Linolenic Acid Artifacts from the Deodorization of Oils[1]

R.G. ACKMAN and **S.N. HOOPER**, Department of the Environment, Fisheries and Marine Service, Halifax Laboratory, Halifax, Nova Scotia, Canada, and **D.L. HOOPER**, Chemistry Department, Dalhousie University, Halifax, Nova Scotia, Canada

ABSTRACT

Gas liquid chromatography on polar open tubular columns of the methyl esters of fatty acids from vegetable oils shows that the linolenic acid in deodorized oils is accompanied by two major artifacts identified as *cis*-9,*cis*-12,*trans*-15 and *trans*-9,*cis*-12, *cis*-15 isomers. Physicochemical studies, isolation, and partial degradation steps showed two additional isomers with *trans*-9,*cis*-12,*trans*-15 and *cis*-9,*trans*-12,*cis*-15 structures. Gas liquid chromatography also showed that linoleic acid was accompanied by the *trans*-9,*cis*-12 isomer. These artifacts were not present in unrefined oils or bleached oils but could be induced by deodorization in the laboratory. Proportions of the two major artifacts in total 18:3ω3 are given for some vegetable oils from the retail market.

INTRODUCTION

There has been a rapid introduction of new varieties of rapeseed oil low in erucic acid (Canbra oils) in Canada (1). The major fatty acids in these oils were determined as part of the breeding programs, but the Department of Industry, Trade, and Commerce requested this laboratory to carry out a screening program for minor fatty acids with the open tubular gas liquid chromatography (GLC) columns previously used with this type of oil (2). Our studies showed that most minor fatty acids belonged to the various homologous series common to most edible oils, including those of the genus *Brassica*. However some samples showed unknown components (artifacts A and C) immediately preceding and following the methyl ester of linolenic acid. Usually in the same samples, one unknown component immediately followed the methyl ester of linoleic acid.

Our studies show that components A and C are geometrical isomers of linolenic acid and that they are produced by the steam deodorization, the process for removing volatile fats from fats and oils, thus producing a bland product. The usual process of degumming, alkali refining, and bleaching does not produce these artifacts. The artifacts are especially easy to detect through the open tubular GLC of the linolenic ester region, and we have directed our oil survey and identification studies toward the two most prominent linolenic acid artifact isomers, which are mono-*trans*, di-*cis* isomers. The third such isomer also has been detected, and a di-*trans*, mono-*cis* artifact also is formed as a modest proportion of the total artifacts.

[1]Presented in part at the AOCS Spring Meeting, New Orleans.

EXPERIMENTAL PROCEDURES

Analytical Technology

All GLC was performed on open tubular wall coated columns 150 ft (46 m) in length and 0.01 in. (0.25 mm) internal diameter (Perkin-Elmer Corp., Norwalk, Conn.) operated in Perkin-Elmer model 900 or 990 GLC units with flame ionization detectors and helium carrier gas. The coatings were either butanediolsuccinate polyester (BDS), SILAR 5CP (a polar phase of silicone with 50% cyanopropyl and 50% phenyl substitution, from Applied Science Laboratories, State College, Pa.), or Apiezon-L (AP-L).

Thin layer chromatography (TLC) was carried out on plates prepared with Supelcosil-12D (Supelco Inc., Bellefonte, Pa.) developed in toluene. The bands were scraped off and extracted with diethyl ether-*n*-hexane 1:1 for recovery of methyl esters. Column chromatography for larger scale preparations (ca. 1-2 g) was based upon Adsorbosil CABN (60/100 mesh) (Applied Science Laboratories) developed with toluene.

NMR spectra of deuterochloroform solutions were recorded on a Varian T-60 spectrometer, operating at 60 MHz.

Raman spectra were recorded on the high resolution laser Raman spectrometer. This spectrometer uses a Carson model 101 argon ion laser, a Spex 1401 double monochro-

TABLE I

Deodorization Artifacts A and C+Y (not including X)[a] Observed in Gas Liquid Chromatographic Analyses of Rapeseed and Canbra Oil Fatty Acids

Seed identification, origin,[b] and date received	Processing[c]	A and C+Y, percent of acids	A and C+Y, percent of 18:3 total
Oro, COOP, Altona, Nov. 5, 1971	Fully refined	0.7	15.6
Oro, COOP, Altona, Nov. 5, 1971	Crude	NSA[d]	NSA
Span, SWP, Saskatoon, Nov. 26, 1971	Degummed	NSA	NSA
RSO, WCSP, Lethbridge, Dec. 14, 1971	Crude	NSA	NSA
Span, WCSP, Lethbridge, Dec. 14, 1971	Crude	NSA	NSA
Span, WCSP, Lethbridge, Dec. 14, 1971	Bleached, deodorized	5.0	53.8
RSO, WCSP, Lethbridge, Dec. 14, 1971	Fully refined	2.2	27.0
Canbra, Monarch, Apr. 4, 1972	Crude	NSA	NSA
Canbra, Monarch, Apr. 4, 1972	Deodorized	2.2	24.2
Span, WCSP (B.R.), Apr. 7, 1972	Likely same refined oil	2.3	26.6
Span, Monarch (B.R.), Apr. 7, 1972	Likely same refined oil	2.6	26.9

[a]The additional artifacts X and Y discovered in the course of this study were minor compared to A and C. In the analysis of unfractionated fatty acids C includes Y.

[b]COOP = Cooperative; SWP = Saskatchewan Wheat Pool; WCSP = Western Canada Seed Processors; Monarch = Monarch Fine Foods; B.R. = BioResearch Laboratories; RSO = regular rapeseed oil; Oro and Span are Canbra strains.

[c]Description from correspondence. Deodorized oils are probably fully refined.

[d]NSA = no significant amount.

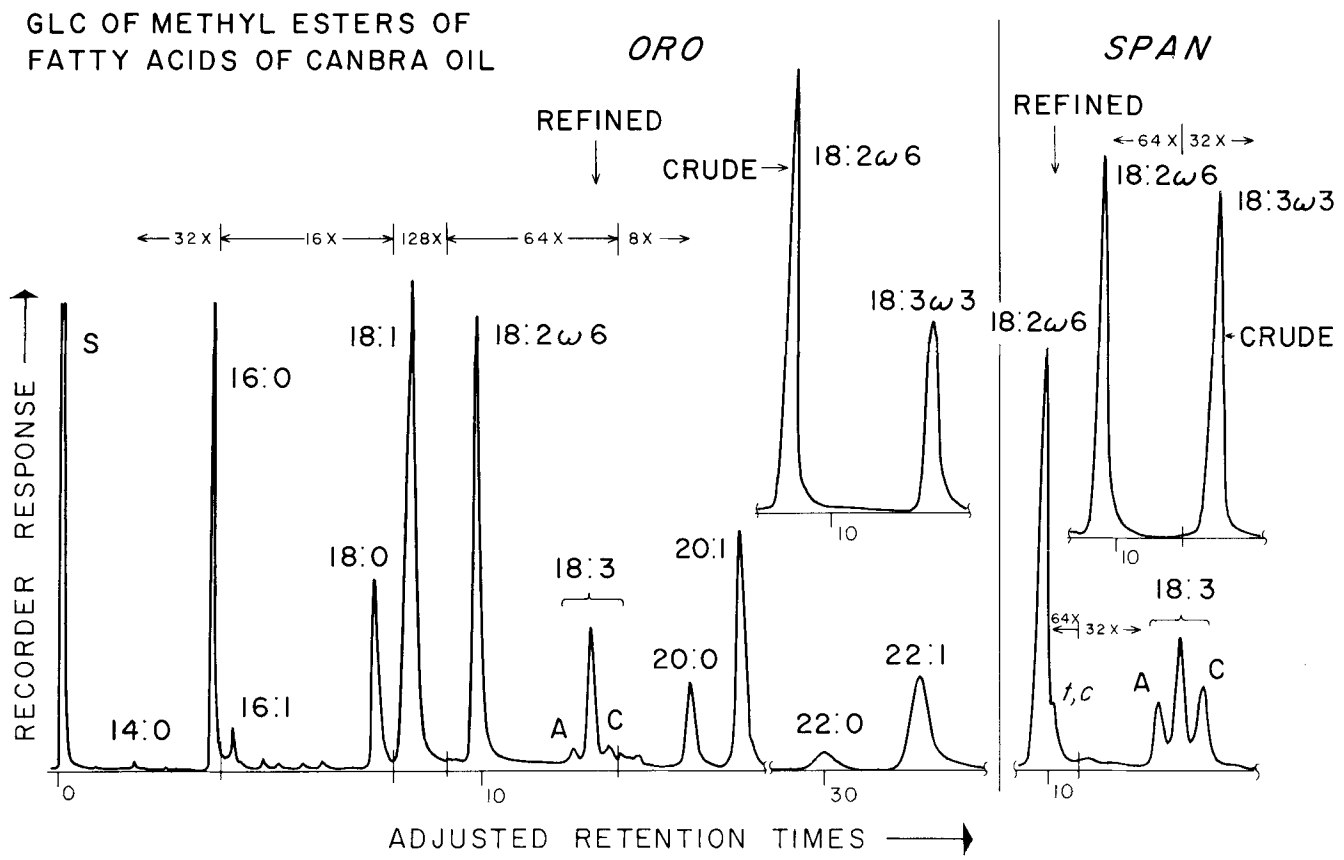

FIG. 1. Comparison of 18:3 fatty acids from Canbra oils. Two Oro oils showing effect of refining upon 18:3 on left; insert shows 18:2ω6 and 18:3ω3 in unrefined oil. On right is shown a similar comparison of refined and crude Span oils of Canbra type. Butanediolsuccinate polyester open tubular column operated at 175 C and 60 psig.

mator, and a photon counting detection system consisting of a Bendix Channeltron photon counter tube and a SSR amplifier-discriminator and rate meter. The samples used in this work were contained in mp tubes, with no attempt made to control sample temperature. Attempts to use an undiluted mixture of A and C gave only extremely high background signal, and chloroform solutions required upward of 1 hr's irradiation before this background was reduced to less than 10% of the intensity of the c=c stretching bands.

Hydrazine reductions were carried out by stirring the esters to be reduced in an open flask in ethanol with hydrazine for ca. 1 hr. A magnetic stirring hot plate permitted gentle heating.

Oils: Origin, Analysis, Bleaching and Deodorization

The initial edible oils studied were samples of Canbra (low erucic acid) rapeseed oil crushed and refined in various locations in Canada. A major oil refiner provided soybean oil from one lot sampled before and after refining. Additional samples were purchased from retail food outlets in Halifax, Nova Scotia, or Seattle, Wash., and included one linseed oil sample. An additional linseed oil was a regular unprocessed oil purchased retail in a hardware store. Esterifications of fatty acids recovered after removal of unsaponifiable materials (AOCS method Ca-6b-53 [3]) were effected by refluxing or heating in a centrifuge tube (Teflon lined screw cap) for 5 min with boron trifluoride-methanol 1:15 (4). The resulting esters were extracted into petroleum ether and the solution washed once with water, once with weak bicarbonate solution, and once again with water prior to drying and concentrating for GLC analyses.

Bleaching was carried out by stirring a crude strain Span rapeseed oil with 2% Vegaclay 1 (a sulphuric acid treated bentonite from Pembine Mountain Clays Ltd., Winnipeg, Manitoba) in a round bottomed glass flask under nitrogen for 30 min at 100 C. After cooling, the oil was filtered and the methyl esters of fatty acids prepared and examined for artifacts.

Deodorization of oils was carried out in a 250 ml round bottomed glass flask with three standard taper necks. These permitted entry of a glass capillary drawn from a S/T 14/23 MF 15/1 inlet tube (Quickfit) carrying nitrogen or water-steam, a vacuum and nitrogen connection (three way stopcock), and a glass thermocouple well. An electric heating mantle connected to a variable transformer was used to adjust the temperature of deodorization. In these laboratory trials a water aspirator vacuum (15 mm pressure vs. 6 mm or less in industrial practice) was applied carefully and intermittently held by closing a stopcock in the line as the oil was warmed. Once it became less viscous, the initial foaming subsided, and the temperature was rapidly raised to 230 C. When the temperature was over 100 C, the run was considered to have started, and water (in lieu of a nitrogen connection) was added to a mark in the bulb at the top of the capillary inlet tube. This water was replaced as necessary from a graduate to record the amount of water admitted. This was typically 2-5 ml/hr, depending upon the capillary used. Samples were withdrawn when required by breaking the vacuum with nitrogen and admitting a current of nitrogen during removal of a few ml of oil via the neck from which the thermocouple well was removed temporarily.

RESULTS AND DISCUSSION

Origin of Artifact

The general fatty acid pattern of the Canbra (low erucic acid) oils supplied for analysis is illustrated in Figure 1 with an open tubular GLC analysis of the methyl esters of fatty acids from oil from strain Oro grown in 1971. The two small peaks (A and C) on each side of the anticipated

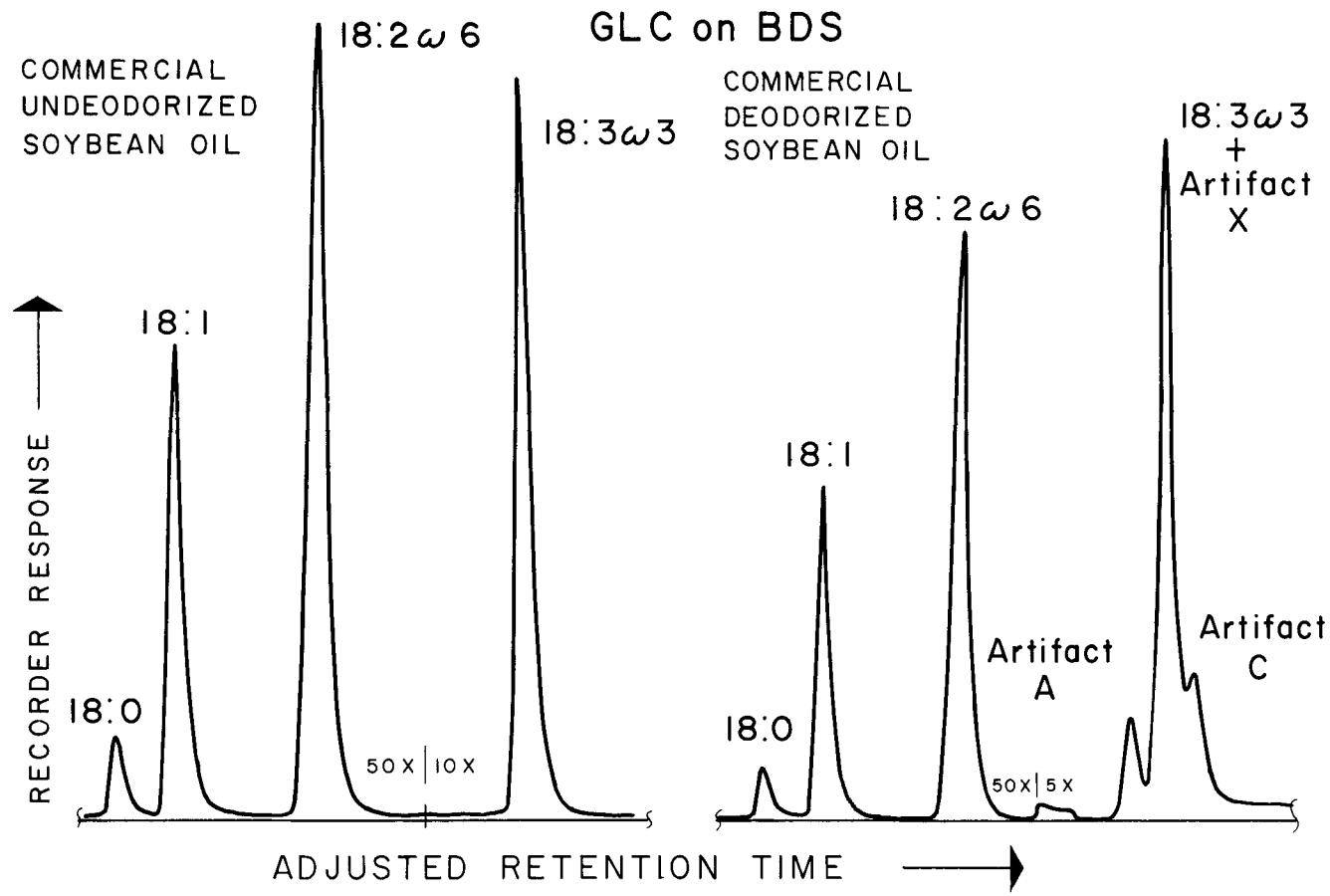

FIG. 2. Comparison of C_{18} group of acids from soybean oils from the same lot of oil before (left) and after (right) ordinary commercial deodorization. Aged butanediolsuccinate polyester open tubular column operated at 170 C and 40 psig. Elution time ca. 15 min for 18:0.

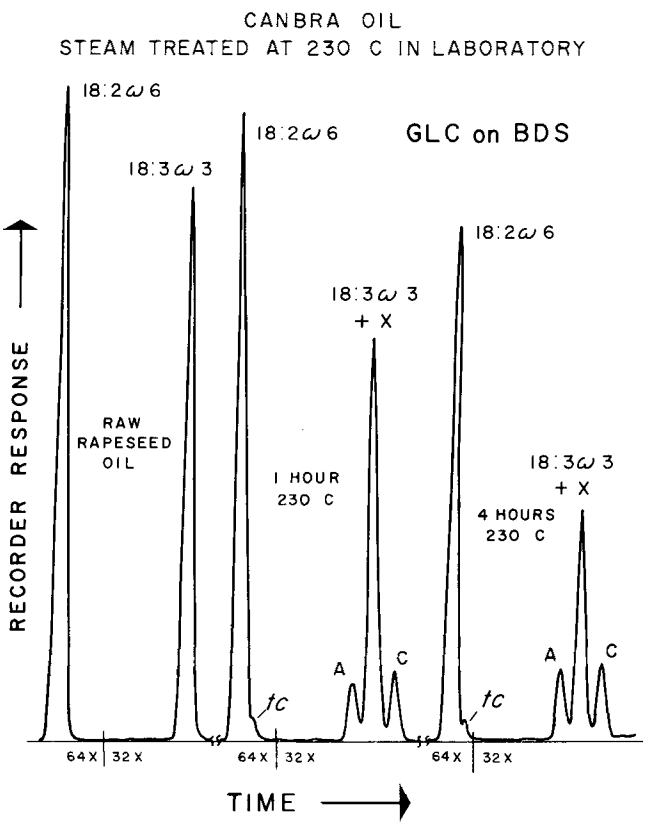

FIG. 3. Comparison of C_{18} polyunsaturated acids from Canbra oil before and after steam deodorization at 230 C at the laboratory. Note development of artifact peak on trailing edge of 18:2ω6 peaks. Butanediolsuccinate polyester open tubular column operated at 170 C and 40 psig. Elution time ca. 15 min for 18:0.

18:3ω3 peak were the novel features. This oil was degummed, alkali refined, bleached, and deodorized. The crude Oro oil from the same plant did not show the presence of the two minor components (Fig. 1 insert). Oil from a different strain, Span, processed in a different plant did show the two minor peaks (A and C) and in a higher proportion relative to 18:3ω3 but again only in the methyl esters of fatty acids from the degummed, alkali refined, bleached, and deodorized oil and not from those from the crude oil (Fig. 1). This pattern overruled other factors, such as strain of seed and origin of oil (Table I). Therefore, A and C appeared to be artifacts from the refining process.

Typical processing of rapeseed oils, including Canbra, would include the admixture of 0.1-0.2% concentrated H_3PO_4 followed by treatment for a few min with 8% NaOH solution. The oil then is water washed once or twice with all of these operations normally being carried out at temperatures of 90 C or less. Bleaching with 0.5-1.5% activated clay for 15-45 min would be at 100-110 C. Vacuum deodorization at 2-8 mm Hg would include exposure to 230-260 C for 1 hr or more in a semicontinuous process or up to 4 hr in batch operations (5).

Initial thinking suggested a possible geometrical isomerization of the original *cis*-ethylenic double bonds since *trans*-double bond formation has been reported to accompany bleaching (6). However, this European study indicated that, under conditions of bleaching similar to those normally employed in western Canada (ca. 100 C, 20-30 min, 1-3% bleaching earth), the newly formed *trans*-acids were conjugated and derived from preexisting hydroperoxides of conjugated diene (principally linoleic acid) origin. The consequent formation of conjugated di- or triethylenic unsaturation from the decomposed hydroperoxides would give artifacts with much longer GLC retention times than the A and C shown in Figure 1. Our experiment with

TABLE II

Artifacts A and C+Y (not including X)[a] and Diene Observed in Gas Liquid Chromatographic Analyses of Fatty Acids from Canbra Oil Sampled During Laboratory Deodorization[b]

Fatty acid[c]	Starting material	1 hr	2 hr	3 hr	4 hr
18:2ω6	20.53	20.21	19.54	19.38	19.77
Isomeric 18:2 (*tc*)	---	0.56	0.52	0.65	0.77
Isomer A (*cct*)	---	0.90	1.09	1.46	1.73
18:3ω3 + X (*tct*)	9.01	6.41	5.72	5.24	4.70
Isomer C (*tcc*) + Y (*ctc*)	---	1.03	1.31	1.57	1.83
Total 18:3		8.34	8.12	8.27	8.21

[a]The additional artifacts X and Y discovered in the course of this study were minor compared to A and C. In the analysis of unfractionated fatty acids C includes Y.

[b]Component as percent of total fatty acids in crude and deodorized oils (time at 230 C).

[c]*t* = *trans*, *c* = *cis*, order is 9,12,15.

TABLE III

Deodorization Artifacts A and C+Y (not including X)[a] Observed in Gas Liquid Chromatographic Analyses of Methyl Esters of Fatty Acids from Edible Oils and Linseed Oil

Origin and processing	A and C+Y, percent of oil	A and C+Y, percent of 18:3 total
Supermarket retail		
Peanut oil (Planters oil)	?	? (18:3 negligible)
Soybean oil (Crisco oil)	0.48	15.2
Corn oil (Mazola oil)	0.04	6.5
Health food store		
Sunflower oil, Pollards, cold pressed	---	10.0
Safflower oil, Nu-Life, cold pressed, no preservative	---	? (18:3 negligible)
Corn oil, Nu-Life, cold pressed, no preservative	---	2.0
Linseed oil, Nu-Life, unbleached, unfiltered, no preservative	---	NSA[b]
Supermarket retail, Seattle		
Soybean oil (Crisco oil)	0.46	16.0
Soybean oil (Napoleon: Magnano & Sons)	0.57	21.5
Corn oil (Mazola oil)	0.06	6.6

[a]The additional artifacts X and Y discovered in the course of this study were minor compared to A and C. In the analysis of unfractionated fatty acids C includes Y.

[b]NSA = no significant amount.

Vegaclay did not produce A and C and effectively eliminated the possibility that these components were artifacts from the bleaching process, although the properties of clays and their activities vary widely (7). It also has been reported (6) that isolated ethylenic bonds (oleic acid) could be isomerized from *cis* to *trans* by more drastic conditions, including both higher temperature and longer time, as well as higher levels (10%) of bleaching earth. A temperature of at least 200 C seemed to be required to produce this isomerization, a significant point, since this temperature range would be expected in deodorization, not bleaching.

The experimental deodorization of a crude Canbra oil (Table II) immediately demonstrated that steam deodorization was, in fact, implicated as the origin of components A and C in Canbra oil. A survey of other types of oils (Table III) and soybean oil sampled before and after regular commercial deodorization (Fig. 2) showed components A and C only after deodorization. Of the other oils, those containing linolenic acid all showed A and C with the exception of the linseed oil sample purchased in a health food store. This oil is not normally refined for edible use; and, although sold for edible use, the sample was found to be similar to industrial raw linseed oil. The oils containing no linolenic acid did not show A and C. It was, therefore, concluded that linolenic acid was the original material; and, as indicated by Figure 3 and detailed in Table II, this acid (18:3ω3) was converted almost quantitatively into A and C by steam-vacuum deodorization. At the same time one small artifact peak also appeared in the trailing edge of the

TABLE IV

Structure of Monoethylenic and Diethylenic Fatty Acids Derived from Various Geometrical Isomers of 9,12,15-Octadecatrienoic Acid[a] and Comparison of Retention Data on SILAR-5CP with Some Published DEGS Retention Data[b]

Peak no.	Structure	SILAR-5CP	DEGS
1	18:0	18.00	---
2	*trans*-9	18.25	---
3	*cis*-9	18.31	---
4	*trans*-12	18.35	---
5	*cis*-12	18.42	---
6	*trans*-15	18.42	---
7	*cis*-15	18.60	---
8	*trans*-9,*trans*-15	18.72	18.98
9	*trans*-9,*trans*-12	18.77	19.00
10	*cis*-9,*trans*-15	18.79	19.04
11	*cis*-9,*trans*-12	18.80	---
12	*cis*-9,*cis*-12	18.81	19.06
13	*trans*-9,*cis*-12	18.90	---
14	*trans*-9,*cis*-15	18.92	19.18
15	*trans*-12,*trans*-15	18.91	19.23
16	*cis*-9,*cis*-15	18.95	19.24
17	*cis*-12,*trans*-15	18.99	19.30
18	*trans*-12,*cis*-15	19.06	19.38
19	*cis*-12,*cis*-15	19.11	19.45
20	*cis*-9,*cis*-12,*trans*-15 (A)	19.35	---
21	*trans*-9,*cis*-12,*trans*-15 (X)	19.41	---
22	*cis*-9,*cis*-12,*cis*-15 (Parent)	19.44	19.91
23	*cis*-9,*trans*-12,*cis*-15 (Y)	19.51	---
24	*trans*-9,*cis*-12,*cis*-15 (C)	19.51	---

[a]Additional data will appear elsewhere (18).

[b]See ref. 11.

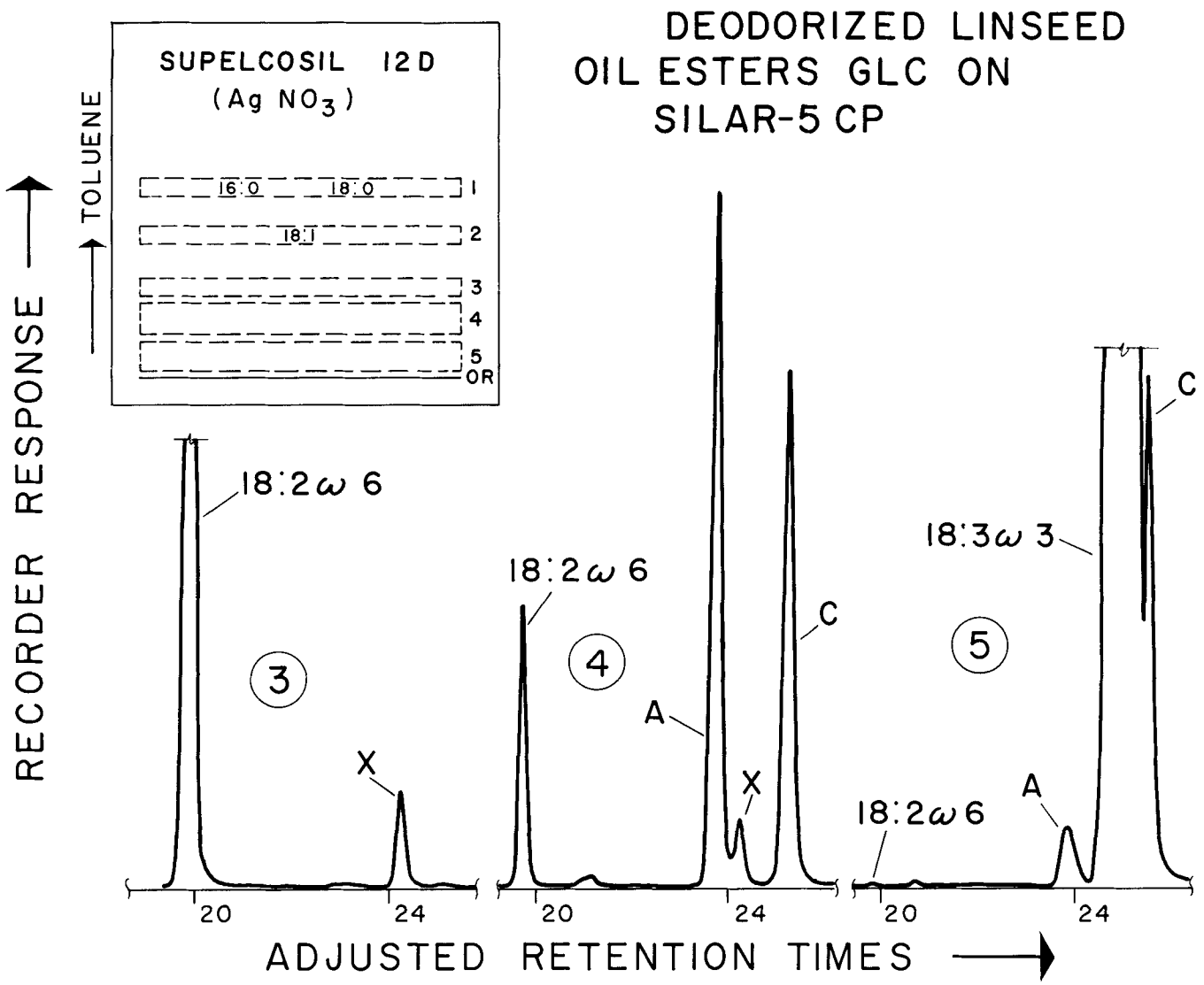

FIG. 4. Results of gas liquid chromatographic examination of thin layer chromatography ($AgNO_3$) bands from methyl esters of fatty acid from laboratory deodorized linseed oil. SILAR-5CP column separates X and 18:3ω3 positions. Open tubular column operated at 180 C and 40 psig.

18:2ω6 peak. However, this was visible only on the more efficient open tubular GLC columns with BDS coatings.

Identification of Artifacts

Initial tests with a concentrate of A and C (admixed with some linolenic acid) isolated from Canbra rapeseed oil deodorized in the laboratory suggested the absence of conjugation (no significant UV absorption), the presence of *trans*-unsaturation (weak IR absorption near 10.36 μ) and an 18:3ω3 structure (mass spectra parent ion predominantly of mass 292 with some indication of a possible component of mass 294). For convenience further isolations and analyses were based upon material prepared from laboratory deodorized linseed oil as the starting material. However, all GLC characteristics described for A and C were also those of the artifacts in all other oils. As a further convenience, urea complexing was used to eliminate methyl esters of most of the saturated and part of the monoethylenic acids before chromatographic study.

TLC of the methyl esters of fatty acids from laboratory deodorized vegetable oils on silver nitrate impregnated silica gel appeared an obvious technique in view of excellent separations of these isomers reported in literature (8,9). Although we were unable to obtain separations adequate to isolate individual isomers completely, considerable information emerged from this attempted fractionation. As shown in Figure 4, band 3 included a single peak (X) in the 18:3 region, coincident with 18:3ω3 on BDS but not on SILAR-5CP. Band 4 immediately behind the 18:2ω6 (linoleic acid) band contained some 18:2ω6, A and C, and the component (X) falling between triene A and the position of authentic *cis*-9,*cis*-12,*cis*-15 (18:3ω3) ester. The next slowest band contained A and C and a major component with the same retention time as *cis*-9,*cis*-12,*cis*-15 ester. Rechromatography of these particular fractions had little effect upon the relative proportions of A and C. Large scale column chromatography of the nonurea adducting methyl esters of fatty acid from laboratory deodorized linseed oil afforded a series of fractions of which a few are shown in Figure 5. The shape of C on BDS in later fractions indicated an extra component (Y) not separable from C on SILAR-5CP. Pooling of appropriate neighboring fractions provided a group mostly of A and C+Y which was examined by Raman and NMR spectroscopy. Raman spectroscopy (Fig. 6) indicated that the ratio of *trans*- to *cis*-bands was ca. 1:2, and NMR indicated that the unsaturated systems included both methylene groups interrupting the ethylenic unsaturation and terminal ethyl groups. The proportions corresponded to a 9,12,15 unsaturated system.

A partial hydrazine reduction of a similar fraction, somewhat enriched in *cis*-9,*cis*-12,*cis*-15 triene and con-

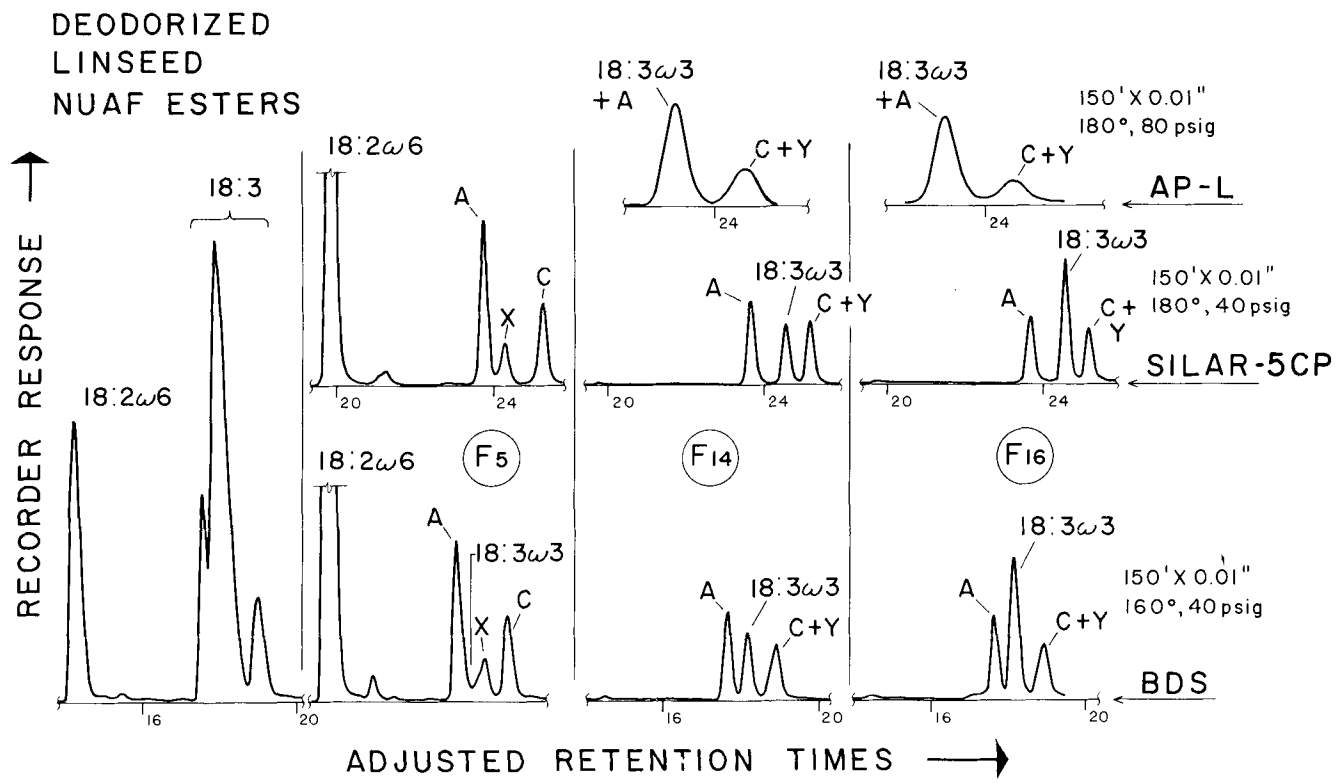

FIG. 5. Some results of fractionation of the methyl esters of fatty acids of a laboratory deodorized linseed oil by column chromatography ($AgNO_3$). F refers to order in which fractions were collected. Gas liquid chromatography runs on butanediolsuccinate polyester, SILAR-5CP, and Apiezon-L open tubular columns are compared. Note absence of 18:3ω3 from F_5 and indication of extra component Y included in C peak in F_{14} and F_{16}. Operating conditions were: Apiezon-L, 180 C, 80 psig; SILAR-5CP, 180 C, 40 psig; butanediolsuccinate polyester, 160 C, 40 psig.

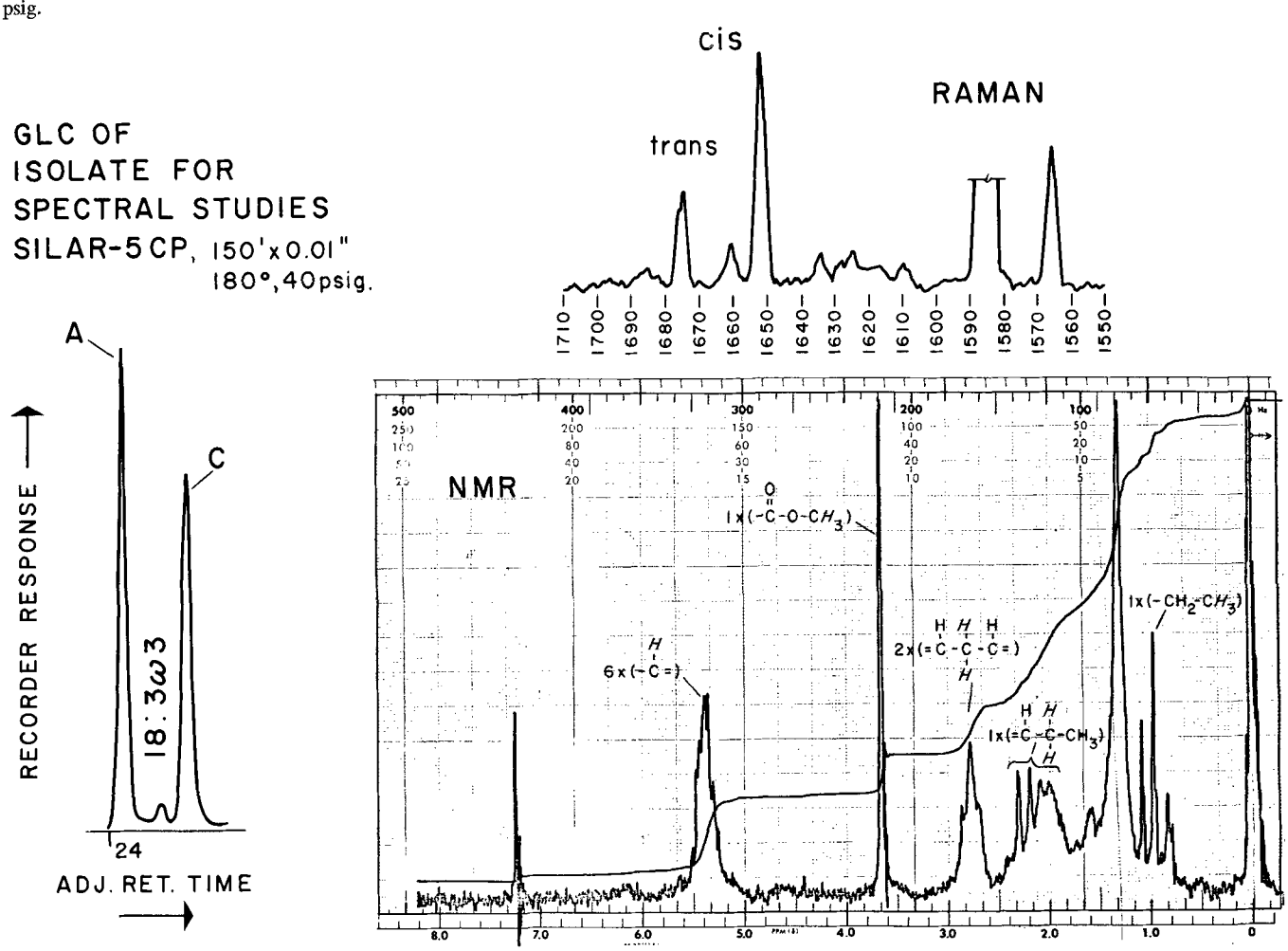

FIG. 6. Raman and NMR spectra of triene ester fraction shown at left as analyzed on SILAR-5CP open tubular column at 180 C and 80 psig. Note quantitative aspects of Raman peaks and NMR integrations.

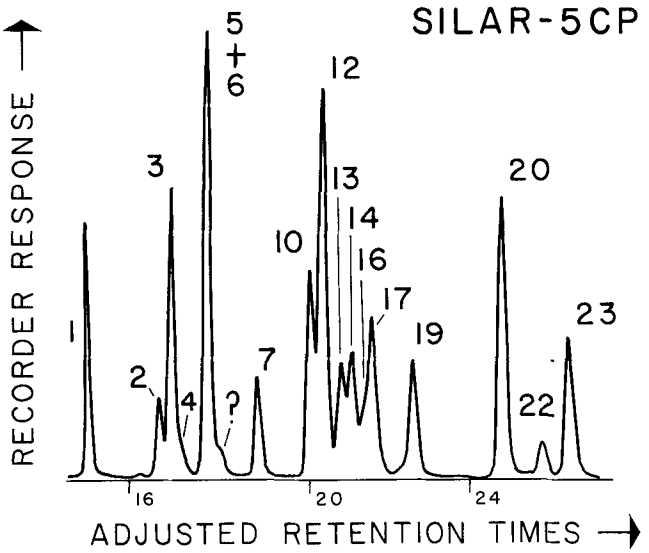

FIG. 7. Products of partial hydrazine reduction of a fraction of A (C+Y) and some 18:2ω6 (note unreduced starting material in peaks 12,20,22,23). Numbers of peaks refer to identifications of Table IV. Analysis on an open tubular SILAR-5CP gas liquid chromatography column at 180 C and 40 psig.

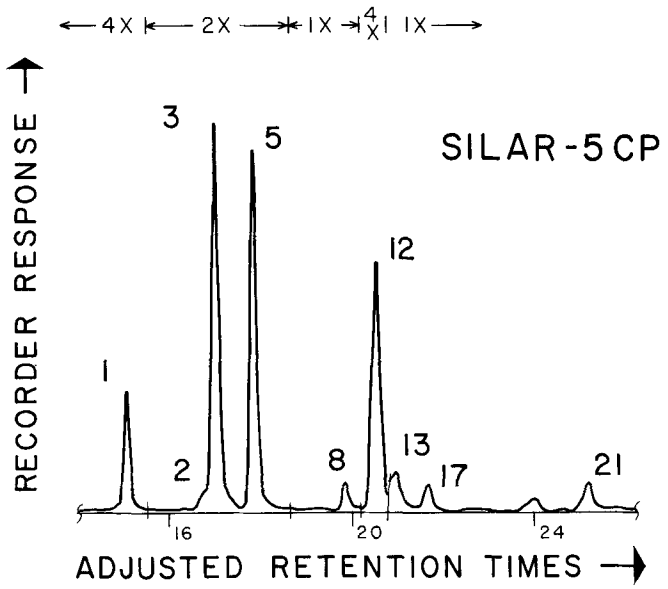

FIG. 8. Partial hydrazine reductions of a mixture of 20% X and 80% 18:2ω6. Note absence of peak for *trans*-12,*cis*-15 octadecadienoate (peak 18) which would fall between peaks 17 and 19. (Compare Fig. 7, shoulder at foot of peak 19 leading edge.) Gas liquid chromatography as for Figure 7.

taining a small amount of *cis*-9,*cis*-12 diene, gave the products shown in Figure 7. In our hands, as reported elsewhere, this technique of reduction of individual ethylenic bands is subject to some positional and geometrical rate differences although original ethylenic bond geometry is specifically preserved (10). The monoethylenic peaks include large *trans*-9 and *trans*-15 components (peaks 2 and 6; peak 6, visible on BDS, is ca. equal to peak 7). These are in ca. equal amounts, although *cis*-15 monoene (peak 7) is less than the *cis*-9 (peak 3), as the 18:2ω6 contributed to the latter. The proportion of *trans*-12 monoene (peak 4) to *cis*-12 monoene (peak 5) is relatively much less. This indicates that, subject to allowance for the *cis*-12 monoene from the *cis*-9,*cis*-12 diene impurity, A and C included *trans*-15 and *trans*-9 bonds. The order of the diethylenic peaks could be established from the publications of Scholfield, et al., (11) by esters of three reference 9,12-octadecadienoic acids supplied by this group, and by the hydrazine reduction products of *cis*-9,*cis*-12,*cis*-15 octadecatrienoic acid (21). The order of elution of mono-*trans*-octadecatrienoic acids on diethyleneglycolsuccinate polyester (DEGS) indicated by Litchfield, et al., (12) included one isomer eluting well before the all *cis* isomer and two close together immediately after it, these respectively corresponding to A and (C+Y) (Fig. 5). Isomer Y, a minor component, is, therefore, most probably *cis*-9,*trans*-12,*cis*-15 in structure, as indicated by the relative dearth of *trans*-12 monoene (peak 4) reduction product. The elution order on AP-L (Fig. 5) was shown by quantitation to be essentially A(+18:3ω3) before C(+Y). Literature reports (9,13-15) shows elution orders and equivalent chain length (ECL) values on AP-L of

	(13)	(15)
cis-9,*cis*-12,*cis*-15	17.57	17.49
cis-9,*cis*-12,*trans*-15	17.57	17.47
cis-9,*trans*-12,*cis*-15	17.72	17.60
trans-9,*cis*-12,*cis*-15	17.73	17.64

Our ECL values (Fig. 5) were (A) 17.55 and (C+Y) 17.73 and, therefore, confirm that

A is *cis*-9,*cis*-12,*trans*-15
Y is *cis*-9,*trans*-12,*cis*-15
C is *trans*-9,*cis*-12,*cis*-15

The cochromatography of Y and C (Fig. 5) resulted in dienoic acid identification difficulties (Fig. 7), since diene product *cis*-9,*trans*-12 (peak 11) is not recognized easily on the leading edge of the *cis*-9,*cis*-12 peaks, and *cis*-9,*cis*-15 (peak 16) could be produced by any all *cis* isomer present before hydrazine reduction. However, the column chromatography fraction ostensibly containing only A,X,C+Y and *cis*-9,*cis*-12 octadecadienoic impurity showed some *trans*-12 monoene (peak 4), a probable *cis*-9,*trans*-12 shoulder (peak 11, not numbered) between the *cis*-9,*trans*-15 (peak 10) and *cis*-9,*cis*-12 (peak 12) isomers, an identifiable *cis*-9,*cis*-15 shoulder (peak 16), and a *trans*-12,*cis*-15 shoulder (peak 18, not numbered) preceding the *cis*-12,*cis*-15 isomer (peak 19) in a reasonable proportion.

The structural details of X were indicated by its chromatographic behavior with silver ions vis a vis the other trienes. The extra mobility indicated two *trans* bonds (9,14), and this agreed with the GLC retention order on DEGS suggested by Litchfield, et al. (12). A partial, but extensive, hydrazine reduction of the fraction, which unfortunately also contained an excess of 18:2ω6, gave products compatible with a *trans*-9,*cis*-12,*trans*-15 structure for artifact X (Fig. 8). The absence of any component including a *trans*-12 structure, especially *trans*-12 monoene and a *trans*-12,*cis*-15 diene, and the GLC peak purity for X on BDS and SILAR-5CP are strongly suggestive that X was a *trans*-9,*cis*-12,*trans*-15 octadecatrienoic acid free of other isomers.

This report shows that standard conditions of edible vegetable oil dedorization, as employed in North America, produce up to 25% conversion of *cis*-9,*cis*-12,*cis*-15 linolenic acid to isomers having one or more geometrically isomerized ethylenic bonds. The excess of *trans*-9 and *trans*-15 over *trans*-12 suggests that either the 12 portion is sterically protected from attack or, more likely, that attack on a *cis*-12 bond often results in positional isomerization, producing conjugated isomers which are not included in this study.

Human milk and butterfat have some 18:3 components which could be purely geometrical isomers, such as A,C,X, or Y (8,16). Bromination-debromination also should produce geometrical isomers, and two mono-*trans*, di-*cis* isomers have been found in a reference material stated to be 99% linolenic acid (17). Slight hydrogenation, which is applied to Crisco oil to reduce the 18:3 content, is unlikely to generate methylene interrupted geometrical isomers of

these types in any high proportion of all *cis* 18:3, but winterization might reduce the proportion of geometrical isomer artifacts in an oil once they were formed.

The shoulder on the back of the *cis*-9,*cis*-12-octadecedienoic ester peaks, e.g. Figure 3, was *trans*-9,*cis*-12 in structure, as shown by cochromatography on BDS and SILAR 5CP with authentic material. The separation of the *cis*-9,*trans*-12 isomer from the dominant *cis*-9,*cis*-12 isomer is poor on polar liquid phases (Table IV); and, therefore, it is unlikely to be observed on inefficient open tubular polar column analyses, e.g. Figure 2, although it should be observed in highly efficient AP-L open tubular GLC analyses owing to its position after the all *cis* isomer and before the other two isomers (9,12,14). Other liquid phases may be preferred, and polyphenyl ether has been especially recommended (11).

As an extension of this work, we have established the elution orders of all eight 9,12,15-octadecatrienoic acids on SILAR-5CP and on BDS columns of differing polarity (18,19), as well as on AP-L (18,20). However, our operations were on wall coated open tubular columns at moderate temperatures. We have not carried out an in-depth study of SILAR-5CP at the higher temperatures which might shift the relative retention times of the mono-, di-, and triethylenic fatty acid isomers. For example, it is possible by temperature manipulation to study artifact X in deodorized oils, if the proportion relative to 18:3ω3 is high enough, by causing the *trans* containing A,X,C+Y complex to elute earlier than the all *cis* 18:3ω3. However, this then leads to a poorer separation of all *cis* 18:3ω3 and C, since the shift affects all fatty acids with *trans* components.

The extensively isomerized octadecadienoic and octadecatrienoic fatty acids which may occur in severely hydrogenated edible oils of either marine or vegetable origin are not known to have any important biochemical or nutritional consequences, provided essential fatty acids are in adequate supply (21,22), although experiments involving the effects of toxic peroxides sometimes are included under heated oils and the distinction from deodorization should be emphasized (23). The importance of isomers more closely resembling the natural all *cis* octadecatrienoic acid perhaps should be singled out for further study in addition to those already executed at the gross physiological response level, as these acids could, perhaps, act to block an obscure or unknown biochemical pathway based upon *cis*-9,*cis*-12,*cis*-15 octadecatrienoic acid. It is possible to shorten deodorization time, and this might reduce the occurrence of these artifacts (24). Otherwise, this study indicates a convenient means of determining whether most liquid vegetable oils containing linolenic acid (or with less sensitivity, linoleic acid) used for edible purposes have been subjected to steam-vacuum deodorization. This process is a virtual guarantee of the destruction of intact protein which might otherwise be implicated as the cause of allergenic reaction (25). The artifacts are also a convenient means of detecting false labeling in cases of liquid edible oils which are sold with claims of not having been subjected to normal refining processes.

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