OCCURRENCE OF *trans*-9-*trans*-12-OCTADECADIENOIC ACID AS A SEED OIL COMPONENT[1,2]

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ABSTRACT

trans-9-*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 and removal of conjugated acids as their adducts with maleic anhydride. Identification was made by absorption spectra and by preparation of derivatives and degradative products. The acid is estimated to constitute about 15% of the total fatty acids of the oil. Linoleic acid was also identified.

The occurrence of one or more positional isomers of linoleic acid in natural oils and fats has been suspected for a long time but was established only recently by the isolation of 10,12-octadecadienoic acid from a seed oil (1, 2). It has been considered less likely that geometric isomers of linoleic acid would be found in seeds since nearly all monoene and diene fatty acids of vegetable oils so far discovered have had the cis configuration. However, in the present work, examination of the seed oil of *Chilopsis linearis* provided infrared spectral evidence for the presence of a fatty acid containing isolated trans double bonds. It proved to be a geometric isomer of linoleic acid.

The freshly extracted oil had infrared absorption at 986 cm^{-1}, mainly due to the conjugated triene acid component, *trans,trans,cis*-9,11,13-octadecatrienoic acid (2). The subsidiary peak at 960 cm^{-1}, however, was more intense than would be expected for a trans–trans–cis acid. The conjugated trans–trans diene acid also known to be present (2) would not contribute to the absorption in the 960 cm^{-1} region. It was presumed, therefore, that part of this absorption must be due to an isolated trans linkage. This was confirmed by fractional crystallization of the mixed fatty acids at low temperatures, which effected a substantial separation of the various components. Three fractions were shown by infrared spectra (Fig. 1) to consist of concentrates of conjugated diene acid (F21), isolated trans-unsaturated acid (F22), and conjugated triene acid (F23).

Fraction F22 was crystallized further until free of conjugated triene acid but it continued to retain a little conjugated diene acid. It was found that the conjugated diene acid could be removed as its adduct with maleic anhydride. This treatment left the acid with isolated trans bonds unchanged. The latter acid melted when pure at 26.5–27.0° and was free of conjugated material as determined by ultraviolet absorption. Its infrared spectrum, max. 963 cm^{-1}, also showed the absence of conjugated diene and triene linkages since there was no peak or shoulder in the region of 986 cm^{-1} (Fig. 2). The intensity of the peak at 963 cm^{-1} was approximately double that of the same peak in the spectrum of elaidic acid; hence the substance must contain two isolated trans double bonds.

Hydrogenation of the acid resulted in the absorption of 2 moles of hydrogen and formation of stearic acid. Oxidative splitting of the acid gave two fragments identified by gas chromatography as hexanoic and azelaic acids, showing that the diene grouping is 9,12. These reactions, along with the spectral data, established the structure and configuration of the substance as *trans*-9-*trans*-12-octadecadienoic acid. To confirm its

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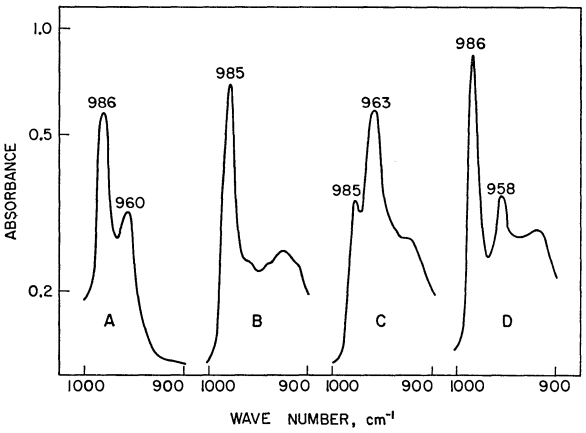

FIG. 1. Infrared absorption at 900–1000 cm^{-1}. A, chilopsis oil. B, fatty acids fraction F21, mainly trans–trans conjugated diene. C, fraction F22, mainly trans–trans non-conjugated diene. D, fraction F23, mainly trans–trans–cis conjugated triene.

identity an authentic sample was prepared by stereomutation of linoleic acid to the trans–trans form (linelaidic acid). This substance and the chilopsis acid had the same melting point and mixed melting point. The *p*-phenylphenacyl esters were also identical. The natural acid was converted by alkaline permanganate to di-*threo*-9,10,12,13-tetrahydroxystearic acid, m.p. 148°, and this was identical with an authentic sample.

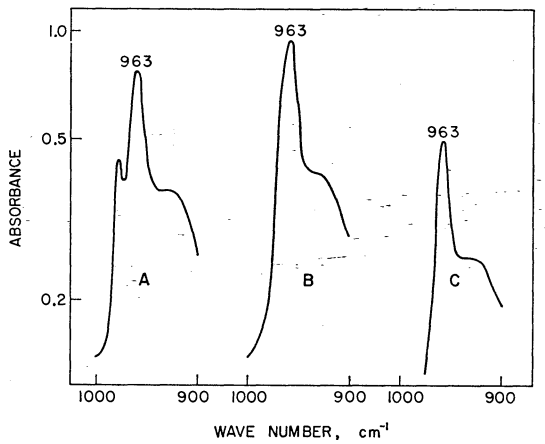

FIG. 2. Infrared absorption at 900–1000 cm^{-1}. A, fatty acids fraction, mainly trans–trans non-conjugated diene. B, pure *trans*-9-*trans*-12-octadecadienoic acid from chilopsis oil. C, *trans*-9-octadecenoic (elaidic) acid.

The most soluble fraction of the fatty acids of the oil (F4) was examined (as methyl esters) by gas chromatography. The emergence time of the major constituent was identical with that of methyl linoleate. Its identity as linoleate was confirmed by oxidizing a part of fraction F4 by alkaline permanganate. The product was the expected di-*erythro*-9,10,12,13-tetrahydroxystearic acid, m.p. 173–174°.

EXPERIMENTAL

Ultraviolet absorption measurements were made in cyclohexane solution with a Beckman DU spectrophotometer. Infrared spectra were determined in carbon disulphide 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 b.p. 30–60°.

Chilopsis Seed Oil

Seed of *C. linearis* (Cav.) Sweet was obtained from a reliable commercial source. It was ground and extracted by stirring with petroleum ether at 25°. The solvent was removed at 25–30° in a current of nitrogen. The oil had iodine value 149, refractive index 1.4923 at 25°, and 1.3% of free fatty acid as oleic. Ultraviolet absorption maxima were at 233, 263, 272, and 283 mμ; infrared maxima were at 960 s and 986 vs cm^{-1}.

Isolation of trans-9-trans-12-Octadecadienoic Acid

The oil (54 g) was hydrolyzed at 25° by allowing it to stand for 18 hours with 6% ethanolic sodium hydroxide under nitrogen. The unsaponifiable matter was removed by extraction with petroleum ether. The fatty acids were collected in petroleum ether and the solvent was removed under reduced pressure at 25° in a current of nitrogen. The acids (47 g) were dissolved in acetone (20 ml/g) and the solution was cooled stepwise to −15, −50, and −70°. Fatty acid crystals were collected at each stage and their infrared spectra were taken (Table I).

TABLE I

Crystallization of acids of chilopsis oil

Fraction	Crystallizing temp., °C	Yield, g	Main component, as shown by infrared absorption
F1	−15	2.6	Saturated acids
F2	−50	17.8	Acid with isolated trans bonds
F3	−70	12.5	Conjugated triene acid
F4	(Filtrate)	11.3	Ordinary cis-unsaturated acids
F2 recrystallized:			
F21	−19	3.7	Conjugated trans–trans diene
F22	−40	7.1	Isolated trans–trans diene
F23	(Filtrate)	6.7	Conjugated trans–trans–cis triene
F22 recrystallized:			
F221	−20	3.4	Isolated trans–trans diene
F222	−45	2.6	Isolated trans–trans diene

Fractions F221 and F222 were mainly isolated trans–trans diene acid. The ultraviolet spectra showed that they were free of conjugated triene acid but there was 27% of conjugated diene acid in F221 and 11% in F222. Fraction F222 (2.6 g) was treated with maleic anhydride in benzene (3) to form the adduct of the conjugated acid. The benzene was evaporated in a current of nitrogen and the residue was leached with pentane. The pentane solution, cooled to −20°, yielded 1.96 g of *trans*-9-*trans*-12-octadecadienoic acid, m.p. 26.5–27.0°, n_D^{27} 1.4655; (lit. m.p., 25° (4), 27.8–28.4° (5); n_D^{27} 1.4641 (4)). The melting point was unchanged in admixture with linelaidic acid prepared by stereomutation of pure linoleic acid with Poutet's reagent (6) or with selenium. The ultraviolet spectrum had no peaks in the regions 233 mμ or 272 mμ and the content of conjugated acids was calculated to be less than 0.3%. The infrared spectrum had ν_{max} 963 vs cm^{-1} and no other peaks in the region 900–1000 cm^{-1} except the carboxyl peak at 930 cm^{-1} (Fig. 2). The intensity of the peak at 963 cm^{-1} was approximately twice that of pure elaidic acid, determined under the same conditions.

Linelaidic acid, prepared by the usual stereomutation of linoleic acid with selenium, was found by ultraviolet absorption to contain about 16% of conjugated acids. They were removed by adduction with maleic anhydride. It was necessary to start with linoleic acid entirely free from oleic acid, otherwise the product was contaminated with elaidic acid, which is very difficult to remove by crystallization.

The amount of *trans*-9-*trans*-12-octadecadienoic acid isolated initially was 4% of the total fatty acids of chilopsis oil. More was obtained by working up other fractions, especially fraction F221. The intensity of the infrared peak at 963 cm^{-1} for fractions containing little or no conjugated triene gives an indication of their content of the *trans*-9-*trans*-12 acid. Taking these into account and allowing for losses in crystallization, it is estimated that the oil contains about 15% of this acid.

Hydrogenation and Oxidative Splitting

The diene acid (0.12 g) was hydrogenated in ethanol with Adams catalyst. It absorbed 2 moles of hydrogen. The resulting solution was concentrated and cooled, giving stearic acid, m.p. and mixed m.p. 68.5–69.5°.

A portion of the diene acid (0.14 g) was subjected to oxidative splitting by permanganate–periodate (7). The acidic products were recovered and esterified by our usual procedure (8) and the methyl esters were examined by gas chromatography. Large peaks corresponding to hexanoate and azelate were observed. There were no other peaks of significant area.

p-Phenylphenacyl Ester

The diene acid (0.2 g) was treated with 2-bromo-4′-phenylacetophenone by the ordinary method (9). The product, crystallized from ethanol and then from acetone, was *p*-phenylphenacyl-*trans*-9-*trans*-12-

octadecadienoate, m.p. 51.5–52.5°. The melting point was unchanged in admixture with an authentic sample prepared from linelaidic acid. Anal. Calc. for $C_{32}H_{42}O_3$: C, 80.97; H, 8.92. Found: C, 80.86; H, 8.94. The melting point of 73–75° reported by earlier workers (10) is considered to be incorrect. Their sample, prepared from linelaidic acid, was not characterized by analysis.

p-Phenylphenacylelaidate was prepared and analyzed. It melted at 71.5–72.5° (lit. 72–73° (10)). Anal. Calc. for $C_{32}H_{44}O_3$: C, 80.61; H, 9.30. Found: C, 80.67; H, 9.12.

Tetrahydroxy Derivative

The diene acid (1.0 g) was oxidized by alkaline permanganate (11) for 20 minutes at 10° and the product was crystallized twice from ethanol, giving the higher-melting racemic form of di-*threo*-9,10,12,13-tetrahydroxystearic acid, m.p. 148°. The melting point was unchanged in admixture with an authentic sample of the acid prepared from pure linelaidic acid.

Linoleic Acid

Fraction F4 (see Table I), containing the most soluble acids from the oil, was freed from conjugated diene and triene acids by treating it twice with maleic anhydride in benzene and discarding the adducts. Two grams of the remaining acids, which were soluble in petroleum ether, was oxidized by alkaline permanganate for 10 minutes at 7°. The crude product was washed with hot ethyl acetate, crystallized from ethanol, and washed successively with ethyl acetate and acetone. The product was pure di-*erythro*-9,10,12,13-tetrahydroxystearic acid, m.p. 173–174°, alone and mixed with an authentic sample.

DISCUSSION

Although most unsaturated fatty acids in seed oils occur in the cis form, exceptions are known. Until recently, all of the exceptions were acids which had one or two trans bonds in a conjugated system, e.g. α-eleostearic acid. In 1962, however, Bagby and co-workers (12) reported the occurrence of two acids having a trans bond but not having conjugated unsaturation. They were *trans*-5-octadecenoic acid and *trans*-5-*cis*-9-*cis*-12-octadecatrienoic acid, both found in the seed oil of *Thalictrum polycarpum*.

The present work describes the occurrence of an acid containing two non-conjugated trans bonds, viz. *trans*-9-*trans*-12-octadecadienoic acid. It is notable that this acid and *trans*-10-*trans*-12-octadecadienoic acid (2) are the only two isomers of linoleic acid known so far to occur in seed oils and that both are found in the oil of *C. linearis*, along with linoleic acid itself. Hilditch has pointed out (13) that seeds containing conjugated triene acid (eleostearic) do not seem to synthesize the corresponding non-conjugated acid, linolenic. It is evident that this apparent incompatibility does not extend to the diene acids since chilopsis oil is shown to have both conjugated and non-conjugated octadecadienoic acids.

The *trans*-9-*trans*-12-acid is considered to be a natural component of the glycerides and not an artefact, for the following reasons. The seed showed no evidence of deterioration. The oil was freshly extracted and had a normal content of free fatty acid, 1.3%. The infrared spectrum of the oil showed the presence of isolated trans unsaturation when examined immediately after extraction. Furthermore, the oil and its acids were handled under very mild conditions throughout the work. The estimated content of *trans*-9-*trans*-12-octadecadienoic acid is about 15% of the total fatty acids, hence most or all of it must have been in combination in the glycerides.

A rough calculation of the composition of the total fatty acids of the oil, based mainly on the spectral analysis of the various fractions, gives the following percentages: saturated acids, 5; *trans*-10-*trans*-12-octadecadienoic, 12; *trans*-9-*trans*-12-octadecadienoic, 15; *trans*-9-*trans*-11-*cis*-13-octadecatrienoic, 25; linoleic, 25; undetermined, 18.

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