Hydrogenolysis Products of the Minor Fatty Acids From *Euphoria Longana* Seed Oil

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Abstract

The behavior of shorter-chain cyclopropane fatty acid components of *Euphoria longana* seed oil and some other minor components during hydrogenolysis has been studied by open-tubular gas liquid chromatography on butanediolsuccinate polyester and Apiezon L substrates. The retention data and degree of resolution of the pairs of monomethylbranched fatty acids resulting from the hydrogenolysis of cyclopropane rings are used to indicate the positions of the latter in a series of fatty acids as ω9 relative to the terminal methyl group. An instance of possible isomerization of a cyclopropene fatty acid has been detected. The probable positions of monoethylenic unsaturation in fatty acids are discussed.

Introduction

Publication of the fatty acid composition of *Euphoria longana* seed oil indicated that dihydrosterculic (9,10-methyleneoctadecanoic) acid was a major (17.4%) component (1). It was thought that a continuing project of investigation of the gas liquid chromatography (GLC) behavior of monomethylbranched fatty acids in our laboratory (2) could benefit from examination of the pairs of adjacent isomers produced by hydrogenolysis of any cyclopropanoid fatty acid of chain length shorter than dihydrosterculic.

Because of the limited aim of the study, hydrogenolysis was carried out on nearly the total methyl ester sample prepared, and the evidence for unusual minor components and their alteration products is therefore based solely on GLC retention behavior and hydrogenolysis considerations. Among other minor components indicated there was a series of components (C_{17}, C_{15}, C_{13}) considered to be cyclopropane fatty acids with the methylene groups in the ω9 position (conforming to the ethylenic shorthand identification system where unsaturation is measured from the center of the functional group to and including the terminal methyl group). Unexpected GLC evidence suggested a C_{11} chain with a cyclopropene function near the methyl end capable of undergoing unusual isomerizations during esterification (or GLC), and hydrogenolysis. Ethylenic fatty acids of various chain lengths were also scrutinized for structure. Lack of sample material precluded further confirmatory structural studies.

Experimental Procedures

Approximately 30 seeds, 26 g, were extracted with 300 ml of *n*-hexane in a Waring Blendor. The re-

TABLE I

Known and Proposed Fatty Acid Components of *Euphoria longana* Seed Oil With Retention Data on BDS and APL Columns[a]

Identification assigned to component in GLC analysis	BDS Analysis				APL Analysis			
	ECL Value	Per cent of peak from sample analysis			ECL Value	Per cent of peak from sample analysis		
		Raw	0.5 hr	5 hr		Raw	0.5 hr	5 hr
10-Methylundecanoate	11.58	NP[b]	0.01	0.05	11.62	NP	0.01	0.05
Unknown	11.64	<0.01	0.15	0.19	?	?	?	?
9-Methylundecanoate	11.71	NP			11.70	NP	0.12	0.16
Dodecanoate	12:0	0.06	0.08	0.10	12:0	0.06	0.08	0.09
3-Methyldodecanoate	12.12	NP	0.01	0.04	12.37	NP	0.01	0.04
Unknown	12.22	NP	0.10	NP	?	?	?	?
4-Methyldodecanoate	12.44	NP	Trace	0.02	12.48	NP	Trace	0.02
9,10-Methyleneundecanoate	12.88	NP	0.13	NP	12.22	NP	0.13	NP
Tridecanoate	13:0	~0.01	0.01	0.01	13:0	?	0.01	0.01
Possible diene	13.35	0.40			12.15	0.26	Trace	NP
3,4-Methylenedodecanoate	13.35		NP	NP	12.86	0.12	0.12	0.06
Tetradecanoate	14:0	0.38	0.41	0.41	14:0	0.41	0.41	0.40
Tetradecenoate (ω9?)	14.18	0.04	NP	NP	13.69	0.03	NP	NP
5- and 6-Methyltetradecanoate	14.33	NP	0.03	0.48	14.38	NP	0.02	0.49
13-Methyltetradecanoate	14.56	0.01	0.01	0.01	14.62	?	0.01	0.01
Pentadecanoate	15:0	0.04	0.09	0.15	15:0	0.04	0.07	0.16
Pentadecenoate	15.14	0.01	NP	NP	?	?	?	?
5,6-Methylenetetradecanoate	15.28	0.70	0.63	0.05	14.82	0.73	0.59	0.05
Hexadecanoate	16:0	19.0	19.0	19.0	16:0	19.0	19.0	19.0
Hexadecenoate (ω9?)	16.24	0.44	NP	NP	15.67	0.40	NP	NP
Hexadecenoate (ω7?)	16.30	0.26	NP	NP	15.74	0.29	NP	NP
7- and 8-Methylhexadecanoate	16.32	NP	0.07	0.82	16.34	NP	0.02	0.84
Heptadecanoate	17:0	0.23	0.34	0.37	17:0	0.21	0.32	0.39
7,8-Methylenehexadecanoate	17.24	0.99	1.01	0.02	16.75	1.01	0.99	0.01
Octadecanoate	18:0	6.8	.....	.....	18:0	6.5	.....	.....
Unknown	18.03	0.52	?	?	?	?	?	?
Octadecenoate (ω9)	18.17	29.1	NP	NP	17.57	29.2	NP	NP
Octadecenoate (ω7)	18.24	0.53	NP	NP				
Unknown	?	?	?	?	17.84	0.58	NP	NP
Octadecadienoate (18:2ω6)	18.65	7.9	NP	NP	17.50	10.1	NP	NP
Octadecatrienoate (18:3ω3)	19.30	2.6	NP	NP				
9,10-Mthyleneoctadecanoate	19.18	19.0	.....	.....	18.72	19.4	.....	.....
Unknown	.....	.....	.....	.....	13.13	NP	0.01	Trace
Unknown	.....	.....	.....	.....	14.15	NP	0.01	NP
Unknown	16.32?	.....	.04?	.....	15.15	NP	0.05	0.02
Unknown	.....	.....	.....	.....	16.10	?	0.03	NP
Unknown	.....	.....	.....	.....	18.10	0.04	?	?

[a] Observed recorder peak areas are normalized to 16:0 = 19.0% to facilitate comparisons.
[b] NP, not present.

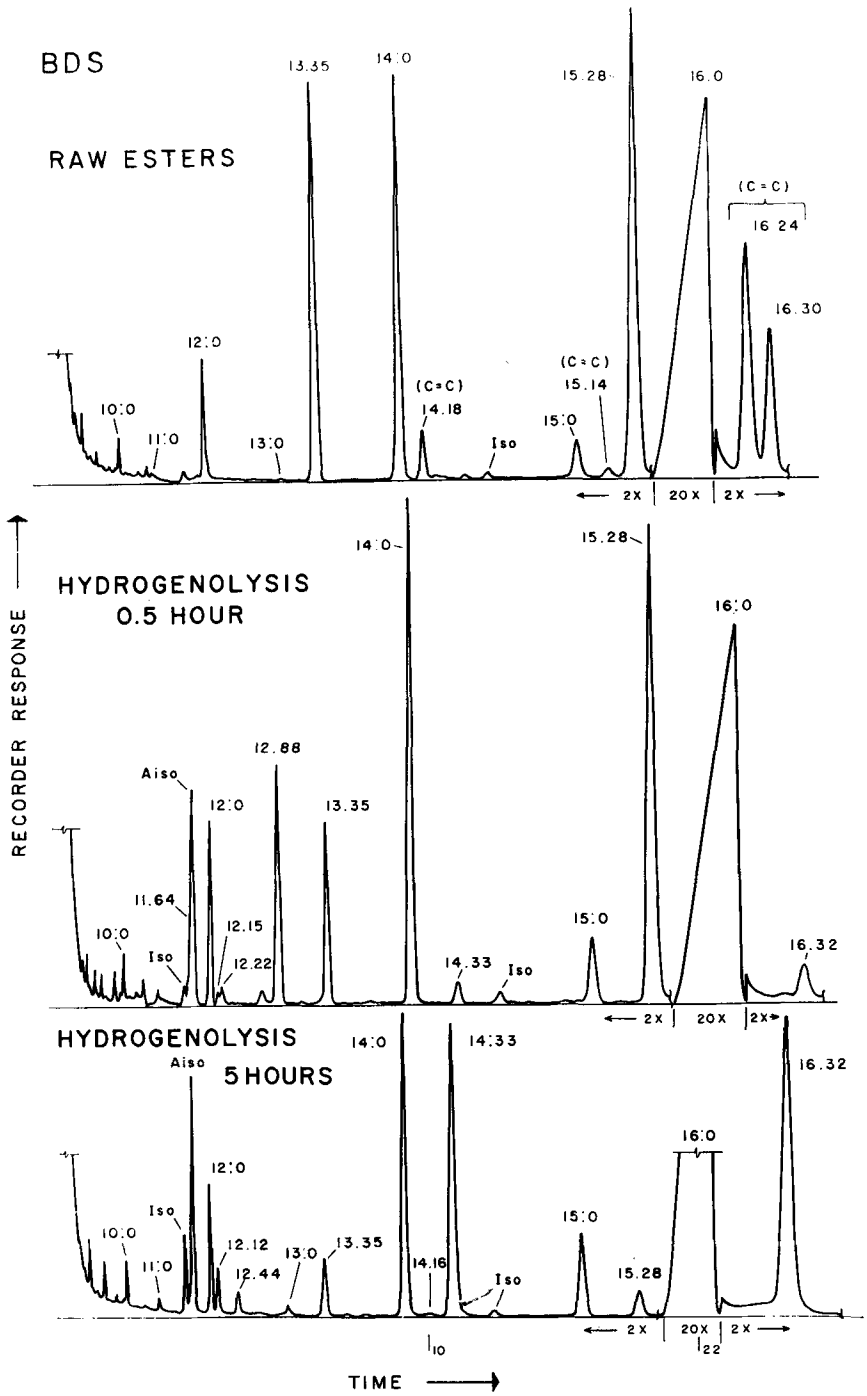

FIG. 1. Parts of GLC charts from analyses of raw and partially hydrogenolyzed *E. longana* fatty acid esters on BDS column. Operating conditions given in text. Times (min) at bottom, attenuations at each baseline. Wavy lines denote gaps in chart reproduction.

covered oil, 0.70 g, was saponified and unsaponifiable materials removed by AOCS methods. The fatty acids were recovered and converted to methyl esters (recovery 0.37 g) with 5% BF_3-MeOH (3).

GLC studies on the raw esters and subsequent products were carried out on a Perkin-Elmer model 226 fitted with either butanediolsuccinate polyester (BDS) or Apiezon L (APL) coated on stainless steel open-tubular columns 150 ft (50 m) in length and 0.01 in (0.25 mm) i.d. Operating conditions were: injection port 250 C; BDS columns 150 C and 40 psig helium; APL columns 190 C and 60 psig helium. GLC records were obtained with a 1 mv recorder fitted with a ball and disc type integrator.

Hydrogenolysis was carried out qualitatively in the isolated agitation cell of a laboratory hydrogenator (4). Esters, 0.29 g, were dissolved in glacial acetic acid, 10 ml, and Adams' platinum catalyst (Matheson, Coleman and Bell, 0.12 g) was added. At 0.5 and 1.5 hr the cell was opened and 0.25 ml of solution removed for examination of the esters. At 5.0 hr the contents were rinsed into 40 ml of distilled water with the aid of a little EtOH and an extraction was performed with 10 ml of neohexane. The aqueous solution was re-extracted once and the combined neohexane solutions were washed once with distilled water before concentration for GLC. The 0.25 ml samples were extracted similarly on a smaller scale.

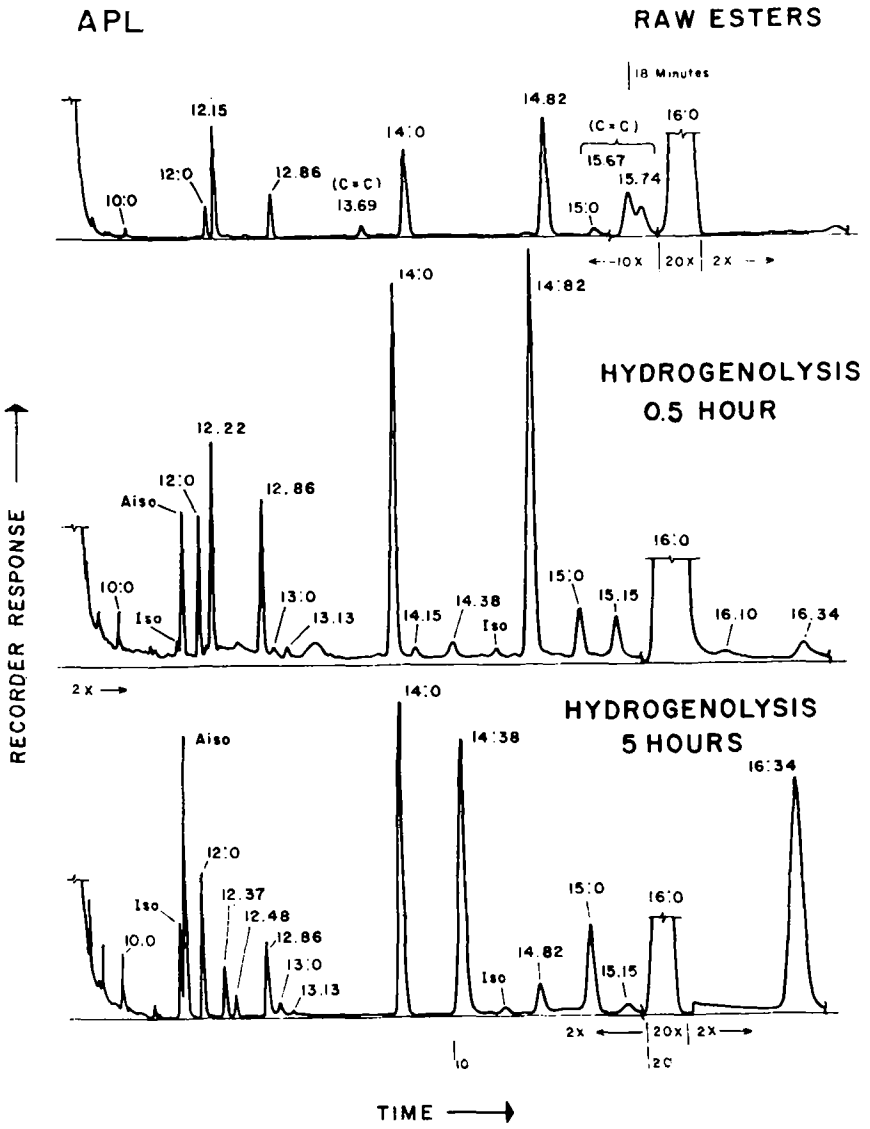

FIG. 2. Parts of GLC charts from analyses of raw and partially hydrogenolyzed *E. longana* fatty acid esters on APL column. Operating conditions given in text. Details as for Figure 1. Note that in top graph part of the baseline is at 10 × attenuation and that there is an isolated baseline discontinuity to show (C = C) components which is not used elsewhere.

Results and Discussions

Parts of the GLC charts obtained have been reproduced in Figures 1 and 2. The observed components are listed, with equivalent chain length (ECL) values and suggested identifications, in Table I, generally in the order observed in the BDS analyses. The area percentages (uncorrected) have been computed without respect to C_{20} and higher components known to be present (1), and have been further adjusted to 19.0% for methyl palmitate (1) to facilitate internal comparisons.

Normal Cyclopropane Materials

The esters prepared from the seed oil fatty acids included a series with ECL values on BDS of 13.35, 15.28, 17.24 and 19.18, with peaks corresponding on APL, with ECL values of 14.82, 16.75 and 18.72 observed for the last three. The first component in this series unexpectedly split into two peaks on APL, with ECL values of 12.15 and 12.86.

The higher components (i.e., ECL values on BDS of 15.28, 17.24 and 19.18) yielded on hydrogenolysis single peaks, with ECL values on BDS of 14.33, 16.32 and 18.32, and on APL of 14.38, 16.34 and 18.33. These specific comparisons could be verified quantitatively (Table I). The largest hydrogenolysis product other than 18:0, derived from ethylenic unsaturation, would be C_{19} and, derived from known 9,10-methyleneoctadecanoate, and therefore a mixture of 9- and 10-methyloctadecanoates (not included in Table I). [Unless otherwise specified plain numbers refer to carbons counted from the carboxyl group.] These two isomers cannot be separated (2) on polar open-tubular GLC columns of even high efficiency (~50,000 plates). The next lowest pair of homologous compounds which we had under study, the 9- and 10-methylhexadecanoates (C_{17}), can be partially separated on even moderately efficient polar open-tubular GLC columns (~25,000 plates)--the 9-methyl isomer (ECL 16.31) preceding the 10-methyl isomer (ECL 16.34) and, although no useful separation of equal size peaks can be detected on APL (ECL 16.38), it is known that the 10-methyl isomer is in the latter portion of the peak which is usually noticeably broader than an adjacent peak of comparable size containing only one component (Ackman and Hooper, unpublished data).

Neither of these separation effects could be observed in the *E. longana* hydrogenolysis product, although on BDS the peak was possibly somewhat wider than

TABLE II

Comparisons of Experimental and Literature Retention Data for Proposed Structures of Components From GLC of Esters of *Euphoria longana* Fatty Acids[a]

Chain length	Structure	Polyester data reference			APL data reference				
		Euphoria longana BDS	BDS[b]	NPGS[c,d]	*Euphoria longana*	*Cis*		*Trans*	
Cyclopropane									
11	ω1(10Δ11)	.....	.....	(20.04)	.....	.....	(19.38)[c]	.....	.....
11	ω2(9Δ10)	12.88?	.....	(19.80)	12.22?	.....	(19.22)	.....	.....
12	ω9(3Δ4)	13.35	.....	(19.32)	12.86	.....	(18.85)	.....	.....
14	ω9(5Δ6)	15.28	.....	(19.20)	14.82	.....	(18.76)	.....	.....
16	ω9(7Δ8)	17.24	.....	(19.18)	16.75	.....	(18.75)	.....	.....
18	ω9(9Δ10)	19.18	.....	19.24	18.72	.....	18.73	.....	.....
Monoethylenic									
14	ω9	14.18	(16.22)	(18.14)	13.69	(17.63)[e]	(17.63)[d]	(17.70)[e]	17.74[d]
16	ω9	16.24	16.22	(18.14)	15.67	(17.63)	(17.63)	(17.70)	17.74
18	ω9	18.17	18.18	18.14	17.57	17.63	17.63	17.70	17.74
16	ω7	16.31	16.28	(18.23)	15.74	(17.70)	17.68	(17.74)	17.78
18	ω7	18.24	18.27	18.23	?	17.70	17.68	17.74	17.78
?	?	.....	.....	.....	17.84	.....	.....	.....	.....

[a] Where no exact reference data is available that for the nearest available homologue is lisited in parenthesis.
[b] Reference 26.
[c] Reference 6 for cyclopropanoids.
[d] Reference 19 for monoethylenics.
[e] Reference 25.

would be expected for a single component. The lowest pair of homologues in our other series of 9- and 10-methyl isomers (C_{15}) is known to give separate peaks on both BDS and APL columns, ECL values being respectively 14.38 and 14.45 on BDS, and 14.45 and 14.49 on APL (Ackman and Hooper, unpublished data).

No separation was observed in the *E. longana* hydrogenolysis product peak in the current work, although the peaks were definitely slightly wider than would be expected for single components (e.g., compared to 14:0). It is therefore concluded that these C_{19}, C_{17} and C_{15} components derive from a series of *E. longana* cyclopropane fatty acids with the methylene group across the 9 and 10 carbons numbered from the terminal methyl group ($CH_3 = 1$). The corresponding pairs of methyl-branched fatty acids would be 9- and 10-, 7- and 8-, and 5- and 6-methyl, numbered from the carboxyl group. The next lowest component (C_{13}) in this series (ECL on BDS, 13.35; on APL 12.86) is therefore the 3,4-methylenedodecanoate, yielding 3-methyl- and 4-methyldodecanoates on hydrogenolysis. These can be readily identified (Table I; Fig. 1 and 2) as they separate on both BDS and APL open-tubular columns, and have ECL values quite different from most of the other methyl branched fatty acids, including a specific retention shift with column polarity changes for the 3-methyl isomers (2). The different proportions may represent a steric effect or isomerization based on the proximity to the carboxyl group (Table I). This acid has been reported in rats fed longer chain cyclopropane acids (5).

The presence of this series of *cis* cyclopropane fatty acids is further substantiated by agreement with literature ECL retention data (6) for the parent acids (Table II), especially for the more reproducible APL liquid phase. The BDS values are similar to neopentylglycolsuccinate (NPGS) (also a low polarity polyester) data but are less exactly relatable, ECL values differing due to polarity, to modification by chain length, or to difference in operating temperature, etc. Seeds of the plant order Malvales have been reported to contain lower homologues of sterculic and dihydrosterculic acid (7). If ω9 is the structure in these acids it would be of considerable biological interest as differing from most microbial cyclopropane functions which are 9,10 relative to the carboxyl group (8,9) because of the predominance of this type of parent ethylenic unsaturation.

Precision open-tubular GLC of at least some of the pairs of methyl-branched fatty acids derived from cyclopropane fatty acids can thus be a valuable and simple adjunct to more sophisticated instrumental techniques (1,10) for the study of structural details. In some cases direct GLC study of cyclopropane esters could also be rewarding (11).

Unusual Cyclopropane Materials

The balance of the peak with ECL of 13.35 on BDS had ECL 12.15 on APL. After 0.5 hr of hydrogenolysis, little alteration of the normal cyclopropane fatty acids had been effected, but this unusual short chain component had altered in retention characteristics to ECL 12.88 on BDS and 12.22 (except for a trace remaining at 12.15) on APL. Concurrently there was substantial production of anteisododecanoate (9-methylundecanoate) and some production of 12:0.

The 0.5 hr products are categorized without difficulty on a GLC basis. The ECL values of 12.88 (BDS) and 12.22 (APL) are very close in fractional chain length units (FCL, the two digits after the decimal in ECL, equivalent chain length, values) to literature values (respectively 19.80 and 19.22) for 16,17-methyleneoctadecanoate (6). The breakdown of this component to iso- and anteisododecanoates, and to 12:0 on further hydrogenolysis, suggests that the structure of this intermediate was 9,10-methyleneundecanoate. Hydrogenolysis was rapid and apparently complete in 5 hr, in contrast to the other cyclopropane fatty acid esters, where part remained even after this longer period of hydrogenolysis.

It has been suggested that an intact parent cyclopropenoid acid would not survive treatment with BF_3-MeOH (12,13), although another report is more favorable regarding the use of this esterification system (14). Thermal isomerization during GLC needs to be considered separately from acid-catalysis effects. The shifts of ECL values (13.35 to 12.88, 12.15 to 12.22) are consistent with the original ester having polarity based on a moderately unsaturated structure. The rapid production of anteisododecanoate suggests, for example, that 9-methylene-10,11-undecenoate might have been in the raw esters as a plausible alteration product derived from a 9,10-cyclopropenoid fatty acid precursor. Two similar isomeric conjugated dienes are formed from methyl sterculate on $AgNO_3$-silica gel chromatography (15). Formation of conjugated dienes reportedly occurs during GLC analysis (16). Heating methyl sterculate with a palladium hydrogenation-type catalyst (but in a nitrogen atmosphere) has been

shown to produce conjugated dienes of this type (17).

A structurally similar hydrocarbon diene derived from phytane has been studied by programmed-temperature GLC on several liquid phases, the retention data being reported as retention indices (18). Subtraction of the indices for the parent hydrocarbon from this data, and of parent ECL value 12.00 from our experimental methyl ester data gives comparable differences: 1.35 for our ester on BDS vs. 1.23 (Carbowax 20 M) and 1.52 (FFAP) for the phytadiene on polar phases, and 0.15 (our ester) vs. 0.25 (for the phytadiene) on APL. These values are reasonable GLC-based confirmation of the proposed diene structure considering the experimental differences.

In the present instance the terminal position of the vinyl group would favor specific formation of the isomer proposed. The original 9,10 functional position is attractive since it corresponds to a desaturation position found generally in plant fatty acids. [Malvalic (8,9-methylene-8,9-heptadecenoic) acid, an exception to this principle, is a secondary product (7). Comparative FCL values suggest an unusual structure for an unknown cyclopropenoid fatty acid in *Althaea rosea cav* lipids (19).] Although we are unaware of a precise precedent, hydrogenation of the projected 9-methylene-10,11-undecenoate system in the 1,4 positions could conceivably generate the 9,10-methyleneundecanoate believed to be present after 0.5 hr hydrogenolysis, and some 12:0 would also likely be produced by rearrangement of intermediates.

Since insufficient starting material remained, it was not possible to investigate the functional groups in the materials with ECL values of 13.35 (and 12.88) on BDS and 12.15 (and 12.22) on APL, but all known ethylenic unsaturation, for example, disappeared in the initial 0.5 hr of hydrogenolysis. Terminal cyclopropane involvement is difficult to prove as the cyclopropane fatty acids appear to be unusual in that a terminal position for the functional group continues to increase GLC retention times slightly, relative to the penultimate position, whereas ethylenic (20–22) and acetylenic (23) terminal functions decrease retention time on this basis. This phenomenon is discussed elsewhere (24). An APL retention value of 11.20 listed for a presumed 10,11-methyleneundecanoate (25) is low by one FCL unit.

Certain other trace components were apparent in the 5 hr hydrogenolysis product on APL, those with ECL 13.13 and 15.15 being of special interest. Superficially similar components (by ECL value) observed on APL at 0.5 hr with ECL 14.15 and 16.10 disappeared on further hydrogenolysis. At the same time most of a rather obvious component present at 0.5 hr, but not present in the raw lipid esters, with ECL on APL of 15.15, disappeared; it is possible that the residue with the same ECL value was part of the different series mentioned above. The relatively large component in the 0.5 hr analysis of ECL 15.15 could not be definitely located in the BDS analysis, but may have had ECL 16.33 and thus have been coincident with the methyl-branched hexadecanoates. Possibly this component could be related structurally to the proposed undecanoate diene, but no specific hydrogenolysis products could be identified.

Ethylenic Methods

The genesis of cyclopropane fatty acids by insertion of a methylene group across a pre-existing ethylenic double bond is accepted (7,26). There is evidence (based on ECL values for octadecenoates; Table II) for the anticipated related series of ω9 ethylenic structures (tetradec-5- and hexadec-7- as well as octadec-9-enoic). Additionally hexadec-9-enoic acid (ω7) could be identified with some confidence and a very small proportion of octadec-11-enoic acid (ω7) apparently accompanied the octadec-9-enoic acid in the BDS analysis but could not be confirmed in the APL analysis due to the lesser separation of these isomers on this liquid phase (26). Conversely a presumed ethylenic component (completely eliminated in 0.5 hr hydrogenolysis) with ECL value of 17.84 on APL in the raw material analysis fell after any likely ethylenic component (ω7, ω6 or ω5) (20,27; also Table II) but could have an ethylenic group in a position closer to the end of the chain. It is considered likely to be a C_{18} acid and quantitatively was very similar to the proposed octadec-11-enoate, but is believed to be of more unusual structure (rearrangement diene?) than the tentatively identified common *cis* isomers.

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