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ADDITIVITY OF RETENTION DATA FOR ETHYLENIC FUNCTIONS IN ALIPHATIC FATTY ACIDS

I. APIEZON L

R. G. ACKMAN and S. N. HOOPER

Department of the Environment, Fisheries and Marine Service, Halifax Laboratory, Halifax, Nova Scotia (Canada)

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SUMMARY

The equivalent chain lengths of the methyl esters of eight isomeric 9,12,15-octadecatriethylenic acids for Apiezon L are compared with values calculated from fractional chain length values determined for methyl esters of monoethylenic C₁₈ acids with Δ^9 , Δ^{12} and Δ^{15} unsaturation. The necessity of further correction based on the appropriate two diethylenic couplings is shown, confirming published concepts, and the desirability of "particular" corrections (*i.e.* based on the same column and same temperature) is discussed.

INTRODUCTION

Since retention data are very conveniently presented in the form of equivalent chain length (ECL) values¹⁻⁴, the tabulation of accurate gas-liquid chromatographic (GLC) retention data for isomeric mono- and polyethylenic fatty acids has led to several attempts to calculate retention data as ECL values from fractional chain length (FCL) values^{5,6}. As Apiezon L (AP-L) is often considered an ideal liquid phase for interlaboratory comparisons^{3,5} we chose to evaluate it in an application of the Σ FCL approach to part of another study.

Recent studies on geometrical and positional isomers of C₁₈ fatty acids with three ethylenic double bonds in the positions 9, 12 and 15 were based on four geometric isomers of natural all-*cis*-octadecatriethylenic acid formed in vegetable oils on steam/vacuum deodorization⁷, and their degradation products obtained by partial reduction with hydrazine⁸. We included in these studies an alternative series of four geometrical octadecatriethylenic acid isomers produced by nitrous acid elaidinization⁹ of all-*cis*-octadecatriethylenic acid, and are thus able to compare retention data for some hitherto relatively inaccessible geometrical and positional isomers with those for the more readily available materials.

Reference mono- and diethylenic positional and geometrical isomers were prepared as a basis for study and to supplement previous attempts to organize calculation of retention times of isomers of polyethylenic fatty acid from structural elements^{5,6}. The success of this approach is illustrated with a practical problem of

locating peaks in a complex GLC envelope. Necessarily we have included certain comparisons with literature data which may already have been discussed at length^{5,6,10-15}. Recent and homogeneous data are generally preferred for such comparisons.

EXPERIMENTAL

Material origins and preparations are described elsewhere^{7,8}, except for the hydrazine reduction products of 6-*cis*,9-*cis*,12-*cis*-octadecatriethylenic acid, which were prepared as for the corresponding 9,12,15-isomer. Most identifications have been validated by cross-checking peaks on chromatograms based originally on two different parent structures, or occasionally by comparison with literature data. However, with a few exceptions individual compounds were normally not completely isolated for positive identification by degradation identification, or prepared by specific methods of synthesis.

GLC was carried out on wall-coated open-tubular stainless-steel columns, 150 ft. (46 m) × 0.01 in. (0.25 mm) I.D., purchased already coated from Perkin-Elmer (Norwalk, Conn., U.S.A.). They were operated in a Perkin-Elmer Model 990 gas chromatograph under conditions given in table headings and figure captions. The injection/split system was held at 270°, the manifold connections at 250°. The carrier gas was helium.

RESULTS AND DISCUSSION

A typical and relatively simple analytical problem is shown by the complex envelope of four basic sections obtained on AP-L for the hydrazine reduction products of 9*t*,12*t*,15*t*-18:3* (Fig. 1). The same eight materials gave eight specific peaks on the polar liquid phase SILAR-5CP (50% cyanopropyl, 50% phenyl-substituted silicone liquid phase from Applied Science Labs., State College, Pa., U.S.A.) (Fig. 2). This illustrates a basic deficiency in work with methyl esters of fatty acids on AP-L, which is otherwise outstanding as a liquid phase for reproducible interlaboratory comparisons^{3,5}. Recourse to a thin-layer plate of silica gel impregnated with silver nitrate and developed in toluene gave the fractions also examined on butanediol succinate polyester (BDS) in Fig. 1. However, we have not attempted a complete separation of *cis* and *trans* combinations in all the possible diethylenic acids with 9, 12 or 15 bond positions on silver nitrate as they would fall within the same general areas as other triethylenic and monoethylenic isomers in GLC examination of mixtures on AP-L as shown below. Such complex chromatograms would be relatively meaningless compared to those obtained on polar columns such as SILAR-5CP or BDS, where these particular groups of 18:1, 18:2 and 18:3 acids occupy specific and separate areas of the chromatograms (*cf.* Figs. 1 and 2, or 3 and 4, or see more detailed discussions³).

Our results (Table I) are comparable to and supplement the more comprehensive sets of literature values. For example the ECL values listed by Strocchi *et al.*¹⁵ for octadecatriethylenates from their own work are on the average 0.045 ECL unit

* A shorthand notation for position (from the carbonyl group), geometry (*t*=*trans*, *c*=*cis*), chain length and number of double bonds.

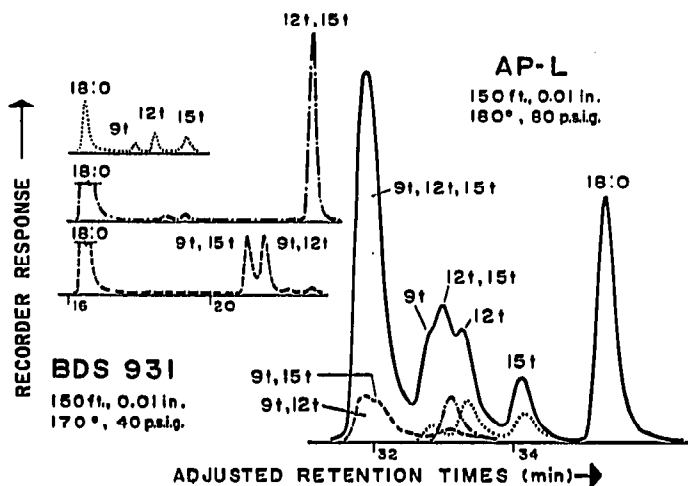


Fig. 1. Comparison of GLC envelope on AP-L for methyl esters of a group of $9t,12t,15t$ -octadecatriethylenic acids and mono- and diethylenic partial hydrazine reduction products, with silver nitrate TLC fractions on AP-L (within envelope) and on BDS (on left). Additional 18:0 has been added for identification purposes. The $9t,12t$ versus $9t,15t$ AP-L relationship is unconfirmed (*cf.* ref. 6). Compare envelope in Fig. 2.

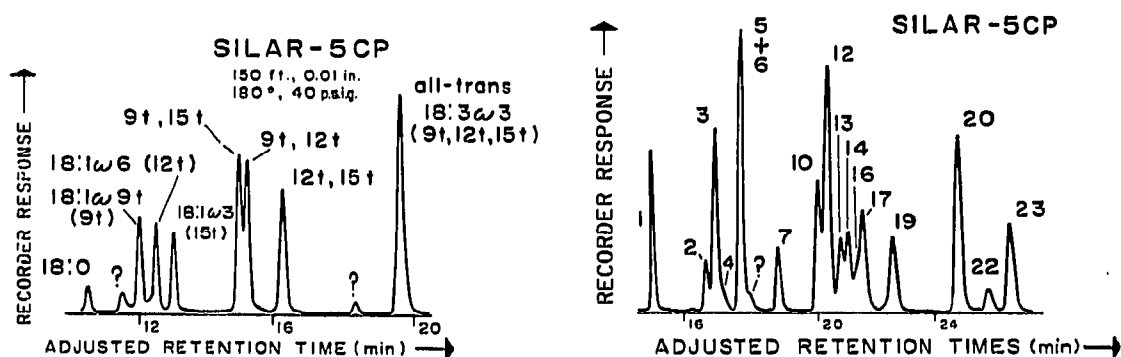


Fig. 2. Partial hydrazine reduction products of $9t,12t,15t$ -octadecatriethylenic acid (compare Fig. 1) examined on SILAR-5CP. The $9t,15t$ versus $9t,12t$ relationship is confirmed independently (*cf.* refs. 8 and 17).

Fig. 3. Partial hydrazine reduction products of three-component mixture of octadecatriethylenic acids of isomer geometry $9c,12c,15t$ (20); $9c,12c,15c$ (22) and $9t,12c,15c$ (23) examined on SILAR-5CP. Refer to Table I and text for details and compare Fig. 4. Peak 1 is 18:0.

higher than ours. Barve *et al.*⁵ noted the same effect relative to their values, which tend to be lower than ours, especially for the octadecatriethylenic acids. The results of Scholfield and Dutton¹² for diethylenic acids are close but very slightly higher, except for the values for the diethylenic isomers $12c,15c$ and $12t,15t$, which seem excessively high.

As unsaturated acids emerge before the corresponding saturated acids on AP-L it is usual in calculation to apply the FCL values for a single ethylenic bond

TABLE I
COMPARISON OF ECL VALUES FOUND EXPERIMENTALLY FOR METHYL ESTERS OF VARIOUS MONO- AND POLYETHYLENIC FATTY ACID ISOMERS ON AP-L WITH LITERATURE DATA, INCLUDING THE BASES FOR CALCULATION OF SUCH DATA FROM ELEMENTAL ETHYLENIC BOND RESULTS

Peak No.*	Fatty acid isomer	ECL values and origin				Mono-ethylenic 1.00-FCL, this study	Calculated ECL values from $\Sigma(1.00-FCL)$ uncorrected	Difference	Barve et al. calculated ECL values, corrected	Diff. Barve calc. value relative to this experimental value
		Found experimentally (180°)	Barve et al. ⁵ (210°)	Schofield and Dutton ⁶ (200°)	Strocchi et al. ¹⁵ (180°)					
—	6t	—	17.72	—	17.76	—	—	—	—	
2	9t	17.74	17.72	17.73	17.78	0.26	—	—	—	
4	12t	17.80	17.78	17.79	—	0.26	—	—	—	
6	15t	17.88	17.86	17.88	—	0.20	—	—	—	
9	9t,12t	17.63	17.63	17.65	17.70	0.12	17.54	-0.09	—	
8	9t,15t	17.65	—	17.68	—	—	17.62	-0.03	—	
15	12t,15t	17.76	—	17.85	—	—	17.68	-0.08	—	
—	6c	17.65	17.65	—	17.69	0.35	—	—	—	
3	9c	17.65	17.63	17.66	17.70	0.35	—	—	—	
5	12c	17.73	17.73	17.76	—	0.27	—	—	—	
7	15c	17.93	17.89	17.96	—	0.07	—	—	—	
—	6c,9c	17.43	—	—	—	—	17.30	-0.31	—	
—	6c,12c	17.43	—	—	—	—	17.38	-0.05	—	
12	9c,12c	17.50	17.50	17.50	17.57	—	17.38	-0.12	—	
16	9c,15c	17.61	—	17.66	(17.66)	—	17.58	-0.03	—	
19	12c,15c	17.73	—	17.83	(17.87)	—	17.66	-0.07	—	
—	6c,9c,12c	17.29	17.27	—	—	—	17.03	-0.26	—	
22	9c,12c,15c	17.53	17.49	—	17.57	—	17.27	-0.26	-0.02	
20	9c,12c,15t	17.53	17.47	—	17.57	—	17.24	-0.29	+0.02	
26	9c,12t,15t	17.61	—	—	17.66	—	17.31	-0.30	-0.03	
25	9t,12t,15t	17.63	—	—	17.67	—	17.42	-0.21	-0.04	
23	9c,12t,15c	17.68	17.60	—	17.72	—	17.34	-0.34	+0.01	
27	9t,12t,15c	17.69	—	—	17.72	—	17.45	-0.24	+0.01	
24	9t,12c,15c	17.71	17.64	—	17.73	—	17.38	-0.33	-0.04	
21	9t,12c,15t	17.73	—	—	17.75	—	17.35	-0.38	-0.03	

* From SILAR-5CP order^{8,17} (e.g. Figs. 2 and 3).

** Revised by original authors from ref. 5.

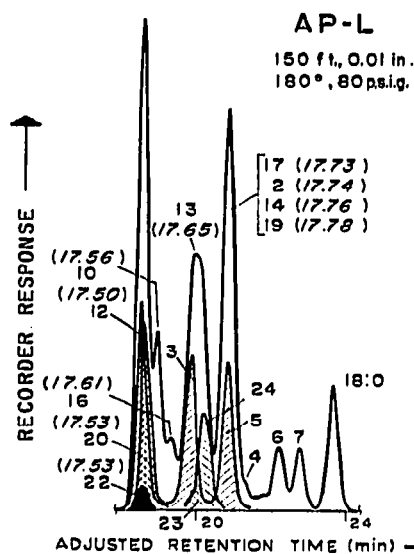


Fig. 4. GLC envelope on AP-L for partial hydrazine reduction products of mixture shown in Fig. 3. Peaks 4, 6 and 7, and shaded areas derive from distinct parts of GLC analyses of experimental or known reference materials examined under identical conditions. Black area is 9*c*,12*c*,15*c* (22) but most of leading major peak is 9*c*,12*c* (12). Refer to Table I for other structural details and to text for discussion of balance of components, mostly of combined *cis*,*trans* geometry.

as $1.00 - \text{FCL}\Delta^n$. Thus in theory one should be able, for a diethylenic compound Δ^x, Δ^y , to use $1.00 - [(1.00 - \text{FCL}\Delta^x) + (1.00 - \text{FCL}\Delta^y)]$ to arrive at the FCL value of $\Delta^x\Delta^y$. For example, to calculate the ΣFCL for the 9*t*,12*t*-diethylenic isomer:

$$1.00 - [(1.00 - 0.74) + (1.00 - 0.80)] = 1.00 - [0.26 + 0.20] = 0.54$$

It is well known that this does not fit very precisely^{5,6} and a correction based on the number of methylene groups has been proposed⁵. Thus a Δ^6, Δ^{12} or Δ^9, Δ^{15} isomer has four interposed methylene groups ($n=4$) and a Δ^6, Δ^9 or Δ^9, Δ^{12} isomer has one interposed methylene group ($n=1$). Assembling the data in groups to emphasize this factor has been done previously, and our data give essentially the same results (Table II).

TABLE II

COMPARISON OF DIFFERENCES FOUND BETWEEN OBSERVED AND CALCULATED ECL VALUES FOR DIETHYLENIC ISOMERS

Octadeca- diethylenic acid isomer		Difference $ECL_{\text{obs.}} - ECL_{\text{calc.}}$			
		This study		Barve et al. ⁵	
		<i>cis</i>	<i>trans</i>	<i>cis</i>	<i>trans</i>
$n=1$	Δ^6, Δ^9	-0.13	-	-0.14	-0.13
	Δ^9, Δ^{12}	-0.12	-0.09	-0.12	-0.10
	Δ^{12}, Δ^{15}	-0.07	-0.08	-	-
$n=4$	Δ^6, Δ^{12}	-0.05	-	-0.03	-0.01
	Δ^9, Δ^{15}	-0.03	-0.03	-0.06	-

This is in superficial agreement with the view that for $n=1$ isomers the diethylenic correction to be applied to the Σ FCL values is about +0.1 while for $n=4$ isomers it is relatively insignificant (≤ 0.05). However, it is important to note that the difference is also apparently related to the position of the total ethylenic unsaturation system in the chain. To extend this viewpoint, the values for two all-*cis*-triethylenic isomers may be compared with values made simply by summing monoethylenic (1.00-FCL) values (Table III).

TABLE III

COMPARISON OF OBSERVED AND CALCULATED ECL VALUES FOR ALL-*cis*-TRIETHYLENIC ISOMERS

Octadecatriethylenic acid isomer	$ECL_{obs.}$	$ECL_{calc.}$	$ECL_{obs.} - ECL_{calc.}$
$\Delta^6, \Delta^9, \Delta^{12}$	17.29	17.03	-0.26
$\Delta^9, \Delta^{12}, \Delta^{15}$	17.53	17.31	-0.22

Despite the partial overlap the larger difference is with the more centrally located unsaturation as observed for the diethylenic isomers above. The greater difference is a reflection of the shorter retention time for centralized structures (*cf.* Fig. 5) and has been discussed elsewhere for monomethyl-branched fatty acid isomers³. It may only be easily detectable with isomers of only one type of geometry (*i.e.* all-*cis* or all-*trans*). Implications of molecular structural rigidity on elution time have been discussed in detail by Strocchi *et al.*¹⁵.

The eight octadecatriethylenic isomers (Table I) show differences ranging from -0.21 to -0.38. The elution order is made complex by the inclusion of both *cis* and *trans* elements. This is not without some system, as three isomers containing 9*c* elements elute first, and three isomers with 9*t* elements elute last. Also three of the first four to elute contain 15*t* elements. On a gross scale this reflects the relative monoethylenic elution positions of 9*c* before 9*t* and of 15*t* before 15*c* (Fig. 5). Interestingly enough the four 12*t* elements occupy a solid block in the middle of the elution order and the eight ECL values arrange themselves into four sets of two each.

Two different approaches to the problem of including *cis* and *trans* elements in these molecules have been proposed. In the one proposal⁶ octadecadiethylenic acids with one or more *trans* elements have a Σ FCL correction (to be added) on AP-L of +0.15 (Group A) whereas all others (Group B) have a correction of +0.03 to +0.04. For octadecatriethylenic acids the correction is approximately 1.5 times the appropriate dienoic acid correction. The other approach⁵, when applied to the octadecatriethylenic acids, adds the three FCL values and incorporates the two corrections for diene Σ FCL values as well permitting particular structural definition. This latter set of literature calculations works extremely well within the one group of isomers (Table I). A revision of the published⁵ calculated ECL value for the all-*trans*-octadecatriethylenic isomer gives very reasonable agreement with the experimental value.

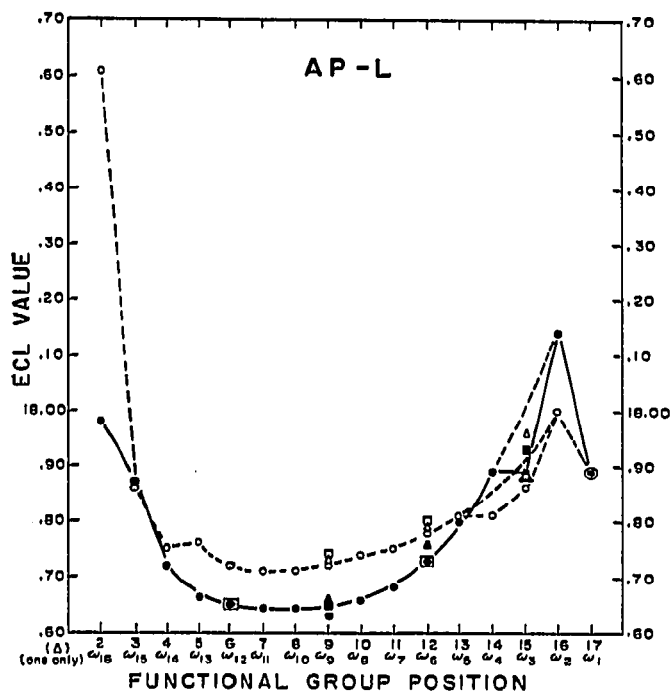


Fig. 5. Compilation of ECL values for monoethylenic acids from other sources^{5, 6} and this study. Actual lines break at $\Delta^{14}(cis)$ and $\Delta^{13}(trans)$. Dotted extension of curves into Δ^{13} - Δ^{17} positions indicates extent of anomalous retention data for this region. Note crossover of *cis* and *trans* lines at about Δ^{13} position. ■—■, Experimental values for *cis*-isomers; □---□, experimental values for *trans*-isomers; ●—●, Barve *et al.*⁵, *cis*; ○---○, Barve *et al.*⁵, *trans*; ▲—▲, Scholfield and Dutton⁶, *cis*; △---△, Scholfield and Dutton⁶, *trans*.

Adopting our deduced correction values for all-*cis*- or all-*trans*-octadecadiethylenic acids permits this type of calculation for the two all-*cis*- and one all-*trans*-octadecatriethylenic acids with data of homogeneous origin:

$$\text{ECL for } 6c, 9c, 12c = 18.00 - (0.35 + 0.35 + 0.27) + 0.13 + 0.12 = 17.28 \text{ (vs. 17.29 found)}$$

$$\text{ECL for } 9c, 12c, 15c = 18.00 - (0.35 + 0.27 + 0.07) + 0.12 + 0.07 = 17.50 \text{ (vs. 17.53 found)}$$

$$\text{ECL for } 9t, 12t, 15t = 18.00 - (0.26 + 0.20 + 0.12) + 0.09 + 0.08 = 17.59 \text{ (vs. 17.63 found)}$$

As our data provide good fits for these three octadecatriethylenates, including the all-*trans*-isomer, it is likely that particular rather than general corrections are indeed required for accurate work. "Particular" implies that retention data have been gathered on one column for both reference compounds and unknowns within a close time period in the life of the column and preferably under identical temperature conditions. A plot of ECL values *versus* isomer position (Fig. 5) shows irregularities in the Δ^{14} - Δ^{15} area possibly arising from this kind of experimental inhomogeneity. Previous experience with methyl-branched fatty acids (which are admittedly apolar) indicated the likelihood of smooth curves in this region¹⁶. It is, however, well es-

tablished that the *cis* and *trans* lines cross in the vicinity of the Δ^{13} isomer for plots of ECL data on AP-L.

As we did not have accurate experimental values for the *cis,trans*-diethylenic acids we have not been able to calculate FCL values for the rest of the octadecatriethylenic isomers. However, with the revision of the all-*trans*-isomer, the ECL values calculated by Barve *et al.*⁵ from their data agree quite well with our experimental results (Table I).

A test of this additivity principle was possible with the partial hydrazine reduction product from a mixture of *9c,12c,15t* (peak 20), *9t,12c,15c* (peak 23), with small amounts of *9c,12c,15c* (peak 22) and *9c,12c* (peak 12), which had been analyzed on SILAR-5CP (Fig. 3). The mixture of reduction products on AP-L gave a complex envelope (Fig. 4) in which a few components such as *15t* (peak 6) and *15c* (peak 7) and 18:0, were clearly isolated. Direct comparison with the materials tabulated in Table I (ECL values in parentheses are from Table I) showed that the first major peak (frontal ECL value 17.50) included SILAR-5CP components 12 (17.50), 20 (17.53) and 22 (17.53). The second major peak (frontal tangent ECL value 17.65) included 3 (17.65) and 24 (17.68). The third major peak (frontal tangent ECL value about 17.75) included 5 (17.73). It was particularly desirable to account for the diethylenic acids represented by SILAR-5CP peaks 12, 16 and 19 (ECL known on AP-L, Table I) and 10, 13, 14 and 17. The ECL values were calculated for the corresponding structures with addition to monoethylenic FCL values of diethylenic corrections of 0.03 for $n=4$ and 0.12 for $n=1$ (Table IV).

TABLE IV

ECL VALUES FOR OCTADECADIETHYLENIC ACIDS PRESENT IN THE GLC ENVELOPE SHOWN IN FIG. 4

Peak No.	Structure	ECL _{calc.}	ECL _{known}
10	<i>9c,15t</i>	17.56	—
12	<i>9c,12c</i>	17.50	17.50
13	<i>9t,12c</i>	17.65	—
14	<i>9t,15c</i>	17.76	—
16	<i>9c,15c</i>	17.61	17.61
17	<i>12c,15t</i>	17.73	—
19	<i>12c,15c</i>	17.78	17.73

It was possible to locate appropriate peaks for SILAR-5CP components 10 and 16 with respective *measured* ECL values on AP-L of 17.57 and 17.61. SILAR-5CP peak 13 evidently coincided with SILAR-5CP peak 3 (*9c*) in the front of the second major AP-L peak, the balance of this peak being SILAR-5CP peak 23 (verified by comparison of areas). Finally, diethylenic SILAR-5CP peaks 17, 14 and 19, with the *9t* component of SILAR-5CP peak 2, accompanied SILAR-5CP monoethylenic peak 5 (*12c*) and this accounted for most of the third large peak on AP-L.

The necessity of including the diethylenic functional correction for calculation of triethylenic ECL data also applies to polar liquid phases¹⁷ and has an interesting analogy in the relative susceptibility to oxidation of these types of acids. Thus linoleic acid (*9c,12c-18:2 ω 6*) is possibly twenty to forty times as susceptible to

oxidation as oleic acid (9c-18:1 ω 9), but linolenic acid (9c,12c,15c-18:3 ω 3) is only about twice as susceptible as linoleic acid^{18,19}. The initial stress factor from the linking of the first two ethylenic bonds in a methylene-interrupted grouping is thus the major factor in oxidation susceptibility. In adding FCL values for triethylenic acids of this type the diethylenic corrections are less than the monoethylenic FCL increments, but represent an adjustment for the stress from interaction similar to that of the oxidation susceptibility.

Thus we can confirm that the choice of AP-L as a liquid phase offers not only unique opportunities for the interlaboratory application of retention data but also for calculation of reasonably accurate retention data for unknowns from precise reference data obtained under the same laboratory conditions.

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