⁂Gas Chromatographic Equivalent Chain Lengths of Fatty Acid Methyl Esters on a Silar 10C Glass Capillary Column

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ABSTRACT

Gas chromatographic equivalent chain lengths have been determined for a number of fatty acid methyl ester isomers on a Silar 10C glass capillary column at 170 C. The results are comparable with others published for polycyanopropylsiloxane columns.

INTRODUCTION

Polycyanopropylsiloxane coatings in glass capillary columns are finding increasing use for gas chromatographic separation of isomeric fatty acid methyl esters (FAME) (1-4). At our laboratory, a Silar 10C column has been used to analyze FAME from hydrogenated oils. To aid in identification of peaks, we have determined equivalent chain lengths (ECL) (5) for a number of 18-carbon FAME, including many not previously reported for polycyanopropylsiloxane columns. Our values are reported here.

EXPERIMENTAL

Chromatograms were run on a 50 m x 0.25 mm id Silar 10C Quadrex glass capillary column (Applied Science Division, Milton Roy Company Laboratory Group) installed in a Perkin-Elmer Model 3920 gas chromatograph. Column temperature was set at 170 C with injector ca. 255 C and column-detector interface ca. 200 C. Column helium pressure was 30 psi. Gas flow through the column was 1.06 ml/min and through the split outlet 175 ml/min, corresponding to a split ratio of 165. Attenuation was times 8 with an amplifier range of times 1. Samples were either prepared in the course of other work at this laboratory or donated by other workers. They were run as isooctane solutions (usually 1%) mixed with reference compounds. Total solution injected was in the range 0.2 to 0.6 μl. Except when peak positions coincided, each sample solution contained two commercial methyl ester mixtures: 16:0, 18:0, 20:0, 22:0 for calculation of ECL and 16:0, 18:0, 18:1*c*9, 18:2*c*9*c*12, 18:3*c*9*c*12*c*15, 20:0 for comparison of the ECL value with that of the commonly occurring oleate, linoleate or linolenate.

The detector signal from the gas chromatograph was transmitted to an on-line computer, and retention times were determined with the same program ordinarily used for quantitation of peaks (6). Because the solvent peak was off scale, the retention time for an unretained compound was determined each day by injections of <0.1 μl isooctane at a reduced sensitivity to keep the peak on scale. ECL values were calculated by comparison with the 18:0 and 20:0 peaks.

RESULTS AND DISCUSSION

Under the conditions used for these chromatograms, approximate retention times were 4.48 min for an unretained compound, 18.5 min for oleate and 22.5 min for linoleate, corresponding to a capacity ratio of 3.1 for oleate and 4 for linoleate and an oleate-linoleate separation factor of 1.3. Values of 120,000 theoretical plates were calculated for oleate and linoleate using either the peak width at the base or at one half peak height and using a chart speed of 5 cm/min to obtain peaks wide enough for satisfactory measurement.

Our ECL values are listed in Table I. Unsaturation in all samples is designated by numbering from the carboxyl—the Δ notation. Each is an average of at least two runs. To correct for any run-to-run variations, each monoene value was compared to oleate run at the same time and corrected for the average oleate value, so that corrected ECL = average ECL_{oleate} + (ECL - ECL_{oleate}). Similar corrections were made by comparing dienes with linoleate and trienes with linolenate. Corrected values in the table generally differ from the original value by less than 0.01 unit.

The 18:1 *c*8, *c*10, *c*13, and *t*13 and 18:2 *t*12*c*15 and *t*12*t*15 samples contained deuterium. Patton and Lowenstein (7) have reported that saturated deuterium-containing esters are eluted faster than their nondeuterium-containing counterparts. We have confirmed that for methyl oleate and elaidate d_6, the ECL is 0.062 smaller, and have made appropriate corrections.

Samples for linolenate geometric isomers are fractions from an argentation countercurrent distribution (8). These are all mixtures of two or more isomers, and identification is based on their relative position in the countercurrent distribution series and on comparison with values published by Ackman and Hooper (9) for Silar 5CP. Identification of some of these isomers is therefore only tentative.

Values to three decimal places were carried through the calculations and rounded to two for inclusion in the table. For esters run repeatedly as reference compounds, in which 65-80 values were collected, averages and standard deviations were as follows: oleate 18.609 ± 0.040; linoleate 19.509 ± 0.008; linolenate 20.524 ± 0.012. As an indication of accuracy, palmitate was 16.002 ± 0.022. ECL for methyl docosanoate was 22.033 ± 0.011. This value is high because negative skew of this slow-moving peak caused the position of the first moment to precede slightly the measured peak maximum.

The minimal difference in ECL that can be distinguished depends somewhat on the relative size of the peaks. On our curves, a difference in ECL (Δ ECL) of 0.05 generally resulted in two clearly separated individual peaks. With the 5 cm/min chart speed used for theoretical plate determinations, resolution (10) between methyl oleate d_6 and methyl oleate (Δ ECL 0.06) was 1.3; between *cis*-9, *trans*-12- and *trans*-9, *cis*-12-octadecadienoate isomers (Δ ECL 0.08), it was 1.6. For Gaussian curves, a resolution of 1.5 corresponds to baseline separation; however, this was not quite achieved for the octadecadienoate isomers, apparently because of slight negative skew and tailing evident at the higher chart speed.

Our values are comparable to other published values. They are 0.10 to 0.29 higher than those of Myher et al. (11) on a packed Silar 10C column at 180 C. They range from 0.01 to 0.44 lower than those of Conacher and Iyengar (12) on a Silar 10C packed column at 200 C, and from 0.11 to 0.33

TABLE I

Equivalent Chain Length Values for Fatty Acid Methyl Esters on Silar 10C Column at 170 C

	18:1 Δ		18:2 Δ		18:3 Δ	
	c	*t*			9,12,15	
2	18.76	19.73	*c5c9*	18.94	*ttt*	19.94
5	18.43	18.35	*c5c12*	19.20	*tct*	20.15
6	18.56	18.40	*c6c10*	19.29	*ctt*	20.12[a]
7		18.43	*c6c11*	19.19	*ttc*	20.22[a]
8	18.56	18.41	*c6c12*	19.34	*tcc*	20.42[a]
9	18.61	18.41	*c7c12*	19.26	*cct*	20.38[a]
10	18.64	18.48	*c8c12*	19.34	*ctc*	20.31
11	18.70	18.49	*c9c12*	19.51	*ccc*	20.52
12	18.75	18.54	*c9c15*	19.61		
13	18.84	18.59	*c12c15*	19.81	*c6c9c12*	20.15
15	18.96	18.66	*c9t12*	19.32		
17	19.00		*t9c12*	19.40	Conjugated	
			t9t12	19.13	*t10t12t14*	23.89
			c12t15	19.52	*t9t11t13*	24.02
			t12c15	19.62	*c9t11t13*	23.62
			t12t15	19.32		
			c9t15	19.29		
			t9c15	19.44		
			t9t15	19.10		
			Conjugated			
			t9t11	21.22		
			c9t11	20.72		
			t10c12	20.86		
			t10t12	21.23		
			t11t13	21.19		

[a]Tentative identification based on elution order in argentation countercurrent distribution (8) and comparison with Ackman and Hooper (9) values for Silar 5CP.

lower than values calculated from retention data of Henly et al. (13) on a packed Silar 10C column at 180 C. Our values vary from the same to 0.13 lower than values calculated from retention data of Slover and Lanza (3) on a glass capillary coated with SP2340, another polycyanopropylsiloxane material, at 180 C. They are 0.14 to 0.66 higher than values we previously published (14,15) for a cyanoethyl silicone, XF1150, in a stainless-steel capillary. Jamieson et al. (16) have suggested that their tables and equations with polyester phases for C_{18}, C_{20} and C_{22} polyunsaturated FAME (17) can be extended to cyanoalkyl phases. For 6,9,12-octadecatrienoate, the only ester for which comparative data are available, their equation predicts 20.09, which is only 0.06 lower than our value. Therefore, their equations seem to be suitable for use with our linolenate value and similar data.

The values in Table I indicate that the more abundant isomers expected in a hydrogenated vegetable oil should be separated on the Silar 10C column. Among isomers expected in small amounts, 18:1 *t*13 elutes very close to oleate. However, for a complete study of all isomeric esters, fractionation and ozonization would still be necessary.

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