

A PROPOSED BASIS FOR THE SYSTEMATIC IDENTIFICATION OF UNSATURATED FATTY ACID ESTERS THROUGH GAS-LIQUID CHROMATOGRAPHY ON POLYESTER SUBSTRATES

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INTRODUCTION

The linear relation obtained when the log retention times for methyl esters of certain unsaturated fatty acids possessing the same number of double bonds, but different chain lengths, are plotted against the number of carbon atoms in the fatty acid chain has been shown^{1,2} to be correlated with the end carbon chain (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*). This relationship may depend on the contribution made by the end carbon chain to some form of modification of the vapour pressure, since saturated esters having the same overall chain length display increasing retention times as either the alcohol or acid moiety is shortened³, and this correlates with vapour pressure changes known for this type of ester⁴. In octadecenoic acid esters this also appears to be the case, since as the double bond moves from the central 9-position towards the carboxyl end⁵ or towards the terminal methyl end^{6,7} the retention time increases. It is therefore instructive to compare the effect of the end carbon chain with that of what we may call the carboxyl end chain (the number of carbon atoms from the carboxyl group to the first carbon atom of the first double bond, inclusive), and to examine the variables which may affect these relationships.

LINEAR LOG PLOT RELATIONSHIP

On the basis of the proposed linear relation it would at first appear that the role of the carboxyl end chain is a neutral one, the addition of two carbon atoms (for pairs of commonly occurring fatty acids of even chain lengths) contributing the same effect independently of the actual size of the carboxyl end chain. This appears to be the case for the range covered by the linearly related pairs with carboxyl end chains as short as 5 and 7, or as long as 9 and 11, or even 11 and 13 if the unknown listed by FARQUHAR *et al.*⁸ with a retention time relative to stearate of 4.38 is the 13,16-docosadienoic ester. Although for esters of saturated acids the log of properly corrected retention times when plotted against the number of carbon atoms in the chain usually gives a straight line over a wide range of chain lengths^{9,10}, there is some indication that for the shorter chain lengths this line may inflect upwards as the chain length decreases^{11,12}. In

* One more carbon atom than as originally defined¹.

the latter instance¹² the average line for the points representing dodecanoic, tetradecanoic and hexadecanoic esters is parallel to the lines joining the unsaturated ester points, and both converge with the line joining the points for octadecanoic, eicosanoic and docosanoic esters as the chain length increases. On the other hand in the data of FARQUHAR *et al.*⁸ at three temperatures all the saturated ester points from dodecanoic to eicosanoic fall on a straight line, while the unsaturated lines and the saturated line become more convergent as the operating temperature decreases. Depending on operating conditions, it is therefore possible that the contribution of the carboxyl end chain may reflect a modification of retention time paralleling that observed with certain of the shorter chain saturated acids, the precise range of chain lengths and slope of the line of these acids being influenced by many operating variables. This could explain why the unsaturated ester lines appear to be parallel to each other as the range of saturated acids corresponding to the carboxyl end chains is fairly narrow and therefore would give a nearly linear log plot. Moreover since the linear log plot usually relates only pairs of acids the error associated with any slight change in the slope of the log plot of the lower saturated fatty acids will not seriously affect the apparent parallel linear relationship between such pairs as the carboxyl end chains vary in length. Unless this range is co-linear with the higher saturated fatty esters the latter line will not be parallel with the unsaturated acid lines.

SEPARATION FACTORS

In a study of the separation factors of acids of the same chain length three main types of separation factors have been established¹³. In type I separation factors the end carbon chains are fixed and the separation factors may be grouped on the basis of the three-carbon ratios of the carboxyl end chains commonly occurring in natural lipid systems (Table I). These separation factors may not be completely independent of the length of the end carbon chain, with some indication that as the latter increases, for a given fatty acid chain length, the type I separation factors also increase for those pairs with the same carboxyl end chain ratio. This also corresponds to fewer double bonds, but the type II separation factors (see below) indicate that the number of double bonds should be relatively unimportant.

In comparing the separation factors for the pairs of acids which may be linearly related by the log plot relationship it is seen that as the fatty acid chain length increases (or the respective carboxyl end chains are each increased) there is some evidence of diminution in the separation factors, particularly in the case of the more reliable EGA data. This supports the contention that the magnitude of the carboxyl end chains may affect the parallel linearity of the log plot relationship.

In addition to the separation factors for the adjacent pairs of acids listed in Table I very good agreement is observed in those based on pairs differing by two double bonds but having the same end carbon chains. Thus for a 4/10 carboxyl end chain ratio the EGA values are 1.21 and 1.22. For the 5/11 carboxyl end chain ratios the EGA values are 1.24 and 1.24, and the EGS values are 1.31 and 1.31.

In type II separation factors (Table II) the chain length and carboxyl end chain are fixed and the separation factors may be grouped on the basis of the end carbon chain ratios. These factors are of a higher order of magnitude than the type I factors and correspondingly more accurate. The correlation is very good, independent of the

TABLE I
TYPE I SEPARATION FACTORS BASED ON THE RATIOS OF CARBOXYL
END CHAINS WITH FIXED FATTY ACID CHAIN LENGTH AND END CARBON CHAINS

| Carboxyl end chain ratio | Fatty acid | End carbon chain | Polyester | | | | |
|--------------------------------|------------|------------------------|-------------|----------------------|-------------|----------------------|------|
| | | | EGA (197°) | | EGS (205°) | | |
| | | | $r_{12:10}$ | Separation factor | $r_{12:11}$ | Separation factor | |
| 4/7 | 22:6 | 4, 7, 10, 13, 16, 19 | 3 | 7.75 | | 6.20 | |
| | 22:5 | 7, 10, 13, 16, 19 | 3 | 7.00 | 1.10 | 5.47 | 1.13 |
| 4/7 | 22:5 | 4, 7, 10, 13, 16 | 6 | 6.09 | | — | |
| | 22:4 | 7, 10, 13, 16 | 6 | 5.50 | 1.11 | — | — |
| 5/8 | 20:5 | 5, 8, 11, 14, 17 | 3 | 3.85 | | 3.34 | |
| | 20:4 | 8, 11, 14, 17 | 3 | 3.51 | 1.10 | 3.05 | 1.10 |
| 5/8 | 20:4 | 5, 8, 11, 14 | 6 | 3.04 | | 2.58 | |
| | 20:3 | 8, 11, 14 | 6 | 2.76 | 1.10 | 2.32 | 1.11 |
| 6/9 | 18:4 | 6, 9, 12, 15 | 3 | 1.97 | | 1.83 | |
| | 18:3 | 9, 12, 15 | 3 | 1.72 | 1.14 | 1.56 | 1.17 |
| 6/9 | 18:3 | 6, 9, 12 | 6 | 1.54 | | 1.41 | |
| | 18:2 | 9, 12 | 6 | 1.34 | 1.15 | 1.21 | 1.17 |
| 6/9 | 18:2 | 6, 9 | 9 | 1.29 | | 1.18 | |
| | 18:1 | 9 | 9 | 1.12 | 1.15 | 1.00 | 1.18 |
| 6/9 | 16:3 | 6, 9, 12 | 4 | — | | 0.925 | |
| | 16:2 | 9, 12 | 4 | — | — | 0.773 | 1.19 |
| 6/9 | 16:2 | 6, 9 | 7 | — | | 0.730 | |
| | 16:1 | 9 | 7 | — | — | 0.612 | 1.19 |
| 7/10 | 22:5 | 7, 10, 13, 16, 19 | 3 | 7.00 | | — | |
| | 22:4 | 10, 13, 16, 19 | 3 | 6.40 | 1.09 | — | — |
| 7/10 | 22:4 | 7, 10, 13, 16 | 6 | 5.50 | | — | |
| | 22:3 | 10, 13, 16 | 6 | 5.00 | 1.10 | — | — |
| 8/11 | 20:4 | 8, 11, 14, 17 | 3 | 3.51 | | 3.05 | |
| | 20:3 | 11, 14, 17 | 3 | 3.10 | 1.13 | 2.54 | 1.20 |
| 8/11 | 20:3 | 8, 11, 14 | 6 | 2.76 | | 2.32 | |
| | 20:2 | 11, 14 | 6 | 2.45 | 1.13 | 1.97 | 1.18 |
| 8/11 | 20:2 | 8, 11 | 9 | 2.32 | | — | |
| | 20:1 | 11 | 9 | 2.02 | 1.15 | — | — |

chain length and number of double bonds, and the types follow closely those found in naturally occurring lipid mixtures.

The type III separation factors (Table III) are based on pairs of acids with the same chain length and the same number of double bonds. The correlation between these factors apparently depends on the respective pairs of acids being linearly related by the log plot system. The type III factors can be obtained by dividing the applicable type II factor by the appropriate average type I factor (inverting the carboxyl end chain ratio, but not the numerical value). The most highly centralized systems of polyethylenic unsaturation have the lowest type III separation factors (*cf. ref. 14*),

TABLE II
 TYPE II SEPARATION FACTORS BASED ON THE RATIOS OF END CARBON
 CHAINS WITH FIXED FATTY ACID CHAIN LENGTH AND CARBOXYL END CHAINS

| Fatty acid | End carbon chain | Polyester | | | | | | | | | | |
|------------|----------------------|------------|--------------------|------|------|------------|--------------------|-------|------|------|------|------|
| | | EGA | | | | EGS | | | | | | |
| | | $r_{18:0}$ | Separation factors | | | $r_{18:1}$ | Separation factors | | | | | |
| | | | 3/6 | 6/9 | 3/9 | | 3/6 | 6/9 | 3/9 | 4/7 | | |
| 22:6 | 4, 7, 10, 13, 16, 19 | 3 | 7.75 | | | | | 6.20 | | | | |
| 22:5 | 4, 7, 10, 13, 16 | 6 | 6.09 | 1.27 | | | | 4.64 | 1.33 | | | |
| 22:4 | 4, 7, 10, 13 | 9 | 5.10 | | 1.19 | 1.52 | | — | — | — | | |
| 22:5 | 7, 10, 13, 16, 19 | 3 | 7.00 | | | | | — | | | | |
| 22:4 | 7, 10, 13, 16 | 6 | 5.50 | 1.27 | | | | — | — | | | |
| 22:4 | 10, 13, 16, 19 | 3 | 6.40 | | | | | — | | | | |
| 22:3 | 10, 13, 16 | 6 | 5.00 | 1.28 | | | | — | — | | | |
| 20:5 | 5, 8, 11, 14, 17 | 3 | 3.85 | | | | | 3.34 | | | | |
| 20:4 | 5, 8, 11, 14 | 6 | 3.04 | 1.27 | | | | 2.58 | 1.30 | | | |
| 20:3 | 5, 8, 11 | 9 | 2.53 | | 1.20 | 1.52 | | 2.11 | | 1.22 | 1.58 | |
| 20:4 | 8, 11, 14, 17 | 3 | 3.51 | | | | | 3.05 | | | | |
| 20:3 | 8, 11, 14 | 6 | 2.76 | 1.27 | | | | 2.32 | 1.31 | | | |
| 20:2 | 8, 11 | 9 | 2.32 | | 1.19 | 1.51 | | — | — | — | — | |
| 18:4 | 6, 9, 12, 15 | 3 | 1.97 | | | | | 1.83 | | | | |
| 18:3 | 6, 9, 12 | 6 | 1.54 | 1.28 | | | | 1.41 | 1.30 | | | |
| 18:2 | 6, 9 | 9 | 1.29 | | 1.19 | 1.53 | | 1.18 | | 1.20 | 1.55 | |
| 18:3 | 9, 12, 15 | 3 | 1.72 | | | | | 1.56 | | | | |
| 18:2 | 9, 12 | 6 | 1.34 | 1.28 | | | | 1.21 | 1.29 | | | |
| 18:1 | 9 | 9 | 1.12 | | 1.19 | 1.54 | | 1.00 | | 1.21 | 1.56 | |
| 16:3 | 6, 9, 12 | 4 | — | | | | | 0.925 | | | | |
| 16:2 | 6, 9 | 7 | — | | | | | 0.730 | | | | 1.27 |
| 16:2 | 9, 12 | 4 | — | | | | | 0.773 | | | | |
| 16:1 | 9 | 7 | — | | | | | 0.612 | | | | 1.26 |

particularly evident in the case of the 6,9- and 9,12-octadecadienoates. In this case although the numerical index ratios of the two types of end chains are the same the greater influence of the end carbon chains gives the necessary change in volatility to ensure some separation. It must be presumed that the carboxyl end chain ratio contribution is reduced in effect by the ester linkage and alcohol moiety.

The gas-liquid chromatography of complex lipid mixtures such as marine oils is greatly assisted by the use of these separation factors, supplementing in a more precise way the linear log plot system. Thus a suspected component may be identified by its various relations to any of several different acids which have been identified. Alternatively the retention time may be predicted and the location on the chromatogram examined for a component. When even less information is available trial and error procedures including several components may be necessary.

In the tabulated data drawn from that of FARQUHAR *et al.*⁸ a number of component fatty acids (in italics) have been identified through the use of these systematic separation factors¹³, as well as by the linear log plot relationship². In addition a few others

TABLE III

TYPE III SEPARATION FACTORS WITH FIXED FATTY ACID CHAIN LENGTH AND NUMBER OF DOUBLE BONDS, COMPARING FOUND AND CALCULATED VALUES

| Fatty acid | EGA Polyester | | | | | EGS Polyester | | | | | | | | |
|------------|-------------------|------------------|---------|--------|------------------|---------------|------------------|---------|--------|------------------|------|------|------|------|
| | $r_{18:0}$ | Type III (found) | Type II | Type I | Type III (calc.) | $r_{18:1}$ | Type III (found) | Type II | Type I | Type III (calc.) | | | | |
| 22:5 | 7, 10, 13, 16, 19 | 7.00 | | | | 5.47 | | | | | | | | |
| 22:5 | 4, 7, 10, 13, 16 | 6.09 | 1.15 | 3/6 | 1.27 | 7/4 | 1.10 | 1.15 | 1.18 | 3/6 | 1.31 | 7/4 | 1.13 | 1.16 |
| 22:4 | 10, 13, 16, 19 | 6.40 | | | | — | | | | | | | | |
| 22:4 | 7, 10, 13, 16 | 5.50 | 1.16 | 3/6 | 1.27 | 10/7 | 1.10 | 1.15 | — | — | — | — | — | — |
| 20:4 | 8, 11, 14, 17 | 3.51 | | | | 3.05 | | | | | | | | |
| 20:4 | 5, 8, 11, 14 | 3.04 | 1.15 | 3/6 | 1.27 | 8/5 | 1.10 | 1.15 | 1.18 | 3/6 | 1.31 | 8/5 | 1.11 | 1.18 |
| 20:3 | 11, 14, 17 | 3.10 | | | | 2.54 | | | | | | | | |
| 20:3 | 8, 11, 14 | 2.76 | 1.12 | 3/6 | 1.27 | 11/8 | 1.13 | 1.12 | 1.09 | 3/6 | 1.31 | 11/8 | 1.19 | 1.10 |
| 18:3 | 9, 12, 15 | 1.72 | | | | 1.56 | | | | | | | | |
| 18:3 | 6, 9, 12 | 1.54 | 1.12 | 3/6 | 1.27 | 9/6 | 1.15 | 1.10 | 1.11 | 3/6 | 1.31 | 9/6 | 1.18 | 1.11 |
| 22:4 | 7, 10, 13, 16 | 5.50 | | | | — | | | | | | | | |
| 22:4 | 4, 7, 10, 13 | 5.10 | 1.08 | 6/9 | 1.19 | 7/4 | 1.10 | 1.08 | — | — | — | — | — | — |
| 20:2 | 11, 14 | 2.45 | | | | — | | | | | | | | |
| 20:2 | 8, 11 | 2.32 | 1.05 | 6/9 | 1.19 | 11/8 | 1.13 | 1.05 | — | — | — | — | — | — |
| 18:2 | 9, 12 | 1.34 | | | | 1.21 | | | | | | | | |
| 18:2 | 6, 9 | 1.29 | 1.04 | 6/9 | 1.19 | 9/6 | 1.15 | 1.03 | 1.18 | 1.02 | 6/9 | 1.21 | 9/6 | 1.18 |
| 18:2 | 11, 14 | 1.45 | | | | — | | | | | | | | |
| 18:2 | 8, 11 | 1.33 | 1.09 | 4/7 | 1.23 | 11/8 | 1.13 | 1.09 | — | — | — | — | — | — |
| 16:2 | 9, 12 | — | | | | 0.773 | | | | | | | | |
| 16:2 | 6, 9 | — | — | — | — | 0.730 | 1.06 | 4/7 | 1.26 | 9/6 | 1.18 | 1.18 | 1.06 | |

have been listed with the retention time and structure both in italics, indicating hypothetical acids with predicted retention times. These are included to complement certain fatty acids of known structure which could not otherwise be included in a particular table. The excellent correlation of all of these acids and retention times in the various types of separation factors indicates the reliability of this systematic approach to fatty acid identification. The data for the EGS polyester are drawn from a preliminary identification study¹² on the component fatty acids of seal blubber and virtually all of the acids were tentatively identified by both the linear log plot and separation factors. All the fatty acids employed in the EGS data (Tables I, II and III) have been associated with peaks excepting 6,9-octadecadienoic. An Aerograph A-90 was employed in these studies, still air bath temperature 205°, injection port temperature 250°. Columns were copper tubing, 1/4 in. O.D. and 10 ft. in length, packed with 20% commercial EGS on GC-22 "Super-Support". Helium at 90 ml/min was employed as the carrier gas.

The type I and type III separation factors are particularly susceptible to small errors in retention time. Thus the data of FARQUHAR *et al.*⁸ giving the temperature variation of retention time for 9,12,15-octadecatrienoic acid, in comparison for that for 6,9,12,15-octadecatetraenoic acid, suggests that 1.72 at 197.5° is slightly high, and a value of 1.70 improves the correlation for all three types of separation factors. The retention time of 5.10 for 4,7,10,13-docosatetraenoate has been adopted instead of the previous tentative identification¹³ of this acid with a component with the value of 5.30, since the latter obviously does not fit the separation factors, and also since there is reason to believe that this may be a heneicosapentaenoate tentatively identified in seal oil¹². In the EGS series reasonable correlation is obtained excepting with the 4,7,10,13,16,19-docosahexaenoate, for which it is difficult to determine a retention time accurately owing to limitations of the apparatus. A value of 6.10 for the retention time of this ester would give very satisfactory results with all three types of separation factors.

The above data are all based on the normally occurring fatty acids related to 9-hexadecenoic, 9-octadecenoic, 11-eicosenoic and 13-docosenoic acids. An opportunity to extend this to other C₁₈ monoethylenic acids and acids related to the latter is provided by some hitherto unpublished information⁷ provided through the courtesy of Dr. C. R. SCHOLFIELD. The relative retention times in Table IV represent composite

TABLE IV
TYPE I AND TYPE II SEPARATION FACTORS FOR FATTY ACIDS
RELATED TO 9-, 12- AND 15-OCTADECENOIC ACIDS ON AN EGS POLYESTER

| Fatty acid | Type I | | | | | Fatty acid | Type II | | | |
|------------|--------------------------|--------------------|-------|-----------|--------------------------|------------|--------------------|------|------|-----------|
| | <i>r</i> _{18:1} | Separation factors | | | <i>r</i> _{18:1} | | Separation factors | | | |
| | | 9/12 | 12/15 | 9/15 | | | 3/6 | 6/9 | 3/9 | |
| 18:3 | 9, 12, 15 | 1.71 | | | | 18:3 | 9, 12, 15 | 1.71 | | |
| 18:2 | 12, 15 | 1.43 | 1.20 | | | 18:2 | 9, 12 | 1.28 | 1.34 | |
| 18:1 | 15 | 1.10 | | 1.30 1.56 | | 18:1 | 9 | 1.00 | | 1.28 1.71 |
| 18:2 | 9, 12 | 1.28 | | | | 18:2 | 12, 15 | 1.43 | | |
| 18:1 | 12 | 1.04 | 1.23 | | | 18:1 | 12 | 1.04 | 1.37 | |

data from different analyses on an EGS polyester and therefore must be regarded as only approximately correct, but general agreement is indicated between the pairs in the type I and type II factors, while the type III separation factor for the 9,12- and 12,15-octadecadienes calculated from the average of each (1.12) agrees with the value found from the retention times (1.12). Although no direct comparison may be made, this type III separation factor is much higher than the corresponding factor (Table III) obtained on EGS polyester for the highly centralized 6,9- and 9,12-octadecadienes, agreeing with the view that the most highly centralized unsaturated fatty acid ester structures have the shortest retention time and the lowest type III separation factors.

VARIABLES AFFECTING SEPARATION FACTORS

In general the separation factors between unsaturated fatty acids are improved slightly by a reduction in operating temperatures. This conclusion is based on the temperature dependent data listed by FARQUHAR *et al.*⁸ and other work¹⁵ (see Table V).

TABLE V

COMPARISON OF TYPE II SEPARATION FACTOR PROPORTIONS OBTAINED BY DIFFERENT AUTHORS

| Separation factors | | | Temp. | Polyester | % | Support | Mesh | Wash | | Gas | Ref. |
|--------------------|------|------|-------|-------------|-----|--------------|---------|------|------|-----|------|
| 3/9 | 3/6 | 6/9 | | | | | | Acid | Base | | |
| 1.45 | 1.22 | 1.18 | 205 | BDS | 5 | Celite 545 | 40-60 | Yes | No | He | 16 |
| 1.45 | 1.24 | 1.17 | 203 | DEGS | 20 | Celite 545 | 60-80 | Yes | No | He | 17 |
| 1.46 | 1.24 | 1.18 | 205 | EGS | 5 | Chromosorb W | 40-60 | | | He | 16 |
| 1.46 | 1.23 | 1.18 | 203 | DEGS | 20 | Celite 545 | 60-80 | Yes | Yes | He | 18 |
| 1.48 | 1.26 | 1.18 | 221 | LAC-2R-446 | 25 | Chromosorb W | 30-60 | | | He | 19 |
| 1.48 | 1.24 | 1.19 | 200 | Reoplex 400 | 30 | Celite 545 | 60-80 | Yes | Yes | He | 9 |
| 1.49 | 1.24 | 1.20 | 205 | BDS | 7 | Chromosorb W | 40-60 | | | He | 16 |
| 1.50 | 1.26 | 1.19 | 197 | Reoplex 400 | 18 | Celite 545 | 100-120 | Yes | Yes | Ar | 8 |
| 1.52 | 1.27 | 1.20 | 177 | EGA | 2.3 | Embacel | 60-100 | | | Ar | 20 |
| 1.52 | 1.27 | 1.20 | 196 | EGA | 15 | Chromosorb W | 80-100 | | | Ar | 14 |
| 1.53 | 1.26 | 1.21 | 205 | EGS | 20 | GC-22 | 60-80 | | | He | 12 |
| 1.54 | 1.28 | 1.20 | 197 | DEGS | 20 | Chromosorb | 35-80 | | | He | 11 |
| 1.54 | 1.28 | 1.20 | 197 | EGA | 18 | Celite 545 | 100-120 | Yes | Yes | Ar | 8 |
| 1.54 | 1.28 | 1.20 | 184 | EGA | 18 | Celite 545 | 100-120 | Yes | Yes | Ar | 8 |
| 1.57 | 1.30 | 1.21 | 173 | EGA | 18 | Celite 545 | 100-120 | Yes | Yes | Ar | 8 |
| 1.58 | 1.31 | 1.21 | 193 | LAC-2R-728 | 30 | Chromosorb | 48-65 | | | Ar | 21 |
| 1.58 | 1.29 | 1.23 | 200 | DEGS | 20 | Celite 545 | 48-85 | Yes | Yes | H | 10 |
| 1.59 | 1.30 | 1.22 | 180 | EGA | 25 | Celite 545 | 100-210 | | | Ar | 5 |
| 1.60 | 1.30 | 1.22 | 200 | DEGS | 20 | GC-22 | 60-80 | | | He | 15 |
| 1.64 | 1.33 | 1.24 | 180 | DEGS | 20 | GC-22 | 60-80 | | | He | 15 |

This is the reverse of the usual criterion of the efficiency of polyester substrates, the separation of the corresponding saturated and monounsaturated acid esters, which normally improves as the operating temperature is increased.

The type II separation factors are independent of carrier gas flow rate^{15,16} but for a particular polyester on a particular support the variation with the ratio of support to substrate is an important factor in most cases¹⁶. This is shown in Fig. 1 where it is apparent that for the type II 3/9 separation factor, under otherwise identical conditions, a BDS polyester is less sensitive to concentration change on Chromosorb W than on C-22 firebrick, and on both is less sensitive to change than is

EGS polyester. The other type II separation factors appear to be roughly proportional to the 3/9 ratio factor.

An attempt was made to determine the general influence of operational variables on the type II separation factors by surveying the literature separations reported for the series 9-octadecenoate, 9,12-octadecadienoate, and 9,12,15-octadecatrienoate (Table V). Since the 3/9 end carbon chain ratio has the largest numerical value the

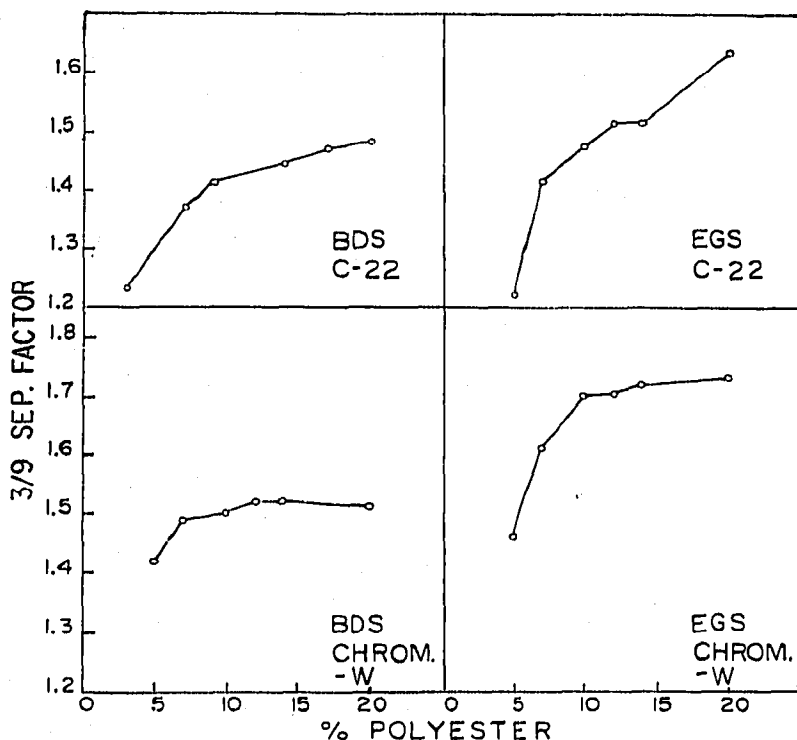


Fig. 1. Variation of type II separation factors (3/9 end carbon chain ratio) with concentration of polyester for different polyesters and supports.

results have been arranged in order of increase of this value. The dependent values for 3/6 and 6/9 end carbon chain ratios seem to increase in proportion, and when plotted an apparently linear relation is obtained (Fig. 2). Owing to the scale needed to express the small quantities involved there is considerable scatter of points, especially for the 6/9 values, but the 3/6 values are moderately consistent. It is very difficult to assess the merits of the data chosen, since there is little indication in the literature in many cases of whether the data are based on corrected or adjusted retention times, or whether the retention times were determined by the frontal tangent or half-base width systems, or by the less desirable method of dropping a perpendicular from the peak top. However, where several groups of ratios are taken from the data of one author^{8,15,16} the agreement with the plotted lines is very good. In general, however, it appears that there is no general correlation in terms of polyester, percentage polyester, support, support size, temperature or carrier gas. In the absence of any correlating factor, the graph might be used as an approximate guide to determine any two of the separation factors if the other is known, but this should be restricted to the range of values plotted, since it has not been shown that a true linear relation exists and extrapolation would be dangerous.

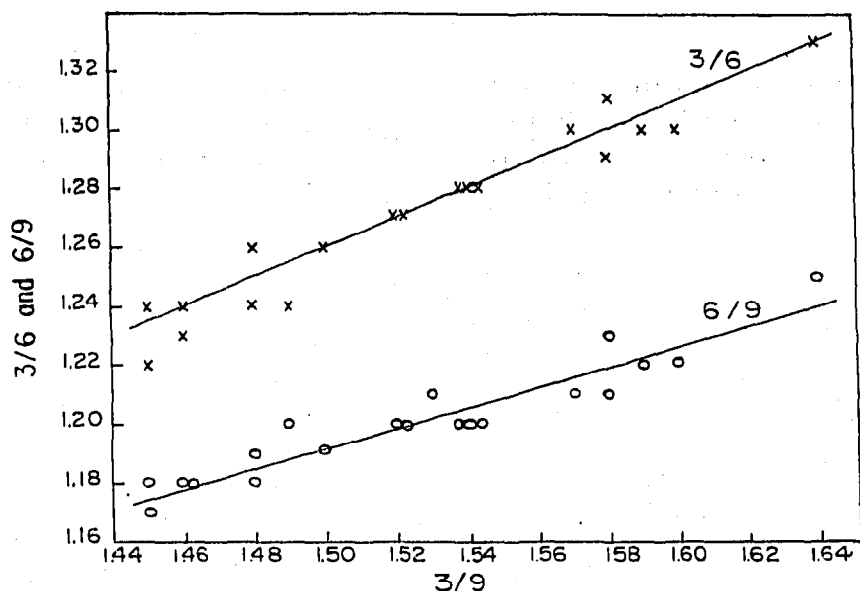


Fig. 2. Plot of data (Table V) relating type II separation factors for methyl 9-octadecenoate, 9,12-octadecadienoate and 9,12,15-octadecatrienoate.

The above discussion applies only to the type II separation factors, but clearly shows that the complex interchange of variables, while affecting the magnitude of these separation factors, has a lesser effect on the proportional differences between separation factors of this type. This is in agreement with the proposed source of this particular relationship, that of differences in volatility induced by the size of the end carbon chain. The latter should not be influenced by polarity of the support for example, yet this would have some effect on the carboxyl end chain, hence modifying the relationship between the carboxyl end chain and the end carbon chain. This would affect the magnitude of the type II separation factors, but would not necessarily have a serious effect on the proportional differences which are due solely to the end carbon chains.

It is therefore probable that derivatives of the unsaturated acids produced by modifying the carboxyl group will have the same type II separation factors as the corresponding methyl esters analysed on the identical column. The data for such a comparison are not immediately available, but the separation factors for the dimethyl acetals of the aldehydes corresponding to 9-octadecenoic, 9,12-octadecadienoic and 9,12,15-octadecatrienoic acids²², respectively 1.25, 1.19 and 1.50 for end carbon chain ratios of 3/6, 6/9, and 3/9, fit the plot (Fig. 2) derived from methyl esters very well. Moreover the separation factor for 9,12-octadecadienyl acetate and 9-octadecenyl acetate²³ on EGA at 184.8° is 1.21, the same as the value reported by the same author⁸ for the corresponding methyl esters on EGA at 184.5° under presumably comparable conditions.

Considering the three types of separation factors in general as applied to fatty acid ester separations, it is obvious that improvement in one separation between particular acids may hinder separation in other cases. Thus the type I and type II separations are obviously slightly better with the EGS polyester on GC-22 (Tables I and II), but the type III separations (Table III) for certain critical pairs such as the 6,9- and 9,12-octadecadienoates are apparently superior with the EGA polyester on Celite 545.

SUMMARY

Systematic separation factors may be established among various unsaturated fatty acid methyl esters analysed by gas-liquid chromatography on polyester substrates. These are apparently dependent on differences in the magnitude of the carboxyl end chain and end carbon chain parts of the fatty acid chain.

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