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Identification of the Major Polyunsaturated C_{16} Acids of Marine Oils by GLC Separation Factors on Normal and Organosilicone Polyesters

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Abstract

The tentative identification of the unsaturated C_{16} acids of marine oils is facilitated through analysis on both normal and organosilicone polyester substrates. Two different separation factors can then be approximated from the more accessible separation factors appropriate to unsaturated acids of longer chain lengths.

Introduction

THE MAJOR unsaturated C_{16} acids of marine oils, such as menhaden and herring oils, have been identified as 9-hexadecenoic, 6,9- and 9,12-hexadecadienoic, 6,9,12-hexadecatrienoic and 6,9,12,15-hexadecatetraenoic (1–3). These are distinguished from the C_{18},C_{20}, and C_{22} acids in that the end carbon chains (4–8) are 1,4 or 7, whereas in the other chain lengths the end carbon chains are normally 3,6 or 9 (3,9). Some minor C_{16} isomers do have the latter end carbon chains, but occur in such small proportions that they are not normally evident by GLC of esters from whole oils. Thus systematic separation factors based on end carbon chains which are interchangeable with acids of other chain lengths are, at first glance, of little use with the C_{16} acids.

The terms "separation" or "separation factor" have been used in a number of meanings in GLC by different authors (cf. 3). The "systematic separation factors" (6,8,10) referred to in the present study apply to ratios of adjusted relative retention times (the greater divided by the lesser) for particular pairs of monounsaturated or methylene interrupted polyunsaturated fatty acids of a given chain length, characterized by having in common either the same number of carbon atoms between the carboxyl group and the center of the first double bond (Type II), or between the ultimate double bond and the terminal carbon atom of the chain (Type I).

A further complication is that the normal chain length overlap of marine oil methyl esters on polyester substrates will result in one or more of the materials in question probably coinciding with heptadecanoate, heptadecenoate, or octadecanoate, which are present in roughly the same proportions, and on highly polar polyesters the trienoate and tetraenoate frequently are masked by the very large octadecenoate peak.

In a previous study of systematic separation factors applied to the identification of the peaks of a menhaden oil, analysis of the data for the C_{16} acids was inadequate for examination of the separation factors (3,8). By employing a concentrate of these acids obtained from seal oil (10), experiments have revealed certain properties of these acids which give significant variations in separation factors on normal polyester substrates as compared with organosilicone polyester substrates and enable comparisons to be made which greatly assist in the identification of these materials.

Experimental

Columns and operating conditions employed with either Wilkens Aerograph A-90 or Barber-Colman Model 10 gas chromatographs are given in Table I. The organosilicone polyester packing was from Applied Science Laboratories, Inc., while all other columns except No. 5 were prepared from commercial subtrates and support materials in this laboratory.

A high iodine value fraction obtained from urea complex fractionation of seal methyl esters (10) was employed and identifications previously obtained on

TABLE I

Type and Operating Data of Columns Used in Determining Separation Factors.

Column	% Polyester [a]	Support	Column		Temp °C
			Length	Diam	
1 [b]	20% EGS	100–120 Chromosorb W	6 ft	3 mm I.D.	200
2 [c]	20% EGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	205
3 [c]	20% DEGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	220
4 [c]	20% DEGS	60–80 GC-22 Super-Support	10 ft	¼ in. O.D.	220
5 [b]	18% EGA	100–120 Celite 545	5 ft	4 mm I.D.	197
6 [b]	10% EGSS-X	100–120 Sil. Gas-Chrom P	6 ft	3 mm I.D.	200
7 [b]	12% EGSS-Y	100–120 Sil. Gas-Chrom P	6 ft	3 mm I.D.	200

[a] Ethylene glycol succinate (EGS)
Diethylene glycol succinate (DGS)
Ethylene glycol adipate (EGA)
Ethylene glycol succinate silicone—X (EGSS-X)
Ethylene glycol succinate silicone—Y (EGSS-Y)

[b] Argon carrier gas, ionization detector, glass columns.

[c] Helium carrier gas, thermal conductivity detector, metal columns.

ethylene glycol succinate and neopentyl glycol succinate polyesters were satisfactorily confirmed on a variety of other polyesters. Separation factors for these components were drawn up for all chain lengths and the data in Table II selected as typical of the Type II factors. The normal range of a particular separation factor is ± 0.01 or less in a given complete analysis of a marine oil. Linear log plots of relative retention time were drawn on 358-61 semilog graph paper (2 cycle × 70 divisions) with the aid of a set of parallel rules.

Results and Discussion

Ordinary Polyesters. Employing the concentrate of polyunsaturated esters of seal oil, it was possible to verify that the approximate retention times of the diene and triene C_{16} acids could be estimated from a linear log plot, since they normally lie between the points for various real or hypothetical isomers whose points may be obtained by extrapolation of known structures in the C_{18} acids. If 6,9,12-octadecatrienoate or 6,9,12,15-octadecatetraenoate are present, comparison of these respectively with 9,12-octadecadienoate and 9,12,15-octadecatrienoate will establish the Type I 6/9 separation factor, and from the relative retention time of 9-hexadecenoate that of 6,9,-hexadecadienoate can then be calculated.

Although the Type II 4/7 separation factor normally lies between the 3/6 and 6/9 values (Table II), either the 9,12-hexadecadienoate or the 6,9,12-hexadecatrienoate must be identified in order to fix the other, since there is no unique mathematical approach by comparison of the Type I 6/9 and Type II 4/7 separation factors for the various materials under discussion. It is usually possible to distinguish one of these, often the larger 9,12-hexadecadienoate, by comparison of the peak sizes on polyester columns of differing polarity and consideration of the possible values read off the linear log plot. Thus the relative retention times for 9-hexadecenoate, 6,9- and 9,12-hexadecadienoates and 6,9,12-hexadecatrienoate may usually be obtained on normal polyester columns through the use of the two types of separation factors.

The 6,9,12,15-hexadecatetraenoate is unique among methylene interrupted unsaturated esters, since it has a terminal vinyl group. Experimental values with ordinary polyesters (Table II) indicate that, possibly because of this structure, the Type II 1/4 separation factor is normally the same, or very slightly lower, than the 3/6 separation factor. Thus it is possible to calculate the position for this tetraenoic ester if that for 6,9,12-hexadecatrienoate is known or can be calculated. On the linear log plot this point lies well above the point predicted and found for 4,7,10,13-hexadecatetraenoate by extrapolation from the C_{18} tetraene.

Organosilicone Polyesters. These materials are composed of an integral copolymer of silicone and polyester and are supplied on a silanized support (11,12). They have been found in this laboratory to have much greater thermal stability and lower bleed rates than ordinary polyesters. The performance of two organosilicone polyester columns (Tables I and II) has been tested successfully by the linear log plot and separation factor procedures as applied to mixtures of polyunsaturated fatty acid esters which had been previously analyzed on ordinary polyesters. The experimental values for the C_{16} acid esters revealed two differences which offer certain advantages in the tentative identification of the polyunsaturated acids of this chain length. The procedure previously employed for the four less highly unsaturated C_{16} acids is also still applicable.

TABLE II

Typical separation factors (Type II) obtained with various polyesters

Polyester	Column	Separation Factors				
		1/4	3/6	4/7	6/9	3/9
EGS	1	1.33	1.33	1.31	1.27	1.67
EGS	2	1.29	1.30	1.27	1.22	1.57
DEGS	3	1.26	1.27	1.25	1.21	1.53
DEGS	4	1.25	1.25	1.23	1.20	1.51
EGA[a]	5	......	1.27	1.23?	1.19	1.53
EGSS-X	6	1.29	1.33	1.33	1.27	1.68
EGSS-Y	7	1.20	1.26	1.26	1.20	1.51

[a] Data of Farquhar et al. (3,6–8).

A significant feature of the Type II separation factors for these materials (Table II) is that the 4/7 separation factor is increased to the same value as the 3/6 factor, while the 1/4 factor is appreciably reduced. Thus, the approximate 4/7 separation factor is indirectly obtainable from other chain lengths, while the linear log plot point for 6,9,12,15-hexadecatetraenoate is very nearly colinear with the points for 6,9,12,15-octadecatetraenoate and 8,11,14,17-eicosatetraenoate. This shift is apparently proportional to the silicone content, since on EGSS-X, described as having a low silicone content (11), there is fair separation from 4,7,10,13-hexadecatetraenoate, whereas on EGSS-Y, with a medium silicone content, the two are barely separated. The positions for the other three polyunsaturated C_{16} acids in relation to the lines drawn through the common C_{16} acids are not significantly altered.

The reason for the shift in the 4/7 separation factor is not clear. The data in Table II suggest that this is a genuine increase and not a relative decrease in the 3/6 separation factor. However the order of appearance of polyunsaturated acid esters on methyl silicone substrates such as SE-30 is the reverse of that on organic polyesters, the more highly unsaturated appearing first and hence probably in order of boiling point (13). The hexadecatetraenoate, with a terminal vinyl group, may be particularly affected by this effect, and by the silicone component of the organosilicone polyester. This is suggested by the lower bp of 1-monoalkenes in relation to other monoalkenes, and on non-polar substrates such as methyl silicones reasonable correlation of the monoalkene bp and GLC retention time is obtained at moderate temp (14). Experimental verification of this point on an SE-30 column was not successful, the separation between respective C_{16},C_{18}, and C_{20} monoenoic and tetraenoic acid esters being nominally the same and sufficiently small so that any significant difference was within the error of determination.

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