CALCULATION OF ECL VALUES IN THE GAS–LIQUID CHROMATOGRAPHY OF MULTIPLE-BRANCHED FATTY ACIDS

R. G. ACKMAN
Fisheries Research Board of Canada, Halifax Laboratory, Halifax, N.S. (Canada)
(Received November 14th, 1966)

The carbon number[1] or equivalent chain length[2,3] (ECL) systems for identifying esters in gas–liquid chromatographic analyses have been used with some success for unique fatty acids[4]. The original concept of the mobility of a given material in a gas–liquid chromatographic separation as due to the contribution of a basic skeleton plus contributions from non-interacting substituents[5–11] has, however, not been used very successfully with fatty acids since in naturally occurring fatty acids there is often interaction between neighbouring substituents or groups. Additionally, the lack of success has been because the position of a given substituent or functional group on or in the chain is of considerable significance in determining the fractional chain length (FCL) contribution due to the particular substituent or group[12–14]. It is generally recognized, however, that FCL values are normally nearly constant in the middle portion of an aliphatic chain. At the end of a fatty acid chain remote from the carboxyl group, FCL values in any chain may be modified in a more or less predictable fashion[15,16] depending on the nature of the group or substituent, but when near the carboxyl group may be subject additionally to the possibility of interaction with the carboxyl group[13,17–19].

In our laboratory, a gas–liquid chromatographic study of the saturated fatty acids of marine lipids gave evidence for the occurrence of three multiple-branched fatty acids in the lipids from all higher species[20]. Subsequent work in another laboratory indicated that these were probably 4,8,12-trimethyltridecanoic, 2,6,10,14-tetramethylpentadecanoic and 3,7,11,15-tetramethylhexadecanoic acids[21]. More recently, further studies in our laboratory have been carried out with both butanediol succinate (BDS) polyester and silicone (SE-30) open-tubular (capillary or Golay) columns[22]. Esters of authentic 2,6,10,14-tetramethylpentadecanoic and 3,7,11,15-tetramethylhexadecanoic acids (courtesy of R. P. HANSEN, D.S.I.R., Wellington, New Zealand) confirmed the identity of these two multiple-branched acids on both substrates. Petroleum has been shown to contain 3,7,11-trimethyldodecanoic acid in association with 2,6,10,14-tetramethylpentadecanoic acid[23] but comparative analyses with the ester of an authentic sample (courtesy of Dr. L. H. SARRETT, Merck, Sharp and Dohme, N.J.) showed that only traces of 3,7,11-trimethyldodecanoic acid were present in the marine lipids examined.

These studies provided accurate retention times on BDS for esters of three known multiple-branched fatty acids and one tentatively identified acid (Table I). The possibility of calculating the retention time of the ester of 4,8,12-trimethyltridecanoic acid was investigated since three model fatty acids were available for

TABLE I

ECL DATA FOR BRANCHED-CHAIN FATTY ACIDS ON BDS OPEN-TUBULAR COLUMNS OF HIGH EFFICIENCY (NO. 1) OR MODERATE EFFICIENCY (NO. 2)

Fatty acid		*BDS column No. 1; 150°*		*BDS column No. 2; 170°*	
		$r_{18:0}$	*ECL*	$r_{18:0}$	*ECL*
	12:0	0.088	12.00	—	—
	3,7,11-trimethyldodecanoic	0.125[a]	12.87	—	—
Iso	14:0	—	—	0.199	13.55
	14:0	0.198	14.00	0.233	14.00
	4,8,12-trimethyltridecanoic (?)	0.210[b]	14.14	~ 0.250[c]	~ 14.18
Iso	15:0	0.247	14.52	0.286	14.54
Anteiso	15:0	0.264	14.70	0.305	14.73
	15:0	0.298	15.00	0.337	15.00
Iso	16:0	0.370	15.53	0.413	15.56
Anteiso	16:0	0.398	15.72	0.444	15.75
	2,6,10,14-tetramethylpentadecanoic	0.413[a]	15.82	0.450	15.80
	16:0	0.446	16.00	0.485	16.00
Iso	17:0	0.555	16.55	0.597	16.56
Anteiso	17:0	0.589	16.71	0.633	16.75
	17:0	0.666	17.00	0.700	17.00
	3,7,11,15-tetramethylhexadecanoic	0.673[a]	17.02	~ 0.715[c]	~ 17.08
Iso	18:0	0.825	17.52	0.852	17.56
	18:0	1.000	18.00	1.00	18.00

[a] Determined independently of marine lipid fatty acids.
[b] Accurate value for component completely separated from other marine lipid fatty acids.
[c] Estimated value for component incompletely separated from other marine lipid fatty acids.

validating the procedures. The isoprene skeletons of these acids made this appear particularly feasible since there should be no interaction among the methyl groups.

The higher branched-chain fatty acids have been exhaustively studied from various viewpoints and the published material includes a table of separation factors for all of the methyloctadecanoic acids[24]. This data had to be converted into FCL units but no retention data for the appropriate linear fatty acids were provided to complement the tabulated data which were obtained on Reoplex 400 at 216°. To prepare FCL units a log plot line was drawn based on linear fatty acid ester data[25] obtained on Reoplex 400 at 197° ($r_{19:0}$ of 0.431 for methyl hexadecanoate). The FCL units obtained from this line by using the separation factors from the literature[24] in the normal fashion are listed in Table II and plotted against position of methyl substituent in Fig. 1. An apparent discrepancy occurs between the separation factor originally tabulated for 15-methyloctadecanoate and a plot similar to Fig. 1 in the same publication[24]. The FCL value for this acid in Table I and Fig. 1 is therefore an interpolation.

It is particularly evident from Fig. 1 that methyl groups in the 2-5 and 12-17 positions give FCL values differing significantly from the rest. They may be designated by types respectively as carboxyl end (C_x) and terminal end (ω_y) numbers, with the less critical positions in between designated m_x. The FCL values in Table II were then tested as shown in Table III. It will be noted that in the two shorter chain acids ω_5 FCL values have been employed in preference to m_8 and m_7 respectively. This usage arises from the obviously greater significance to be assigned to C_x or ω_y FCL values.

The excellent agreement obtained between the calculated and observed ECL values for these four acids was most unexpected since the FCL values were determined

TABLE II

SEPARATION FACTORS AND FCL VALUES FOR METHYL-BRANCHED OCTADECANOIC ACID METHYL ESTERS WITH TYPE DESIGNATIONS

Methyl position	*Literature*[24] $r_{19:0}$[a]	*FCL*[b]	*Type designation*
2	0.713	(—) 0.19	C_2
3	0.764	0.05	C_3
4	0.833	0.36	C_4
5	0.808	0.24	C_5
6	0.804	0.22	m_6
7	0.806	0.23	m_7
8	0.799	0.21	m_8
9	0.805	0.22	m_9
10	0.806	0.23	m_{10}
11	0.811	0.26	m_{11}
12	0.819	0.29	ω_6
13	0.812	0.27	ω_5
14	0.844	0.41	ω_4
15	0.672	(0.43)[c]	ω_3
16	0.898	0.62	ω_2
17	0.870	0.51	ω_1

[a] Reoplex 400 at 216°.
[b] Generated from a log plot line with $r_{19:0}$ as 1.00, $r_{16:0}$ as 0.431.
[c] Interpolated value.

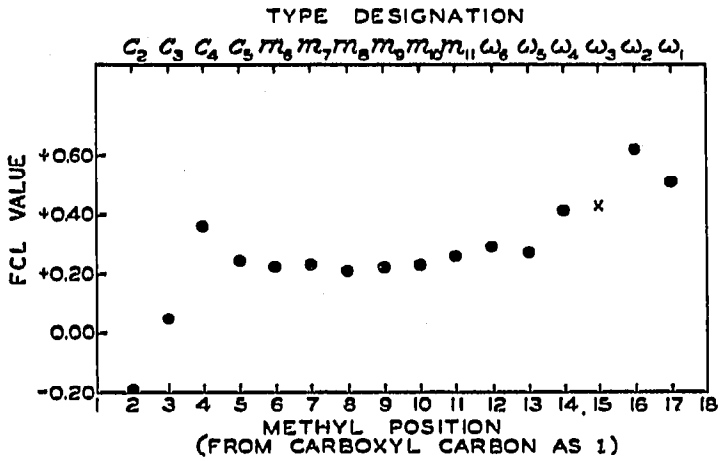

Fig. 1. Plot of FCL value against methyl positions on octadecanoate chain. Type designations at top. Value for ω_3 interpolated.

from a linear log plot based on a line chosen more or less at random from the literature and the arbitrary use of another plot line would generate a quite different family of FCL values. The ECL value for 3,7,11-trimethyldodecanoic acid at 197° on ethylene glycol-adipate (EGA) polyester[26] is 12.80, also close to the calculated value. The probable reason for the good agreement in these examples is that the Reoplex 400 and EGA columns are of comparable polarity to the BDS open-tubular column. Thus octadeca-6,9,12-15-tetraenoate ester falls before eicosenoic ester on the packed columns[25] and also on the BDS open-tubular columns[27]. Retention data obtained on β-cyclodextrin esters as gas–liquid chromatographic substrates were also examined

TABLE III

APPLICATION OF FCL VALUES (TABLE II) TO THE CALCULATION OF ECL VALUES FOR MULTIPLE BRANCHED FATTY ACIDS OBSERVED IN MARINE LIPIDS

Experimental data given at 150° in Table I.

3,7,11,15-Tetramethylhexadecanoate		
Basic chain		16.00
FCL contribution	C_3	0.05
FCL contribution	m_7	0.23
FCL contribution	ω_5	0.27
FCL contribution	ω_1	0.51
Calculated	ECL	17.06
Experimental	ECL	17.02
4,8,12-Trimethyltridecanoate		
Basic chain		13.00
FCL contribution	C_4	0.36
FCL contribution	ω_5	0.27
FCL contribution	ω_1	0.51
Calculated	ECL	14.14
Experimental	ECL	14.14
2,6,10,14-Tetramethylpentadecanoate		
Basic chain		15.00
FCL contribution	C_2	(—)0.19
FCL contribution	m_6	0.22
FCL contribution	ω_5	0.27
FCL contribution	ω_1	0.51
Calculated	ECL	15.81
Experimental	ECL	15.82
3,7,11-Trimethyldodecanoate		
Basic chain		12.00
FCL contribution	C_3	0.05
FCL contribution	ω_5	0.27
FCL contribution	ω_1	0.51
Calculated	ECL	12.83
Experimental	ECL	12.87

since three multiple-branched acids were included[28]. From the FCL values in Table II the ECL values calculated for the multiple-branched fatty acids were found to be in good agreement (Table IV) with the experimental ECL values obtained with β-cyclodextrin acetate at 220°. This material is, however, of somewhat greater polarity with respect to unsaturated materials. Another experimental log plot line drawn by trial and error (with $r_{19:0}$ of 0.354 for methyl hexadecanoate) generated respective FCL values of 0.04, 0.23, 0.38 and 0.61 for methyl 2-, 3-, 6- and 15-hexadecanoates. As shown in Table IV this set of FCL values gave good agreement with experimental ECL values determined on β-CDX butyrate.

As a rough guide to the choice of log plot lines for generating FCL values from the literature data (Table II) for use in a particular analysis, the reasonably accessible ω_1 (*iso* acid) FCL values may be used to establish a preliminary line. Thus, the typical FCL value of 0.53 found for ω_1 acids at 150° in marine oils (Table I) is very close to that (0.51) inadvertently generated for Table II. The experimental β-CDX acetate FCL value of 0.55 is also fairly close to this value but the experimental β-CDX butyrate value of 0.65 differs considerably from these figures. The trial and error line used to generate the FCL values for β-CDX butyrate (Table IV) was in fact based on the observation that the difference in the ω_1 FCL values for the acetate FCL values was 0.04.

The validity or accuracy of the FCL values given in Table II is not guaranteed beyond the original data. As previously pointed out the 15-methyloctadecanoate figure is an interpolation. Some reservations must also be held regarding the 16-methyloctadecanoate (ω_2) FCL value of 0.62. As shown in Table I the ω_1 and ω_2 FCL values obtained with authentic esters on the open-tubular BDS column at 150° were consistent at respectively about 0.53 and 0.71. These figures are in good agreement with typical values obtained from literature data[25] of about 0.52 for ω_1 and about 0.72 for ω_2 on EGA at 197°. A survey of all available literature data gave respective

TABLE IV

APPLICATION OF FCL VALUES TO THE CALCULATION OF ECL VALUES FOR MULTIPLE-BRANCHED FATTY ACIDS ANALYSED ON β-CDX ACETATE AND BUTYRATE SUBSTRATES[28]

	β-CDX	
	Acetate[a]	*Butyrate*[b]
3,6,13-Trimethyltetradecanoate		
Basic chain	14.00	14.00
FCL contribution C_3	0.05	0.23
FCL contribution m_6	0.21	0.38
FCL contribution ω_1	0.51	0.61
Calculated ECL	14.77	15.22
Experimental ECL	14.75	15.20
3,6-Dimethylpentadecanoate		
Basic chain	15.00	15.00
FCL contribution C_3	0.05	0.23
FCL contribution m_6	0.21	0.38
Calculated ECL	15.26	15.61
Experimental ECL	15.15	15.60
2,14-Dimethylpentadecanoate		
Basic chain	15.00	15.00
FCL contribution C_2	(—)0.19	0.04
FCL contribution ω_1	0.51	0.61
Calculated ECL	15.32	15.65
Experimental ECL	15.25	15.70

[a] FCL values from Table II.
[b] For derivation of FCL values see text.

average FCL values of 0.57 (from 32 figures) and 0.73 (from 19 figures), but no pattern could be detected associating variations with polyester polarity or operating temperature.

The large FCL value of a C_4 isomer is substantiated by the calculation for 4,8,12-trimethyltridecanoate and by gas–liquid chromatographic analyses of certain methyl-branched fatty acids on a polypropylene glycol open-tubular column[29,30]. In these reports it is shown that 4-methyloctadecanoate falls between the 12- and 14-methyloctadecanoates, but 4-methylhexadecanoate falls between the 10- and 12-methylhexadecanoates. This means, in effect, that the C_4 FCL value in this analysis would be numerically between the FCL values for ω_4 and ω_6. This is in fact clearly indicated in Fig. 1 and Table II and may be a general rule. The importance of viewing isomers with methyl branches near the terminal end of the chain in terms of ω_y is supported by the similar relationship of the C_4 to the ω_5 position in both the methyloctadecanoate and methylhexadecanoate series.

The impression may be gained from the data in Table I and the close similarity of literature ECL values for 3,7,11-trimethyldodecanoic acid on EGA that the ECL values on polyester substrates for multiple-branched fatty acids are subject to very little variation. This is not necessarily correct, as shown by the literature data for the β-CDX esters[28] and other studies[22,31].

Apolar substrates have not been evaluated for additive FCL values since it has been shown that on SE-30 and QF-1 columns[22,32] the effect of relative concentration

makes retention times for small components unreliable. It is not known if this is true of the widely used Apiezon substrates.

EXPERIMENTAL

The data in Table I were obtained with BDS columns (150 ft. in length × 0.01 in. I.D.) in a Perkin-Elmer 226 gas chromatograph. Injection port temperature was 260°, carrier gas helium at 40 p.s.i.g. A No. 2 inlet splitter was employed. The marine oil sample was obtained by hydrogenating the methyl esters of cod liver oil triglycerides. Methyl esters of several authentic *iso* and *anteiso* fatty acids were found to coincide with appropriate peaks, and homologues were identified by plotting procedures. Authentic multiple-branched fatty acids were also co-chromatographed with pure linear fatty acids. Column No. 1 was new, column No. 2 had lost considerable efficiency with prolonged use[18].

SUMMARY

The generation from literature data of fractional chain length (FCL) values for single methyl substituents in esters of isomeric monomethyl-branched fatty acids is described. The addition of FCL values to the basic chain length gives good agreement between calculated and experimental equivalent chain length (ECL) values for esters of several multiple-branched fatty acids of known structure and is used to verify the structure tentatively assigned to another multiple-branched fatty acid ester.

NOTE ADDED IN PROOF

The tabulated retention data[24] for 15-methyloctadecanoate are in error, but the associated figure is correct[33]. The FCL value for an ω_3 acid can therefore be taken as marginally greater than that for the ω_4 acid.

REFERENCES

1 F. P. Woodford and C. M. van Gent, *J. Lipid Res.*, 1 (1960) 188.
2 T. K. Miwa, K. L. Mikolajczak, F. R. Earle and I. A. Wolff, *Anal. Chem.*, 32 (1960) 1739.
3 T. K. Miwa, *J. Am. Oil Chemists' Soc.*, 40 (1963) 309.
4 H. H. Hofstetter, N. Sen and R. T. Holman, *J. Am. Oil Chemists' Soc.*, 42 (1965) 537.
5 R. B. Clayton, *Nature*, 192 (1961) 524.
6 F. A. Vandenheuvel, G. J. Hendreks, J. C. Nixon and W. C. Layng, *J. Am. Oil Chemists' Soc.*, 42 (1965) 283.
7 W. J. A. Vandenheuvel and E. C. Horning, *Biochim. Biophys. Acta*, 64 (1962) 416.
8 B. A. Knights and G. H. Thomas, *Anal. Chem.*, 34 (1962) 1046.
9 B. A. Knights and G. H. Thomas, *Chem. Ind. (London)*, (1963) 43.
10 B. A. Knights and G. H. Thomas, *J. Chem. Soc.*, (1963) 3477.
11 I. S. Hartman and H. H. Wotiz, *Biochim. Biophys. Acta*, 90 (1964) 334.
12 J. S. O'Brien and G. Rouser, *Anal. Biochem.*. 7 (1964) 288.
13 A. P. Tulloch, *J. Am. Oil Chemists' Soc.*, 41 (1964) 833.
14 G. Schomburg, *J. Chromatog.*, 14 (1964) 157.
15 R. D. Swisher, E. F. Kaelble and S. K. Liu, *J. Org. Chem.*, 26 (1961) 4066.
16 F. Baumann, A. E. Straus and J. F. Johnson, *J. Chromatog.*, 20 (1965) 1.
17 R. E. Anderson and H. Rakoff, *J. Am. Oil Chemists' Soc.*, 42 (1965) 1102.
18 R. G. Ackman and J. D. Castell, *J. Gas Chromatog.*, in press.
19 E. A. Napier, Jr., *Anal. Chem.*, 35 (1963) 1294.

20 R. G. ACKMAN AND J. C. SIPOS, *Comp. Biochem. Physiol.*, 15 (1965) 445.
21 A. K. SEN GUPTA AND H. PETERS, *Fette, Seifen, Anstrichmittel*, 68 (1966) 349.
22 R. G. ACKMAN, J. C. SIPOS AND C. S. TOCHER, *J. Fisheries Res. Board Can.*, in press.
23 J. CASON AND D. W. GRAHAM, *Tetrahedron*, 21 (1965) 471.
24 S. ABRAHAMSSON, S. STALLBERG-STENHAGEN AND E. STENHAGEN, in R. T. HOLMAN (Editor), *Progress in the Chemistry of Fats and Other Lipids*, Vol. 7, Part 1, Pergamon (Macmillan), New York, 1963.
25 J. W. FARQUHAR, W. INSULL, JR., P. ROSEN, W. STOFFEL AND E. H. AHRENS, JR., *Nutr. Revs. (Suppl.)*, 17 (1959) 1.
26 G. POPJÁK AND R. H. CORNFORTH, *J. Chromatog.*, 4 (1960) 214.
27 R. G. ACKMAN, J. C. SIPOS AND P. M. JANGAARD, *Lipids*, in press.
28 H. SCHLENK, J. L. GELLERMAN AND D. M. SAND, *Anal. Chem.*, 34 (1962) 1529.
29 G. ODHAM, *Arkiv Kemi*, 22 (1964) 417.
30 G. ODHAM, *Arkiv Kemi*, 23 (1965) 431.
31 G. J. KREMER, *Klin. Wochschr.*, 43 (1965) 517.
32 R. G. ACKMAN, *J. Gas Chromatog.*, 3 (1965) 15.
33 E. STENHAGEN, *private communication.*