OCCURRENCE OF TRANS-3-HEXADECENOIC ACID IN A SEED OIL[1]

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ABSTRACT

trans-3-Hexadecenoic acid was found to be a component of the glyceride oil of seeds of *Helenium bigelowii* A. Gr. It constituted about 10% of the total fatty acids. The acid was isolated and identified by oxidative cleavage and by its infrared and nuclear magnetic resonance (n.m.r.) spectra. Reaction with alkaline permanganate gave 3-hydroxypalmito-γ-lactone. Mild alkaline treatment of methyl *trans*-3-hexadecenoate caused a shift of the double bond to the 2-position. A sample of *Helenium hoopesii* seed oil did not contain any *trans*-unsaturated acid.

Glyceride acids with unsaturation in the 3-position were not known to occur in seed oils until very recently. Two such acids were reported in 1964, viz. a 3,9,12,15-tetraene acid, found by Hopkins and Chisholm in a species of *Tecoma* (Bignoniaceae) (1), and a 3,9,12-triene acid, found by Bagby and co-workers in a species of *Calea* (Compositae) (2). Both of these are C_{18} acids.

In the present work, the seed oil of *Helenium bigelowii* A. Gr. (family Compositae) was found to contain a substantial amount of a *trans*-monoene acid which proved to be *trans*-3-hexadecenoic acid. This species of *Helenium* is a herbaceous plant, native to North America, and is grown as a flowering perennial. A sample of a related species, *H. hoopesii* A. Gr., did not contain any *trans* fatty acid.

The seed oil of *H. bigelowii* had no conjugated unsaturation but its infrared spectrum revealed the presence of isolated *trans* olefinic unsaturation (ν_{max} = 960 cm^{-1}, C—H out-of-plane bending mode (3)). The oil was converted to methyl esters and submitted to gas–liquid chromatography (g.l.c.). The chromatogram indicated the presence of 12% of hexadecenoic acid.

The mixed acids were crystallized fractionally from acetone at low temperatures and the fractions were examined by g.l.c. and by infrared absorption. It was evident that the component responsible for *trans* absorption was in the hexadecenoic acid fraction. Repeated crystallization of this fraction from acetone at low temperatures gave a pure acid, m.p. 53–54°. Its methyl ester had an infrared maximum at 962 cm^{-1}, equal in intensity to the same peak in the spectrum of methyl elaidate, confirming the presence of one *trans* double bond.

Hydrogenation of the acid gave palmitic acid, establishing its structure as a straight chain, C_{16} acid. Oxidative cleavage gave tridecanoic acid, showing that the double bond is at the 3-position. The compound is therefore *trans*-3-hexadecenoic acid (I). The melting point is in agreement with recorded data (3). The structure was confirmed by the n.m.r. spectrum, which had the following bands (tetramethylsilane = 0):

Chem. shift, δ	Type of band	No. protons	Assignment
5.56	Quintuplet	2	$CH{=}CH$
3.07	Essentially doublet	2	$=CHCH_2CO$
2.04	Multiplet	2	$CH_2CH=$
0.89	Multiplet	3	CH_3

$CH_3(CH_2)_{11}CH{=}CHCH_2COOH$

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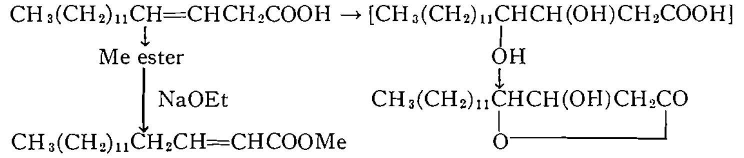
The band at $\delta = 3.07$ is assigned to the methylene protons in the grouping $=CHCH_2CO$ on the basis of its chemical shift and its essentially doublet structure. This is supported by the absence of the band characteristic of the ordinary methylene group adjacent to carboxyl, which would appear at $\delta = 2.3$ (4). Furthermore, the band given by methylene protons adjacent to an isolated double bond ($\delta = 2.04$) represents only two protons.

Hydroxylation of the acid by alkaline permanganate gave a lactone, judged to be 3-hydroxypalmito-γ-lactone (II), because of the known ease of formation of γ-lactones from hydroxy acids. It had infrared maxima at 3540s (OH stretching) and 1763, 1785vs (C=O stretching in γ-lactone (5)).

I



$$CH_3(CH_2)_{11}CH{=}CHCH_2COOH \rightarrow [CH_3(CH_2)_{11}\underset{OH}{CH}CH(OH)CH_2COOH] \rightarrow CH_3(CH_2)_{11}CHCH(OH)CH_2CO\ (\text{lactone, }O\text{ bridge})$$

$$CH_3(CH_2)_{11}CH{=}CHCH_2COOH \rightarrow \text{Me ester} \xrightarrow{\text{NaOEt}} CH_3(CH_2)_{11}CH_2CH{=}CHCOOMe$$

II

III

The ester of *trans*-3-hexadecenoic acid was heated with ethanol containing sodium ethoxide according to the procedure of Linstead and Noble (6). This caused a shift of the double bond to the 2-position (III), as shown by the infrared and n.m.r. spectra. A strong C=C stretching band appeared at 1653 cm^{-1} in the infrared (αβ-unsaturated ester (7)). This bond shift, produced under relatively mild conditions, is further proof that the original double bond is at Δ^3. The n.m.r. spectrum of the product had a typical ABX_2 pattern with multiplets centered at $\delta = 5.7$ and 7.0, representing the two olefinic protons. The coupling constant, J_{AB}, was 16 c.p.s., showing that the double bond is *trans* (8).

The amount of *trans*-3-hexadecenoic acid was about 10% of the total fatty acids of the oil. There was some evidence of a *trans* polyenoic acid.

EXPERIMENTAL

Infrared spectra were determined in carbon disulphide in a Perkin–Elmer model 21 spectrophotometer with sodium chloride prism. Nuclear magnetic resonance spectra were measured at 60 Mc/s on a Varian model A-60 spectrometer in deuterochloroform solution with tetramethylsilane as internal standard. Gas–liquid chromatography was carried out with a diethylene glycol – succinic acid polyester as liquid phase, on Celite, and a thermal conductivity detector. Petroleum ether refers to the fraction of b.p. 30–60°. Melting points were determined in capillary tubes and are corrected.

Helenium bigelowii Seed Oil

Seed of *Helenium bigelowii* A. Gr. was obtained from a reliable commercial source. Extraction of the ground seed with petroleum ether yielded 15% of oil. The oil had an infrared absorption peak at 960 cm^{-1} with intensity equivalent to a content of about 14% of *trans*-monoene fatty acid. There was no peak in the region 980–995 cm^{-1} (no conjugated unsaturation). A portion was converted to methyl esters. The mixed esters were analyzed by g.l.c. at 185°. The percentages of the various acids were (mean of three determinations): palmitic 10, hexadecenoic 12, octadecenoic 8, octadecadienoic 65, octadecatrienoic 5. There were no acids in the C_{20}–C_{22} region.

Isolation of the Acid

The oil was hydrolyzed by refluxing for 30 min with 6% ethanolic potassium hydroxide and the unsaponifiable matter was removed. The acids (31.2 g) were dissolved in 280 ml of acetone and the solution was cooled to −17° and then to −37°. Fatty acid crystals were filtered off at each stage and were examined by g.l.c. and by infrared absorption.

Fraction	Crystallizing temp., °C	Yield, g	Main component
F1	−17	3	Palmitic acid
F2	−37	4	*trans*-Hexadecenoic acid
F3	Filtrate	23	Octadecadienoic acid

Most of the *trans* acid was in fraction F2, which was solid. This fraction was crystallized from 30 ml of acetone at −20°. The crystals (2.55 g) consisted of 85% hexadecenoic acid and 15% palmitic acid, determined by g.l.c. One gram of this product, crystallized twice from acetone at −5°, yielded 118 mg of pure *trans*-3-hexadecenoic acid, m.p. 53–54° [lit. 53.5–55.0° (3)].

Anal. Calcd. for $C_{16}H_{30}O_2$: C, 75.5; H, 11.9. Found: C, 75.7; H, 12.25. Its n.m.r. spectrum is described above.

The methyl ester was prepared. It had ν_{max} = 962vs cm^{-1}, with intensity equivalent to one *trans* double bond.

Hydrogenation and Oxidative Cleavage

A portion of the acid (0.1 g) was hydrogenated in methanol with Adams platinum catalyst. It gave palmitic acid in quantitative yield, m.p. 62.0–62.5° alone and mixed with palmitic acid.

The monoenoic acid (0.06 g) was treated with permanganate–periodate by von Rudloff's procedure (9). On acidification, the reaction mixture gave a precipitate which was collected and crystallized once from acetone and once from pentane. It was tridecanoic acid, m.p. 38–39° alone and mixed with an authentic sample. The other products were extracted from the filtrate, esterified, and examined by g.l.c. There was no appreciable amount of any other monobasic acid.

3-Hydroxypalmito-γ-lactone

trans-3-Hexadecenoic acid (0.5 g) was dissolved in 500 ml of water containing 0.5 g of sodium hydroxide, mixed with 50 ml of 1% aqueous potassium permanganate, and allowed to stand at 20° for 6 min. The excess permanganate was destroyed, the solution was acidified and cooled in ice, and the precipitate was filtered off, dried, and washed with pentane to remove non-hydroxylic by-products. The residual product (0.14 g) was crystallized three times from ethanol at −20°, giving 3-hydroxypalmito-γ-lactone, m.p. 71–72°.

Anal. Calcd. for $C_{16}H_{30}O_3$: C, 71.1; H, 11.2. Found: C, 70.8; H, 11.0.

It formed glistening white flakes, moderately soluble in ethanol but insoluble in petroleum ether. It had ν_{max} = 3540s (OH), 1763, and 1785vs (γ-lactone). There was no peak in the region of acid carbonyl (ca. 1705 cm^{-1}).

Isomerization of the Ester

Methyl *trans*-3-hexadecenoate (0.3 g) was dissolved in 50 ml of absolute ethanol to which 0.035 g of sodium metal had been added. The solution was refluxed for 12 h, diluted with water, acidified, and extracted with petroleum ether. The extract contained some free acid. It was reesterified by methanol – hydrogen chloride. The liquid product (0.22 g) had ν_{max} 1653s (αβ-unsaturated ester). A model compound, methyl *trans*-2-octadecenoate, had a similar strong band at 1653 cm^{-1}.

Helenium hoopesii Seed Oil

Seed of *H. hoopesii* A. Gr. yielded 16% of oil, n_D^{25} 1.4753. Its ultraviolet spectrum had no maxima in the region 230–320 mμ (no conjugated diene or triene). The infrared spectrum showed much unsaturation (ν_{max} = 3000 cm^{-1}) but no *trans* double bonds (ca. 962 cm^{-1}). Analysis by g.l.c. showed the following percentages of fatty acids: palmitic 9; hexadecenoic, less than 1; octadecenoic 8; octadecadienoic 83.

DISCUSSION

Occurrence of a mono-unsaturated Δ^3 acid in seed oil fatty acids has not been observed previously. However, *trans*-3-hexadecenoic acid was detected by Debuch in 1961 in the leaves of two plant species (10) (not Compositae) and the same acid was isolated from an alga by Klenk and Knipprath in 1962 (3). Two polyenoic acids with unsaturation at Δ^3 are known to occur in seed oils (1, 2). The Δ^3 double bond in these three natural acids has the *trans* configuration. The 5-octadecenoic acid of *Thalictrum* seed oil is also *trans* (11). It is possible that ethylenic groupings near the carboxyl end of the chain tend to form in the *trans* configuration.

Natural monoenoic fatty acids include a group with various chain lengths in which the double bond is at position 3, 5, 9, 11, or 13, counting from the carboxyl end. This series, complete except for a Δ^7 acid, may be of significance in relation to the mechanism of biosynthesis. There is a strong likelihood that the longer chain monoenoic acids are formed by the addition of a 2-carbon unit at the carboxyl end of a shorter chain acid, e.g. 11-octadecenoic acid from 9-hexadecenoic acid (12), and 5-octadecenoic acid from 3-hexadecenoic acid.

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