

## Constitution and Stereochemistry of the Kamlolenic Acids.

By L. CROMBIE and J. L. TAYLER.

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Kamlolenic acid is a conjugated 18-hydroxyoctadecatrienoic acid. There is no evidence for the presence of a keto-group. The triene system is in the 9 : 11 : 13-position, since ozonolysis of the methyl ester gives methyl 8-formyl-octanoate. Kamlolenic acid is thus (II). Infra-red and ultra-violet light-absorption data enable the stereochemistry of  $\alpha$ - and  $\beta$ -kamlolenic acid to be equated with that of  $\alpha$ - and  $\beta$ -elæostearic acid. The  $\beta$ -form is the all-*trans*-compound whilst the  $\alpha$ -form is probably the *cis*-9 : *trans*-11 : *trans*-13-stereoisomer.

THE fruit of *Mallotus philippinensis* Muell. Arg. (a member of the *Euphorbiaceae*) has long been known as the source of a dye, rottlerin, which has anthelmintic and purgative properties, but only recently has the nature of the seed oil been investigated. This oil (kamala oil) has drying properties similar to those of tung oil (also obtained from a member of the *Euphorbiaceae*), and as the small evergreen tree from which it comes is widely distributed in Asia it has potential commercial interest [Puntambekar, *Current Sci.*, 1942, **11**, 464; Krishna, Puntambekar, and Raizada, *Indian Forest Record (New Ser.)*, 1936, **1**, 2]. Seeds examined by us contained 42% of kernel which yielded 30% of oil. An unstable unsaturated acid has been isolated from kamala oil and named  $\alpha$ -kamlolenic acid (Aggarwal, Bhatnagar, Narain, and Karimullah, *J. Sci. Ind. Res., India*, 1948, **7B**, 136; Gupta, "Proc. Symp. on Indian Oils and Fats and their Utilisation," 1951, p. 33). The present communication is concerned with its structure and stereochemistry.

On iodine-catalysed ultra-violet irradiation,  $\alpha$ -kamlolenic acid, in light petroleum, is transformed into a less soluble stereoisomeric  $\beta$ -form with a higher melting point. When hydrogenated, both acids yield 18-hydroxyoctadecanoic acid and three mols. of hydrogen are absorbed (Gupta, Sharma, and Aggarwal, *J. Sci. Ind. Res., India*, 1951, **10**, B, 76; Gupta, *loc. cit.*). Our observations are in agreement with these facts and we are unable to support the view of Puntambekar (*Proc. Indian Acad. Sci.*, 1952, **35**, A, 57) that  $\alpha$ -kamlolenic acid is not a pure compound or that it contains a keto-group which is reduced to a hydroxylic function during hydrogenation. Thus, the infra-red spectra of both kamlolenic acids (and their methyl esters) show strong primary hydroxyl absorption bands in the OH stretching and bending regions (near 3200 and 1060  $\text{cm}^{-1}$ ), whilst for the  $\alpha$ -acid there is no indication of a C=O grouping beyond that formally derived from the carboxyl grouping. Furthermore, both methyl esters yield crystalline  $\alpha$ -naphthylurethanes (Table 1) and recently Gupta, Gupta, and Aggarwal (*J. Sci. Ind. Res., India*, 1953, **12**, B, 240) described acetyl derivatives. The two acids form crystalline methyl and *p*-bromophenacyl esters.

TABLE 1.

Acid	Acid, m. p.	Micro- hydrog.	Me ester, m. p.	<i>p</i> -Bromo- phenacyl ester, m. p.	$\alpha$ -Naphthyl- urethane of Me ester, m. p.	Acetyl deriv., m. p.*
$\alpha$ -Kamlolenic .....	78°	2.9H <sub>2</sub>	24°	86°	58°	43—44°
$\beta$ -Kamlolenic .....	89	3.0H <sub>2</sub>	45	96—98	61	58—59

\* Data of Gupta *et al.* (*loc. cit.*).

Location of the triple unsaturation in the 18-hydroxyoctadecanoic chain has occasioned some confusion. Aggarwal *et al.* at first believed that kamlolenic acid was either the 18-hydroxy-9 : 12 : 14- or the -9 : 13 : 15-trienoic acid (see also Gupta, Sharma, and Aggarwal, *ibid.*, 1952, **11**, B, 463). Puntambekar (*loc. cit.*) favoured 3-oxo-5 : 7-diene or 8-oxo-9 : 11 : 13-triene structures but his degradative evidence does not warrant such conclusions. Very recently, Calderwood and Gunstone (*Chem. and Ind.*, 1953, 436) and Gupta, Gupta, and Aggarwal (*loc. cit.*) reported that kamlolenic acid absorbs ultra-violet light in the manner typical of a triene (see also Table 2), and yields azelaic acid on permanganate oxidation.

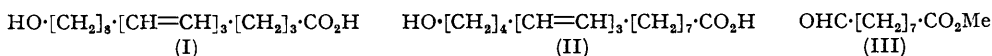
This confines the unsaturation to the 9 : 11 : 13- or 5 : 7 : 9-positions (II or I). A decision can now be made as methyl  $\alpha$ -kamlolenate yields, on ozonolysis, the aldehyde-ester (III) which can come from (II) but not from (I). The aldehyde was isolated as its 2 : 4-dinitro-

TABLE 2. *Ultra-violet light absorption data (solvent : ethanol).*

	$\lambda_{\max.}$ (m $\mu$ )	$10^{-3} \epsilon$		$\lambda_{\max.}$ (m $\mu$ )	$10^{-3} \epsilon$
$\alpha$ -Kamlolenic acid <sup>a</sup> .....	261	40.5	$\beta$ -Kamlolenic acid <sup>a</sup> .....	258	45.0
	271	52.0		268	60.0
	282	42.0		279	47.0
Methyl $\alpha$ -kamlolenate .....	261	38.0	Methyl $\beta$ -kamlolenate ...	258	42.0
	270.5	49.0		268	57.0
	282	40.0		279	44.5
$\alpha$ -Elæostearic acid <sup>b</sup> .....	261	36.0	$\beta$ -Elæostearic acid <sup>b</sup> .....	259	47.0
	271	47.0		268	61.0
	281	38.0		279	49.0

<sup>a</sup> Gupta, Gupta, and Aggarwal (*loc. cit.*) give max. at 270.5 ( $\epsilon$  53,000) and 268 m $\mu$  ( $\epsilon$  58,500) for the main band of the  $\alpha$ - and  $\beta$ -acid respectively. <sup>b</sup> O'Connor and Heinzelman (*J. Amer. Oil Chem. Soc.*, 1947, **24**, 212) give 270 ( $\epsilon$  47,000) and 268 m $\mu$  ( $\epsilon$  60,300) for the main band of the  $\alpha$ - and  $\beta$ -acid respectively.

phenylhydrazone and compared with an authentic specimen obtained by the ozonolysis of methyl oleate. Kamlolenic acid is thus 18-hydroxyoctadeca-9 : 11 : 13-trienoic acid and it remains to deal with its stereochemistry.



The ready  $\alpha \rightarrow \beta$  transformation is reminiscent of elæostearic and licanic acid and ultra-violet absorption data support the analogy.\* On passing from  $\alpha$ -kamlolenic or  $\alpha$ -elæostearic acid to the corresponding  $\beta$ -form, there is a similar shift of the triplet to slightly shorter wave-lengths and a similar increase in the extinction coefficients (Table 1). But on this evidence only the resemblance might be superficial. There are two other reported stereoisomers of the elæostearic structure—punicic acid from pomegranate oil (Toyama and Tsuchiya, *J. Soc. Chem. Ind., Japan*, 1935, **38**, 182B; Farmer and Van den Heuvel, *J.*, 1936, 1809), and the little-investigated trichosanic acid from the oil of *Trichosanthes cucumerioides* (Toyama and Tsuchiya, *J. Soc. Chem. Ind., Japan*, 1935, **38**, 185B), which by stereomutation yield  $\beta$ -elæostearic acid and would certainly show a similar increase in extinction coefficient in the process. Further evidence was therefore sought from infra-red spectra.

Comparison of the spectra (paraffin mulls) of kamlolenic with elæostearic acids affords strong justification for equating the stereochemistry in the  $\alpha$ - and  $\beta$ -forms. There is a remarkable similarity (Table 3) especially in the region 1000—700 cm.<sup>-1</sup>, and this is of

TABLE 3. *Infra-red comparison at 1000—700 cm.<sup>-1</sup>.*

Acid	993 s	967 ms	932 ms	916 ms	875 w	818 w	783 w	768 w	747 ms	728 m
$\alpha$ -Kamlolenic	993 s	967 ms	932 m	917 ms	874 w	822 w	784 w	767 w	747 ms	727 m
$\alpha$ -Elæostearic	999 m	986 s	929 w	899 m	876 m	834 mw	766 w	—	730 m	719 m
$\beta$ -Kamlolenic	998 s	987 vs	929 w	899 m	877 m	833 mw	766 w	—	729 m	719 m

vs = very strong; s = strong; m = moderate; w = weak.

particular interest as regularities in the C=C-H out-of-plane bending region may prove useful for identification of trienes of unknown configuration. It is significant that the two  $\beta$ -acids have a doublet (near 1713 and 1702 cm.<sup>-1</sup>) in the carboxylic C=O stretching region whilst the  $\alpha$ -acids have but one resolvable maximum (1687 cm.<sup>-1</sup>). This seems to be due to differing arrangements in the crystal (and hence of the hydrogen-bonding pattern), which are imposed by different geometrical stereochemistry.

Recent work by Paschke, Tolberg, and Wheeler (*J. Amer. Oil Chem. Soc.*, 1953, **30**, 97) indicates that  $\beta$ -elæostearic acid is the all-*trans*-9 : 11 : 13-isomer, whilst  $\alpha$ -elæostearic acid is the *cis*-9 : *trans*-11 : *trans*-13-stereoisomer. On the basis of the arguments above, the same stereochemistry is assigned to  $\alpha$ - and  $\beta$ -kamlolenic acids, though the reservation must

be made that the *trans*-9 : *trans*-11 : *cis*-13 might not be readily distinguishable from the *cis*-9 : *trans*-11 : *trans*-13 by any of the methods used. The placing of two *trans*-double bonds in a contiguous position in both molecules accords with the almost theoretical diene numbers of the acetylated acids (Gupta, Gupta, and Aggarwal, *loc. cit.*). The fact that the split C=C stretching vibrations are easier to detect in methyl  $\alpha$ -kamlolenate than in methyl  $\beta$ -kamlolenate is in agreement with the higher symmetry of the latter molecule.

#### EXPERIMENTAL

Microanalyses were carried out in the microanalytical laboratories of Imperial College (Mr. F. H. Oliver).

*Isolation of  $\alpha$ - and  $\beta$ -Kamlolenic Acids.*—Kamala kernels (50 g.) were crushed gently and continuously extracted with light petroleum (b.p. 40—60°) to give, on evaporation of the solvent, a clear yellow oil (15 g.). This was heated under reflux in nitrogen for 30 min. with ethanolic potassium hydroxide (10%; 100 ml.). After concentration *in vacuo*, the acids were isolated in the usual way. When these were set aside in ether at 0°,  $\alpha$ -kamlolenic acid separated as white crystals: concentration yielded a second crop (total 3.5 g.; m.p. 72—75°). The acid was purified by recrystallisations from ether (Found: C, 73.5; H, 10.4. Calc. for C<sub>18</sub>H<sub>30</sub>O<sub>3</sub>: C, 73.45; H, 10.25%). For further data see Tables.

$\alpha$ -Kamlolenic acid (2.0 g.) was dissolved in a mixture (200 ml.) of 1 : 1 light petroleum (b. p. 40—60°)-ether, and a trace of iodine was added. This solution was irradiated (3 hr.) at 20° (quartz flask) with ultra-violet light.  $\beta$ -Kamlolenic acid (1.0 g.; m. p. 87—88.5°) separated, and a further crop (0.25 g.) was isolated by concentration (Found: C, 73.55; H, 10.45%). Prolonged irradiation leads to the formation of oil.

*Derivatives of  $\alpha$ - and  $\beta$ -Kamlolenic Acids.*—By treatment with diazomethane in ether, crystalline methyl esters were isolated. The  $\alpha$ - (Found: C, 73.9; H, 10.45. C<sub>19</sub>H<sub>32</sub>O<sub>2</sub> requires C, 74.0; H, 10.45%) and the  $\beta$ -form (Found: C, 74.15; H, 10.5%) were purified by low-temperature crystallisation from pentane. These esters yielded  $\alpha$ -*naphthylurethanes*. Both the  $\alpha$ - (Found: C, 75.45; H, 8.3. C<sub>36</sub>H<sub>39</sub>O<sub>4</sub>N requires C, 75.45; H, 8.25%) and the  $\beta$ -urethanes (Found: C, 75.50; H, 8.4%) crystallised from light petroleum (b. p. 60—80° or 80—100°). Despite their similar m. p.s (Table 1) these were depressed by more than 8° on admixture.

*p*-*Bromophenacyl* esters were isolated in the usual way and crystallised from ethanol (Found:  $\alpha$ -Form: C, 63.5; H, 7.3.  $\beta$ -Form: C, 63.6; H, 7.2. C<sub>26</sub>H<sub>35</sub>O<sub>4</sub>Br requires C, 63.55; H, 7.2%).

The two acids react with maleic anhydride in benzene at 100° but the products are not easily purified since reaction seems to occur at the hydroxyl grouping as well as with the dienic system.

*$\alpha$ - and  $\beta$ -Elæostearic Acids.*—The  $\alpha$ -acid (m. p. 47.5°) was purified by low-temperature crystallisation from pentane (Found: C, 77.5; H, 11.0. Calc. for C<sub>13</sub>H<sub>20</sub>O<sub>2</sub>: C, 77.65; H, 10.85%). The  $\beta$ -acid (m. p. 72°) was similarly purified (Found: C, 77.4; H, 10.9%).

*Hydrogenation of  $\alpha$ -Kamlolenic Acid.*—The acid (105 mg.), dissolved in ethyl acetate (10 ml.), was added to pre-reduced palladium-carbon (100 mg.) in ethyl acetate (10 ml.). Hydrogen (3 mols.) was absorbed at atmospheric pressure. The catalyst was removed by filtration and washed with hot ethyl acetate. Cooling of the filtrate gave 18-hydroxystearic acid (60 mg.) which when twice recrystallised from ethyl acetate had m. p. 98° (Chuit and Hausser, *Helv. Chim. Acta*, 1929, 12, 463, give m. p. 96.6—97.2°).

*Ozonisation of Methyl  $\alpha$ -Kamlolenate.*—Ozonised oxygen was bubbled through a solution of methyl  $\alpha$ -kamlolenate (1.26 g.) in glacial acetic acid (15 ml.) until the solution no longer decolourised bromine in acetic acid. Ether (15 ml.) and zinc dust (1.5 g. in portions) were added, together with a few drops of water, and the mixture was refluxed gently until it no longer reacted with starch-iodide paper. The solid was removed by filtration and washed with ether, and the united ethereal extracts were washed with water. The ether was removed under reduced pressure, the residue dissolved in ethanol (20 ml.), and 2 : 4-dinitrophenylhydrazine (800 mg.) added. After addition of concentrated hydrochloric acid (1 ml.) to the boiling solution, heating was maintained for 2 min. and the mixture cooled (0°) for 18 hr. The orange crystals were filtered off and extracted with benzene. Evaporation of the ethanolic filtrate gave a red material which was also extracted with benzene. The two extracts were washed, dried, evaporated, and chromatographed on alumina. At least nine bands were formed but attention was confined to those more easily eluted. The first three fractions were oily and semisolid (250 mg.) and the later ones oils or glues. Fraction 2 (80 mg.) was washed with a little ethanol and yielded a yellow crystalline solid containing traces of orange material (45 mg.). This was crystallised five times from ethanol and had m. p. 64—65° (Found: C, 52.3, 53.05; H, 6.4, 6.4; N, 14.75%), un-

depressed on admixture with an authentic specimen of the 2:4-dinitrophenylhydrazone of methyl 8-formyloctanoate (see below).

*Methyl 8-Formyloctanoate*.—Methyl oleate was purified by distillation through a Stedman gauze-packed column: we are indebted to Mr. R. Ackman, Mr. B. W. Baker, and Mr. A. G. Jacklin for this purification. The ester (2.2 g.) was ozonised as described above and gave a mixture of 2:4-dinitrophenylhydrazones (2.2 g.). Chromatography of the mixture (1 g.) on alumina gave crude nonaldehyde 2:4-dinitrophenylhydrazone in the first benzene fractions (630 mg. crude; 330 mg. pure, m. p. 103–105°). The next group of fractions gave the crude 2:4-dinitrophenylhydrazone of methyl 8-formyloctanoate (250 mg. crude; m. p. 66.5°, pure). This crystallised from ethanol as yellow needles (Found: C, 52.25; H, 6.05; N, 15.35.  $C_{16}H_{22}O_6N_4$  requires C, 52.45; H, 6.05; N, 15.3%).

*Ultra-violet Light Absorption Data*.—These were determined with a Unicam photoelectric instrument immediately after dissolution of the sample in ethanol. Solutions kept for a few hours showed a marked decline in extinction coefficient. The solid kamlolenic acids themselves are stable at 0° in stoppered tubes for at least one week (m.p. and light absorption) but deteriorate after some days at 20°. The  $\alpha$ -methyl ester sealed under nitrogen became very resinous after 18 hr. at 20°.

*Infra-red Spectra*.\* —The spectra of  $\alpha$ - (C.S. no. 128) and  $\beta$ -kamlolenic acid (C.S. no. 129), the  $\beta$ -methyl ester (C.S. no. 130) and  $\alpha$ - (C.S. no. 131) and  $\beta$ -elæostearic acid (C.S. no. 132) are deposited with the Chemical Society. We are indebted to Dr. W. C. Price for the use of a single-beam Grubb-Parsons spectrometer (rock salt optics).

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DEPARTMENT OF ORGANIC CHEMISTRY, IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY,  
SOUTH KENSINGTON, LONDON, S.W.7. [Received, March 19th, 1954.]

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