213. *The Structures of Two Acids from Olive Leaves.*

By G. D. Meakins and R. Swindells.

Two acids from olive leaves have been identified as 10 : 16-dihydroxyhexadecanoic and 9 : 10 : 18-trihydroxyoctadecanoic acid.

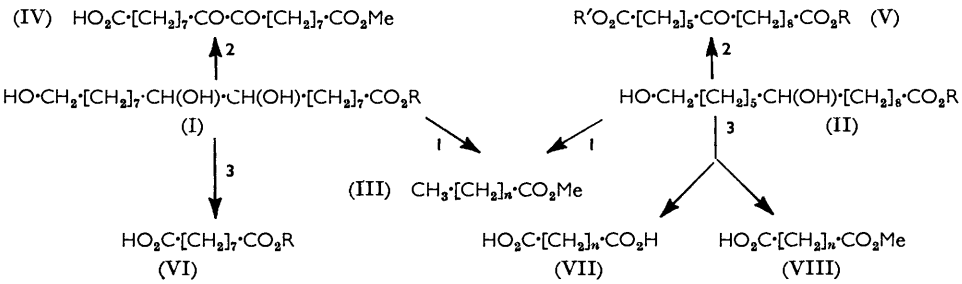
M. Passerini and his collaborators isolated two apparently new acids from leaves of the olive (*Olea europaea*) [1,2] and of Japanese privet (*Ligustrum japonicum*).[3] These acids, designated I and II, were separated by fractional precipitation of the sodium salts [1] or by fractional crystallisation of the acids after preliminary purification with baryta. Microanalytical data on the acids I and II and their methyl esters indicated formulæ $C_{14}H_{28}O_4$ and $C_{15}H_{30}O_4$ respectively, but no structural investigations were carried out.

Working with olive leaves we separated the acids I and II efficiently by chromatography of the mixed methyl esters on alumina. The constants of the acids and their derivatives (see Table) suggested that they might be identical with previously known compounds, isolated for example from the cutin of *Agave americana*.[4] Proof that acid I is 9 : 10 : 18-trihydroxyoctadecanoic acid (I; R = H, $C_{18}H_{36}O_5$) and acid II is 10 : 16-dihydroxyhexadecanoic acid (II; R = H, $C_{16}H_{32}O_4$) was obtained by slight modifications of the methods used by Matic with the hydroxy-acids from *Agave* cutin.[4]

Acid I (I; R = H) on reduction with phosphorus and hydriodic acid, followed by zinc and hydrochloric acid in methanol, gave methyl stearate (III; $n = 16$). Chromic oxidation of the methyl ester I (I; R = Me) in acetone at 20° produced the 9 : 10-dioxo-compound (IV). Fission of the methyl ester (I; R = Me) with chromic oxide in acetic acid at 40° afforded azelaic acid and its monomethyl ester (VI; R = H and Me) which were separated with pentane and methylated to products identified in both cases as

[1] Passerini, Ridi, and Ceccherelli, *Sperimentale, Sez. Chim. biol.*, 1951, **2**, 63.
[2] Passerini, Ridi, and Papini, *Ann. Chim.* (*Italy*), 1953, **43**, 201.
[3] Passerini and Papini, *Sperimentale, Sez. Chim. biol.*, 1951, **2**, 72; *Ann. Chim.* (*Italy*), 1953, **43**, 204.
[4] Matic, *Biochem. J.*, 1956, **63**, 168.

dimethyl azelate by vapour-phase chromatography. [In the present work vapour-phase chromatography of methyl esters [5] was convenient for characterising various oxidation and reduction products of acids I and II. Mixtures of esters $MeO_2C\cdot[CH_2]_n\cdot CO_2Me$ with $n = 4$—8 gave separate peaks whose times of emergence and areas allowed semiquantitative analyses to be made.]



Reagents: 1, HI–P, then Zn–HCl–MeOH. 2, CrO_3–Me_2CO at 20°. 3, CrO_3–AcOH at 40°.

Reduction of acid II (II; R = H) gave methyl palmitate (III; $n = 14$), and mild oxidation of methyl ester II (II; R = Me) afforded the 7-oxo-ester (V; R = Me, R′ = H) which was converted into the diester (V; R = R′ = Me) and the diacid (V; R = R′ = H). Fission of the ester (II; R = Me) yielded adipic and pimelic acid (VII; $n = 4$ and 5) and the monoesters of azelaic and sebacic acids (VIII; $n = 7$ and 8). A sample of 10 : 16-dihydroxyhexadecanoic acid kindly supplied by Dr. M. Matic was converted into the methyl ester and the 7-oxo-compounds (V): mixed melting points with derivatives prepared from acid II showed no depression. There is no obvious explanation for the one case of melting point discrepancy, *viz.*, that for the parent acids.

Melting points of hydroxy-acids and derivatives.

	Acid I [1,2]	9 : 10 : 18-Trihydroxy-octadecanoic acid [4]	Present work
Acid	100—102°	102—103°	99—102°
Methyl ester	81—82	81—82	80—82
	Acid II	10 : 16-Dihydroxy-hexadecanoic acid	
Acid	95—97	75—76	95—98
Methyl ester	73—75	67·5—68·3	68—69
7-Oxo-diacid (V; R = R′ = H)	—	112·5—113·5	110—112
,, monomethyl ester (V; R = Me, R′ = H)	—	64—66 *	64—66
,, dimethyl ester (V; R = R′ = Me)	—	39—43 *	40—43

* Prepared from 10 : 16-dihydroxyhexadecanoic acid kindly supplied by Dr. M. Matic.

Experimental

M. p.s were determined on a Kofler block and are corrected. Neutral alumina was prepared by stirring Peter Spence material (grade H) with an excess of ethyl acetate at 20° for 2 days, filtration, washing repeatedly with hot water, and heating at 250° for 2 days. Vapour-phase chromatography was carried out on a Griffin (mark 2) apparatus using a column (6′ × 0·25″) packed with Celite impregnated with Silicone (Griffin and George " S.E.30 "): with the column temperatures specified below and a constant nitrogen flow rate (2·1 l./hr.) the times of emergence were characteristic of the various compounds examined.

Isolation of Acids from Olive Leaves.—Dry leaves (200 g.) were minced and stirred with boiling 5% aqueous sodium hydroxide (2 l.) for 30 min. The cooled mixture was filtered through muslin and then through Whatman's No. 541 filter paper. After acidification of the

[5] James and Martin, *ibid.*, p. 144.

filtrate with 4N-hydrochloric acid the insoluble material was isolated by centrifugation, washed with water, dried, and continuously extracted with ether for 12 hr. Evaporation of the ether afforded a dark green gum (*ca.* 18 g.) which was shaken with 5% aqueous sodium carbonate (1 l.). The mixture was washed with ether (2 × 500 c.c.), warmed (to remove ether), cooled, and acidified with 4N-hydrochloric acid. The material collected by filtration was washed and dried to give a pale green solid (*ca.* 16 g.).

A portion of the above solid (5 g.) was treated with an excess of diazomethane in ether. The solution was concentrated and adsorbed on neutral alumina (300 g.). Elution with ether (500 c.c.) gave a green oil which was discarded. Further elution with ether (1·5 l.) afforded a solid (1·4 g.) which crystallised from light petroleum–ethyl acetate to give methyl 10 : 16-dihydroxyhexadecanoate (II; R = Me) (1·1 g.) as white needles, m. p. 68—69° (Found: C, 67·45; H, 11·05. Calc. for $C_{17}H_{34}O_4$: C, 67·5; H, 11·25%), $\nu_{max.}$ (in $CHCl_3$) 3625, 3450, 1730, and 1047 cm.$^{-1}$. Hydrolysis with ethanolic potassium hydroxide yielded 10 : 16-dihydroxyhexadecanoic acid (II; R = H), m. p. 95—98° (from aqueous methanol) (Found: C, 66·6; H, 11·25. Calc. for $C_{16}H_{32}O_4$: C, 66·6; H, 11·1%).

Elution with ether–methanol (9 : 1) gave a green gum which was rejected, followed by solid material (1·2 g.). Crystallisation of this from light petroleum–ethyl acetate afforded material (1 g.; m. p. 73—75°) which was hydrolysed with ethanolic potassium hydroxide. The product crystallised from acetone–chloroform and then from methanol, to give 9 : 10 : 18-trihydroxyoctadecanoic acid (I; R = H) (0·8 g.), m. p. 99—102° (Found: C, 65·0; H, 10·9. Calc. for $C_{18}H_{36}O_5$: C, 65·0; H, 10·85%). Treatment of the acid in ether–methanol with ethereal diazomethane afforded the methyl ester (I; R = Me), m. p. 80—82° after crystallisation from light petroleum–ethyl acetate (Found: C, 66·0; H, 11·05. Calc. for $C_{19}H_{38}O_5$: C, 65·9; H, 11·0%), $\nu_{max.}$ (in $CHCl_3$) 3610, 3430, 1724, and 1050 cm.$^{-1}$.

Reduction of Acids (I and II; R = H).—The acid (200 mg.) was refluxed with hydriodic acid (*d* 1·7; 6 c.c.) and red phosphorus (70 mg.) for 16 hr. After dilution with water and extraction with ether, the ether solution was washed with 5% aqueous sodium hydrogen sulphite, dried, and evaporated. The product was dissolved in methanol (10 c.c.) and boiled with 10N-hydrochloric acid (2 c.c.) and zinc (300 mg.) for 6 hr. Filtration, dilution with water, and extraction with ether afforded the methyl ester of the appropriate fatty acid. 9 : 10 : 18-Trihydroxyoctadecanoic acid gave methyl stearate (150 mg.), m. p. 36—38° (from acetone) (time of emergence 190 min. from vapour-phase chromatography column at 190°). This ester was hydrolysed to stearic acid, m. p. and mixed m. p. 67—69°. 10 : 16-Dihydroxyhexadecanoic acid gave methyl palmitate (170 mg.) (time of emergence 140 min. at 190°), which was hydrolysed to palmitic acid, m. p. and mixed m. p. 60—63°.

Oxidation of Methyl Esters (I and II; R = Me).—(a) *With chromic acid in acetone.* The methyl ester (400 mg.) in acetone (25 c.c.) was treated with a slight excess of 8N-chromic acid at 20°. After dilution with water the product was isolated with ether in the usual way. Oxidation of methyl 9 : 10 : 18-trihydroxyoctadecanoate for 2 min. gave the *methyl hydrogen* 9 : 10-*dioxo-octadecanedioate* (IV), yellow plates (85 mg.), m. p. 80—83° (from ethyl acetate) (Found: C, 63·7; H, 8·95. $C_{19}H_{32}O_6$ requires C, 64·05; H, 9·0%), $\nu_{max.}$ (in CCl_4) 3000 (very broad), 1738, and 1709 cm.$^{-1}$. Azelaic acid (100 mg.), m. p. 103—106°, was isolated from the ethyl acetate mother-liquor.

Oxidation of methyl 10 : 16-dihydroxyhexadecanoate for 5 min. gave the 16-*methyl hydrogen* 7-*oxohexadecanedioate* (V; R = Me, R′ = H), plates (240 mg.), m. p. 64—66° (from ethyl acetate) (Found: C, 65·2; H, 9·75. $C_{17}H_{30}O_5$ requires C, 65·0; H, 9·55%), $\nu_{max.}$ (in CCl_4) 3000 (very broad), 1740, and 1710 cm.$^{-1}$. Treatment of the ester with an excess of diazomethane afforded *dimethyl* 7-*oxohexadecanedioate* (V; R = R′ = Me), m. p. 40—43° (Found: C, 65·6; H, 9·5. $C_{18}H_{32}O_5$ requires C, 65·8; H, 9·75%), $\nu_{max.}$ (in CCl_4) 1739, and 1708 cm.$^{-1}$. Saponification of both esters gave 7-oxohexadecanedioic acid (V; R = R′ = Me), m. p. 110—112° (Found: C, 64·2; H, 9·35. Calc. for $C_{16}H_{28}O_5$: C, 64·0; H, 9·4%).

(b) *With chromic oxide in acetic acid.* The ester (70 mg.) and chromic acid (90 mg.) were dissolved in acetic acid (3 c.c.), and the solution was kept at 40° for 24 hr. The product obtained after dilution with water and thorough extraction with ether was refluxed with pentane (25 c.c.) and then cooled. Two fractions ("pentane-insoluble" and "pentane-soluble") were obtained, and separately methylated with diazomethane. Methyl 9 : 10 : 18-trihydroxyoctadecanoate afforded dimethyl azelate (time of emergence 29 min. from column at 177°) in both fractions. Methyl 10 : 16-dihydroxyhexadecanoate gave dimethyl adipate

and dimethyl pimelate (times of emergence 8 min. and 12·5 min. respectively) from the pentane-insoluble fraction, and dimethyl azelate and dimethyl sebacate (times of emergence 29 min. and 44 min. respectively) from the pentane-soluble fraction.

These emergence times agree with those found with authentic specimens: dimethyl suberate emerges after 18·5 min.

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