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# NEW TRITERPENES FROM THE LEAVES OF *OLEA EUROPAEA*

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**Key Word Index**—*Olea europaea*, Oleaceae, triterpenes; maslinic acid, oleanane hydroxy acids

**Abstract**—The terpene fraction from the leaves of *Olea europaea* has been shown to contain the known oleanolic and maslinic acids, together with oleanane hydroxy acids which have never been found before in nature.

## INTRODUCTION

VERY recently, we reported[1] that oleanolic acid (**1a**) is practically the only detectable triterpene of olive husks. Maslinic acid (**1b**), earlier reported[2] as a second triterpene of the husks, was in fact proved to be an artefact arising from oleanolic acid itself, possibly via microbiological hydroxylation occurring during the ageing of the husks. Since maslinic acid has also been isolated from other natural sources[3] where its formation from oleanolic acid seemed unlikely, we extended our chemical study to the leaves of *Olea europaea* in order to check the presence of maslinic acid in other organs of the plant.

## RESULTS

The leaves, collected in the summer from different plants, were dried at room temperature* and then exhaustively extracted with both cold hexane and cold acetone. Hexane afforded a large fraction (5%) consisting of fats which was not further considered. The acetone extract (2%) was instead submitted to several chromatographic fractionations which gave more fats and waxes together with two terpene fractions mainly consisting of oleanolic acid (**1a**) and of maslinic acid (**1b**) respectively.

The crude maslinic acid (**1b**), purified through chromatography and crystallization from MeOH, gave the pure product m.p. 267–70°, $[\alpha]_D$ +40°, identical with authentic material. Evaporation of the mother liquors of crystallization of **1b**, followed by treatment of the resulting solid with ethereal diazomethane, afforded an oil whose NMR examination evidenced the marked enrichment of a second substance. Crystallization of the mixture from hexane afforded a solid m.p. 183–7°, $[\alpha]_D$ −28° $C_{31}H_{50}O_4$, isomeric with methyl maslinate (**1c**). The NMR spectrum differed from that of **1c** by the lack of any proton signal and, moreover, exhibited resonance of the carbomethoxyl protons at 219 Hz, slightly shifted downfield in comparison with the resonance of the same protons in the spectrum

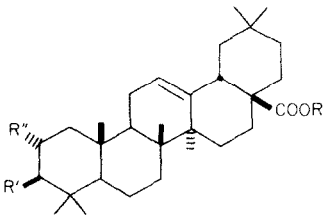
* A check on fresh leaves led to comparable results, though the experimental operations became more difficult owing to appreciable quantities of hydrophilic substances solubilized by water–acetone mixtures

[1] CAPUTO, R., MANGONI, L., MONACO, P. and PREVITERA, L. (1974) *Phytochemistry* **13**, 1551

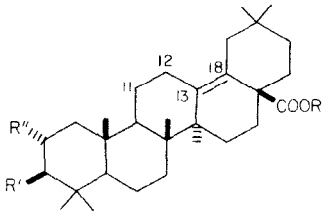
[2] CAGLIOTI, L., CAINELLI, G. and MINUTILLI, F. (1961) *Gazz. Chim. Ital.* **91**, 1387

[3] CHEUNG, H. T. and FENG, M. C. (1968) *J. Chem. Soc.* (C) 1047

of **1c**. Elemental composition and spectral features suggested the structure **2c**, in which the double bond is located at the $\Delta^{13(18)}$ tetrasubstituted position, thus also accounting for the shift of the carbomethoxyl resonance




(**1a**) R, R'' = H, R' = OH
(**1b**) R = H, R', R'' = OH
(**1c**) R = Me, R', R'' = OH
(**1d**) R = Me, R' = OH, R'' = H




(**2a**) R, R'' = H, R = OH
(**2b**) R = H, R', R'' = OH
(**2c**) R = Me, R', R'' = OH
(**2d**) R = Me, R' = OAc, R'' = H

We have called this substance methyl $\delta$-maslinate, in analogy with $\delta$-amyrin[4] which has the same tetrasubstituted double bond The possibility that **2c** is an artefact, formed from **1c** through acid catalysed isomerization occurring in the isolation procedure, seems to be unlikely Oleanolic acid (**1a**) is in fact known[5] not to undergo the acid catalysed isomerization $\Delta^{12} \rightarrow \Delta^{13(18)}$, owing to the much faster reversible formation of the corresponding hydroxy lactone. In addition, as far as we are aware, such isomerizations even in the $\beta$-amyrin series have been accomplished only in very few cases[6] and then under strongly acidic conditions

The assignment of the structure **2c**, based on spectroscopic evidence, was then confirmed by conversion of methyl maslinate (**1c**) into **2c** according to the procedure used by Ruzicka *et al.*[7] for $\beta$-amyrin. Methyl maslinate was in fact acetylated and then refluxed with $SeO_2$ in acetic acid to afford the $\Delta^{11,13(18)}$ conjugate diene. Direct hydrogenation of this latter on a prereduced $PtO_2$ catalyst, followed by alkaline hydrolysis and re-esterification of the carboxyl group, then led to a product m.p. 182–4°, $[\alpha]_D$ −26°, identical with the natural sample

Oleanolic acid (**1a**), the second substance in the acetone extract, after crystallization from MeOH, has m.p. 307–9°, $[\alpha]_D$ +78° and was identical with authentic material A check on the mother liquors of crystallization of **1a** also showed the enrichment of a second substance, then isolated by further crystallization from hexane The substance melted over a wide range and was therefore acetylated and esterified, thus being characterized as the corresponding acetoxy methyl ester m.p. 237–9°, $[\alpha]_D$ −65° The close similarity of its spectral characteristics—mainly the NMR spectrum—with those of **2c** immediately suggested structure **2d***, subsequently confirmed by comparison with authentic **2d** obtained from oleanolic acid (**1a**) as above described for (**2b**)

* Actually **2d** was already described as a synthetic product prepared from methyl oleanolate (**1d**)[8]

[4] MUSGRAVE, O C, STARK, J and SPRING, F S (1952) *J Chem Soc* 4393
[5] BARTON, D H R and HOLNESS N J (1952) *J Chem Soc* 78
[6] AMES T R HALSALL, T G and JONES, E R H (1951) *J Chem Soc* 450
[7] RUZICKA, L GROB, A and VAN DER SLUYS-VELER, F C (1939) *Helv Chim Acta* **22**, 788
[8] JEGER O NORYMBERSKI J and RUZICKA L (1944) *Helv Chim Acta* **27**, 1532

These results show that, in the leaves of *Olea europaea*, maslinic acid exists as such and may be considered a true metabolite; on the other hand, in the fruits of the same plant, it is only formed during the ageing of the husks. Its mode of formation in the husks appears still to be an open question, though the hydroxylation is probably accomplished by a cellular enzyme rather than by a microbial system

## EXPERIMENTAL

General experimental procedures have been reported elsewhere [1]

*Extraction of the leaves* Powdered dry leaves (800 g) were continuously extracted with cold hexane for 8 hr and with cold acetone for additional 5 hr The reddish yellow acetone extract was concentrated, HCl-washed silica-gel (10 g) added and this was evaporated to dryness The solid residue was then put onto a column filled with HCl-washed silica-gel (530 g) Elution with $C_6H_6$ (5 l) afforded a mixture (10 g) consisting of fats and waxes (NMR, GLC), further elution with $Et_2O$ (2 l) gave a second fraction (6 g) which was treated with 2 N NaOH aq (5 × 100 ml) Acidification of the combined aq layers with conc $H_2SO_4$ then afforded a brown waxy mixture (3 g) which, chromatographed on HCl-washed silica-gel, gave three fractions (A) (0 9 g, $C_6H_6$–$Et_2O$ 9 1) consisting of fats and waxes, (B) (1 5 g, $C_6H_6$–$Et_2O$ 3 2), (C) (0 6 g. $Et_2O$)

*Oleanolic acid* (**1a**) *and acetoxy ester* (**2d**) Fraction (B) was reabsorbed on HCl-washed silica-gel (40 g) $C_6H_6$–$Et_2O$ (4 1) eluted oleanolic acid (**1a**) (1 2 g) m p 307–9° (from MeOH), $[\alpha]_D$ +78° (c 1 2), identical with authentic material Crystallization from hexane of the mother liquors of **1a** gave a solid melting 288–302° which was treated with ethereal $CH_2N_2$ and then directly acetylated ($Ac_2O$–Py overnight) to give the pure acetoxy ester **2d** m p 237–9° (from MeOH), $[\alpha]_D$ −65° (c 1), (Found C, 77 15, H, 10 17, $C_{33}H_{52}O_4$ requires C, 77 29, H, 10 22%), $\delta$ 3 65 (–COOMe, *s* partially overlapping 3-H signal)

*Maslinic acid* (**1b**) *and methyl δ-maslinate* (**2c**) Fraction (C), reabsorbed on HCl-washed silica-gel (20 g) eluting with $C_6H_6$–$Et_2O$ (7 3), gave maslinic acid (**1b**) (320 mg), m p 267–70° (from MeOH), $[\alpha]_D$ +40° (c 0 9), identical with an authentic sample The mother liquors of crystallization of **1b**, treated with ethereal $CH_2N_2$ and chromatographed on silica-gel (eluent $C_6H_6$–$Et_2O$ 4 1), gave the dihydroxy ester (**2c**) m p 183–7° (from hexane), $[\alpha]_D$ −28° (c 2), (Found C, 76 37, H, 10 41 $C_{31}H_{50}O_4$ requires C, 76 50, H, 10 36%), $\nu_{max}$ 3350, 3480 $cm^{-1}$, $\delta$ 3 65 (–COOMe, *s*, partially overlapping 2-H and 3-H signals)

*Partial synthesis of* **2c** Methyl maslinate (**1c**) (300 mg) was treated with $Ac_2O$ in anhydrous pyridine overnight and the product was directly dissolved in HOAc (10 ml), $SeO_2$ (60 mg) added and refluxed for 3 days Work up of the reaction mixture gave an oil ($\lambda_{max}^{EtOH}$ 250 nm)[9] directly submitted to catalytic hydrogenation ($PtO_2$ catalyst in MeCOOH) for 2 hr Alkaline hydrolysis (KOH in MeOH 10%) of the product, followed by treatment with ethereal $CH_2N_2$, afforded a brown oil which was chromatographed on silica-gel Elution with $C_6H_6$–$Et_2O$ (4 1) gave a solid (110 mg) m p 182–4° (from hexane), $[\alpha]_D$ −26° identical with natural **2c**

*Partial synthesis of* **2d** Methyl oleanolate (**1d**) was acetylated and the product submitted to the same sequence of reactions described for the preparation of **2c** Purification of the final product, by crystallization from MeOH, gave the pure **2d** m p 237–9°, $[\alpha]_D$ −65°, identical with the natural compound

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[9] RAMART-LUCAS, P and HOCH, M J (1935) *Bull Soc Chim* **2**, 327