# Isolation of a Natural Isomer of Linoleic Acid from a Seed Oil[1, 2]

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## Abstract

*trans*-10-*trans*-12-Octadecadienoic acid was found to be a component of the glyceride oil of the seeds of *Chilopsis linearis* (Cav.) Sweet. It was isolated by fractional crystallization of the acids at low temperatures. Identification was made by absorption spectra, by the adduct with maleic anhydride, and by identification of degradative products.

The conjugated triene of the oil was also isolated and identified as *trans*-9-*trans*-11-*cis*-13-octadecatrienoic acid. In two samples of seed, the diene acid constituted about 9% of the oil and the triene acid 18% and 25%.

## Introduction

ALTHOUGH LINOLEIC ACID is almost a universal component of seed oils, no other isomer of octadecadienoic acid has been identified in such oils until now. Indications of non-conjugated isomers have been observed from time to time. Strong evidence of their occurrence was obtained by Sreenivasan et al., who examined linoleic acid concentrates from seed oils, but the isomeric acids were not isolated or characterized (10).

There have been fewer indications of conjugated isomers of linoleic acid in nature. This is surprising in view of the fairly common occurrence of conjugated isomers of linolenic acid. However, Markman and Bodnya reported spectral evidence of a conjugated diene substance in the oil of *Catalpa bignonioides* (2). Earle et al. found similar evidence for conjugated diene in the oil of *Chilopsis linearis* but not in *Catalpa bignonioides* (3).

Sixteen species of Bignoniaceae, including the two mentioned above, were examined during our studies of conjugated trienoic acids (4,5,6). Six of these species had a conjugated trienoic acid but only *Chilopsis linearis* had indisputable ultraviolet absorption at 233 mμ, indicative of a conjugated diene. Efforts to isolate this component were successful and it was identified as *trans*-10-*trans*-12-octadecadienoic acid. A preliminary report of the work has been made (7).

## Procedure

### Isolation and Identification

The mixed fatty acids of *Chilopsis linearis* oil were crystallized fractionally from acetone at low temperatures to separate the conjugated diene from the conjugated triene. Fractions rich in the diene were crystallized repeatedly from pentane to remove saturated acids and other congeners; the pure acid melted at 55.5–56.0C. Its absorption in the ultraviolet, $\lambda_{max}$ 232 mμ, denoted a conjugated diene structure. The infrared spectrum had a single very strong peak at 985 $cm^{-1}$ in the region of conjugated unsaturation, showing that the configuration is *trans, trans*.

Hydrogenation gave stearic acid, proving that the substance is a straight-chain, $C_{18}$ acid. Oxidative splitting gave hexanoic and sebacic acids, thus fixing the double bond structure as $\Delta^{10,12}$. The acid is, therefore, *trans*-10-*trans*-12-octadecadienoic acid. It formed adducts readily by the Diels-Alder reaction with maleic anhydride and with tetracyanoethylene, confirming the *trans,trans* configuration. The melting point of the maleic anhydride adduct was not changed by mixing it with an authentic sample of the same adduct from another source.

The most soluble portion of the original mixed acids yielded a conjugated triene on recrystallization. It proved to be *trans*-9-*trans*-11-*cis*-13-octadecatrienoic acid, previously known to occur only in the oils of *Catalpa ovata* (4) and *Catalpa speciosa* (6). The pure acid melted at 31–32C. Its ultraviolet and infrared spectra indicated a conjugated grouping of two *trans* and one *cis* double bond. Stereomutation to the all-*trans* form gave β-eleostearic acid, showing that the acid is a straight-chain, octadecatrienoic acid and that the triene grouping is 9,11,13. This was confirmed by oxidative splitting of the original acid and identification of the products as valeric and azelaic acids. Ready formation of an adduct of the acid with maleic anhydride showd that the two *trans* bonds were adjacent to each other. The adduct was identical (mp and mixed mp) with the adduct previously prepared from the *Catalpa* triene (4) and hence the *Chilopsis* triene must also be *trans*-9-*trans*-11,*cis*-13-octadecatrienoic acid. The non-identity of this acid and α-eleostearic acid was apparent from the melting points of the acids and their adducts.

## Experimental

Ultraviolet spectra were determined in cyclohexane solution with a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulfide in a Perkin Elmer model 21 spectrophotometer with sodium chloride prism. Melting points were determined in capillary tubes and are corrected. Petroleum ether refers to the fraction of bp 30–60C.

### Chilopsis Seed Oil

Seed of *Chilopsis linearis* (Cav.) Sweet was obtained from a reliable commercial source. It was ground and extracted at room temperature by stirring with petroleum ether. The solvent was removed at 25–30C under nitrogen. The oil had peroxide value 4.4; ultraviolet spectrum: $\lambda_{max}$ 233, 263, 272, 283 mμ, absorptivity $E^{1\%}_{1\,cm}$ 116 at 233 mμ; $E^{1\%}_{1\,cm}$ 315 at 272 mμ, equivalent to about 17.8% of conjugated trienoic acid (Fig. 1).

The proportion of the absorptivity at 233 mμ due to the content of conjugated triene was calculated from the absorption curve for pure trienoic acid to be $E^{1\%}_{1\,cm}$ 23. Hence the net absorptivity due to the diene was $116 - 23 = 93$, equivalent to 8.5% of conjugated dienoic acid, employing the factor 0.091 (8). The absorptive characteristics of the total mixed acids, freed from unsaponifiable matter, were almost the same as those of the oil. The oil from a second sample of seed had corrected absorptivity 102 at 233 mμ, equivalent to 9.3% of conjugated diene, and 453 at 272 mμ, equivalent to 25.6% of conjugated

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triene. This oil was treated with dilute alkali to remove the free fatty acids. Ultraviolet absorption analysis of the neutral oil then showed conjugated diene, 9.4%, and conjugated triene, 25.9%. The infrared spectrum of the oil had maxima at 960 and 986 cm$^{-1}$ (conjugated unsaturation) but none in the hydroxyl region, 3300–3600 cm$^{-1}$.

### Isolation of trans-10-trans-12-octadecadienoic acid

The oil (26 g) from the first sample of seed was hydrolyzed by refluxing it for 30 min with 5% ethanolic potassium hydroxide under nitrogen. The unsaponifiable matter was removed by extraction with petroleum ether. The soap solution was acidified and the mixed fatty acids were collected in petroleum ether and recovered by evaporation of the solvent under nitrogen at room temperature and reduced pressure. The acids (24 g) were dissolved in acetone (225 ml) and the solution was cooled stepwise to −10C and −40C. Fatty acid crystals were filtered off at each stage.

Fraction $F_1$, taken at −10C (1.1 g), contained a little diene but $F_2$, taken at −40C (7.0 g), had 23.5% of conjugated diene, determined by ultraviolet absorption and calculated as $C_{18}$ acid. The filtrate ($F_3$) contained most of the triene acid. Fraction $F_2$ was dissolved in acetone (63 ml) and cooled to −12C. Crystalline fatty acids were filtered off (fraction $F_{21}$, 2.6 g, 47.6% conjugated diene). This fraction was crystallized 4 times from pentane, giving nearly pure dienoic acid (0.2 g), mp 55–56C.

The filtrate from fraction $F_{21}$ was cooled to −30C, giving 2.2 g of crystals ($F_{22}$) which contained 14.4% of conjugated diene. This product was crystallized 4 times from pentane. It gave the pure dienoic acid (0.08 g), mp 55.5–56.0C, $E^{1\%}_{1\,cm}$ 1169 at 232 mμ $\nu_{max}$ 985 (vs). Jackson (9) reports mp 55.5C. Other workers report mps up to 57C. The absorptivity has been given as 1137 (10) and 1150–1170 (11).

### Hydrogenation and Oxidative Splitting

The diene (0.1 g) was hydrogenated in methanol with Adams catalyst and crystallized from methanol, giving stearic acid, mp and mixed mp 69.0–69.5C.

Oxidative splitting of the acid (0.14 g) by permanganate-periodate (12) was carried out. The acidic products were recovered and esterfied by our usual procedure (13). The resulting methyl esters were examined by gas chromatography. Large peaks corresponding to hexanoate and sebacate were observed. There were no other peaks.

### Adducts with Maleic Anhydride and Tetracyanoethylene

A fraction (0.35 g) of the crystallized fatty acids of the oil, containing 67% of conjugated diene, was refluxed in benzene for 6 hr with a slight excess of maleic anhydride. The solvent was evaporated and the product was washed with petroleum ether and with water. After two crystallizations from petroleum ether:ethyl ether (4:1), it gave the pure adduct, 3-8′-carboxyoctyl-6-pentylcyclohexene-4,5-dicarboxylic acid anhydride, mp and mixed mp 102.5–103.0C. von Mikusch gives mp 102C (14).

*Anal.* Calcd. for $C_{22}H_{34}O_5$: C, 69.8; H, 9.05. Found: C, 69.5; H, 9.09. This substance has apparently not been characterized previously by elemental analysis.

The adduct (0.18 g) was hydrogenated in methanol with Adams catalyst and the product was crystallized from methanol, giving 3-8′-carboxyoctyl-6-pentylcyclohexane-1,2-dicarboxylic anhydride, mp 81.5–82.0C.

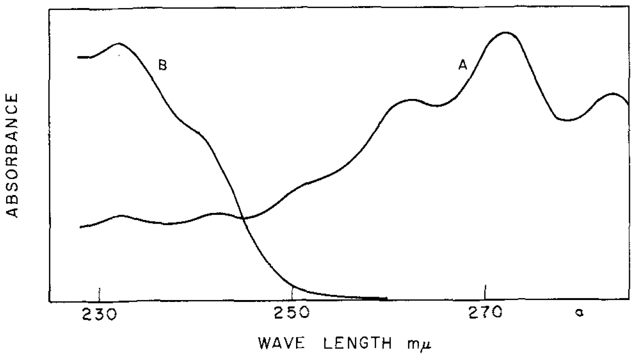

FIG. 1. Ultraviolet absorption spectra. A. *Chilopsis* oil. B. *trans*-10-*trans*-12-octadecadienoic acid isolated from the oil.

*Anal.* Calcd. for $C_{22}H_{36}O_5$: C, 69.4; H, 9.54. Found: C, 69.2; H, 9.40.

A fraction of the mixed fatty acids containing 0.3 g of conjugated diene was mixed with 0.2 g of tetracyanoethylene in 10 ml of benzene and allowed to stand for 1¾ hr (4). After removal of the solvent under reduced pressure, the residue was washed with petroleum ether and crystallized three times at 0C from petroleum ether:ethyl ether (4:1). The product, 3-8′-carboxyoctyl-4,4,5,5-tetracyano-6-pentylcyclohexene, formed white crystals, mp 75.5–76.5C.

*Anal.* Calcd. for $C_{24}H_{32}N_4O_2$: N, 13.7. Found: N, 13.9.

### Identification of the Triene

The filtrate ($F_3$) from the main portion of conjugated dienoic acid was cooled to −55C. It deposited 7.1 g of acids which contained 33% of conjugated triene as determined by ultraviolet absorption. The mixture was crystallized from pentane at −25C, yielding 1.2 g of solid acid containing 76% of conjugated triene. Two further crystallizations from pentane gave the pure acid, mp 31–32C; $\lambda_{max}$ 262, 272, 283 mμ; $\nu_{max}$ 986 vs, 957 m cm$^{-1}$. A portion, treated with iodine in pentane, deposited crystals of β-eleostearic acid, mp 68.5–69.5C. The mp was unchanged in admixture with an authentic sample.

A second portion of the acid was converted to its adduct with maleic anhydride (4). The product melted at 73.0–73.5C, alone and mixed with the corresponding adduct of *trans*-9,*trans*-11, *cis*-13-octadecatrienoic acid, prepared from the acid of *Catalpa ovata* seed oil (4).

A third portion of the acid was subjected to oxidative splitting by permanganate-periodate (12) and the products were esterified (13) and examined by gas chromatography. Large peaks corresponding to valerate and azelate were observed.

## Discussion

*Chilopsis linearis* (Cav.) Sweet, syn. *Bignonia linearis* Cav., is a small flowering tree, native to the southern United States. It is grown for ornament under the common name of desert willow or flowering willow. The tree attains a height of 20 ft and bears long pods containing many winged seeds. The seeds occur in longitudinal pairs with a tuft of fibres at each end.

The ultraviolet spectrum of the seed oil showed a definite peak at 233 mμ. The oil of one other species had a very small peak at 233 mμ but the remaining 14 species of Bignoniaceae that were studied had no evidence of conjugated diene in their seed oils. The dienoic acid must therefore be relatively uncommon.

Removal of the free fatty acids from the *Chilopsis* oil did not alter the absorbance of the oil at 233 mμ, hence the conjugated acid is considered to be a component of the glycerides. It is also believed that the acid exists in the *trans,trans* form in the oil since no change in configuration would be expected under the mild conditions employed for its isolation.

The *trans*-10-*trans*-12-Octadecadienoic acid was made in 1942 by von Mikusch by dehydrating and isomerizing the ricinoleic acid of castor oil (15). It has also been prepared from alkali-isomerized linoleic acid (16). The ultraviolet spectrum of the methyl ester, determined by Tolberg and Wheeler (17) is similar to the spectrum in Figure 1 and includes the inflection at 241–242 mμ.

A closely-related substance is 9-hydroxy-*trans*-10-*trans*-12-octadecadienoic (dimorphecolic) acid, found by Smith and co-workers in the seed oil of *Dimorphotheca* (Compositae) (18). Although there was no evidence of an hydroxy acid in the *Chilopsis* oil, it is possible that such an acid could be an intermediate in the biosynthesis of the unsubstituted dienoic acid. Occurrence of a reducing enzyme in the seed that would convert dimorphecolic acid to the simple dienoic acid is likely. The conjugated trienoic acid of *Chilopsis* is probably formed by a separate pathway in the plant since its unsaturated grouping is $\Delta^{9,11,13}$.

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# Reaction of Acetyl Nitrate with Alcohol Derivatives of Fatty Acids: A Synthesis of Nitrate Esters[1]

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## Abstract

Acetyl nitrate was found to react with fatty alcohols, hydroxy esters, and vicinal glycols to give good yields of the corresponding nitrates. The reaction was applied to the synthesis of *n*-octadecyl nitrate, methyl 2-nitrato-octadecanoate, methyl 12-nitrato-octadecanoate, and 18,19-dinitrato-hexatricontane. Alkyl nitrates and nitrato esters were also prepared from alcohols derived from commercially available marine oils. In addition, the long-chain nitrate derivatives were analyzed by infrared spectroscopy and thin-layer chromatography (TLC).

The potential use of nitrate esters for the synthesis of derivatives is discussed, together with their possible effects on human physiology.

## Introduction

A NUMBER OF METHODS have been reported for the preparation of short-chain organic nitrates ($R{-}O{-}NO_2$), but the instability of many of these compounds and their derivatives has discouraged their use in chemical syntheses. Nevertheless, interest has increased recently in the preparation and properties of organic nitrates, and the chemistry of these compounds has become an important part of the field of organic chemistry (2,7,12,13). The use of nitrate esters for the preparation of a wide variety of derivatives has recently been reviewed by Boschan et al. (2).

It was found in this laboratory that nitrate derivatives of fatty acids are relatively stable, even at elevated temperatures, and are useful intermediates for the preparation of compounds of possible industrial importance. Despite their potential usefulness, however, nitrate derivatives of fatty acids have received little attention in the literature.

A previous communication from this laboratory reported that acetyl nitrate reacts quantitatively with the double bond of methyl oleate to form nitro, acetoxy-nitro, and nitro-nitrate derivatives (9). Further work with this reagent showed that it reacts under similar conditions with fatty alcohols, hydroxy-esters, and vicinal glycols to give good yields of the nitrate derivatives. The present paper summarizes this work and describes a convenient method for the synthesis of the following previously unreported long-chain nitrates: *n*-octadecyl nitrate, methyl 2-nitrato-octadecanoate, methyl 12-nitrato-octadecanoate, and 18,19-dinitrato-hexatricontane. The preparation of alkyl nitrates and nitrato esters from alcohols obtained from commercially available marine oils is also described. In addition, infrared spectroscopy and TLC were found to be desirable complementary methods for the analysis of the long-chain nitrate derivatives reported here.

## Experimental

### Infrared Spectroscopy and TLC

All materials and products were examined by infrared spectroscopy and TLC. Infrared spectra of thin films of liquids on sodium chloride plates were ob-

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