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ADDITIVITY OF RETENTION DATA FOR ETHYLENIC FUNCTIONS IN ALIPHATIC FATTY ACIDS

II. POLAR LIQUID PHASES

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SUMMARY

The elution order of two mono-*cis*,di-*trans*-isomers of the methyl esters of 9,12,15-octadecatriethylenic acids for two polar liquid phases is indicated by the equivalent chain lengths calculated from fractional chain length values determined for methyl esters of monoethylenic C₁₈ acids with Δ^9 , Δ^{12} and Δ^{15} ethylenic unsaturation. The application of appropriate diethylenic coupling corrections is demonstrated. All eight octadecatriethylenic isomers, and related mono- and diethylenic isomers, were examined on the polar liquid phases butanediol succinate and SILAR-5CP.

INTRODUCTION

The basic concepts of additivity of fractional chain length (FCL) values for single functional groups to make up equivalent chain lengths (ECL) of methyl esters of fatty acids have been discussed elsewhere^{1,2}. Others have discussed the necessity for various corrections applicable to polyethylenic fatty acids³⁻⁵ and a study of some mono-, di- and triethylenic C₁₈ acids on Apiezon L (AP-L) is presented in an accompanying paper⁶. For AP-L the location of certain *cis*,*trans*-diethylenic acids occurring in mixtures was successfully worked out in the absence of specific authentic or isolated acids as part of a problem involving the eight octadecatriethylenic acids with bonds in the Δ^9 , Δ^{12} , and Δ^{15} positions.

For polar liquid phases the same materials were available, but because on polar liquid phases, unlike AP-L, mono- and diethylenic acids expand in separate groups after the corresponding saturated acid² the basic experimental retention data (ECL values) for all of the *cis*,*cis*-, *cis*,*trans*- and *trans*,*trans*-diethylenic C₁₈ isomers were available⁷. A minor problem was that two mono-*cis*,di-*trans*-isomers of 9,12,15-octadecatriethylenic acid prepared from the all-*cis*-isomer with nitrous acid^{8,9} had not been isolated, and although separated by gas-liquid chromatography (GLC) on polar columns it was uncertain which was which. We have, therefore, calculated the retention times as ECL values for all eight triethylenic isomers from the diethylenic data for butanediol succinate (BDS), a standard polyester liquid phase in continuous use in our laboratory, and SILAR-5CP, a new liquid phase (a 50% cyanopropyl, 50%

phenyl silicone; Applied Science Labs., State College, Pa., U.S.A.) with novel features with respect to *cis*- and *trans*-isomers⁷.

EXPERIMENTAL

The basic GLC operations were those described elsewhere^{6,7,10} with wall-coated, open-tubular stainless-steel columns 150 ft. (46 m) \times 0.01 in. (0.25 mm) I.D. These were coated by Perkin-Elmer (Norwalk, Conn., U.S.A.) and used in a Perkin-Elmer Model 900 gas chromatograph with an injection/split system at 270° and a manifold at 250°. Helium was the carrier gas. Other operating details are given with the tables and figures.

RESULTS AND DISCUSSION

In Fig. 1 is shown the product of nitrous acid elaidinization of all-*cis*-9,12,15-octadecatriethylenic acid (solid envelope) on two polar liquid phases. From thin-layer chromatography, and following the precepts of Litchfield *et al.*⁸, it was thought that the major product would be all-*trans*, and the next most likely isomers, indicated by relative areas of the minor peaks (and the two peaks in the BDS analysis) to be three in number, would be mono-*cis*, di-*trans*-isomers. Of these, 9*t*,12*c*,15*t** had been found in a steam-deodorized vegetable oil^{7,10}. It was necessary to determine the location of the 9*t*,12*t*,15*c* and 9*c*,12*t*,15*t* isomers. On AP-L, although these two isomers were not isolated, the elution order was provided by literature data¹¹ and by calculation⁶.

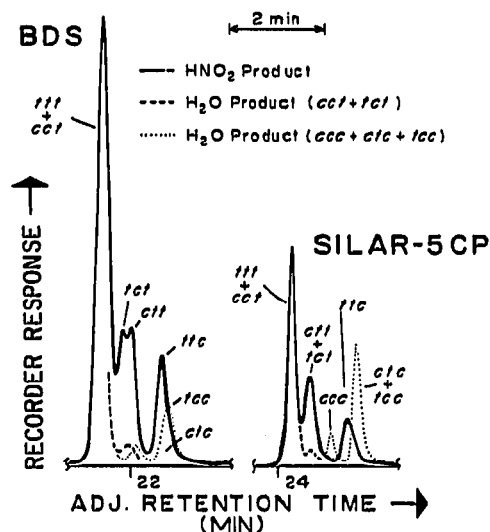


Fig. 1. Comparison of methyl esters of some isomeric octadecatriethylenic acids on open-tubular polar columns. Outer envelope is total product of nitrous acid elaidinization of all-*cis*-isomer; dotted inserts show parts of other products from different analyses run under the same conditions. BDS column operated at 170°, SILAR-5CP column at 180°. *t c t*-*c t t* elution order tentative.

* Shorthand for 9-*trans*,12-*cis*,15-*trans*-octadecatriethylenic acid, further abbreviated to *t c t* etc. where applicable. In monoethylenic acids the Δ^n notation is followed.

The experimental retention data for a good (normal polarity) BDS column (No. 920) and a SILAR-5CP column (No. 902) in Table I are taken from a more extensive tabulation of data⁷, including analyses on another BDS column (No. 928) of reduced polarity. Partial data are also given elsewhere⁷ for BDS column No. 928 when newer, and for another SILAR-5CP column (No. 901). The FCL values, which had to be treated mathematically for AP-L, could be added directly for the polar liquid phases. The additional diethylenic correction principles discussed elsewhere⁶ for AP-L were also used and are shown in Table I. Thus, any two monoethylenic FCL units could be added to give an uncorrected diethylenic ECL value. Typically these differed from measured retention values by as much as 0.24 FCL unit on BDS or 0.23 FCL unit on SILAR-5CP (Table I). The order of magnitude of the differences for diethylenic isomers does not seem to be related to the presence or absence of *trans* unsaturation. The latter point has been used as a basis for two proposed average corrections for diethylenic acids on diethyleneglycol succinate (DEGS) polyester of 0.20 (if a *trans* function was present) or 0.07 (if no *trans* function was present)⁵.

The eight triethylenic isomers were treated similarly with differences of up to 0.39 FCL unit on BDS and 0.33 FCL unit on SILAR-5CP. The two experimental differences for the appropriate diethylenic isomers were then added to the sum of the FCL values for the three monoethylenic isomers to give the corrected triethylenic values. For example, on BDS, $\Sigma FCL (9c + 12c + 15t) = 1.26$, giving the uncorrected ECL value of 19.26 for the $9c, 12c, 15t$ isomer. The two diene corrections are the differences for $9c, 12c$ and $12c, 15t$ (0.12 and 0.14, respectively) and $19.26 + 0.12 + 0.14 = 19.52$, the corrected ECL value. This approach gave corrected calculated ECL values which clearly suggest that on BDS the $9c, 12t, 15t$ isomer will elute near the $9t, 12t, 15t$ isomer and also near the $9t, 12c, 15t$ isomer. Calculated octadecatriethylenic ECL differences for BDS average 0.03 ECL unit, hence the precise elution order of the *cit* and *tct* isomers, for which the ECL values differ by only 0.02 ECL unit, cannot be predicted with confidence. The *tct* isomer is tentatively identified as the first peak from another sample⁷, and with the *cit* isomer accounts for the poorly separated pair shown in Fig. 1. However, the difference between the calculated *cit* and *tct* ECL values of 0.15 ECL unit seems large enough to warrant the assumption that the $9t, 12t, 15c$ isomer elutes much later. This agrees with the GLC data of Fig. 1 where only one extra peak thought to be of the di-*trans*, mono-*cis*-type had not been accounted for. On SILAR-5CP the same general picture emerged from the calculations of Table I. The two isomers were thus satisfactorily accounted for, permitting full identification of all eight possible isomers.

An attempt was made to incorporate a further correction for the 9,15 isomers which are marked with $n=4$, since they have four methylene units between the ethylenic bonds. These $n=4$ corrections are very small on AP-L^{4,6} and also on polar columns (Table I). Although a few individual calculated octadecatriethylenic acid ECL values were improved by this addition, others were thrown out to about the same extent so that a net gain in accuracy could not be measured.

The peculiar retention properties of SILAR-5CP concerning *cis*- and *trans*-isomers have been discussed elsewhere⁷. Temperature is probably a significant factor in this type of separation, as it is with other polar liquid phases¹². We have not evaluated this point but, as SILAR-5CP is reportedly very thermostable^{13,14}, it might be possible to exploit such a property to improve separation of particular combinations

TABLE I
COMPARISON OF ECL VALUES FOR METHYL ESTERS OF DI- AND TRIETHYLENIC FATTY ACIDS CALCULATED FROM FCL VALUES FOR MONOETHYLENIC ACIDS WITH EXPERIMENTAL VALUES, AND WITH CORRECTED TRIETHYLENIC VALUES

Liquid phase fatty acid	BDS No. 920				SILAR-5CP No. 902				
	Experimental ECL values	FCL and Σ FCL values	Difference Σ FCL - ECL	Corrected triethylenic ECL values	Difference	FCL and Σ ECL values	Difference Σ FCL - ECL	Corrected triethylenic ECL values	Difference
9t	18.29	(0.29)	-	-	-	18.25	-	(0.25)	-
9c	18.30	(0.30)	-	-	-	18.31	-	(0.31)	-
12t	18.36	(0.36)	-	-	-	18.35	-	(0.35)	-
12c	18.42	(0.42)	-	-	-	18.42	-	(0.42)	-
15t	18.54	(0.54)	-	-	-	18.42	-	(0.42)	-
15c	18.69	(0.69)	-	-	-	18.60	-	(0.60)	-
9t,15t (<i>n</i> =4)	18.81	18.83	+0.02	-	-	18.72	-0.05	18.67	-
9t,12t	18.82	18.65	-0.17	-	-	18.77	-0.17	18.60	-
9c,15t (<i>n</i> =4)	18.83	18.84	+0.01	-	-	18.79	-0.06	18.73	-
9c,12t	18.84	18.66	-0.18	-	-	18.80	-0.14	18.66	-
9c,12c	18.84	18.72	-0.12	-	-	18.81	-0.08	18.73	-
9t,12c	18.95	18.71	-0.24	-	-	18.90	-0.23	18.67	-
9t,15c (<i>n</i> =4)	19.01	18.98	-0.03	-	-	18.92	-0.07	18.85	-
12t,15t	19.05	18.90	-0.15	-	-	18.91	-0.14	18.77	-
9c,15c (<i>n</i> =4)	18.99	18.99	0.00	-	-	18.95	-0.04	18.91	-
12c,15t	19.10	18.96	-0.14	-	-	18.99	-0.15	18.84	-
12t,15c	19.22	19.05	-0.17	-	-	19.06	-0.11	18.95	-
12c,15c	19.21	19.11	-0.10	-	-	19.11	-0.09	19.02	-
9c,12c,15t	19.52	19.26	-0.26	19.52	0.00	19.35	-0.20	19.15	+0.03
9t,12t,15t	19.52	19.19	-0.33	19.51	-0.01	19.35	-0.33	19.02	-0.02
9t,12c,15t	19.57*	19.25	-0.32	19.63	+0.06	19.41	-0.32	19.09	+0.06
9c,12t,15t	19.59*	19.20	-0.39	19.53	-0.06	19.41	-0.33	19.08	-0.05
9c,12c,15c	19.61	19.41	-0.20	19.63	+0.02	19.44	-0.11	19.33	-0.06
9t,12t,15c	19.68	19.34	-0.34	19.68	0.00	19.50	-0.30	19.20	-0.02
9c,12t,15c	19.68	19.35	-0.33	19.69	+0.01	19.51	-0.25	19.26	0.00
9t,12c,15c	19.70	19.40	-0.30	19.74	+0.04	19.51	-0.24	19.27	+0.08

* Peak identification tentative.

of *cis*- and *trans*-ethylenic unsaturation. We have compared our BDS and SILAR-5CP data for the *cis*- and *trans*- Δ^9 , $-\Delta^{12}$ and $-\Delta^{15}$ monoethylenic isomers (Fig. 2). Both BDS *cis*-isomer curves can be thought to swing smoothly upwards from the Δ^9 to Δ^{15} positions, paralleling recent data for DEGS^{4,15}. The *trans*-isomer behaves similarly although apparently with a lesser slope. The curve for the three *cis*-isomers on SILAR-5CP behaves similarly, but although column "polarity" is about the same as for BDS, the slope from Δ^{12} to Δ^{15} seems slightly less than for BDS. For the *trans*-isomers on SILAR-5CP a downturn through the Δ^{12} to Δ^{15} positions appears probable. This behaviour must account for the superior separation of certain *cis*- and *trans*-isomers from others^{7,10}, especially those including *trans*- Δ^{15} bonds. However, it is of interest to note that on BDS and SILAR-5CP the last four octadecatriethylenic isomers to elute (Table I) have 15-*cis* functions, as did three out of four on AP-L⁶. Comparing the eight triethylenic isomer elution orders on BDS and AP-L it is notable that the four eluting in about the same rank have two adjacent bonds of similar geometry (*cct*, *ctt*, *ttc*, *tcc*). Three out of four which elute earlier on AP-L (*ccc*, *ctc*, *tcc*) share a 15-*cis* function. Conversely, a 15-*trans* function causes three (*cct*, *ttt*, *tet*) to elute later on AP-L than on BDS. Thus the special significance of *trans*-monoethylenic unsaturation in the Δ^{15} position, if not obvious for these eight polyethylenic

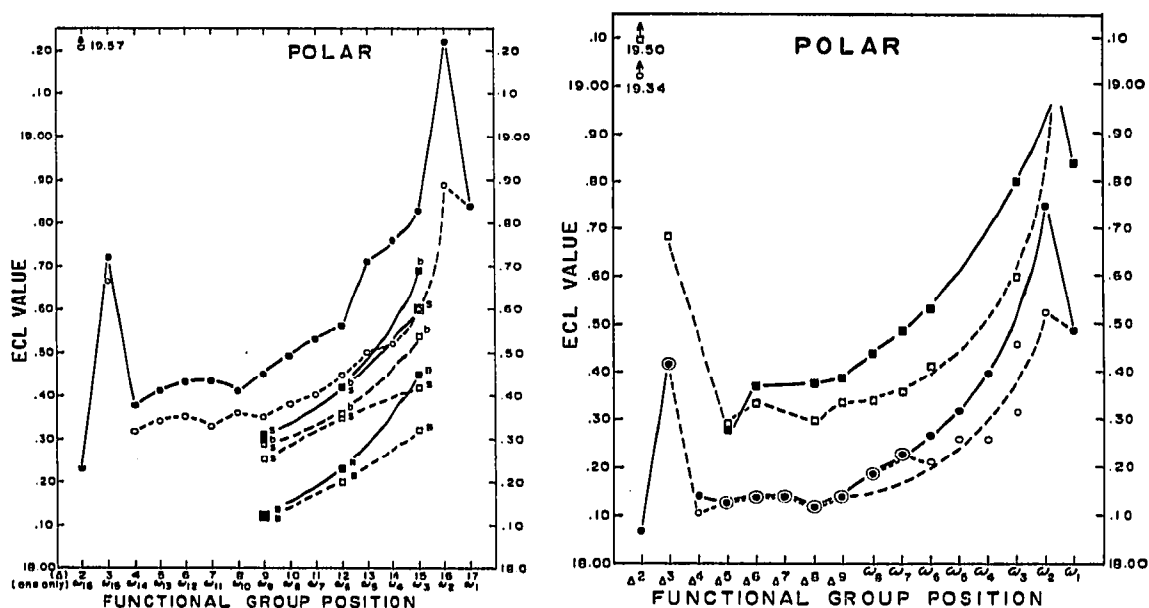


Fig. 2. Plots of experimental retention data (Table I) for Δ^9 , Δ^{12} , and Δ^{15} monoethylenic isomers compared to data for all isomers examined on DEGS. BDS 928 refers to "low polarity" wall-coated open-tubular column, BDS 920 to "normal polarity". ■—■, Experimental values for *cis*-isomers; □—□, experimental values for *trans*-isomers; ●—●, values by Barve *et al.*⁴ for *cis*-isomers on DEGS; ○—○, values by Barve *et al.*⁴ for *trans*-isomers on DEGS; B=BDS 928; b=BDS 920; S=SILAR-5CP.

Fig. 3. Plots of retention data for all monoethylenic isomers on NPGS³ and several isomers on DEGS¹⁵. □—□, *cis*-isomers on NPGS; ○—○, *trans*-isomers on NPGS; ■—■, *cis*-isomers on DEGS; ●—●, *trans*-isomers on DEGS.

acids on SILAR-5CP, is apparent on AP-L (see ref. 6). However, these distinctions are in some cases rather arbitrary as ECL values are identical, or nearly so.

As pointed out in our application with AP-L, this type of curve for FCL value *versus* position of function is expected to be fairly smooth except for ethylenic unsaturation near the two ends of the fatty acid chain. We have, therefore, also prepared curves for other published data (Fig. 3) and our three low-polarity points for column No. 928 (aged BDS) of Fig. 2 coincide almost exactly with the corresponding points on the neopentylglycol succinate (NPGS) curves of Fig. 3.

Both a general viewpoint and point-by-point comparison indicate that irregularities in centrally located unsaturation such as the low ECL value on DEGS of the *cis*- Δ^8 -isomer (Fig. 2), are probably experimental irregularities. The behaviour of the Δ^9 , Δ^{12} and Δ^{15} positions for DEGS and BDS appear regular and coherent in pattern from laboratory to laboratory, an important point in view of the biological significance of the ethylenic unsaturation in these positions¹⁶. The fact that SILAR-5CP, while different for unsaturation in the specific area of Δ^{12} and Δ^{15} , still permits accurate calculation of ECL values is therefore promising for analytical work in biochemistry.

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