close to the 0.740 predicted for the 6,9 isomer (see discussion on centrally located unsaturation). The rule of increased retention time with shorter end carbon chain (10) is probably applicable here, hence comparison of the listed value (19) of *r*, 0.904 for 6,9,12-hexadecatrienoate with the Group IV line, suggests that the listed value is approximately correct. [An independent check by separation factors from (15) gives 0.910, agreeing with this value (25).] This is further supported by the predicted value of 0.860 for the 4,7,10-hexadecatrienoate. The latter is possibly the unknown listed at *r* 0.825. Similarly the extrapolated value of 1.08 for 4,7,10,13-hexadecatetraenoate (15) suggests that the 6,9,12,15 isomer will have a longer retention time. The latter is unique as it has a terminal vinyl group, which may modify the normal response pattern. Farquhar states that these acids do not separate from the octadecanoate peak, hence the *r* values must both lie between 1.00 and 1.12. A probable value for the 6,9,12,15 isomer based on separation factors, is 1.11 (25). The interpolated value for 10,13-octadecadienoate (Table I) cannot be verified.

A significant acid, 5,13-docosadienoic, with a non methylene-interrupted structure, is listed by Bagby et al. (23) with *r* of 3.90 by MECL. This point lies well below any other diene line, but distinct from the monoethylenic line. The separation factor (1.18) from the corresponding saturated acid is not, however, twice that of 13-docosenoate (1.11). It is possible that the 20:(unknown) listed by Farquhar (*r* at 197C 2.18 by interpolation) is of the same type [cf. discussions (15,23)]. The 19-carbon acid, structure unknown, with *r* at 197C of 1.64, does not fall on the Group I line, but also apparently correlates by linear plot at both 197C and 173.5C with the above 20-carbon unknown. The shift from the Group I line appear excessive in both cases for a monoethylenic isomer with a double bond close to the normal position: it is presumed that if these are monounsaturated fatty acids the double bond position would be at least three carbon atoms removed from the normal [e.g. 14-eicosenoate, see text (25)]. The figures of Scholfield et al. (11) suggest that in non methylene-interrupted diene fatty acid esters of a slightly different type, with double bonds in positions n and n + 6, the retention time should be slightly longer than that for the normal diene with n, n + 3 structure, but distinctly less than that of the normal diene structure n + 3, n + 6. The latter authors also agree with Daniels and Richmond (24) and others that conjugation produces a drastic increase in retention time, but this refers to *cis-trans* types of structure.

The 4,7,10,13,16,19-docosahexaenoate point is unique, but two docosapentaenoate esters of interest are listed (19). Separation factors (25) suggest that with *r* of 6.09 will have a 4,7,10,13,16 structure, in which case the end carbon chain rule gives 5,8,11,14,17 or 6,9,12,15,18 as probable structures for the other. The identification of these and other highly unsaturated un-

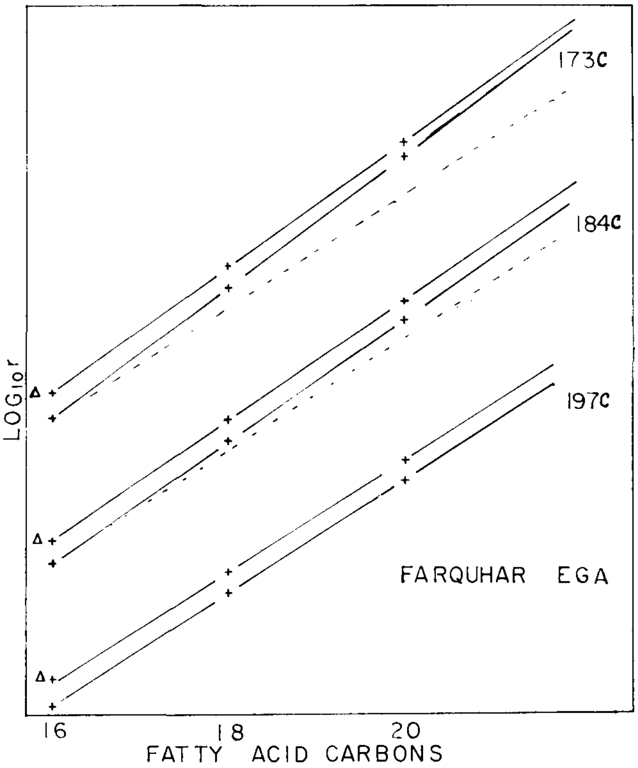

FIG. 3. Composite plot of log r data for saturated and monounsaturated fatty acid esters, showing relative changes in slopes with change in temperature. Dotted line parallels saturated acid line at 197C.

knowns on the basis of separation factors is considered elsewhere (25).

Modified Equivalent Chain Length System

In the present study all the data transferred have been obtained on ethylene glycol-adipic acid type (EGA) polyesters. Since the separations obtained between esters of unsaturated fatty acids are based on polar interactions between the double bonds and the polyester ester linkages, this factor should be nearly constant for these various samples of polyester, although modified to some extent by operating conditions and age of columns. With other types of polyesters the interaction effects may well be sufficiently different to prevent wide application of the MECL system in relating data obtained on completely different polyesters.

The basis of separations on polyesters has been discussed by various workers, but essentially two effects may be considered: that of the London forces necessary to separate the esters on a molecular weight-solubility-volatility basis and superimposed on this, the polar interaction with the double bonds. There is no reason to suppose that these two effects will change at the same rate with temperature change, and this is illustrated in Figure 3, where the relative slopes of the saturated and monounsaturated acid esters change at different rates. As shown by this figure, the parallel linearity for these two lines is therefore an abnormal circumstance. On the other hand plots of the data of Farquhar for 173.5C and 197C indicate that the parallel relationship among the various groups is valid, although of different slope, at both temperatures.

On this reasoning, and on the experimental evidence, the modified equivalent chain length system has an inherently sounder basis than the original concept (21), founded on the possible chance parallel relationship between the two lines.

TABLE III

Comparison of Modified and Original ECL Values for Methyl Esters of Various Acids at Different Temperatures [a]

Fatty acid [b]		ELC Values			
		Modified		Original	
		173.5C	197C	173.5C	197C
18:2	9,12	18.60	18.62	18.90	19.04
18:3	9,12,15	19.38	19.49	19.67	19.90
18:4	6,9,12,15	19.80	19.90	20.05	20.38
20:2	8,11	20.40	20.48	20.63	20.92
20:2	11,14	18.60	18.64	20.83	21.11
20:4	5,8,11,14	21.30	21.35	21.50	21.82
20:5	5,8,11,14,17	22.10	22.15	22.24	22.62

[a] From data of Farquhar (19), EGA.
[b] Notation after Farquhar (19).

A further advantage is that the occurrence of eicosanoic and docosanoic acids in animal lipids is limited, while the monounsaturated acids of these chain lengths occur widely. Hence the basic data for the modified equivalent chain length system is probably more readily available than the saturated acid data necessary for the original system.

The effect of position of the double bond in monoethylenically unsaturated fatty acids is small where the double bond is centrally located (see below), and therefore relatively small errors would be introduced by employing points based on most isomers of the more widely occurring unsaturated acids with the same end carbon chain as listed in Group I. The use of the commonly occurring 9-hexadecenoic acid, e.g., would be feasible provided the line were drawn slightly below this point on the log r plot. 7-hexadecenoic acid does occur as a minor component in certain lipids (27).

Marine oils are a convenient source of monounsaturated fatty acids of the Group I type, together with 9-hexadecenoic acid. If the methyl esters of readily available cod liver oil are treated with urea to remove the bulk of the saturated esters, the second urea complex will consist substantially of the monounsaturated esters with minor amounts of tetradecanoic and hexadecanoic esters. Although the structural purity of these esters has not been established, they provide an excellent basis for the MECL system. Dogfish liver oil is reported (28) to contain moderate amounts of different isomers of octadecenoic, eicosenoic and docosenoic acids; but this oil is generally abnormal in composition, and Stoffel and Ahrens report 9-octadecenoic acid as the only octadecenoic acid in menhaden oil (29).

The influence of temperature changes on the MECL system is slight in comparison with the large changes in values determined by the original ECL system (Table III). In comparing the data of different authors by the two systems the temperature differences must therefore be considered, as well as slight differences in column polarity with age. The range of values obtained by different authors, all using EGA-type polyesters, is fairly wide even with the MECL system, but falls within a narrower range than with the ECL system (Table IV).

In general, the publication of retention data by carbon number (30), ECL (21) or MECL should be discouraged. The internal use of such systems for comparisons of structure, etc., may be desirable, but for comparisons of tabulated data the original adjusted retention times or relative retention times is more satisfactory, avoiding the compounding of errors and the necessity of choosing an arbitrary slope for the basic line when plotting such data.

TABLE IV

Comparison of Modified and Original ECL Values for Methyl Esters of Certain Fatty Acids from Data of Different Workers (in order 19,19,19,21,26,7)

Fatty acid [a]	EGA	EGA	Reoplex 400	Reoplex 400	LAC-2R-446	EGA	Avg	Avg deviation
	173.5C	197C	197C	190C or 210C	221C	180C		
Equivalent chain length based on monounsaturated ester line								
18:2 9,12	18.60	18.62	18.60	18.58	18.60	18.60	18.60	0.01
18:3 9,12,15	19.38	19.49	19.38	19.38	19.43	19.44	19.41	0.04
20:4 5,8,11,14	21.30	21.35	21.40	21.20	21.40	21.30	21.32	0.06
Equivalent chain length based on saturated ester line								
18:2 9,12	18.90	19.04	19.98	19.00	18.92	18.95	18.96	0.06
18:3 9,12,15	19.67	19.90	19.75	19.80	19.72	19.72	19.76	0.06
20:4 5,8,11,14	21.50	21.82	21.70	21.60	21.50	21.58	21.62	0.09

[a] Notation of Farquhar (19).

Measurement of Retention Time

It has been observed in this laboratory (22) (employing conventional catherometer equipment and DEGS columns) that peaks obtained for the major monounsaturated component esters of marine oils are invariably more sharply skewed than adjacent peaks for polyunsaturated esters present in the same proportion. This effect is therefore not due to normal overloading of the column. While the midpoint of the peak is a tempting basis for retention time determination (19), Table V shows that in this case more constant results are to be obtained by using either the frontal tangent or half base width (31) procedures. This is evident in the separation factors, relative to the monounsaturated esters, determined for eicosapentaenoate and docosapentaenoate. Unfortunately, many authors fail to report the system employed, or even if retention times are adjusted. Unadjusted retention times do not normally give a straight line for the saturated esters in a log *r* plot in the acid range of interest.

Discussion

In general the consolidation of data in Table II and Figure 2 provides a sound basis for the rules listed below, relating the GLC retention times of the methyl esters of the common higher unsaturated fatty acids through plots of log *r*, against number of carbon atoms in the fatty acid chain.

1) The number of carbon atoms in the chain, reckoned from the center of the double bond farthest removed from the carboxyl group, and including the terminal methyl group, shall be designated the end carbon chain.

2) Esters of monounsaturated fatty acids with the same end carbon chain, on a plot of log *r* against number of carbon atoms in the fatty acid chain, can be joined by a straight line.

3) In the case of esters of the normal methylene-interrupted polyunsaturated acids, a straight line, drawn through any ester point plotted from log *r* against number of carbon atoms in the chain, and drawn parallel to the line established by rule 2, joins all points representing fatty acids with the same number of double bonds and the same end carbon chain.

4) In the case of acids of one chain length and with the same number of methylene-interrupted double bonds, or with one double bond only, generally those esters with the shorter end carbon chains will have the longer retention times.

It is apparent that once the necessary overall suitability of the plotted points has been established (i.e., that three of any group such as Group I do give a straight line), there exists a factor which is constant in a given plot for any pair of points belonging to one group, but applicable to any group. Thus the separation factor for 9-octadecenoate and 11-eicosenoate is 2.02 ÷ 1.12 = 1.80. Applying this to an extreme example, the *r* of 3.85 for 5,8,11,14,17-eicosapentaenoate predicts that for 7,10,13,16,19-docosapentaenoate the *r* will be 1.80 × 3.85 = 6.94 (known 7.00).

While these rules have been developed on data determined on an ethylene glycol-adipic acid polyester, there is no reason why they would not be applicable to any polyester (see below), but the data necessary to verify this are inadequate. In the case of the data of Lindgren et al. (20), employing a diethylene glycol-succinic acid polyester, the general pattern is the same, but no good line can be drawn through the points representing the monoethylenic acids. This suggests a change in operating conditions during the analysis, or possibly the influence of other factors, such as the peak shape affecting the retention times. On the other hand, DEGS data obtained here give consistently straight lines for the critical points presumed to be 9-octadecenoic, 11-eicosenoic and 13-docosenoic acids; and a parallel line in each case for tentatively identified eicosapentaenoate and docosapentaenoate of the Group X type. The odd-numbered monounsaturated acids with 17 and 19 carbons, occurring in marine oils, appear to fall on the line for Group I type acids, suggesting an end carbon chain of 9. The DEGS data of Hawke et al. (16) also give the same general pattern of lines as that in Figure 1.

The significance of the end carbon chain in relating structure and GLC retention time is not immediately apparent. The data of Landowne and Lipsky (Table I) and the illustrations of Scholfield et al. (11), indicate that this effect is not linear with end carbon chain variation. The various centrally located monoethylenic double bonds or polyethylenic double bond systems appear to show only a modest difference in retention time between isomers, but this effect increases rapidly as the end carbon chain shortens. James (7) has indicated that 6- and 4-octadecenoic

TABLE V

Comparison of Methods for Determining (Adjusted) Retention Times on a DEGS column at 225C.

Fatty acid [a]	Tangent	½ Base width	Peak top
Relative to methyl octadecanoate			
16:0	0.623	0.625	0.619
16:1	0.725	0.730	0.722
18:0	1.00	1.00	1.00
18:1	1.16	1.16	1.19
20:0	1.61	1.60	1.59
20:1	1.84	1.83	1.84
22:0	2.60	2.54	2.57
22:1	2.86	2.87	2.91
20:5	3.59	3.54	3.52
22:5	5.55	5.54	5.47
22:6	6.33	6.26	6.28
Relative to monounsaturated acid			
20:5	1.94	1.93	1.91
22:5	1.94	1.93	1.88
22:6	2.21	2.18	2.16

[a] Notation after Farquhar (19).

esters have progressively longer retention times when compared to 9-octadecenoic ester, and on this basis it is possible to find parallels strongly suggesting that volatility must be the determining factor. In a study of the effect of varying the acid and alcohol carbon chain lengths, while keeping the total carbon atoms constant, Lefort et al. (32) observed only slight differences in retention time on a polyester substrate where only relatively long acid and alcohol chains were involved, but an increase in retention time with a short chain in either the alcohol or the acid. It is known that the vapor pressures of esters of this type, with centrally located ester linkages, are higher than those with short chains (33); hence the shorter retention times. The opposing results of James and Scholfield for the monoethylenic octadecenoic fatty acids are thus explained.

It seems reasonable that the separation of unsaturated fatty acid esters into groups of the same structural type, with the same end carbon chain, depends on three separatory effects: a London force separation by chain length; a polar interaction with the single or methylene-interrupted double bond systems; and finally a slight modification in the volatility introduced by the length of the end carbon chain. Under these circumstances the application of the present linear plot system to any polyester seems feasible.

REFERENCES

1. Orr, C. H., and J. E. Callen, J. Am. Chem. Soc., *80*, 249 (1958).
2. Orr, C. H., and J. E. Callen, Ann. N. Y. Acad. Sci., *72*, 649–665 (1959).
3. Lipsky, S. R., and R. A. Landowne, *Ibid.*, *72*, 666–674 (1959).
4. Lipsky, S. R., and R. A. Landowne, Biochim. Biophys. Acta, *27*, 666–667 (1958).
5. James, A. T., and J. Webb, Biochem. J., *66*, 515–520 (1957).
6. Stoffel, W., W. Insull, Jr., and E. H. Ahrens, Jr., Proc. Soc. Exp. Biol. Med., *99*, 238–241 (1958).
7. James, A. T., J. Chromatog., *2*, 552–561 (1959).
8. Magidman, P., S. F. Herb, R. A. Barford, and R. W. Riemenschneider, JAOCS, *39*, 137–142 (1962).
9. Landowne, R. A., and S. R. Lipsky, Biochim. Biophys. Acta, *47*, 589–592 (1961).
10. Landowne, R. A., and S. R. Lipsky, *Ibid.*, *46*, 1–6 (1961).
11. Scholfield, C. R., E. P. Jones, J. Nowakowska, E. Selke, and H. J. Dutton, JAOCS, *38*, 208–211 (1961).
12. Ackman, R. G., Nature, *194*, 970–971 (1962).
13. Lipsky, S. R., R. A. Landowne, and M. R. Godet, Biochim. Biophys. Acta, *31*, 336–347 (1959).
14. Smith, L. M., J. Dairy Sci., *44*, 607–622 (1961).
15. Stoffel, W., and E. H. Ahrens, Jr., J. Am. Chem. Soc., *80*, 6604–6608 (1958).
16. Hawke, J. C., R. P. Hansen, and F. B. Shorland, J. Chromatog., *2*, 547–551 (1959).
17. Craig, B. M., and N. L. Murty, Can. J. Chem., *36*, 1297–1301 (1958).
18. Hallgren, B., S. Stenhagen, A. Svanborg, and L. Svennerholm, J. Clin. Invest., *39*, 1424–1434 (1960).
19. Farquhar, J. W., W. Insull, Jr., P. Rosen, W. Stoffel, and E. H. Ahrens, Jr., Nutrition Rev. (Suppl.), *17*, 1–30 (1959).
20. Lindgren, F. T., A. V. Nichols, and R. D. Wills, Am. J. Clin. Nutrition, *9*, 13–23 (1961).
21. Miwa, T. K., K. L. Mikolajczak, F. R. Earle, and I. A. Wolff, Anal. Chem., *32*, 1739–1742 (1960).
22. Ackman, R. G., and R. D. Burgher, Unpublished work.
23. Bagby, M. O., C. R. Smith, Jr., T. K. Miwa, R. L. Lohmar, and I. A. Wolff, J. Org. Chem., *26*, 1261–1265 (1961).
24. Daniels, N. W. R., and J. W. Richmond, Nature, *187*, 55–56 (1960).
25. Ackman, R. G., JAOCS, *40*, 564–567 (1963).
26. Richardson, T., A. L. Tappel, and E. H. Gruger, Jr., Arch. Biochem. Biophys., *94*, 1–6 (1961).
27. Klenk, E., and H. Steinbach, Zeit. physiol. Chem., Hoppe-Seyler's, *316*, 31–44 (1959).
28. Malins, D. C., and C. R. Houle, Proc. Soc. Exp. Biol. Med., *108*, 126–129 (1961).
29. Stoffel, W., and E. H. Ahrens, Jr., J. Lipid Research *1*, 139–146 (1960).
30. Woodford, F. P., and C. M. van Gent, J. Lipid Research *1*, 188–190 (1960).
31. Gerson, T., J. Chromatog., *6*, 178–181 (1961).
32. Lefort, D., C. Paquot, and A. Pourchez, Oléagineux, *16*, 253–259 (1961).
33. Markley, K. S., Fatty Acids, Their Chemistry and Physical Properties, 1st ed., p. 169, Interscience Publishers, New York, 1947, p. 169.

[Received May 9, 1962—Accepted April 16, 1963]

An Analysis of Separation Factors Applicable in the Gas-Liquid Chromatography of Unsaturated Fatty Acid Methyl Esters on a Polyester Substrate

R. G. ACKMAN, Fisheries Research Board of Canada, Technological Station, Halifax, Nova Scotia

Abstract

The employment of gas-liquid chromatography (GLC) separation factors between methyl esters of unsaturated fatty acids is feasible as a means of tentative identification, either between acids of one chain length and differing numbers of double bonds, or between acids of one chain length and the same number of double bonds in differing positions, provided the acid structures are appropriately grouped by end carbon chain. The modification of separation factors by temperature, chain length, number of double bonds, or position of double bonds is apparent from examination of a larger number of examples than was hitherto available. Examples of the usefulness of separation factors in identifying unknowns or predicting retention times are given.

Introduction

THE OBSERVATION has been made (1,2) that methyl esters of monoethylenic fatty acids, or of polyethylenic fatty acids with normal methylene-interrupted double bond systems, can be linearly interrelated when plotted on the basis of log retention time against number of carbon atoms in the fatty acid chain through the mutual number of double bonds and the same "end carbon chains." This correlating factor, defined as the number of carbon atoms from the center of the double bond farthest removed from the carboxyl group, to and including the terminal methyl group, is one carbon more than as originally conceived (1). This system permits the tentative identification of GLC peaks of esters of unsaturated fatty acids by extrapolation from components of a different chain length whose structure is known. The rationalization of certain literature data on this basis has permitted a listing, essentially an analysis on ethylene glycol-adipic acid polyester (EGA), of accurate retention times for methyl esters of a larger number of fatty acids with known and presumed structures than was hitherto available (2).

Systematic retention time relationships may also exist between the methyl esters of unsaturated fatty acids of one chain length and differing numbers of double bonds, or between those of the same chain length and the same number of double bonds, in different isomeric arrangements. This approach is based on the use of "separation factors" obtained by dividing the retention time or relative retention time of one fatty acid methyl ester by the lesser