CHROM. 4081

TEMPERATURE EFFECTS IN THE CALCULATION OF EQUIVALENT CHAIN LENGTH VALUES FOR MULTIPLE-BRANCHED FATTY ACID ESTERS AND KETONES ON POLAR AND NON-POLAR OPEN TUBULAR COLUMNS

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(Received March 24th, 1969)

SUMMARY

Accurate equivalent chain length (ECL) values were determined for methyl esters of a variety of methyl-branched fatty acids. Only those with methyl substituents in the C_2, C_3 and ω_1 (iso) position were particularly affected by changes in operating temperature on one type of polar open-tubular column, or were otherwise significantly affected by the polarity of different polyester columns. In other positional isomers ECL values dropped only very slightly with increasing temperature. On Apiezon L columns only the C_3 isomer was significantly affected. Adjustments for temperature effects improved accuracy in calculating ECL values for multiple methyl-branched fatty acids and some corresponding ketones.

INTRODUCTION

It has been shown that equivalent chain length (ECL) values may be calculated for isoprenoid fatty acid methyl esters with some degree of precision when appropriate adjustments for column polarity are made in relative fractional chain length (FCL) values[1]. The associated experimental work was limited in scope and the derivation of the fundamental data (FCL values) based on results obtained on a packed GLC column at a single temperature[2]. Additional information is now available to indicate the effects of operating temperature for polyester-coated (butanediol succinate) open-tubular columns, to extend the entire concept to non-polar (Apiezon L) open-tubular columns, and to show applicability to structurally related materials such as ketones.

EXPERIMENTAL

All columns were purchased from the manufacturer of the GLC equipment and were nominally 150 ft. (50 m) in length and 0.01 in. (0.25 mm) in internal diameter. Purchase specifications were normally limited to the type of material employed as a coating, although a minimum number of theoretical plates were specified in some cases. Column BDS (butanediol succinate) P-1 was installed in a Perkin-Elmer 900; all others, including those coated with AP-L (Apiezon L), were installed in a Model 226 (columns BDS 7 and BDS 10 were operated in series). Column temperatures are given in the tables; carrier gas pressures were varied to suit other operating conditions (*e.g.* for BDS P-1: 10 p.s.i.g. at 150° and 80 p.s.i.g. at 100°) and the nature of the sample. The injection port temperature was normally 270° and a relatively fine splitter (No. 1) was installed. Samples were injected as solutions in neohexane by a Hamilton 701 microsyringe (normally 0.002 ml). Retention times were measured from the leading edge of the solvent peak and ECL values read off plots on Keuffel and Esser 46-4970 semi-logarithmic (2-cycle) graph paper. Values should be accurate to ± 0.02 ECL units.

RESULTS AND DISCUSSION

Polyester liquid phases

The samples available included the critical isomers with methyl branches at each end of the chain where FCL values were largest and the maximum variations in

TABLE I

EXPERIMENTAL AND LITERATURE ECL VALUES FOR ESTERS OF A NUMBER OF MONOMETHYL-SUBSTITUTED ALIPHATIC ACIDS AND OF SOME SIMPLE KETONES ON POLYESTER COLUMNS AND AT DIFFERENT TEMPERATURES

Material and FCL notation	*Experimental*					*Literature*[2]
	BDS P-1			*BDS No. 12*	*BDS 7 + 10*	*Reoplex*[a]
	100° $r_{16:0}$ = *2.98*[b]	*130°* $r_{16:0}$ = *2.50*	*150°* $r_{16:0}$ = *2.25*	*130°*	*130°*	*216°*
Esters						
Methyl 2-methyloctadecanoate (C_2)	18.00	17.93	17.89	—	—	17.91
Methyl 3-methyloctadecanoate (C_3)	18.21	18.18	18.13	—	—	18.13
Methyl 4-methyloctadecanoate (C_4)	18.41	18.40	18.39	—	—	18.41
Methyl 5-methyloctadecanoate (C_5)	—	—	—	—	—	18.30
Methyl 8-methyloctadecanoate (m_8)	18.33	18.31	18.30	—	—	18.28
Methyl 13-methyloctadecanoate (ω_5)	—	—	—	—	—	18.33
Methyl 15-methyloctadecanoate (ω_3)	—	18.53	18.53	—	—	—[c]
Methyl 14-methylheptadecanoate (ω_3)	17.56	17.55	17.55	—	—	17.58
Methyl 16-methyloctadecanoate (ω_2)	18.70	18.71	18.70	—	—	18.64
Methyl 15-methylheptadecanoate (ω_2)	—	—	—	—	—	17.70
Methyl 14-methylhexadecanoate (ω_2)	16.71	16.69	16.71	—	—	—
Methyl 17-methyloctadecanoate (ω_1)	—	—	—	—	—	18.58
Methyl 15-methylhexadecanoate (ω_1)	16.61	16.58	16.56	—	—	—
Methyl 14-methylpentadecanoate (ω_1)	15.59	15.57	15.55	—	—	—
Ketones						
2-Ketopentadecane	14.10	14.20	14.27	14.20	14.20	—
3-Ketohexadecane	14.80	14.86	14.91	14.87	14.85	—
3-Ketooctadecane	—	—	16.90	16.85	16.85	—

[a] Generated from a log plot line with $r_{10:0}$ as 1.00 and $r_{16:0}$ as 0.400.
[b] For $r_{14:0}$ = 1.00.
[c] See Discussion in ref. 1.

magnitude and/or sign were expected, and also a representative example (m_8) from the center of the chain. It should however be noted that the *small* variations in FCL values for the positions from C_5 to ω_5 in the methyl-substituted octadecanoates (Table I) would not necessarily be strictly applicable in other chain lengths, especially those of shorter basic chain lengths.

A set of FCL data generated[1] from the complete set of original retention data[2], determined at 216° on Reoplex 400, with $r_{18:0}$ as 1.00 and $r_{16:0}$ as 0.400, fits the experimental polyester data for 150° with remarkable agreement in most cases (Table I). It appears that the literature-derived ω_2 value for 16-methyloctadecanoate is somewhat low, although that for 15-methylheptadecanoate is very satisfactory. Other than this instance, it is possible to assume that the literature values not verified experimentally are reasonably valid, although it would have been of particular interest to check the ω_4 and ω_5 values. The large temperature differences involved in the experimental work, and also between these and the literature work (Table I) should be noted.

There is a definite temperature influence on FCL values in the C_2 and C_3 isomers, and to a lesser degree in the ω_1 isomers. A very slight change could be discovered in the FCL values for C_4 and m_8, but the ω_3 and ω_2 values were affected very little (within plotting error) or possibly not at all. The data of FARQUHAR *et al.*[3] support these findings as with a packed EGA (ethylene glycol adipate) column at 173°, 182° and 197°, results from different chain lengths indicated that ω_1 FCL values averaged for each temperature changed only from 0.53 to 0.55 and average ω_2 FCL values were precisely constant at 0.71. Moreover in a programmed-temperature study on a range of acids carried out with a packed FFAP* (polar) column, operated from 115° to 250°, it is reported that ω_2 ECL values altered only from 8.65 for a C_9 acid to 16.69 for a C_{17} acid, and FCL values for corresponding ω_1 isomers ($\sim$ 0.51) were much less affected, although possibly decreasing[4].

There is also a pronounced temperature effect on the FCL values of 2- and 3-ketones (Table I). In this instance the dissimilarity in structure from the reference fatty acid esters makes this result not unexpected. However, it is of interest to note that exactly the same ECL values were obtained at 180° on a column packed with 15.5% EGSS-Y on Gas-Chrom P as for the open-tubular BDS column at 130°.

The results of the calculations for ECL values for several fatty acids with multiple-branched chains, and of two structurally related ketones, are given in Table II. The accommodation of the calculated values to experimental values through the use of appropriate temperature FCL values illustrates that such corrections are desirable, although the corrections involved are not extremely large in comparison with variations in ECL values for different open-tubular BDS-coated columns. These columns were in normal use and not depolarized or otherwise severely affected by age. The difference in polarity from column to column for this presumably homogenous type of substrate (it is not known if a surface-active material was employed in column coating) in a thin film is surprising. Significant FCL value variations are to be expected, especially for the C_2 and C_3 isomers, in using polar columns of differing polarity[1], but it is possible that this could be offset experimentally in some instances by judicious choice of temperature to attain FCL values desired for correlation with

* Varian-Aerograph trade mark.

TABLE II

EXPERIMENTAL AND CALCULATED ECL VALUES FOR ESTERS OF A NUMBER OF MULTIMETHYL-SUBSTITUTED (ISOPRENOID) ALIPHATIC ACIDS, AND OF RELATED 2-KETONES

Material	*BDS P-1*						*BDS No. 12*	*BDS 7 + 10*	*Literature*[5]
	Experimental			*Calculated*			*Experimental*	*Experimental*	*FFAP-packed column (programmed)*
	100°	*130°*	*150°*	*100°*	*130°*	*150°*	*130°*	*130°*	
Esters									
3,7,11-Trimethyldodecanoate	13.06	12.98	12.96	13.13	13.06	12.98	—	12.91	—
4,8,12-Trimethyltridecanoate	14.34	14.26	14.24	14.33	14.28	14.24	14.29	14.18	14.03
5,9,13-Trimethyltetradecanoate	—	—	—	15.27	15.22	15.18	—	15.08	—
2,6,10,14-Tetramethylpentadecanoate	16.22	16.05	15.97	16.25	16.12	16.04	—	—	15.76
3,7,11,15-Tetramethylhexadecanoate	17.41	17.32	17.20	17.46	17.37	17.28	—	—	16.96
Ketones									
2-Keto-6,10-dimethylundecane	11.06	11.09	11.15	11.02	11.08	11.12	—	—	—
2-Keto-6,10,14-trimethylpentadecane	15.35	15.37	15.40	15.35	15.39	15.42	15.42	15.28	—

literature data. It is possible that temperature programming could induce variations in otherwise correlatable ECL values for these particular isomers, but interestingly enough the literature data[5] of Table II indicate about the same difference of 0.19–0.24 FCL units for each of the three isoprenoid ECL values when compared to BDS experimental data at 150°, although each has a different "C_n" methyl substituent.

TABLE III

EXPERIMENTAL ECL VALUES FOR ESTERS OF A NUMBER OF MONOMETHYL-SUBSTITUTED ALIPHATIC ACIDS AND OF SOME SIMPLE KETONES ON NON-POLAR COLUMNS

Material and FCL notation		*Experimental*			*Literature*[a] *Silicone*[b] *244°*
		AP-L No. 4		*AP-L No. 3*	
		170° $r_{16:0}$ = *2.54*[a]	*190°* $r_{16:0}$ = *2.34*	*170°* $r_{16:0}$ = *2.60*	
Esters					
Methyl 2-methyloctadecanoate	(C_2)	18.15	18.12	18.21	18.22
Methyl 3-methyloctadecanoate	(C_3)	18.29	18.27	18.28	18.28
Methyl 4-methyloctadecanoate	(C_4)	18.41	18.42	18.42	18.41
Methyl 8-methyloctadecanoate	(m_8)	18.32	18.31	18.31	18.26
Methyl 15-methyloctadecanoate	(ω_3)	18.51	18.51	18.51	—
Methyl 14-methylheptadecanoate	(ω_3)	17.52	17.52	17.54	17.54
Methyl 16-methyloctadecanoate	(ω_2)	—	—	—	18.69
Methyl 14-methylhexadecanoate	(ω_2)	16.72	16.71	16.71	—
Methyl 17-methyloctadecanoate	(ω_1)	—	—	—	18.56
Methyl 15-methylhexadecanoate	(ω_1)	16.61	16.62	16.61	16.56
Methyl 14-methylpentadecanoate	(ω_1)	15.62	15.63	15.60	—
Ketones					
2-Ketopentadecane		14.07	14.06	—	—
3-Ketohexadecane		14.92	14.94	—	—

[a] For $r_{14:0}$ = 1.00.
[b] Generated from a log plot line with $r_{10:0}$ as 1.00, $r_{16:0}$ as 0.475.

The necessity of making adjustments for the polarity of columns is a general requirement for the discriminating use of ECL data based on proportionality related to the structures and hence to the physico-chemical properties of the molecules. This applies, for example, to certain common unsaturated fatty acids where a proportional relationship for GLC retention data has been shown[6,7]. A recent "correlation trial"[8], although restricted to only one polyester liquid phase, failed to take this into account and has been severely criticized[9].

Non-polar liquid phases

Two different columns were used (Table III). It is indicated that these were coated with a *modified* Apiezon L prepared as described by AVERILL[10]. Very little temperature effect was detected, even in the ketones, and this, together with some differences in ECL values due to columns, was only obvious in the fatty acid esters where methyl branching occurred in position 2 (Table III). This emphasizes the special sensitivity of the 2-methyl substituent.

Agreement with literature (silicone) data was excellent, except that ω_1 values were higher on the Apiezon columns than on the silicone column. The latter, however, was run at a much higher temperature. In a study of *unsaturated* fatty acids with terminal branching some unusual alterations in FCL values with different chain lengths have been noted on a silicone column at 220° but not at 185°. It is not known if the unsaturation plays any role in this effect[11]. The extensive data of FARQUHAR *et al.*[3] on AP-L yielded FCL values within the ranges 0.60–0.61 and 0.67–0.70 for ω_1 and ω_2 isomers, respectively, averaged for different chain lengths, at their three temperatures.

Calculated and experimental ECL values for the isoprenoid materials (Table IV) agree moderately well, and are also close to programmed-temperature and literature data[5]. It is interesting to note that the experimental data vary most appreciably from the calculated ones in the instances involving the 2- and 3-methyl substituents. Otherwise "average" or "typical" values could be taken and used with confidence even in temperature-programmed work and Apiezon L appears to be the material

TABLE IV

EXPERIMENTAL AND CALCULATED ECL VALUES FOR ESTERS OF A NUMBER OF MULTIMETHYL-SUBSTITUTED (ISOPRENOID) ALIPHATIC ACIDS AND OF A RELATED 2-KETONE

Material	*AP-L No. 4*				*AP-L No. 3*		*Literature AP-L packed column (programmed)*
	Experimental		*Calculated*		*Experimental 170°*	*Calculated 170°*	
	170°	*190°*	*170°*	*190°*			
Esters							
3,7,11-Trimethyldodecanoate	13.21	13.19	13.22	13.20	—	13.20	—
4,8,12-Trimethyltridecanoate	14.32	14.31	14.34	14.35	14.34	14.34	14.35
2,6,10,14-Tetramethylpentadecanoate	16.32	16.31	16.40	16.37	16.38	16.44	16.41
3,7,11,15-Tetramethylhexadecanoate	17.41	17.41	17.54	17.52	17.44	17.51	17.50
Ketones							
2-Keto-6,10,14-trimethylpentadecane	15.29	15.28	15.33	15.31	—	—	—

of choice in identifying methyl-branched fatty acids for this reason. It is regrettable that the separation of the commonly found *iso* (ω_1) and *anteiso* (ω_2) is rather poorer on AP-L than on most polar columns, and for this reason they may not separate adequately to permit discrimination on AP-L packed columns[4]. The "load effect" also may affect retention times on certain non-polar substrates[12,13] as may simple peak broadening[14,15].

ACKNOWLEDGEMENTS

The author acknowledges the co-operation of S. N. HOOPER in part of this study. E. FRANCIS prepared the 2-keto-6,10-dimethylundecane. The 2-keto-6,10,14-trimethylpentadecane was the gift of A. K. LOUGH, Aberdeen, and most of the isoprenoid acids were donated by A. K. SEN GUPTA, Hamburg. The majority of the longer-chain monomethyl-branched fatty acids were the gift of E. STENHAGEN, Stockholm.

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