THE ANALYSIS OF OILS AND FATS BY GAS CHROMATOGRAPHY

VIII. CORRELATION OF RETENTION DATA WITH POLARITY OF STATIONARY PHASE

GEORGE R. JAMIESON AND ELIZABETH H. REID
Department of Chemistry, Paisley College of Technology, Paisley (Great Britain)
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SUMMARY

A computer has been used to construct tables of equivalent chain-length values for fourteen $C_{18}-C_{22}$ polyolefinic fatty acid methyl esters, with the values for methyl linolenate as references. These tables can be used with retention data from polyester columns of different polarities for the tentative identification of olefinic methyl esters and fatty alcohol derivatives.

The tables can also be used for a comparison of London interactions in a series of related compounds.

INTRODUCTION

In recent years many different types of polyesters and modified polyesters have been used as stationary liquids in the gas chromatographic separation of fatty acid methyl esters. These polyesters are particularly useful for the separation of olefinic esters which are retarded relative to the saturated esters of the same chain length; the degree of retardation depending on the polarity of the polyester. The basis of such separations has been discussed[1-3], and two effects may be considered:

(a) The London dispersion interactions which effect separations on a molecular weight–solubility–volatility basis. These interactions decrease with a decrease in molecular weight and with an increase in branching of the hydrocarbon chain. They are also affected by the number of olefinic bonds, the positions of these bonds in the chain, and by the configurations about these bonds. The London interactions decrease with an increase in polarity of the polyester stationary liquid.

(b) The specific molecular attraction between the polarisable olefinic bonds of the unsaturated methyl esters and the ester groups of the polyester. The magnitude of this attraction increases with the number of olefinic bonds. This polar effect specifically retards unsaturated methyl esters and, in most instances, outweighs the London forces which have an opposing effect.

Recently, JAMIESON[4] has discussed the effect of stationary phase polarity on the

separation of polyolefinic methyl esters and it is found that the apparent polarity of a polyester phase depends on the following:

(i) source and purity of the polyester;

(ii) concentration of the stationary phase (packed, open tubular and support-coated open tubular (scot) columns);

(iii) "age" of the stationary phase;

(iv) nature of the support;

(v) operating temperature.

The correlation of data, especially among different laboratories, would be much simplified if a small number of "standard" phases and support materials were available. However, the production of such standard materials has, so far, not been found feasible.

To help in the tentative identification of polyolefinic methyl esters various correlations of retention data and molecular structure have been proposed and have been successfully employed for methylene-interrupted polyolefinic esters[2, 5-11]. The use of these methods requires the determination of retention data for a number of standard esters for each stationary phase, and for each change in operating conditions.

In the present work the calculation of retention data for methylene-interrupted polyolefinic esters has been simplified further by constructing equations which relate the equivalent chain length (ECL) of methyl linolenate ($18:3\omega3$) with each of fourteen polyolefinic C_{18}–C_{22} esters. From these equations, tables were constructed, with the aid of a computer, giving the ECL values of these fourteen esters for ECL values of methyl linolenate from 18.50 to 21.00 in increments of 0.01. These tables can be used for the tentative identification of these esters from retention data on any polyester phase, of any age and concentration, and at any operating temperature in the normal working range.

RESULTS AND DISCUSSION

There is a large amount of published retention data for methylene-interrupted polyolefinic fatty acid methyl esters and many of these data have been collected and tabulated in ECL form[4]. Although attempts have been made to classify polyester phases in terms of polarity, this has not been entirely successful since the classifications were based on data for a number of different polyesters of varying "age" and at a variety of temperatures. The authors feel that it would be more useful to consider polyester phases as a single class of substances of varying polarity instead of a number of individual substances each of which will show changes in polarity on "ageing" and with changes in operating temperature. A measure of the polarity can be obtained by considering the ECL value of methyl linolenate ($18:3\omega3$). This ester was chosen as the reference ester in the present work since, (a) it is readily available in a pure form to obtain reliable retention data, (b) it is present in many mixtures of methyl esters from biological sources, and (c) it shows acceptable changes in ECL value with changes in stationary phase polarity.

The data used in the present investigation were obtained from the literature[4] and from a large number of results which had been obtained in the authors' laboratory over the past five years. The data include results from a number of different polyester

phases on various supports in packed, support-coated open tubular and open tubular columns operated at various temperatures.

Graphs were constructed by plotting the ECL values of various polyolefinic methyl esters *versus* the ECL values for methyl linolenate ($ECL_{18:3\omega3}$) and it was seen that there were linear relationships with little scatter. The data were then processed by a computer and slope and intercept values were obtained for a series of lines corresponding to each of the fourteen methylene-interrupted polyolefinic methyl esters. These values with their standard errors are shown in Table I.

TABLE I

DATA FOR THE EQUATION $ECL_X = aECL_{18:3\omega3} + b$

Methyl ester (X)	No. of results	a	b	Sq. of standard errors[*]	
				a	b
18:3ω6	68	0.907	1.480	0.0002	0.0794
18:4ω3	77	1.258	−4.661	0.0010	0.3866
20:2ω9	29	0.658	7.690	0.0020	0.7817
20:2ω6	62	0.647	8.127	0.0003	0.1220
20:3ω9	21	0.910	3.080	0.0074	2.9877
20:3ω6	65	0.915	3.208	0.0025	0.9674
20:3ω3	17	0.986	2.189	0.0012	0.4531
20:4ω6	67	1.119	−0.509	0.0034	1.3217
20:4ω3	62	1.350	−4.606	0.0007	0.2712
20:5ω3	73	1.518	−7.602	0.0004	0.1675
22:4ω6	33	1.266	−1.448	0.0090	3.5470
22:5ω6	22	1.441	−4.600	0.0044	1.7278
22:5ω3	38	1.527	−5.843	0.0011	0.4269
22:6ω3	40	1.792	−10.726	0.0018	0.7025

Using the slope and intercept data the computer was programmed to prepare tables of ECL values for each of the fourteen esters corresponding to $ECL_{18:3\omega3}$ values from 18.50 to 21.00 with increments of 0.01. Part of these tables is shown in Table II and the standard errors for certain values of $ECL_{18:3\omega3}$ are shown in Table III.

The ECL tables can then be employed for the tentative identification of the various polyolefinic esters using retention data from any polyester column operating in the normal temperature range. Examples of results obtained from the ECL tables are shown in Table IV. The examples were chosen to include polyesters, silicone-modified polyesters and cyclodextrin acetate (CDXA) and, in all cases, the agreement between the ECL values calculated from retention data and those from the tables is very satisfactory. It is interesting to find that there is a fairly good agreement among the results from the non-polar Apiezon L phase, bearing in mind that the calculated ECL values were obtained by extrapolation from the polyester data.

The ECL tables can also be used to predict the best type of polyester phase for a specific separation, *e.g.* 18:4ω3 and 20:2ω9 have similar ECL values on a phase with $ECL_{18:3\omega3}$ of 20.50 but separations of these two esters could be achieved on phases with $ECL_{18:3\omega3}$ less than 20.00; also, 20:3ω3 has lower ECL values than 20:4ω6 on phases with $ECL_{18:3\omega3}$ above 20.25, and higher ECL values with phases

TABLE II

PART OF TABLE OF EQUIVALENT CHAIN-LENGTH VALUES OF C_{18} AND C_{20} ESTERS

18:3ω3	18:3ω6	18:4ω3	20:2ω9	20:2ω6	20:3ω9	20:3ω6	20:3ω3	20:4ω6	20:4ω3	20:5ω3
19.60	19.27	20.01	20.57	20.80	20.77	21.12	21.50	21.40	21.84	22.14
19.61	19.28	20.02	20.58	20.81	20.78	21.13	21.51	21.41	21.85	22.16
19.62	19.28	20.03	20.58	20.81	20.79	21.14	21.52	21.43	21.87	22.17
19.63	19.29	20.05	20.59	20.82	20.80	21.15	21.53	21.44	21.88	22.19
19.64	19.30	20.06	20.60	20.83	20.81	21.15	21.54	21.45	21.89	22.20
19.65	19.31	20.07	20.60	20.83	20.82	21.16	21.55	21.46	21.91	22.22
19.66	19.32	20.08	20.61	20.84	20.83	21.17	21.56	21.48	21.92	22.23
19.67	19.33	20.10	20.62	20.85	20.84	21.18	21.57	21.49	21.93	22.25
19.68	19.34	20.11	20.63	20.85	20.86	21.19	21.58	21.50	21.95	22.26
19.69	19.35	20.12	20.63	20.86	20.87	21.20	21.59	21.52	21.96	22.28
19.70	19.36	20.14	20.64	20.87	20.88	21.21	21.60	21.53	21.98	22.29
19.71	19.37	20.15	20.65	20.87	20.89	21.21	21.61	21.54	21.99	22.31
19.72	19.38	20.16	20.66	20.88	20.90	21.22	21.62	21.55	22.00	22.33
19.73	19.39	20.18	20.66	20.89	20.91	21.23	21.63	21.57	22.02	22.34
19.74	19.39	20.19	20.67	20.90	20.92	21.24	21.64	21.58	22.03	22.36
19.75	19.40	20.20	20.68	20.90	20.93	21.25	21.65	21.59	22.04	22.37
19.76	19.41	20.22	20.68	20.91	20.95	21.26	21.66	21.61	22.06	22.39
19.77	19.42	20.23	20.69	20.92	20.96	21.27	21.67	21.62	22.07	22.40
19.78	19.43	20.24	20.70	20.92	20.97	21.28	21.68	21.63	22.09	22.42
19.79	19.44	20.25	20.71	20.93	20.98	21.28	21.69	21.64	22.10	22.43
19.80	19.45	20.27	20.71	20.94	20.99	21.29	21.70	21.66	22.11	22.45
19.81	19.46	20.28	20.72	20.94	21.00	21.30	21.71	21.67	22.13	22.46
19.82	19.47	20.29	20.73	20.95	21.01	21.31	21.72	21.68	22.14	22.48
19.83	19.48	20.31	20.74	20.96	21.02	21.32	21.73	21.70	22.15	22.49
19.84	19.49	20.32	20.74	20.96	21.03	21.33	21.74	21.71	22.17	22.51
19.85	19.50	20.33	20.75	20.97	21.05	21.34	21.75	21.72	22.18	22.52
19.86	19.51	20.35	20.76	20.98	21.06	21.34	21.76	21.73	22.20	22.54
19.87	19.51	20.36	20.76	20.98	21.07	21.35	21.77	21.75	22.21	22.56
19.88	19.52	20.37	20.77	20.99	21.08	21.36	21.78	21.76	22.22	22.57
19.89	19.53	20.39	20.78	21.00	21.09	21.37	21.79	21.77	22.24	22.59
19.90	19.54	20.40	20.79	21.00	21.10	21.38	21.80	21.79	22.25	22.60
19.91	19.55	20.41	20.79	21.01	21.11	21.39	21.81	21.80	22.26	22.62
19.92	19.56	20.42	20.80	21.02	21.12	21.40	21.82	21.81	22.28	22.63
19.93	19.57	20.44	20.81	21.02	21.14	21.41	21.83	21.82	22.29	22.65
19.94	19.58	20.45	20.82	21.03	21.15	21.41	21.84	21.84	22.31	22.66
19.95	19.59	20.46	20.82	21.04	21.16	21.42	21.85	21.85	22.32	22.68
19.96	19.60	20.48	20.83	21.04	21.17	21.43	21.86	21.86	22.33	22.69
19.97	19.61	20.49	20.84	21.05	21.18	21.44	21.87	21.88	22.35	22.71
19.98	19.62	20.50	20.84	21.06	21.19	21.45	21.88	21.89	22.36	22.72
19.99	19.62	20.52	20.85	21.06	21.20	21.46	21.89	21.90	22.37	22.74

with $ECL_{18:3\omega3}$ less than 19.60. The two esters would be difficult to separate on phases with $ECL_{18:3\omega3}$ between 19.60 and 20.25.

Recently JAMIESON AND REID[15] published ECL values for the acetyl, trifluoro-acetyl, and trimethylsilyl derivatives of a series of olefinic fatty alcohols and they found that although the trifluoroacetyl and trimethylsilyl derivatives had shorter retention times there was a loss in separation compared with the acetyl derivatives. The methyl ester ECL tables were used to find the ECL values for the alcohol deriva-

TABLE III

SQ. OF STANDARD ERRORS FOR EQUIVALENT CHAIN-LENGTH VALUES FROM TABLES

Methyl ester	$ECL_{18:3\omega3}$					
	18.50	19.00	19.50	20.00	20.50	21.00
18:3ω6	0.019	0.013	0.007	0.006	0.011	0.018
18:4ω3	0.041	0.027	0.014	0.014	0.026	0.041
20:2ω9	0.062	0.041	0.023	0.019	0.035	0.055
20:2ω6	0.023	0.015	0.008	0.008	0.015	0.023
20:3ω9	0.139	0.097	0.056	0.025	0.043	0.082
20:3ω6	0.048	0.033	0.019	0.018	0.024	0.042
20:3ω3	0.046	0.031	0.018	0.018	0.029	0.045
20:4ω6	0.076	0.049	0.027	0.026	0.048	0.076
20:4ω3	0.034	0.022	0.012	0.012	0.023	0.035
20:5ω3	0.027	0.018	0.010	0.009	0.017	0.027
22:4ω6	0.136	0.091	0.050	0.036	0.068	0.111
22:5ω6	0.095	0.064	0.038	0.030	0.050	0.080
22:5ω3	0.046	0.031	0.017	0.014	0.025	0.056
22:6ω3	0.058	0.038	0.021	0.017	0.032	0.052

TABLE IV

COMPARISON OF EQUIVALENT CHAIN-LENGTH VALUES AT DIFFERENT STATIONARY PHASES AND AT DIFFERENT TEMPERATURES

Methyl ester	EGSS-X (ref. 11) 180°			DEGS (ref. 12) 220°			DEGS (ref. 13) 170°			CDXA (ref. 3) 234°		
	Det.	Calc.	Diff.	Det.	Calc.	Diff.	Det.	Calc.	Diff.	Det.	Calc.	Diff.
18:3ω3	20.31			20.33			20.18			20.10		
18:3ω6	19.90	19.92	+0.02	20.00	19.94	−0.06	19.76	19.80	+0.04	19.70	19.73	+0.03
18:4ω3	20.88	20.93	+0.05	20.95	20.96	+0.01	20.74	20.76	+0.02	20.73	20.66	−0.07
20:2ω9	21.14	21.08	−0.06	21.11	21.10	−0.01	20.96	20.93	−0.03	—	—	—
20:2ω6	21.25	21.28	+0.03	21.28	21.29	+0.01	21.19	21.14	−0.05	21.13	21.14	+0.01
20:3ω9	21.58	21.56	−0.02	21.55	21.58	+0.03	21.36	21.44	+0.06	21.53	21.37	−0.16
20:3ω6	21.81	21.73	−0.08	21.96	21.75	−0.21	21.70	21.62	−0.08	21.72	21.55	−0.17
20:3ω3	22.25	22.21	−0.04	22.30	22.23	−0.07	22.13	22.08	−0.05	—	—	—
20:4ω6	22.25	22.31	+0.06	22.35	22.34	−0.01	22.01	22.15	+0.14	22.15	22.04	−0.11
20:4ω3	22.78	22.81	+0.03	22.94	22.84	−0.10	22.69	22.64	−0.05	—	—	—
20:5ω3	23.23	23.23	0	23.32	23.26	−0.05	23.06	23.03	−0.03	22.88	22.91	+0.03
22:4ω6	24.17	24.25	+0.08	24.33	24.28	−0.05	24.00	24.09	+0.09	24.03	23.99	−0.04
22:5ω6	24.69	24.69	0	24.83	24.72	−0.11	24.49	24.49	0	24.43	24.37	−0.06
22:5ω3	25.14	25.16	+0.02	25.25	25.19	−0.06	24.97	24.98	+0.01	24.87	24.84	−0.03
22:6ω3	25.68	25.65	−0.03	25.77	25.69	−0.08	25.47	25.42	−0.05	25.35	25.27	−0.08

tives (Table V) and these values were in good agreement with those determined from retention data from an EGSS-X open tubular column. Since there is such an agreement this would suggest that, for these alcohol derivatives, the magnitude of the polar attractions between olefinic bonds and the polyester phase is similar for each derivative, but since the corresponding retention times decrease in the series acetate, trifluoroacetate, trimethylsilyl ether, the London forces decrease in the same order.

It must be emphasized that the ECL value tables can only be used as a rapid

TABLE V

EQUIVALENT CHAIN LENGTHS OF DERIVATIVES OF UNSATURATED ALCOHOLS SEPARATED ON EGSS-X

Alcohol	Equivalent chain length								
	Acetate			TFA			TMS		
	Det.	Calc.	Diff.	Det.	Calc.	Diff.	Det.	Calc.	Diff.
18:3ω3	20.05			19.87			19.63		
18:3ω6	19.68	19.68	0	19.54	19.51	—0.03	19.37	19.29	—0.08
18:4ω3	20.60	20.59	—0.01	20.34	20.36	+0.02	20.05	20.05	0
20:2ω9	20.98	20.90	—0.08	20.76	20.76	0	20.60	20.59	—0.01
20:2ω6	21.12	21.10	—0.02	21.00	20.98	—0.02	20.89	20.82	—0.07
20:3ω9	21.36	21.45	+0.09	21.19	21.29	+0.10	21.01	21.07	+0.06
20:3ω6	21.59	21.53	—0.06	21.43	21.35	—0.08	21.26	21.15	—0.11
20:3ω3	21.96	21.95	—0.01	21.74	21.77	+0.03	21.51	21.54	+0.03
20:4ω6	22.08	21.98	—0.10	21.88	21.75	—0.13	21.62	21.45	—0.17
20:4ω3	22.48	22.46	—0.02	22.24	22.21	—0.03	21.94	21.89	—0.05
20:5ω6	23.01	22.83	—0.18	22.66	22.56	—0.10	22.27	22.20	—0.07
22:5ω3	24.81	24.77	—0.04	24.50	24.49	—0.01	24.15	24.13	—0.02
22:6ω3	25.16	25.18	+0.02	24.86	24.85	—0.01	24.50	24.41	—0.09

GSS-Y (ref. 12) 10°			BDS (ref. 14) 170°			DEGS (scot) (ref. 4) 180°			ApL (ref. 3) 240°		
et.	Calc.	Diff.	Det.	Calc.	Diff.	Det.	Calc.	Diff.	Det.	Calc.	Diff.
).76			19.50			18.94			17.51		
).38	19.41	+0.03	19.15	19.17	+0.02	18.65	18.66	+0.01	17.30	17.36	+0.06
).13	20.22	+0.09	19.86	19.88	+0.02	19.15	19.14	—0.01	17.30	17.36	+0.06
).69	20.68	—0.01	—	—	—	—	—	—	—	—	—
'.01	20.91	—0.10	20.75	20.73	—0.02	20.35	20.36	+0.01	19.48	19.46	—0.02
).97	21.06	+0.09	20.70	20.82	+0.12	—	—	—	19.15	19.00	—0.15
:.34	21.26	—0.08	21.06	21.03	—0.03	20.51	20.55	+0.04	19.23	19.23	0
'.72	21.63	—0.09	21.45	21.40	—0.05	20.77	20.84	+0.07	—	—	—
:.60	21.57	—0.03	21.29	21.27	—0.02	20.60	20.55	—0.05	19.00	19.09	+0.09
!.08	22.03	—0.05	21.75	21.70	—0.05	21.00	20.93	—0.07	—	—	—
!.32	22.36	+0.04	21.97	21.99	+0.02	21.14	21.13	—0.01	19.00	18.98	—0.02
}.60	23.56	—0.04	23.23	23.23	0	—	—	—	20.93	20.72	—0.21
}.90	23.86	—0.04	23.50	23.47	—0.03	22.62	22.63	+0.01	20.87	20.63	—0.24
｜.30	24.34	+0.04	23.90	23.93	+0.03	23.09	23.07	—0.02	21.00	20.90	—0.10
｜.59	24.65	+0.06	24.18	24.18	0	—	—	—	20.73	20.65	—0.08

method for the tentative identification of the polyolefinic derivatives. For a more complete identification use should be made of separation factors with adequate standards and degradative methods. It is probable that when more experimental data become available the equations would be modified and more accurate tables constructed. Preliminary work indicates that similar tables could be constructed for C_{18} olefinic esters but only a limited amount of retention data is available. It is believed that the method could be applied to fatty esters with other groups than olefinic present.

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