Fatty Acids of Doxantha Seed Oil[1]

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Abstract

The seed oil of *Doxantha unguis-cati* was found to have the following fatty acid composition: *cis*-9-hexadecenoic 64, *cis*-11-octadecenoic 15, oleic 4, hexadecadienoic 1, linoleic 4, palmitic 12 and stearic <1%. The significance of the unusual fat composition is discussed in relation to biosynthetic mechanisms.

Introduction

REMARKABLE VARIATION has been noted in the composition of seed oils of the Bignoniaceae family (1). Examination of the species *Doxantha unguis-cati*, described herein, shows that its seed oil is unique in composition, having 64% of *cis*-9-hexadecenoic acid in the glyceride acids. Previously, the highest known content of this acid in seed oils was 20–23% (2,3).

A second unusual feature of Doxantha seed oil is the presence of 15% of *cis*-11-octadecenoic acid. This acid has been identified with certainty in only one other seed oil, *Asclepias syriaca* (4), although indications of minor amt have been seen in other species.

Doxantha unguis-cati (L.) Miers (syn. *Bignonia tweediana Lindl.*) is a yellow-flowered, climbing plant, native to the West Indies, and is grown in warm climates under the common name Cat's Claw.

Procedure and Results

The oil had a moderate degree of unsaturation and its IR and UV spectra showed no unusual absorption. It was converted to methyl esters and examined by gas-liquid chromatography (GLC), which indicated a large proportion of hexadecenoic acid along with other C_{16} and C_{18} acids. The saturated and dienoic acids were removed by fractional crystallization at low temp. The remaining mixture of monoenoic acids was esterified and separated by preparative GLC into hexadecenoic and octadecenoic acids. Upon oxidative cleavage by permanganate-periodate (5), the hexadecenoic acid portion was found to be almost entirely the Δ^9 isomer. The C_{18} portion was shown by similar treatment to consist of 11-octadecenoic and 9-octadecenoic acids in a ratio of 4:1.

The most-soluble fraction of the mixed acids was also submitted to oxidative cleavage and the octadecadienoic acid was found to be ordinary linoleic acid.

The pure hexadecenoic acid was converted by alkaline permanganate to *erythro*-9,10-dihydroxyhexadecanoic acid, proving that the original acid was the *cis* isomer. The other unsaturated acids also had the *cis* configuration since the IR spectrum of the oil had no maximum in the region of *trans* unsaturation (965 cm^{-1}). The percentage composition of the total fatty acids was estimated as follows:

Acid	Wt %	
	Sample 1	Sample 2
Palmitic	12	12
9-Hexadecenoic	64	69
Hexadecadienoic	1	<1
Stearic	<1	<1
11-Octadecenoic	15	14
Oleic	4	
Linoleic	4	4

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Experimental

GLC was carried out with a diethyleneglycol succinic acid polyester on Celite as the liquid phase, and a thermal conductivity detector. Components of injected samples were identified by comparison of their emergence times with those of reference standards, determined under identical conditions. Petroleum ether refers to the fraction of bp 30–60C.

Doxantha Seed Oil. The seed was obtained from reliable commercial sources. Sample 1 was ground and extracted with petroleum ether, yielding 29% of oil (air-dry basis) with iodine value (I.V.) 78.8. The IR spectrum (in CS_2) was that of a simple glyceride oil with no evidence of cyclic, epoxy, hydroxy or keto groups, conjugated unsaturation, or *trans* unsaturation.

The oil was hydrolyzed by alkali under nitrogen, freed of unsaponifiable matter, and converted to methyl esters. The mixed esters were analyzed by GLC at 185C. The percentages of the various acids were as given above, except that oleic and 11-octadecenoic esters emerged as one peak. There were traces of acids in the C_{20}–C_{22} region.

Identification of Acids. The mixed acids (15.6 g) were dissolved in 140 ml of acetone and the solution was cooled stepwise to −10C, −27C and −40C. Fatty acid crystals were filtered off at each stage. A sample of each fraction was esterified and examined by GLC.

Fraction	Crystallizing temp, °C	Yield, g	I.V.	Main component acids
F1	−10	3.29	22.8	Palmitic and hexadecenoic
F2	−27	0.31	.....	.....
F3	−40	2.25	94.8	Hexadecenoic and octadecenoic
F4	(filtrate)	9.01	.....	C_{18} and C_{18} mono- and di-unsaturated

Fraction F4 was crystallized twice from small volumes of acetone at −40C, yielding a mixture of hexadecenoic and octadecenoic acids, shown by GLC to be free of saturated and dienoic acids. A portion was subjected to oxidative cleavage by permanganate-periodate (5) and the fragments were esterified and examined by GLC. Heptanoic, nonanedioic and undecanedioic acids were the main products, along with a small proportion of nonanoic acid, showing that the original acids were chiefly 9-hexadecenoic and 11-octadecenoic. Cleavage of Fraction F3 gave a similar result.

The remainder of the monoenoic acid mixture was separated by preparative GLC (as methyl esters) into hexadecenoic acid and octadecenoic acid. A portion of the hexadecenoic acid (50 mg) was treated with alkaline permanganate solution for 5 min at 0C and the product was crystallized from ethyl acetate. It was *erythro*-9,10-dihydroxyhexadecanoic acid, mp and mixed mp 126.5–127.5C.

Upon oxidative cleavage, the hexadecenoic acid gave heptanoic and nonanedioic acids with only traces of other fragments. It was thus almost entirely 9-hexadecenoic acid. The octadecenoic acid portion gave, as cleavage products, heptanoic, nonanoic, nonanedioic, and undecanedioic acids. The proportions of the four fragments showed that the C_{18} portion consisted of 11-octadecenoic and 9-octadecenoic acids in a ratio of 4:1.

The most soluble fraction from the crystallization of F4 contained 16% of octadecadienoic acid. Cleavage of this fraction gave a proportionate amt of hexanoic acid in addition to the expected heptanoic and nonanoic acids from the monoenoic acids. Consequently, the octadecadienoic acid is judged to be mainly the 9,12-isomer, i.e. linoleic acid. The dibasic acids from the cleavage reaction were almost entirely nonanedioic and undecanedioic acid.

Discussion

Bignoniaceae seed oils studied previously have had a large proportion of dienoic or trienoic fatty acids but no appreciable amt of hexadecenoic acid. In contrast, *Doxantha* fatty acids are predominantly monoenoic and the major component is *cis*-9-hexadecenoic acid. Occurrence of hexadecenoic acid in seed oils is common in amt under 1% (6) but in large amt (10% or more) the acid has appeared only in a few species of Proteaceae (2,3), in one species of Asclepidacae (4), and in the present example in Bignoniaceae.

11-Octadecenoic acid is rare in seed oils. In *Doxantha* and *Asclepias syriaca* (4), the only major sources known so far, it occurs along with an unusually large proportion of 9-hexadecenoic acid. This is presumptive evidence for the formation of 11-octadecenoic acid in the seed by the addition of a C_2 unit to 9-hexadecenoic acid at the carboxyl end of the chain. Additional evidence was obtained for this conversion during a study of the maturing seed of *A. syriaca* (7). The same mechanism for the biosynthesis of monoenoic acids in seeds has been deduced by Downey and Craig for the sequence: oleic → 11-eicosenoic → 13-docosenoic acids (8).

9-Hexadecenoic acid may also be formed by a chain-lengthening process, starting from a Δ^3 acid, similar to the mechanism observed by Bloch and others in certain bacteria (9). Discovery of a Δ^3 monoenoic acid in seeds (3-hexadecenoic) (10) lends support to this hypothesis. The supposed intermediate acids, 3-decenoic, 5-dodecenoic, and 7-tetradecenoic, which would lead to 9-hexadecenoic, have not been found in seed oils as yet. However, the synthesis may be so rapid that no appreciable amt of the intermediate acids remains in the mature seed.

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A New Acid from *Calea urticaefolia* Seed Oil: *trans*-3, *cis*-9,*cis*-12-Octadecatrienoic Acid[1]

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Abstract

A major constituent fatty acid (31.2%) from *Calea urticaefolia* (Mill.) DC. seed oil is the previously unknown *trans*-3,*cis*-9,*cis*-12-octadecatrienoic acid. The oil also contains 2.2% of an unidentified acid and others with gas-liquid chromatographic characteristics that correspond to the conventional fatty acids: myristic, 0.1%; palmitic, 9.3%; stearic, 2.9%; oleic, 5.3%; and linoleic, 48.9%.

Introduction

IT HAS BEEN POINTED OUT previously (5) that seed oil from the genus *Calea* has acids similar to the *trans*-5,*cis*-9,*cis*-12-octadecatrienoic acid from *Thalictrum polycarpum* seed oil. However, the unknown C_{18}-trienoic acid from *Calea urticaefolia* (Mill.) DC. (family Compositae) seed oil has an equivalent chain length (ECL) (20) which differs slightly from that of the Thalictrum acid. This paper reports the isolation of the Calea acid and its characterization as a previously unknown C_{18}-trienoic acid.

Experimental

General Methods. Gas-liquid chromatographic (GLC) analyses were carried out with a Burrell Kromotog K-5, and the retention values were treated as described by Miwa et al. (20). The operating conditions and description of the columns are the same as those mentioned in other communications (6,27). Except where noted, methyl esters were prepared from methanol with acid catalyst. When desired for characterization work, esters were saponified by refluxing 0.5 hr with 2*N* ethanolic potassium hydroxide. Melting points were determined with a Fisher-Johns block. IR spectra were measured in a 1-mm cell with a Perkin-Elmer model 137-0001 recording spectrophotometer. Quantitative values were obtained in carbon disulfide by the baseline technique (21), and the quantity of isolated *trans*-unsaturation was obtained by comparing the extinction coefficient of the "unknown" with that of methyl elaidate. Except where noted, UV spectra were measured in ethanol with a Beckman DU spectrophotometer. The nuclear magnetic resonance (NMR) spectrum was measured with a Varian A-60 spectrometer on a carbon tetrachloride solution containing tetramethylsilane.

Preparation of Mixed Fatty Acid Methyl Esters. Coarsely ground seed (11.12 g) of *Calea urticaefolia* was extracted overnight in a Soxhlet apparatus with petroleum ether (bp 32–57C). The bulk of the solvent was removed under nitrogen, and the remainder was removed *in vacuo* with a rotating evaporator. The oil contained 1.4% free acid calculated as oleic acid. IR spectroscopy indicated 34.9% of isolated *trans*-unsaturation. The UV spectrum indicated no conjuga-

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