CONJUGATED FATTY ACIDS OF TRAGOPOGON AND CALENDULA SEED OILS[1]

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ABSTRACT

The seed oil of *Tragopogon porrifolius* L. was found to contain about 4% of conjugated diene hydroxy fatty acids, identified as 9-hydroxy-10,12- and 13-hydroxy-9,11-octadecadienoic acids. On dehydration, the products were 8,10,12- and 9,11,13-octadecatrienoic acids. The diene acids were *cis,trans* or *trans,cis* and the triene acids were judged to be all-*trans*.

The conjugated triene acid of *Calendula officinalis* L. seed oil was isolated and identified as *trans*,8:*trans*,10:*cis*,12-octadecatrienoic acid by spectral analysis, by the structure of the maleic anhydride adduct, and by identification of degradation products. All-*trans*-8,10,12-octadecatrienoic acid was found to react with maleic anhydride at both the 8,11 and 10,13 positions.

INTRODUCTION

During the isolation of an epoxy fatty acid from the seed oil of *Tragopogon porrifolius* L. (family Compositae) (1) it was observed that a small proportion of an unsaturated acid of unusual characteristics was also present. It amounted to about 4% of the total fatty acids and had a conjugated diene linkage. On heating with glacial acetic acid it was converted to a conjugated triene acid. Both the diene and triene acids were unstable. Their structures were determined as described below, each proving to be a mixture of two position isomers.

The diene acids were found to have a hydroxy group and were identified as 9-hydroxy-10,12-octadecadienoic acid and 13-hydroxy-9,11-octadecadienoic acid. The triene acids were identified as 8,10,12- and 9,11,13-octadecatrienoic acids.

A naturally occurring triene acid was isolated from the seed oil of *Calendula officinalis* L. (family Compositae) and shown to be *trans*,8:*trans*,10:*cis*,12-octadecatrienoic acid. The isomerized form (all-*trans*) of this acid had been prepared previously by McLean and Clark from the same species (2). The configuration of the natural acid (*trans,trans,cis*) is opposite to that of α-eleostearic acid (*cis,trans,trans*).

Shortly after this work was completed, Morris, Holman, and Fontell reported the occurrence of two hydroxy diene acids in the seed oils of certain other species, apparently identical with the hydroxy acids described herein (3). Their evidence, based on spectral data and thin-layer chromatography, is in agreement with ours, which is based on spectral data and chemical degradative results. All of the results lead to the conclusion that the substances are 9-hydroxy-10,12- and 13-hydroxy-9,11-octadecadienoic acids.

RESULTS AND DISCUSSION

Conjugated Acids of Tragopogon Oil

The oil had ultraviolet absorption characteristic of diene conjugation, indicating the presence of about 4% of a diene acid, based on the total fatty acids. There was no appreciable triene absorption. After saponification in the cold and removal of unsaponifiable matter, the fatty acids were partitioned between petroleum ether and aqueous ethanol (1 volume H_2O: 4 volumes EtOH). The alcoholic portion contained both diene acid and epoxy acid in about equal amounts. Further partitioning and crystallization from acetone

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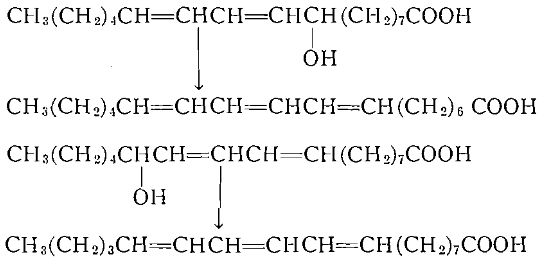
at low temperature yielded a concentrate consisting mainly of conjugated diene acid (ultraviolet max. 233 mμ). It had infrared absorption at 3580 cm^{-1}, indicating an hydroxyl group, and two peaks of nearly equal intensity at 945 and 980 cm^{-1}, characteristic of a conjugated *cis,trans* or *trans,cis* diene (4).

The substance was subjected to oxidative splitting by periodate–permanganate (5) and the acidic products were converted to methyl esters, avoiding loss of volatile components (6). The mixture of methyl esters was examined by gas chromatography. Peaks corresponding to hexanoate and azelate were observed, indicating unsaturated centers at the 12,13 and 9,10 positions, respectively. Since these could not both be present in one conjugated diene acid, it is concluded that the substance was a mixture of two acids, one being $\Delta^{9,11}$ and the other $\Delta^{10,12}$. Neither heptanoic nor sebacic esters appeared, hence the hydroxyl group must have been attached to the terminal fragment in the 9,11 acid and to the carboxyl fragment in the 10,12 acid (III, I).

Boiling the hydroxy diene acid mixture with glacial acetic acid gave a solid acid (or acids), shown by spectral analysis to have a conjugated triene structure and no hydroxyl group. This substance absorbed 3 moles of hydrogen to give stearic acid, hence it had a straight chain of 18 carbon atoms. The triene acid was split by periodate–permanganate and the fragments were shown by gas chromatography to consist of valeric, hexanoic, suberic, and azelaic acids. The substance was therefore a mixture of two octadecatrienoic acids (II and IV) having double bonds in the 8,10,12 and 9,11,13 positions, respectively.



$$CH_3(CH_2)_4CH{=}CHCH{=}CH\underset{\substack{|\\ OH}}{CH}(CH_2)_7COOH \qquad (I)$$

$$\downarrow$$

$$CH_3(CH_2)_4CH{=}CHCH{=}CHCH{=}CH(CH_2)_6COOH \qquad (II)$$

$$CH_3(CH_2)_4\underset{\substack{|\\ OH}}{CH}CH{=}CHCH{=}CH(CH_2)_7COOH \qquad (III)$$

$$\downarrow$$

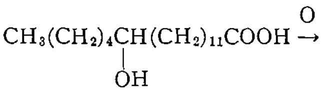
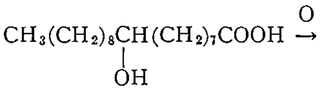
$$CH_3(CH_2)_3CH{=}CHCH{=}CHCH{=}CH(CH_2)_7COOH \qquad (IV)$$

This result confirms the positions of the two double bonds in the diene acids as reported above (10,12 and 9,11). It also provides evidence for the location of the hydroxyl group. Thus, in the 10,12-diene acid, the hydroxyl group must be attached to carbon 8 or 9 to produce an 8,10,12-triene. Because of the ease with which dehydration takes place, it is almost certain that the hydroxyl is at position 9, where it is activated by the adjacent double bond (I). By similar reasoning, the 9,11-diene acid is considered to have the hydroxyl at position 13 (III), giving the 9,11,13-triene on dehydration. 9-Hydroxy-10,12-octadecadienoic is evidently a geometric isomer of dimorphecolic acid, discovered by Smith and co-workers (7), in which the double bonds are *trans,trans*, and which underwent dehydration with the same readiness.

Confirmation of the position of the hydroxyl group was obtained by hydrogenating the mixture of hydroxy diene acids. The product melted at 65–66° and appeared to be a mixture of monohydroxystearic acids. Oxidative splitting by solid permanganate in acetic acid was performed first on a model substance, 12-hydroxystearic acid. The fragments therefrom, identified by gas chromatography, were hexanoic, heptanoic, undecanedioic, and dodecanedioic acids. Thus splitting took place at two positions, 11,12 and 12,13. Minor proportions of azelaic and sebacic acids were also produced. Applying this procedure to the hydrogenated acids gave four monobasic acids, pentanoic, hexanoic, nonanoic, and decanoic (Table I), hence the original hydroxyl group must have been on the 13th

TABLE I

Oxidative splitting of hydrogenated acids

 $CH_3(CH_2)_4\underset{OH}{CH}(CH_2)_{11}COOH \xrightarrow{O}$	$CH_3(CH_2)_3COOH$ $CH_3(CH_2)_4COOH$	+	$HOOC(CH_2)_{11}COOH$ $HOOC(CH_2)_{10}COOH$
 $CH_3(CH_2)_8\underset{OH}{CH}(CH_2)_7COOH \xrightarrow{O}$	$CH_3(CH_2)_7COOH$ $CH_3(CH_2)_8COOH$	+	$HOOC(CH_2)_7COOH$ $HOOC(CH_2)_6COOH$

carbon atom in one acid (III) and on the 9th carbon atom in the other (I). There were minor amounts of heptanoic and octanoic acids. One pair of the corresponding dibasic acids was identified, viz. suberic and azelaic, both in substantial amount. The C_{12} and C_{13} dibasic acids were in smaller amount, apparently having been partially degraded by the oxidizing agent, as occurred in the oxidation of 12-hydroxystearic acid.

The two hydroxy diene acids appeared to be present in about equal amounts in the original oil. This was determined from the relative amounts of the respective fragments of the oxidized hydroxy diene acids and of the oxidized triene acids, estimated from the gas chromatograms.

The possible presence of 11-hydroxy-9,13-octadecadienoic acid was considered but was judged unlikely. Oxidation of the hydrogenated hydroxy acids gave no sebacic acid, one of the expected products from 11-hydroxystearic acid.

The concentrate of the hydroxy diene acids had infrared maxima of nearly equal intensity at 945 and 980 cm^{-1} (Fig. 1). By analogy with the spectra of the simple conjugated diene acids (4), this is strong evidence that both hydroxy acids are mainly, if not entirely, *cis,trans* or *trans,cis* in configuration. The mixture of the two triene acids obtained on dehydration, however, had a single strong infrared band at 990 cm^{-1} and no other maxima in the 900–1000 cm^{-1} region. It is concluded that these acids are all *trans*. Since the diene acids had one *trans* bond and dehydration may be expected to produce a second *trans* bond, the original *cis* bond must have isomerized spontaneously to *trans* during the dehydration to give the all-*trans* triene product. The all-*trans* structure was confirmed when an attempt to isomerize it by iodine in pentane produced no change in melting point or other properties.

Conjugated Acid of Calendula Oil

Saponification of calendula oil and crystallization of the acids from acetone at low temperature yielded a conjugated triene acid, $C_{18}H_{30}O_2$, as determined by elementary analysis and ultraviolet absorption. It gave stearic acid on hydrogenation. Oxidative splitting of the triene acid gave hexanoic and suberic acids, identified by gas chromatography. The double bonds must have been, therefore, at positions 8, 10, and 12 (V).

The infrared spectrum prepared from a solid film of the acid had a very strong peak at 990 cm^{-1} and a medium strong band at 940–975 cm^{-1}. The solution spectrum, in CS_2, had maxima at 987 (vs), 955 (m), and 920 cm^{-1} (m). This type of spectrum suggests a grouping of two *trans* and one *cis* bond, although it is not exactly the same as that of α-eleostearic acid (8). The ultraviolet spectrum showed maxima at 262, 272, and 283 $m\mu$, also indicative of two *trans* and one *cis* bond in conjugation, by analogy with α-eleostearic acid (8, 9).

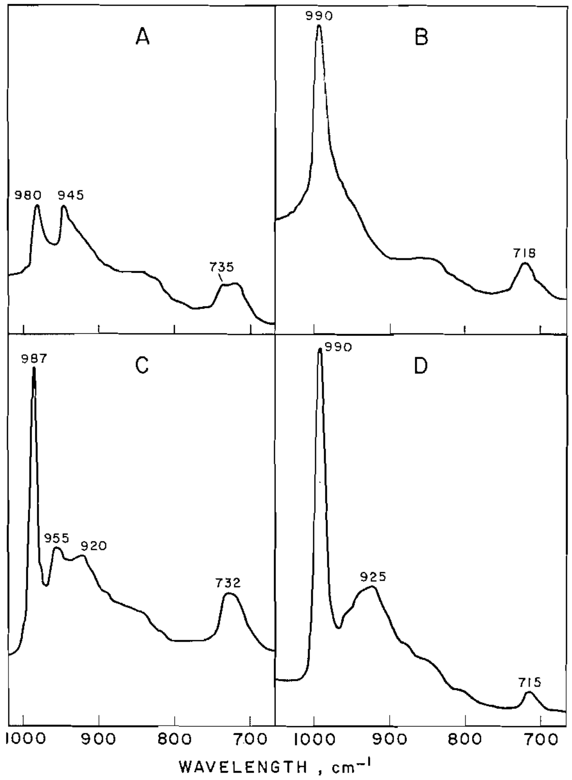
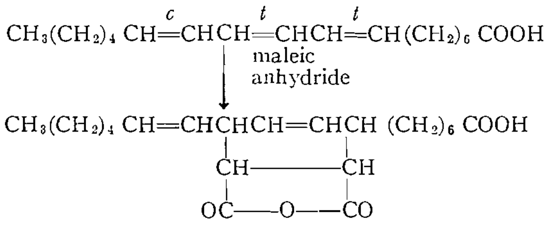


FIG. 1. Infrared spectra, between 700 and 1000 cm^{-1}, determined in CS_2 solution. A, concentrate of hydroxy diene acids from tragopogon; B, methyl esters of triene acids obtained by dehydration of the hydroxy diene acids; C, triene acid from calendula; D, all-*trans* isomer of triene acid from calendula.

The acid reacted readily with maleic anhydride and the adduct was easily purified, showing the presence of two adjacent *trans* double bonds. On splitting the adduct by permanganate–periodate, only one straight chain acid was obtained as a major product. It was identified by gas chromatography as hexanoic acid. There was only a trace of suberic acid. Thus the adduct must have been formed by reaction at the 8 and 11 positions, leaving the 12,13 double bond free (VI).



$$\mathrm{CH_3(CH_2)_4\,CH\overset{c}{=}CHCH\overset{t}{=}CHCH\overset{t}{=}CH(CH_2)_6\,COOH} \qquad (V)$$

$$\Big\downarrow \text{maleic anhydride}$$

$$\begin{array}{l}\mathrm{CH_3(CH_2)_4\,CH{=}CHCHCH{=}CHCH\ (CH_2)_6\,COOH}\\ \qquad\qquad\qquad\quad\ \ |\qquad\qquad\quad\ |\\ \qquad\qquad\qquad\quad \mathrm{CH}\!-\!\!-\!\!-\!\!-\!\!-\!\!-\mathrm{CH}\\ \qquad\qquad\qquad\quad\ \ |\qquad\qquad\quad\ |\\ \qquad\qquad\qquad\ \ \mathrm{OC}\!-\!\!-\mathrm{O}\!-\!\!-\mathrm{CO}\end{array} \qquad (VI)$$

The infrared spectrum of the adduct (VI) had no peak in the region 950–975 cm^{-1} and hence no *trans* exocyclic double bond. The 12,13 double bond was therefore *cis* and the original acid was *trans*,8:*trans*,10:*cis*,12-octadecatrienoic acid. The reasoning is similar to that employed by Paschke and co-workers in determining the configuration of the eleostearic acids (10). The maleic anhydride adduct of all-*trans*-8,10,12-octadecatrienoic acid was prepared for comparison. Its infrared spectrum had a peak at 963 cm^{-1} (*trans* exocyclic double bond).

The *trans*,8:*trans*,10:*cis*,12 acid, m.p. 40–40.5°, was readily converted to the all-*trans* form, m.p. 77–78°, by treatment with iodine in pentane. The infrared spectrum (CS_2 solution) had a strong single peak at 990 cm^{-1}, indicative of the all-*trans* structure, and very little absorption in the vicinity of 950 cm^{-1}. An all-*trans* conjugated triene acid may be expected to react with maleic anhydride in two ways. Thus, Bickford and coworkers found evidence that addition occurs to β-eleostearic acid at both the 9,12 and 11,14 positions (11). In the present work, proof was obtained that two different adducts are formed in the reaction of all-*trans*-8,10,12-octadecatrienoic acid with maleic anhydride. The reaction product was oxidized by permanganate–periodate and the fragments were examined by gas chromatography. Both hexanoic acid and suberic acid were found in substantial amounts, showing that addition of maleic anhydride had occurred at the 10,13 as well as the 8,11 positions, forming two separate adducts.

The name calendic acid is proposed for *trans*,8: *trans*,10: *cis*,12-octadecatrienoic acid.

EXPERIMENTAL

Methods

Infrared spectral data were obtained with a Perkin–Elmer Model 21 infrared spectrophotometer with sodium chloride prism. Samples were in carbon disulphide solution unless stated otherwise. Ultraviolet measurements were made in cyclohexane or isooctane solution. Gas–liquid chromatography of methyl esters was conducted at various column temperatures from 70° to 170°, according to the chain length of the sample. The detector was a thermistor type of thermal conductivity cell. Column length was 1 meter, helium flow rate 95 ml/min, and inlet pressure 7 to 12 p.s.i., depending on the column temperature. Identification of the various esters was made by comparing the emergence time with that of a standard sample of the same ester under the same conditions on the same day.

TRAGOPOGON OIL

Seed of *Tragopogon porrifolius* L. was purchased from a commercial seed house. It was of the current year's stock. The seed (450 g) was ground and extracted with petroleum ether by stirring with successive lots of the solvent at room temperature. The solvent was removed at 25–30° under nitrogen. The oil had ultraviolet absorption maximum at 233 mμ, $E^{1\%}_{1\,\text{cm}}$ 47.5, equivalent to 4.0% conjugated octadecadienoic acid based on the total fatty acids. There was no measurable absorption at 268 mμ (conjugated triene).

The oil (83 g) was saponified at room temperature (25°) by letting it stand for 18 hours with a solution of 25 g of potassium hydroxide in 500 ml of ethanol. The unsaponifiable matter was removed by extraction and the fatty acids were partitioned between petroleum ether and ethanol:water (4 volumes EtOH:1 volume H_2O). The partitioning was carried out in three stages and the products recovered from the alcohol portions were analyzed for conjugated diene by ultraviolet absorption and for oxirane oxygen by Swern's method (12) with the following results:

Portion	Wt. of acids, g	Conjugated diene acid, %	Oxirane oxygen, %
1	7.0	32.0	2.1
2	7.2	8.7	1.2
3	4.4		0.7

The acids from the first portion were estimated to contain 32% conjugated diene acid and 37% epoxy acid, assuming that both are of chain length C_{18}. This mixture was treated further by solvent partition and the alcohol-soluble portion, on crystallization from acetone at −70°, yielded an oil (0.34 g) which was a concentrate of the conjugated diene acid. It had ultraviolet maximum 233 mμ and $E^{1\%}_{1\,\mathrm{cm}}$ 561.

Identification of Diene Acids

The concentrate had an infrared peak at 3580 cm^{-1} (OH) and two peaks of equal intensity at 945 and 980 (conjugated *cis,trans* double bonds). Oxidative splitting by permanganate–periodate (5) and examination of the esterified fragments by gas chromatography (6) showed that they were hexanoate and azelate. There was no heptanoate or sebacate. There was some evidence of other compounds at longer emergence times, possibly fragments carrying the hydroxy group.

On hydrogenation of the diene acid concentrate (1.0 g) with Adams catalyst in ethyl acetate and successive crystallizations from ethyl acetate and from hexane, the product (0.33 g) melted at 65–66° and was evidently a mixture of monohydroxystearic acids. Calculated for $C_{18}H_{36}O_3$: C, 71.95; H, 12.08. Found: C, 71.83; H, 11.70. Infrared maximum, 3600 cm^{-1} (OH). Oxidative splitting of the hydrogenated product by solid permanganate in glacial acetic acid at 50° (13) gave a mixture of monobasic and dibasic acids which was esterified and submitted to gas chromatography. A similar oxidation of 12-hydroxystearic acid gave two monobasic acids, hexanoic and heptanoic, and two dibasic acids, undecanedioic and dodecanedioic, along with minor proportions of azelaic and sebacic acids. The monobasic acids obtained from the oxidation of the hydrogenated diene acids were chromatographed (as methyl esters) at 55°, 70°, and 93°. They were mainly pentanoic (15%), hexanoic (22%), nonanoic (25%), and decanoic (28%), with smaller amounts of heptanoic (5%), and octanoic acids (5%). Suberic and azelaic acids were observed in substantial amount (as esters) when chromatographed at 175°. There were also dodecanedioic and tridecanedioic acids, in smaller proportions, along with pimelic and undecanedioic. There was little or no sebacic acid.

Dehydration to Triene Acids

The mixed hydroxy diene acids were dehydrated as follows. The concentrate (3.4 g) was refluxed with glacial acetic acid (17 ml) for 4 hours, the acetic acid was removed under reduced pressure and the residue was heated for 1 hour with 35 ml of *N* alcoholic KOH. The crude acids were crystallized from 15 ml of acetone at −35°, giving 0.9 g of glistening plates, m.p. 58–60°. Further crystallization from ethyl acetate raised the melting point to 60–61° but no higher. (Found: C, 77.69; H, 10.65. Octadecatrienoic acid, $C_{18}H_{30}O_2$, requires C, 77.65; H, 10.85.) The substance had ultraviolet absorption maxima at 260, 269, 280 mμ (all-*trans* conjugated triene). The infrared spectrum (solid film) had a single strong peak at 985–995 cm^{-1}, also characteristic of the all-*trans* form. There was no OH peak. The spectrum of the methyl ester (in CS_2) showed a similar all-*trans* band at 990 cm^{-1}. When hydrogenated in methanol with Adams catalyst, the acid (0.37 g) absorbed 3.06 moles of hydrogen, and the product was stearic acid (0.34 g), m.p. and mixed m.p. 69–70°, equivalent weight 284.9 (calc. 284.4).

A portion of the triene acid was oxidized by periodate–permanganate (5) and the products were converted to methyl esters (6) for identification by gas chromatography. Valerate and hexanoate were identified at 80°, in approximately equal amounts (53:47). Suberate and azelate were observed at 170°. A larger portion was oxidized by the procedure of McLean and Clark (2). Azelaic acid, m.p. and mixed m.p. 104–105°, was isolated with some difficulty by crystallizing the mixture of products. It was judged that more than one dibasic acid was present.

An attempt to isomerize the triene acids by iodine in hexane solution produced no change in melting point.

CALENDULA OIL

trans,8:trans,10:cis,12-Octadecatrienoic Acid (Calendic Acid)

Seed of *Calendula officinalis* L. was purchased from a commercial seed house. It was ground and the oil was extracted with petroleum ether in a Soxhlet. Ten grams of the oil was saponified by heating for 1 hour with 6% alcoholic potassium hydroxide under nitrogen. The unsaponifiable matter was removed and the fatty acids were crystallized fractionally from acetone at temperatures from 0° to −40°, giving 2.5 g of an acid, m.p. 40–40.5°. The acid was highly unstable in air, becoming sticky in a few minutes if exposed at room temperature. It was kept under nitrogen at the temperature of solid carbon dioxide. Calculated for $C_{18}H_{30}O_2$: C, 77.65; H, 10.85. Found: C, 77.97; H, 10.92. Ultraviolet maxima at 262, 272, and 283 mμ. Infrared maxima (solid film) at 990 (vs), 940–975 (m), and 740 (m); (in CS_2) at 987 (vs), 955 (m), 920 (m), and 732 cm^{-1} (m).

Hydrogenation with Adams catalyst in methanol gave stearic acid, m.p. and mixed m.p. 69.5–70°. The triene acid was oxidized by permanganate–periodate (5) and the products were examined as methyl esters by gas chromatography (6). Peaks corresponding to hexanoate and suberate were observed. There were no other peaks.

The triene acid was heated with maleic anhydride in benzene for 1 hour, forming an adduct, m.p. 73–74° after several crystallizations from ether:petroleum ether (1:5). Calculated for $C_{22}H_{32}O_5$: C, 70.18; H. 8.57. Found: C, 70.18; H, 8.55. The infrared spectrum showed no peak or shoulder in the region of 950–975 cm^{-1}. The adduct was oxidized by permanganate–periodate and the fission products were converted to methyl esters and chromatographed. There was a large peak identified by its emergence time as hexanoate and a very small peak identified as suberate.

The content of conjugated triene acid in the oil was estimated to be 47%.

All-trans-8,10,12-Octadecatrienoic Acid

On treatment with iodine in pentane, the triene acid (2.5 g), m.p. 40–40.5°, was quickly converted to the all-*trans* isomer which melted at 77–78° after crystallization from ethanol at 0° (1.9 g). Infrared maximum 990 cm^{-1} (vs); ultraviolet maxima 259, 269, and 280 mμ. McLean and Clark reported ultraviolet maxima 258, 268, and 280 mμ (2). The maleic anhydride adduct was prepared. It was more difficult to purify than that from the unisomerized acid and melted no higher than 70–71° (McLean and Clark, 71°). The yield was 1.5 g from 1.9 g of the *trans* acid. In admixture with the adduct from the unisomerized acid it melted at 56–58°. Its infrared spectrum showed a small but distinct peak at 963 cm^{-1}. The adduct was oxidized by permanganate–periodate and the fragments were identified by gas chromatography as hexanoate and suberate, both in considerable amount.

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REFERENCES

1. M. J. CHISHOLM and C. Y. HOPKINS. Chem. & Ind. (London), 1154 (1959).
2. JOHN MCLEAN and A. H. CLARK. J. Chem. Soc. 777 (1956).
3. L. J. MORRIS, R. T. HOLMAN, and K. FONTELL. J. Am. Oil Chemists' Soc. **37**, 323 (1960).
4. J. R. CHIPAULT and J. M. HAWKINS. J. Am. Oil Chemists' Soc. **36**, 535 (1959).
5. R. U. LEMIEUX and E. VON RUDLOFF. Can. J. Chem. **33**, 1701 (1955).
6. M. J. CHISHOLM and C. Y. HOPKINS. Can. J. Chem. **38**, 805 (1960).
7. C. R. SMITH, JR., T. L. WILSON, E. H. MELVIN, and I. A. WOLFF. J. Am. Chem. Soc. **82**, 1417 (1960).
8. L. CROMBIE and A. G. JACKLIN. J. Chem. Soc. 1632 (1957).
9. F. D. GUNSTONE. Chemistry of fats and fatty acids. Chapman and Hall Ltd., London. 1958. p. 25.
10. R. F. PASCHKE, W. TOLBERG, and D. H. WHEELER. J. Am. Oil Chemists' Soc. **30**, 97 (1953).
11. W. G. BICKFORD, E. F. DUPRE, C. H. MACK, and R. T. O'CONNOR. J. Am. Oil Chemists' Soc. **30**, 376 (1953).
12. D. SWERN, T. W. FINDLEY, G. N. BILLEN, and J. T. SCANLAN. Anal. Chem. **19**, 414 (1947).
13. K. E. BHARUCHA and F. D. GUNSTONE. J. Chem. Soc. 613 (1957).