166. *The Tetraenoic Acid of* Tecoma stans *Seed Oil.*

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A fatty acid, isolated from the seed oil of *Tecoma stans* (L.) H.B.K., is shown to be the hitherto unknown octadeca-*trans*-3,*cis*-9,*cis*-12,*cis*-15-tetraenoic acid. It constitutes about 19% of the total fatty acids.

Tetraenoic fatty acids are common in fish and animal oils but rare in vegetable oils. Until recently, the only example known in seed oils was the conjugated parinaric acid, octadeca-9,11,13,15-tetraenoic acid,[1] which occurs in several plant species. A non-conjugated tetraenoic acid, all-*cis*-octadeca-6,9,12,15-tetraenoic acid, was found by Craig and Bhatty in 1963 in the seed oil of *Onosmodium occidentale* and other Boraginaceae.[2]

During our investigations of Bignoniaceae seed oils [3-6] evidence of a tetraenoic acid was noted in the glycerides of the seed oil of *Tecoma stans* (L.) H.B.K., syn. *Stenolobium*

[1] Gunstone, " Introduction to the Chemistry of Fats and Fatty Acids," Chapman and Hall, London, 1958, p. 19.
[2] Craig and Bhatty, *J. Amer. Oil Chemists' Soc.*, 1964, **41**, 209.
[3] Hopkins and Chisholm, *J.*, 1962, 573.
[4] Chisholm and Hopkins, *J. Org. Chem.*, 1962, **27**, 3137.
[5] Chisholm and Hopkins, *Canad. J. Chem.*, 1963, **41**, 1888.
[6] Hopkins and Chisholm, *J. Amer. Oil Chemists' Soc.*, 1964, **41**, 42.

stans, Seem., *Bignonia stans*, L. This species is a shrub or small tree, native to tropical America, and is grown as an ornamental under the common name, Yellow Bells.

The oil was highly unsaturated. Its absorption spectra revealed the presence of *trans*-olefinic unsaturation but no double bonds in conjugation. It was converted into methyl esters and submitted to gas–liquid chromatography. The chromatogram showed the ordinary unsaturated fatty acids but also indicated the presence of a non-conjugated tetraenoic acid of 18 carbon atoms. Isomerization of the oil by alkali at 180° gave much conjugated diene and triene but only a trace of conjugated tetraene, suggesting that the tetraene acid, if present, had an unusual arrangement of double bonds.

Data from n.m.r. spectrum.

Chemical shift (δ) *	Type of band	No. of protons	Assignment
1·00	Triplet	3	CH_3·CH_2·CH=
1·40	Multiplet	4	−CH_2·CH_2·CH_2−
2·10	Multiplet	6	−CH_2·CH_2·CH=
2·83	Triplet	4	=CH·CH_2·CH=
3·05	Doublet	2	=CH·CH_2·COO−
3·67	Singlet	3	−COO·CH_3
5·39	Triplet	6	CH_2·CH=CH·CH_2
5·55	Multiplet	2	−CH=$CH$$CH_2$COO−

* Values with reference to tetramethylsilane = 0.

The supposed tetraene acid was concentrated from the total mixture of acids by low-temperature crystallization, followed by adduction with urea. The composition of the fractions was monitored by gas–liquid chromatography and by infrared absorption. It was evident that the unknown acid contained the *trans*-olefinic group. The acid was obtained pure, as methyl ester, by column chromatography of the final concentrate, employing the silica–silver nitrate solid phase of de Vries.[7] On hydrogenation, it absorbed four mols. of hydrogen to give methyl stearate, thus confirming the C_{18} straight chain and the presence of four double bonds or their equivalent. The ultraviolet and infrared spectra were consistent with an arrangement of four non-conjugated double bonds, one of which was *trans*. There was no evidence of other grouping such as allene, cyclopropyl, or cyclopropenyl.

The nuclear magnetic resonance spectrum of the methyl ester provided much structural information (Table). The pattern of the signal centred at δ 1·00 is that of the terminal methyl group in the system CH_3·CH_2·CH=CH− and is superimposable on the corresponding signal of methyl linolenate. Hence the tetraene acid is considered to have a Δ^{15} double bond. The unusual signal at δ 3·05 is assigned to the methylene protons in the grouping =CH·CH_2·CO·O−, on the basis of its chemical shift and its essentially doublet structure, thus placing a double bond at Δ^3. The allylic methylene (=CH·CH_2·CH=) is seen at the usual position, δ 2·83.[8] There is no signal at δ 2·3 as there would be if the ordinary α-methylene group in −CH_2·CH_2·CO·O−, were present.[8]

The signal for the usual olefinic bonds appears at 5·39. Its area represents six protons or three double bonds. The additional peak, centred at δ 5·55, representing two protons, is assigned to the protons at the Δ^3-double bond, whose chemical shift is altered by the proximity of the carbonyl group. The remaining protons are accounted for as shown in the Table. The total proton count was 30, estimated with reference to the methyl ester peak, confirming the formula $C_{17}H_{27}$·CO_2Me.

The occurrence of four protons in the allylic position, =CH·CH_2·CH=, requires at least three of the olefinic linkages to be in the familiar 1,4 relationship (methylene-interrupted), as in linoleic and linolenic acid. Since two of the double bonds are at positions 3 and 15, the only possible arrangements for the four double bonds are 3,6,9,15 or 3,9,12,15 or 3,6,12,15. The choice of the correct one could not be made by simple oxidative splitting,

[7] de Vries, *J. Amer. Oil Chemists' Soc.*, 1963, **40**, 184.
[8] Hopkins, *J. Amer. Oil Chemists' Soc.*, 1961, **38**, 664.

since all three structures would give propionic, malonic, and adipic acids. Accordingly, the acid was partially hydrogenated by hydrazine [9] and the monoene acids were obtained as a mixture of isomers by crystallization of the product at low temperatures. Oxidative splitting of these monoene acids by von Rudloff's method [10] gave hexanoic, nonanoic, azelaic, and dodecanedioic acids. There was no lauric or adipic acid. Hence, the acid must have double bonds at the 9 and 12 positions but not at the 6 position. It is therefore octadeca-3,9,12,15-tetraenoic acid (I). Similar results were obtained by partial hydroxylation, followed by hydrogenation and oxidative splitting, according to the procedure of Gunstone and Sykes.[11]

Further proof of the existence of a double bond at position 3 was obtained by isomerization. The pure tetraene ester was heated with sodium ethoxide in ethanol under conditions known to cause a shift of the double bond from the 3- to the 2-position.[12] The product, although not obtained pure, showed strong infrared absorption at 1650 and 1715 cm.$^{-1}$, characteristic of αβ-unsaturated esters. The spectral peak at 963 cm.$^{-1}$ (*trans*-CH=CH) was of very low intensity in the isomerized ester (II), showing that the newly-formed Δ^2-bond is *cis* and that the original Δ^3-bond must have been *trans*. There was no increase in absorption in the region 980—995 cm.$^{-1}$ during the isomerization, thus there was no shifting of the 9,12,15 double bond group into conjugation. It is concluded, therefore, that the Δ^3-bond in the original acid has the *trans*-configuration and that the other three double bonds are *cis*. The acid is thus octadeca-*trans*-3,*cis*-9,*cis*-12,*cis*-15-tetraenoic acid (I).

$$\mathrm{Me{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}[CH_2]_4{\cdot}\overset{t}{C}H{=}\overset{t}{C}H{\cdot}CH_2{\cdot}CO_2H} \quad \text{(I)}$$

$$\mathrm{Me{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}[CH_2]_4{\cdot}CH_2{\cdot}\overset{c}{C}H{=}\overset{c}{C}H{\cdot}CO_2H} \quad \text{(II)}$$

Natural fatty acids having unsaturation at the 3-position are rare. None has been found until now in a seed oil, although hexadec-*trans*-3-enoic acid is known in plant leaves [13] and in an alga.[14]

Experimental

Ultraviolet (u.v.) spectra were measured for cyclohexane solutions in a Beckman D.U. spectrophotometer. Infrared (i.r.) spectra were determined in carbon disulphide in a Perkin-Elmer model 21 spectrophotometer with sodium chloride prism. The nuclear magnetic resonance (n.m.r.) spectrum was measured at 60 Mc./sec. on a Varian model A-60 spectrometer in $CDCl_3$ 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. Light petroleum refers to the fraction of b. p. 30—60°.

Tecoma Seed Oil.—Seeds of *Tecoma stans* were ground and extracted with light petroleum and the solvent was removed in a current of nitrogen. The oil (23·0% of the seeds) had iodine value 209 (Wijs, 30 min.), n_D^{25} 1·4834, $\nu_{max.}$ 963 cm.$^{-1}$ (*trans*-CH=CH). There were no u.v. absorption maxima in the region 220—320 mμ (no conjugated unsaturation).

A sample of the oil was converted into methyl esters and analysed by gas–liquid chromatography (g.l.c.). The percentages of individual acids were (mean of 3 determinations): palmitic 6, stearic 3, octadecenoic 7, octadecadienoic 24, octadecatrienoic 41, octadecatetraenoic 19 ("carbon number" 19·9). There was less than 1% of hexadecenoic acid and there were no C_{20}–C_{22} acids. A second sample of seeds, obtained from a different source, gave results only slightly different from the above.

Isolation of the Acid (as Methyl Ester).—The oil (25 g.) was hydrolysed at 25° by an excess of 6% ethanolic potassium hydroxide. The unsaponifiable matter was removed by extracting the alkaline solution with light petroleum, and the acids were collected with suitable precautions

[9] Aylward and Sawistowska, *Chem. and Ind.*, 1962, 484.
[10] Lemieux and von Rudloff, *Canad. J. Chem.*, 1955, **33**, 1701.
[11] Gunstone and Sykes, *J.*, 1962, 3058.
[12] Linstead and Noble, *J.*, 1934, 610.
[13] Debuch, *Z. Naturforsch.*, 1961, **16***b*, 561.
[14] Klenk and Knipprath, *Hoppe-Seyler's Zeit. physiol. Chemie*, 1962, **327**, 283.

against oxidation.[4] The acids (21·6 g.) were dissolved in acetone (200 ml.) and the solution was cooled stepwise to −45, −60, and −73°. Fatty acid crystals were filtered off at each stage. Only the acids recovered from the final filtrate (4·5 g.) showed appreciable *trans*-absorption ($\nu_{max.}$ 963 cm.$^{-1}$). A total of 50 g. of oil was treated in this way, yielding 9·1 g. of acids from the filtrate after crystallizing at −73°. The acids were converted into methyl esters and the content of tetraene ester was found to be 45% (g.l.c.).

The esters (9·4 g.) were treated with urea (9·4 g.) in ethanol (170 ml.) and cooled stepwise to −20, −30, and −40°. Crystals of the fatty ester–urea complex were filtered off at each stage and urea (4·9 g.) was added to the filtrate each time. The ester fractions were recovered and analysed. The fraction (3·38 g.) obtained from the filtrate at −40° had 80% of tetraene ester. A portion (0·18 g.) of this fraction was dissolved in light petroleum and placed on a column of silica impregnated with silver nitrate.[7] Fractions were eluted by light petroleum–ether mixtures (90 : 10, 80 : 20, 70 : 30, 60 : 40) and monitored by g.l.c. The last fraction (0·089 g.) gave a single peak on the chromatogram and was judged to be pure *methyl octadecatetraenoate*. It had m. p. below −30°, $\nu_{max.}$ 963vs cm.$^{-1}$, n_D^{25} 1·4800. The absorption coefficient at 963 cm.$^{-1}$ was the same as that of methyl elaidate. The n.m.r. spectrum is described above. The ester was very unstable in air, developing an unpleasant fishy odour (Found: C, 78·2; H, 10·1. $C_{19}H_{30}O_2$ requires C, 78·6; H, 10·4%).

Hydrogenation and Location of Double Bonds.—The ester (52 mg.) was hydrogenated in ethanol with Adams catalyst. It absorbed 3·8 mol. of hydrogen, giving methyl stearate, m. p. and mixed m. p. 38—39°.

Partial reduction of the tetraene acid by hydrazine was carried out as follows.[9] The acid (1·5 g.) was added to hydrazine (2·7 g.) in 95% ethanol (15 ml.). The mixture was kept at 50—55° for 6 hr. while air was bubbled through and ethanol was added to maintain the original volume. The product (1·4 g.), examined by g.l.c. as methyl esters, consisted of 47% saturated, 43% monoenoic, and 10% dienoic esters. It was hydrolysed and the acids were dissolved in acetone and cooled stepwise to 0, −20, and −50°. Crystals were filtered off at each stage and analysed by g.l.c. The crystals obtained at −50° were almost entirely octadecenoic acids. This portion was subjected to oxidative splitting by von Rudloff's method [10] and the resulting mixture of acids was examined by g.l.c. at 90, 160, and 185° as methyl esters. The major fragments observed were hexanoic, nonanoic, azelaic, dodecanedioic, and pentadecanedioic acids. Other peaks were too small to be significant. There was no adipic or lauric acid.

Another portion of the tetraene acid was examined by the oxidation method of Gunstone and Sykes [11] as follows. The acid (0·22 g.) was partially hydroxylated by performic acid and the product was hydrogenated to saturate the remaining double bonds. The resulting hydroxy-acids (0·13 g.) were cleaved by von Rudloff's method and the acidic fragments were analysed (g.l.c.). The results were the same as those obtained by the hydrazine reduction described above.

Isomerization by Sodium Ethoxide.[12]—Sodium (10 mg.) was dissolved in ethanol (50 ml.) and the methyl ester (0·28 g.) of the tetraene acid was added. The solution was refluxed under nitrogen for 4 hr. Water was added and the product was washed and dried. Its i.r. spectrum showed $\nu_{max.}$ 1650s and 1715s cm.$^{-1}$ but only weak absorption at 963 cm.$^{-1}$. The u.v. spectrum had no peak in the region 220—320 mμ.

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