

CHROM. 12,331

GAS CHROMATOGRAPHY OF ESTERS

XIII*. INTERRELATIONSHIP OF EQUIVALENT CHAIN LENGTH (ECL) AND RETENTION INDEX VALUES OF FATTY ESTERS

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(Received August 23rd, 1979)

SUMMARY

The interrelationship of equivalent chain length and retention index values of straight- and branched-chain fatty esters is demonstrated on a typical polyester phase (DEGS), a phenyl-substituted polysiloxane (OV-17) and on two low-polarity, thermally stable hydrocarbons. Hydrogenated Apiezon M and the synthetic hydrocarbon $C_{87}H_{176}$ were used, and have lower polarities than the simple Apiezon greases that have been used with fatty esters. These stationary phases have polarities comparable to that of squalane and retention increments of fatty esters may be considered comparatively with those of simple esters which have been compared with squalane.

INTRODUCTION

A variety of relationships have been used to specify retention behaviour and the interrelation of nine of the commoner structure retention parameters was shown mathematically by Guerin and Banks¹. The growing use of automated gas chromatographic equipment with accompanying computer processing of retention data will continue to eliminate tedious measurements from charts and provide data virtually simultaneously with the elution of compounds. The determination of mathematical dead-time has recently been reviewed² and this, together with the interpolation of

* Part XII: see J. K. Haken, A. Nguyen and M. S. Wainwright, *J. Chromatogr.*, 178 (1979) 471.

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the results for a compound on a graph prepared with homologous compounds, *e.g.*, *n*-alkanes, to provide retention indices are simple procedures that can be rapidly handled by on-line processors.

Retention indices³ are established by the relationship

$$I = \frac{100(\log t_{RS} - \log t_{RN}) + 100N}{\log t_{R(N+1)} - \log t_{RN}} \quad (1)$$

where

- t_{RS} = retention of unknown *n*-substrate;
- t_{RN} = retention of *n*-alkane with *N* carbon atoms;
- $t_{R(N+1)}$ = retention of *n*-alkane with *N* + 1 carbon atoms;
- N* = integer equal to carbon number of t_N .

The slope (*b*) of the semi-logarithmic plot of carbon number *versus* retention of *n*-alkanes is common to many of the schemes considered by Guerin and Banks¹ and is determined from two points on a graph prepared with *n*-alkanes, as shown in eqn. 2, although in a computer plot the slope is determined statistically by considering all of the *n*-alkanes used in the calibration:

$$b = \frac{\log t_{R(N+x)} - \log t_{R(N)}}{x} \quad (2)$$

where

$t_{R(N+x)}$ and $t_{R(N)}$ = retention of *n*-alkanes with *N* and *N* + *x* carbon atoms, respectively

The retention index is therefore given by

$$I = \frac{100(\log t_{RN} + N)}{b} \quad (3)$$

The methylene unit (MU) nomenclature proposed by VandenHeuvel *et al.*⁴ is essentially identical with the retention index system. A calibration using *n*-docosane and *n*-tetracosane was used and the methylene unit multiplied by 100 gives the retention index. The proposal based on a two-point calibration line is less satisfactory than the normally determined multi-point *n*-alkane calibration line.

The difficulty in selecting a reference series suitable for all polarities of solute and solvent is well known. Lorenz and Rogers⁵ considered the effect of sorption phenomena on retention indices and found significant errors when the solute and solvent polarities were widely variant. These findings were similar to those of VandenHeuvel and Horning⁶, who recommended that the polarities of the two species should be similar.

The use of secondary standards with *n*-alkanes has been suggested, and Hawkes⁷ discussed the difficulty of using primary *n*-alkanols as proposed by Grobler⁸, who had criticised the retention index system using polar stationary phases owing to the rapid elution of hydrocarbons and indicated that compounds with very different boiling points cannot be chromatographed under strictly identical conditions. *n*-Propyl ethers were recommended as standards by Hawkes⁷, with ketones and

aldehydes as alternatives. 2-Alkanones had earlier been suggested as standards by Dymond and Kilburn⁹ and the merits of using ketones as secondary reference standards were later detailed by Ackman¹⁰.

The difficulty or impossibility of providing a universally acceptable reference series has been demonstrated by a study of the behaviour of compounds of moderately polar character, *e.g.*, *n*-alkyl esters. With dimethyl polysiloxane, the solvent of lowest polarity included, it is apparent that methyl acetate (b.p. 57.1°) has a retention index of 509, *i.e.*, identical with that of *n*-pentane (b.p. 36.2°), while *n*-hexyl acetate (b.p. 169.2°) has a retention index of 988, *i.e.*, almost identical with that of *n*-decane (b.p. 174.0°), so that for a given retention index the esters have higher boiling points than the comparable *n*-alkanes. With the introduction of two phenyl groups, *i.e.*, using DC-710, the situation is reversed, with methyl acetate ($I = 609$) being comparable to *n*-hexane (b.p. 69.0°) and *n*-hexyl acetate ($I = 1092$) being comparable to *n*-undecane (b.p. 196°). With weaker acceptor phases, *i.e.* XE-60, these retention indices are increased to 737 and 1221, respectively, the former being equivalent to an *n*-alkane with a boiling point of approximately 220°. With a more polar stationary phase (Silar 5CP), retention indices of 894 and 1313 are obtained, which are equivalent to *n*-nonane (b.p. 151°) and *n*-tridecane (b.p. 235°), respectively.

The great differences in boiling points at each end of the scale reflect the differences in the slopes for the *n*-alkanes and simple esters on the various stationary phases. As alternative secondary reference standards, acetate esters and symmetrical esters with equal acid and alcohol chain lengths have been suggested¹¹.

The use of esters as standards has found considerable acceptance with fatty esters in the form of the equivalent chain length (*ECL*) relationship developed by Woodford and Van Gent¹² and Miwa *et al.*¹³. The relationship is equivalent to the retention index system, results for an unknown ester being interpolated on graph prepared using homologous long-chain methyl esters.

The relationship is shown in eqns. 4 and 5 and is identical with eqns. 1 and 3 except for the reference series used.

$$ECL = \frac{\log t_{RS} - \log t_{RN} + N}{\log t_{R(N+2)} - \log t_{RN}} \quad (4)$$

$$ECL = \frac{(\log t_{RN} + N)}{b} \quad (5)$$

The quantities are as shown above but refer to methyl fatty esters, while the calibration line is usually constructed using even-carbon-number esters.

With computer processing of chromatographic data it is possible to provide retention data relative to multiple reference series and with the retention index and *ECL* representations a simple consideration of the slopes of plots for standard homologous series is necessary.

In this paper we consider the retention of straight- and branched-chain fatty esters with the data expressed in terms of both retention indices and *ECL* values. The data were determined on a typical polyester phase (DEGS) and on two low-polarity, thermally stable hydrocarbons. Hydrogenated Apiezon M and the synthetic hydrocarbon C₈₇H₁₇₆ were used, and have lower polarities than the simple Apiezon greases that have been used with fatty esters. These stationary phases are comparable

TABLE I
EQUIVALENT CHAIN LENGTHS (ECL), RELATIVE RETENTIONS (RR) AND RETENTION INDICES (I) OF METHYL ALKANOATES
AND *n*-ALKANES

Compounds	Carbon number	Hydrogenated Apiezon M (230°)			C ₁₇ H ₁₆ (200°)			OV-17 (200°)			DEGS (200°)		
		ECL	RR	I	ECL	RR	I	ECL	RR	I	ECL	RR	I
Methyl esters	C ₁₀	10.00	0.104	1256	10.00	0.075	1266	10.00	0.091	1410	10.00	0.202	1794
	C ₁₂	12.00	0.221	1457	12.00	0.177	1468	12.00	0.211	1613	12.00	0.347	2003
	C ₁₄	14.00	0.471	1659	14.00	0.424	1659	14.00	0.465	1814	14.00	0.585	2201
	C ₁₆	16.00	1.000	1859	16.00	1.000	1860	16.00	1.000	2015	16.00	1.000	2401
	C ₁₈	18.00	2.120	2059	18.00	2.350	2061	18.00	2.170	2215	18.00	1.703	2599
	C ₂₀	20.00	4.479	2257	20.00	5.530	2262	20.00	4.550	2414	20.00	2.907	2796
	C ₂₂	22.00	9.499	2454	22.00	13.109	2460				22.00	4.963	2993
	<i>n</i> -Alkanes	C ₁₀	7.43	0.040	1000								
C ₁₂		9.40	0.083	1200				7.96	0.044	1200			
C ₁₄		11.39	0.176	1400				9.89	0.093	1400			
C ₁₆		13.37	0.371	1600			1600	11.87	0.201	1600			
C ₁₈		15.37	0.789	1800			1800	13.86	0.436	1800	10.05	0.205	1800
C ₂₀		17.36	1.670	2000			2000	15.85	0.943	2000	11.98	0.343	2000
C ₂₂		19.34	3.520	2200			2200				13.98	0.585	2200
											15.98	0.995	2400
											18.03	1.756	2600
											20.15	3.021	2800

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EXPERIMENTAL

Gas chromatography

A Hewlett-Packard 5700A gas chromatograph, fitted with dual flame-ionization detectors, was used. Nitrogen (30 ml/min) was used as the carrier gas, and hydrogen (30 ml/min) and air (200 ml/min) were supplied to the detector. All results were determined isothermally at 200°. Four stationary phases were used, *viz.*, in order of increasing polarity, hydrogenated Apiezon M (refs. 14 and 15), C₈₇H₁₇₅ (ref. 16), OV-17 and DEGS, all coated to the extent of 10% on Chromosorb W AW DMCS (80–100 mesh) and packed into 2 m × 3 mm O.D. stainless-steel columns.

The gas chromatograph was connected to a Hewlett-Packard 3370B integrator with facilities for paper-tape output on a teletype (STC, ASR-33). The chromatograph was also fitted with a Hewlett-Packard 7671A auto-sampler.

Preparation of fatty acid methyl esters from wool wax

Wool from Merino sheep was extracted with light petroleum (b.p. 40–60°), using a Soxhlet apparatus. Small portions of wax were saponified with alcoholic sodium hydroxide solution. Unsaponifiable components were removed with light petroleum, the solution was then acidified and fatty acids together with hydroxy fatty acids were extracted with light petroleum. Fatty acids were separated from hydroxy fatty acids by thin-layer chromatography on silica gel G using *n*-hexane–diethyl ether–acetic acid (70:30:1). The bands were detected under UV light after spraying with 0.1% ethanolic 2,7-dichlorofluorescein and identified using standards (Applied Science Labs., State College, Pa., U.S.A.) run on the same plate. Fatty acid bands were scraped from the plate and extracted with diethyl ether. Methyl esters were prepared by adding diazomethane solution.

Calculations

The data were processed by means of a PDP 11/15 computer fitted with a high-speed paper-tape reader and disc pack (RK05), dead-times being calculated by the procedure of Guardino *et al.*¹⁷.

ECL values were computed from the modified regression equation first obtained by the procedure of Guardino *et al.* from a homologous series of straight-chain fatty acid methyl esters. In order to allow for the normal variations that occur with net retention times with time (as up to 36 samples could be automatically processed), all experimental data were converted into retentions relative to methyl palmitate (C16). The net retention time of methyl palmitate was constantly re-measured in each sample, as it either occurred naturally or, if not, was added as an internal standard. Retention indices were similarly determined using homologous *n*-alkanes.

RESULTS AND DISCUSSION

Retention data for methyl alkanoates and *n*-alkanes on the four stationary phases are shown in Table I as retentions relative to methyl palmitate, *ECL* values

TABLE II
EQUIVALENT CHAIN LENGTHS (ECL), RELATIVE RETENTIONS (RR) AND RETENTION INDICES (I) OF WOOL WAX METHYL ESTERS

Compound ^d	Hydrogenated Aplezon M (230°)			C ₁₇ H _{17.6} (200°)			OV-17 (200°)			DEGS (200°)		
	ECL	RR	I	ECL	RR	I	ECL	RR	I	ECL	RR	I
<i>n</i> -C ₉												
<i>iso</i> -C ₁₀	9.63	0.091	1217				9.56	0.082	1365			
<i>n</i> -C ₁₀	9.99	0.104	1252				9.99	0.096	1407			
<i>n</i> -C ₁₁	10.72	0.137	1325				10.69	0.127	1481	10.78	0.249	1883
U	11.35	0.174	1390									
<i>iso</i> -C ₁₂	11.62	0.192	1417		0.153	1425	11.57	0.179	1571	11.55	0.306	1963
<i>n</i> -C ₁₂	11.99	0.221	1454		0.177	1455	11.98	0.210	1612			
U	12.33		1487									
<i>n</i> -C ₁₃	12.71	0.290	1527		0.243	1528	12.69	0.276	1683	12.75	0.421	2085
U	13.35	0.369	1590				12.99	0.310	1713			
<i>iso</i> -C ₁₄	13.62	0.408	1618		0.360	1619	13.58	0.390	1772	13.52	0.516	2161
<i>n</i> -C ₁₄	14.00	0.471	1656		0.424	1658	13.99	0.458	1814	14.00	0.587	2210
<i>n</i> -C ₁₅	14.71	0.615	1727		0.572	1727	14.70	0.603	1885	14.71	0.709	2280
U	15.00	0.618	1756				15.00	0.677				
U	15.33	0.777	1789									
<i>iso</i> -C ₁₆	15.62	0.867	1818		0.849	1820	15.58	0.849	1973	15.48	0.871	2357
<i>n</i> -C ₁₆	16.00	1.00	1856		1.000	1858	16.00	1.000	2015	16.00	1.000	2409
U	16.31	1.119	1886									
<i>n</i> -C ₁₇	16.71	1.304	1927		1.345	1928	16.69	1.310	2085	16.70	1.206	2478
U	16.99	1.452	1955				17.00	1.476	2115			

U	17.26	1.605	1982	17.24	1.957	1978	2172	17.48	1.484	2555
iso-C ₁₈	17.60	1.823	2016	17.57	1.957	2016	1.840	17.48	1.484	2555
n-C ₁₈	18.00	2.120	2056	18.00	2.340	2059	2.158	17.99	1.701	2605
α-C ₁₉	18.70	2.763	2126	18.68	3.153	2128	2.835	18.71	2.056	2676
n-C ₁₉	19.01	3.101	2157							
U	19.19		2175	19.14	3.033	2175				
iso-C ₁₀	19.60	3.880	2216	19.59	4.636	2220	3.969	19.49	2.533	2753
n-C ₁₀	19.98	4.479	2254					20.00	2.902	2803
U	20.26	4.962	2281							
α-C ₁₁	20.68	5.821	2321							
U	21.18	7.036	2374				2481	20.71	3.511	2873
U	21.31		2386							
iso-C ₁₁	21.61		2412							
n-C ₁₂								21.48	4.310	2948
α-C ₁₃								22.02	4.976	3001
iso-C ₁₄								22.72	5.985	3069
n-C ₁₄								23.49	7.345	3144
α-C ₁₅								24.02	8.476	3197
iso-C ₁₆								24.72	10.198	3265
α-C ₁₇								25.50	12.574	3342
								26.93	17.441	3462

* U = Unknown.

and retention indices, and Table II gives similar data for a series of wool wax fatty esters on the same phases.

From Table II, it is evident that on the DEGS column the iso-esters (terminal isopropyl group) have shorter retention times than the anteiso-esters (terminal isobutyl group); the same elution pattern is, as expected, observed with the non-polar stationary phases as the three homologous series are all of the same functional class.

Semi-logarithmic plots for the different series are essentially parallel, and the separation factors are essentially identical, as indicated previously by Gerson¹⁸ and Haken¹⁹. The separation factors are given in Table III; as expected, the separations are enhanced on the stationary phases of lower polarity.

TABLE III

SEPARATION FACTORS OF BRANCHED- AND STRAIGHT-CHAIN FATTY ESTER SERIES

Structure	Hydrogenated Apiezon M	C ₈₇ H ₁₇₆	OV-17	DEGS
<i>n</i> -	2.11	2.35	2.14	1.76
<i>Iso</i> -	2.11	2.35	2.14	1.76
<i>Anteiso</i> -	2.11	2.35	2.14	1.76
U	2.11			

The polarity has a greater influence on the elution of iso-esters than on that of anteiso-esters; this is shown in Table IV, where variations in *ECL* values are given.

TABLE IV

REDUCTION IN RETENTION (*ECL* UNITS) OF ESTERS ON STATIONARY PHASES OF VARYING POLARITY

Structure	Hydrogenated Apiezon M	C ₈₇ H ₁₇₆	OV-17	DEGS
<i>iso</i> -	-0.37	-0.38	-0.43	-0.53
<i>Anteiso</i> -	-0.28	-0.31	-0.32	-0.30
<i>n</i> -	1.00	1.00	1.00	1.00
U	±0.65			

The improved separations on the non-polar columns are further evident by the appearance of a fourth series of minor components on hydrogenated Apiezon M column shown as U in Tables II-IV. The same separation factors are exhibited as for the other series on this stationary phase, and on a non-polar column separation of an unsaturated species would be very unlikely. The composition of wool wax fatty acids has recently been reviewed²⁰ and, in addition to the saturated esters described, series of normal, iso- and anteiso-hydroxy esters are present and the peaks observed are due to small amounts of the materials that remain after the separation reported.

Tables I and II show that the retention is essentially identical on both of the non-polar phases, with retention indices being approximately 250 units or 2.5 carbon numbers greater than the *ECL* values. With increased polarity of the stationary phase,

retention relative to the *n*-alkanes is increased by 410 units or 4.1 carbon numbers on the OV-17 column and 800 units or 8 carbon numbers on the DEGS column. These values show the same trends as in a previous study in which the retention of simple esters on polysiloxane stationary phases of increasing polar character was considered²¹.

The results indicate that the interrelationship of retention indices and *ECL* values or other indices relative to some other homologous calibration line can be readily achieved by using simple data reduction units or by manual operation.

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