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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TABLE I

GLC Analyses of *Calea urticaefolia* Mixed Esters and Their Hydrogenation Products

Type of acid	Equivalent chain length		Ester, %	
	Apiezon L	LAC-2-R 446	Original	Hydrogenated
14:0	14.0	14.0	0.1	0.1
15:0	15.0	15.0	....	trace
16:0	16.0	16.0	9.3	8.6
17:0	17.0	17.0	....	0.2
18:0	18.0	18.0	2.9	90.3
18:1	17.7	18.4	5.3	....
18:2	17.7	19.0	48.9	....
18:3	17.7	19.6	31.2	....
20:0	20.0	20.0	....	0.4
Unknown	?	20.4	2.2	....

tion: λ max 269 mμ (isooctane) $E^{1\%}_{1cm}$ 1.46; no max λ 232 $E^{1\%}_{1cm}$ 6.40.

C. urticaefolia seed oil (2.03 g) was saponified and the unsaponifiables (0.06 g) and free acids (1.82 g) were obtained in the usual manner. The fatty acids were esterified and IR spectral analysis showed 34.8% of isolated *trans*-unsaturation. Their GLC analyses are shown in Table I. Hydrogenation of the mixed esters in ethanol with platinum catalyst yielded mainly methyl stearate (Table I). Additional esters were prepared as needed.

Fractionation of Methyl Esters. The mixed methyl esters (6.41 g) were dissolved in mutually saturated hexane (30 ml) and acetonitrile (120 ml) (19,26) and subjected to a 600-transfer countercurrent distribution (CCD) in a 200-tube automatic Craig-Post apparatus. The methyl esters were divided evenly among the first three tubes, and 40 ml of hypophase were placed in each of the remaining tubes. The automatic operation of the instrument introduced 10 ml of equilibrated hexane to tube 0 at each transfer stage. As hyperphase progressed past tube 200, it was decanted into a fraction collector. Two transfers/tube were combined and successively collected until 200 fractions had been obtained. The solvent was evaporated under reduced pressure from the content of selected tubes. The weight-distribution plot is shown in Figure 1. On the basis of GLC analyses of significant fractions, transfers 461–582 were combined to give 1.79 g of a fraction containing 96.8% of the C_{18}-trienoate together with 0.5% of C_{16}-monoenoate, 0.7% of C_{18}-monoenoate, 0.2% of C_{18}-dienoate, and 1.8% of an unknown (ELC from the polar column was 20.4). IR analyses indicated 97.3% of isolated *trans*-unsaturation. The conc absorbed 2.9 moles of hydrogen/mole of C_{18}-ester to yield methyl stearate. A Wijs iodine value (I.V.) of 264.6 (theory: $C_{19}H_{32}O_2$, 260.4) was obtained, and the free acid had a neutralization equivalent of 284.9 (theory: $C_{18}H_{30}O_2$, 278.4).

Isomerization of the Octadecatrienoic Acid. The UV spectrum of the octadecatrienoic acid indicated essentially no preformed conjugation: no max 232 mμ $E^{1\%}_{1cm}$ 26.3; λ max 268 mμ $E^{1\%}_{1cm}$ 13.5. Alkali isomerization (2) indicated 100 ± 10% of dienoic acid and 2.5 ± 0.8% of trienoic acids. Treatment of the acid with lipoxidase (18) showed 106.4% of polyunsaturated acid.

Permanganate-Periodate Oxidation of the Acid. A portion (0.277 g) of the free octadecatrienoic acid was oxidized with permanganate-periodate according to the method of Lemieux and von Rudloff (17). The resulting cleavage products were continuously extracted with ethyl ether. A portion (5%) of the ether extract was set aside for GLC analyses. The remainder of the ether extract was concentrated by distillation.

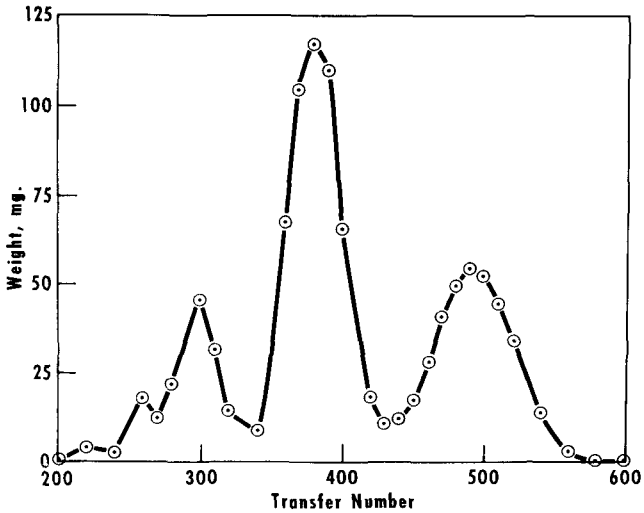

FIG. 1. CCD of *Calea urticaefolia* methyl ester with the solvent system hexane-acetonitrile.

Characterization of Cleavage Products. The conc of the octadecatrienoic acid cleavage products was steam distilled to yield 0.399 mmole of steam-volatile acid (by titration with 0.100*N* sodium hydroxide). The *p*-bromophenacyl ester was prepared from 0.010 g of steam-volatile acid according to the procedure of Stodola (28). The crude product (0.023 g) was recrystallized from 80% ethanol to yield a product melting at 63–65C. An additional recrystallization from 95% ethanol at 0C yielded a product melting at 67–68C. Admixtures with authentic *p*-bromophenacyl esters of hexanoic acid(mp 70.5–71C), pentanoic acid (mp 62C), octanoic acid (mp 63.5–64.5C), and heptanoic acid (mp 69–70C) melted at 69–70C, 56–59C, 42–47C and 59–63C, respectively. The aqueous residue, nonvolatile in steam, was saturated with sodium chloride and extracted with ethyl ether to yield 0.120 g of a semisolid. After the product was triturated with warm chloroform, a white solid (0.031 g) was obtained, mp 146–149C. Additional washing with chloroform yielded a solid melting at 148–150C. An admixture with authentic hexanedioic (adipic) acid (mp 152–153C) melted at 150–150.5C. The bis-*p*-bromophenacyl ester melted at 152.5–153C, and an admixture with authentic bis-*p*-bromophenacyl adipate (mp 153–154.5C) melted at 153–154C.

When a portion of the ethereal solution reserved for GLC analyses was chromatographed as the free acid, hexanoic acid was identified as the principal component together with small amounts (less than 3%) of other monobasic acids. The remainder of the etheral solution was esterified with diazomethane (3), and the solvent was removed *in vacuo*. GLC analyses showed the esters of adipic acid, caproic acid, and what appeared to be malonic acid. There respective area percentages were 40,25 and 20. Other components were present in amounts of less than 5%. Efforts to isolate malonic acid in pure form were not successful.

Partial Reduction of the Octadecatrienoic Acid. A portion (0.849 g) of the octadecatrienoic acid was reduced, in ethanol solution, with hydrazine hydrate as previously described (5). After 5.25 hr, the reaction was stopped by dilution with water and acidification with *N* hydrochloric acid. The product was taken up in the ethyl ether to yield 0.803 g of acid. GLC analyses showed that the product consisted of 10.8% of stearate, 11.9% of octadecatrienoate and 77.3% of a mixture of monoenes and dienes. I.R. analysis indicated 45.2% of isolated *trans*-unsaturation.

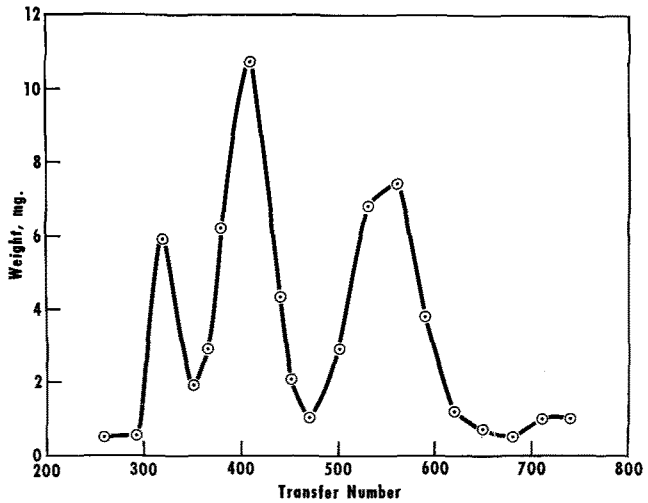

FIG. 2. CCD of the products from the partial reduction of the trienoic acid from *Calea urticaefolia*.

The trans-Unsaturated Monoene Dervied from the Octadecatrienoic Acid. Methyl esters (0.70 g) of the reduction mixture were fractionated by CCD as described previously. The esters were added to the first tube, and 5 ml of hyperphase were added at each transfer stage. The weight-distribution plot is shown in Figure 2. Transfers 368 through 452 were combined to yield 0.212 g of esters. GLC analyses showed 97.3% of C_{18}-monoene, and the IR spectrum indicated 21.9% of isolated *trans*-unsaturation. Crystallization of the monoene mixture from acetone at −45C gave 0.062 g of precipitate (33.8% of isolated *trans*-unsaturation). After the esters were saponified with *N* ethanolic potassium hydroxide, the resulting acids were crystallized from 70% ethanol to yield 0.013 g of a semisolid. An additional recrystallization yielded 0.007 g of a solid melting at 60–61.5C (λ max 10.35 μ K_{cs_2} 0.460, 101% of isolated *trans*-unsaturation).

The free acid was oxidized with permanganate-periodate (17), and the products were treated with diazomethane (3) to yield 0.011 g of esters. GLC analyses showed 74% of methyl pentadecanoate, 10% presumably of pentadecanal, and small amt (less than 4%) of other components.

Results and Discussion

The petroleum ether extract from *Calea urticaefolia* seed showed 34.9% of isolated *trans*-unsaturation. Nearly pure methyl octadecatrienoate was obtained by combination of transfers 461 through 582 from a CCD run (Fig. 1) in which the solvent system hexane-acetonitrile was used.

The presence of three double bonds in the octadecatrienoic acid was confirmed by I.V. (Wijs) and quantitative hydrogenation. IR spectroscopy indicated one isolated *trans* double bond. Alkali isomerization conjugated two of the double bonds; only ca. 2.5% of conjugated triene was noted. Treatment with

TABLE II

Shifts, Assignments, and Number of Protons Observed in the NMR Spectrum of Methyl Octadecatrienoate[a]

Assignment	τ values	Number of protons
CH_3 terminal	9.10	3
CH_2 in chain	8.68	10
CH_2 α to unsatuarted carbon	7.95	6
CH_2 of 1,4-diene	7.25	4
CH_2 α to carboxyl	7.04	
OCH_3	6.36	3
Olefinic H	4.63	6

[a] The chemical shifts are given in terms of τ values as defined by Tiers (30).

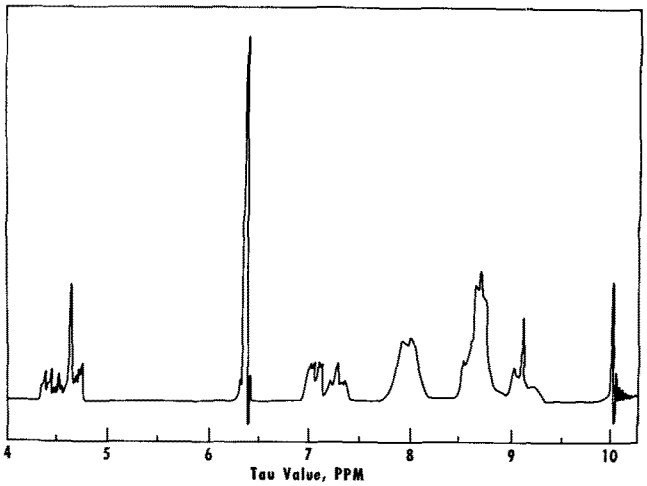

FIG. 3. NMR spectrum of the C_{18}-trienoic ester from *Calea urticaefolia*.

lipoxidase (18) also produced conjugated diene. Since the enzyme is known to be specific for *cis,cis*-methylene interrupted double bonds (14), the presence of this grouping in the trienoic acid was established.

When the octadecatrienoic acid was oxidized with permanganate-periodate, the cleavage products were caproic, adipic and malonic acids. The presence of malonic acid was unexpected because this fragment normally decomposes during oxidation.

Since isomerization data indicate that the one *trans* double bond must be separated from the *cis,cis*-methylene-interrupted double bonds by more than one methylene group [(6) for discussion and leading references], the cleavage products could have come from one of two structures:

cis-3,*cis*-6,*trans*-12-octadecatrienoic acid (A), or

trans-3,*cis*-9,*cis*-12-octadecatrienoic acid (B).

The UV spectrum of the alkali isomerization product favors structure B. There was no maximum at near 260 mμ as would be expected if two double bonds were conjugated with the carboxyl group (22).

The NMR spectrum (Fig. 3) is consistent with either structure A or structure B. The observed peaks were assigned based on these two structures, and the number of protons was determined by the relative areas (Table II). The presence of the 3,4-double bond is confirmed by a doublet at 7.04 τ (15) and by no triplet at 7.8 τ, which is indicative of the more shielded protons on the carbon α to a carboxyl group as in methyl stearate (29).

For further proof of structure, the octadecatrienoic acid was partially reduced with hydrazine hydrate. Such a treatment reduces double bonds in a random fashion without causing isomerization (4,23–25). The reduction mixture was fractionated by a combination of CCD and crystallizations to yield a *trans*-monoene shown to be *trans*-3-octadecenoic acid. This information proves that the C_{18}-trienoic acid is the previously unknown *trans*-3,*cis*-9,*cis*-12-octadecatrienoic acid.

Although the presence of 3,4-unsaturation in naturally occurring fatty acids is known, it has been limited to the *trans*-3-hexadecenoic acid in *Scenedesmus obliquus* (16) and in the leaves of *Spinacia oleracea* and *Antirrhinum majus* (10) and to 3-dodecenoic acid in the waxy constituents of sperm head oil (13).

Our work describing the characterization of a naturally occurring 18-carbon acid with *trans*-3,4-unsaturation extends the list of naturally occurring noncon-

jugated *trans*-unsaturated fatty acids from seed oils. Previously known acids were *trans*-5-octadecenoic acid and *trans*-5,*cis*-9,*cis*-12-octadecatrienoic acid from *Thalictrum polycarpum* (5) and *trans*-9,*trans*-12-octadecadienoic acid from *Chilopsis linearis* (7).

The *trans*-3,*cis*-9,*cis*-12-octadecatrienoic acid from *C. urticaefolia* seed oil is also a member of an expanding new class of naturally occurring fatty acids containing wide separation of double bonds (1,5,6,8,9,11 and 12).

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Chromatographically Homogeneous Lecithin from Egg Phospholipids[1]

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Abstract

Chromatographically homogeneous egg lecithin, as determined by TLC on Silica Gel G, has been isolated from crude egg phosphatides by column chromatography on alumina through modification of existing, lengthy methods. The modified method involved application of crude egg phosphatides to a column of alumina in the proportion of 1 g phosphatide/25 g alumina, and elution of the lecithin fraction with the 2-component solvent system chloroform:methanol, 9:1 by vol. This method of purification separated lecithin from other choline and non-choline components of crude phosphatides, avoided overloading of the alumina column, and made unnecessary the need for a second chromatographic fractionation of partially purified lecithin on silicic acid, which is needed in existing methods of purification of lecithin.

The use of fresh yolks permitted easier removal of pigment from the final product than was possible with commercially dried yolks.

Phosphatides extracted from dried yolks were much more highly colored than were the phosphatides extracted from fresh yolks and the color presisted through chromatography on alumina.

The fatty acid/phosphorus molar ratio of the purified lecithin was 2.00, which is the theoretical FA/P molar ratio of phosphatidylcholine; other materials with this ratio were not present.

[1] Presented at the AOCS Meeting, New Orleans, 1964.
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Introduction

COLUMN CHROMATOGRAPHIC METHODS primarily intended for the analytical fractionation of egg yolk phosphatides have received the attention of several investigators. The initial reports are those of Hanahan et al. (4), and Lea et al. (5). Hanahan et al. used alumina as the adsorbent, and eluted with 95% ethanol to separate choline from non-choline fractions, but were unable to obtain pure phosphatidylcholine free from lysolecithin, as shown by a subsequent report (3). Lea et al. used silicic acid as an adsorbent and eluted pure egg lecithin from a crude egg phospholipid mixture with chloroform:methanol, 8:2 by vol. The latter method is extremely time-consuming. With a more polar solvent system consisting of chloroform:methanol, 1:1 by vol, Rhodes and Lea (10) separated choline and non-choline fractions of egg phospholipids on an alumina column, but had to employ a second fractionation of the choline fraction on a column of silicic acid to remove lysolecithin from lecithin. None of the above-mentioned chromatographic methods were capable of separating pure egg lecithin from a mixture of egg phospholipids on a column of alumina.

Renkonen (9), in an investigation of the breakdown of pure synthetic lecithin on alumina adsorption columns, applied the synthetic lecithin to a column of alumina and successfully separated the pure lecithin from its degradation products (principally lysolecithin) with a solvent system consisting of chloroform:methanol, 9:1 by vol. This ratio of solvents is much less polar than the system employed by Rhodes and