# *The Structure of the Acetate* $C_{32}H_{46}O_5$ ($O_5$ *Acetate*) *derived from β-Amyrin.*

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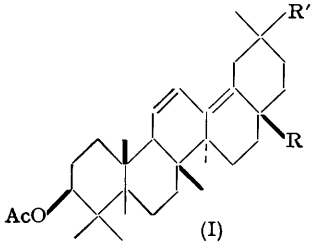
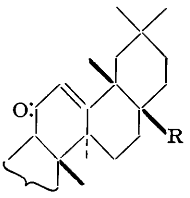
The behaviour of the $O_5$ acetate with alkali has been studied and the nature of the products has been established; a formula (VI) is proposed for the $O_5$ acetate.

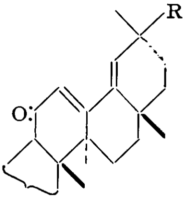
For a number of years it has been known that oxidation of oleana-11 : 13(18)-dienyl acetate (I; R = R′ = Me) with chromic acid gives, in high yield, an acetate, $C_{32}H_{46}O_5$ ($O_5$ acetate). The $O_5$ acetate is also obtained as a major product of the oxidation of 11-oxo-olean-12-enyl acetate (II; R = Me) or of 11-oxo-oleana-12 : 18-dienyl acetate (III; R = Me) with selenium dioxide and of olean-13(18)-enyl acetate (IV; R = R′ = Me) with chromic acid. Compounds directly related to the $O_5$ acetate are obtained by the same methods from corresponding derivatives of oleanolic acid and glycyrrhetic acid. Oxidation of methyl 11-oxo-olean-12-enolate acetate (II; R = $CO_2Me$) with selenium dioxide and of either methyl oleana-11 : 13(18)-dienolate acetate (I; R = $CO_2Me$, R′ = Me) or methyl olean-13(18)-enolate acetate (IV; R = $CO_2Me$, R′ = Me) with chromic acid gives in each case a methyl ester acetate, $C_{33}H_{46}O_7$ (Ruzicka and Jeger, *Helv. Chim. Acta*, 1941, **24**, 1236; Mower, Green, and Spring, *J.*, 1944, 256; Jeger, Norymberski, and Ruzicka, *Helv. Chim. Acta*, 1944, **27**, 1532). The methyl ester acetate $C_{33}H_{46}O_7$ has been obtained by oxidation of methyl 12 : 19-dioxo-oleananolate acetate (V) with selenium dioxide (Barton, Holness, Overton, and Rosenfelder, *J.*, 1952, 3751). Oxidation of the glycyrrhetic acid derivatives, methyl oleana-11 : 13(18)-dienol-30-ate acetate (I; R = Me, R′ = $CO_2Me$) and methyl olean-13(18)-enol-30-ate acetate (IV; R = Me, R′ = $CO_2Me$) with chromic acid and of methyl 11-oxo-oleana-12 : 18-dienol-30-ate acetate (III; R = $CO_2Me$) with selenium dioxide gives, in each case, a methyl ester acetate isomeric with that obtained from the named oleanolic acid derivatives (Jeger, Norymberski, and Ruzicka, *loc. cit.*). The nature of the $O_5$ acetate and its related compounds has not been established or even the subject of conjecture and during the period in which the structure of the β-amyrin–oleanolic acid group of triterpenoids has been established beyond peradventure the formation and structure of this well-defined group of oxidation products has remained an enigma.



(I)



(II)



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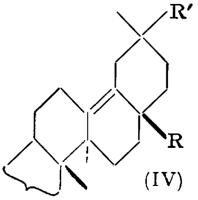

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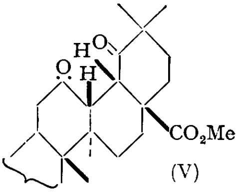
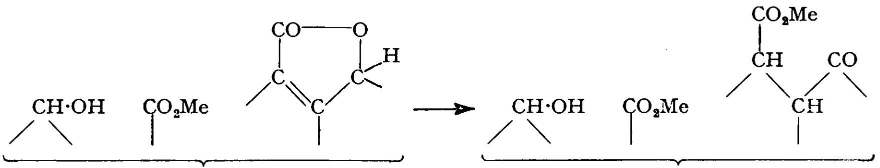


(V)

The $O_5$ acetate gives a faint yellow colour with tetranitromethane and its spectrum shows a broad band with maximum at approximately 2300 Å (ε 3000) together with a low-intensity inflection at 3000 Å. It does not contain a free hydroxyl group or react with carbonyl reagents although the presence of a carbonyl group is suggested by the absorption spectrum. Apart from suffering simple hydrolysis of the $C_{(3)}$-acetoxy-group, the $O_5$ acetate is stable to mineral acid. With alkali, however, the $O_5$ acetate is profoundly changed. In aqueous alkali, an amorphous neutral fraction is obtained, acetylation of which gives a homogeneous acetate formulated by Mower *et al.* (*loc. cit.*) as $C_{33}H_{46}O_5$ (or a near homologue) and by Jeger *et al.* (*loc. cit.*) as $C_{30}H_{46}O_4$. As a minor product of the action of alkali on the $O_5$ acetate Jeger *et al.* (*loc. cit.*) obtained a monobasic acid, $C_{29}H_{46}O_5$, which showed a strong absorption maximum at 2270 Å (log ε 4·26).

We have re-examined the behaviour of the $O_5$ acetate with alkali. Treatment of the $O_5$ acetate with methanolic potassium hydroxide, with exclusion of water, gives in high yield a methyl ester, $C_{31}H_{48}O_5$, which gives the corresponding acetate, $C_{33}H_{50}O_6$. In contrast to the parent $O_5$ acetate, the methyl ester shows strong absorption at 2250 Å (ε *ca.* 15,000) and it gives a positive Légal test although the colour with the nitroprusside reagent develops slowly; these properties suggest that the methyl ester contains an αβ-unsaturated lactone group. Hydrolysis of the $O_5$ acetate by aqueous-alcoholic alkali again gave an amorphous neutral fraction, together with a crystalline acid. Acetylation of the neutral fraction yielded a crystalline acetate, $C_{31}H_{48}O_4$; this is identical with the acetate obtained by the same method by Mower *et al.*, and by Jeger *et al.* Alkaline hydrolysis of the acetate gives the corresponding (crystalline) alcohol, $C_{29}H_{46}O_3$, reacetylated to the parent acetate, $C_{31}H_{48}O_4$. Analysis of the crystalline acid, which is identical with that obtained by Jeger *et al.*, agrees with $C_{30}H_{46}O_5$. It shows a well-defined absorption band at 2260 Å (ε 12,700) and with diazomethane gives a methyl ester, $C_{31}H_{48}O_5$, identical with the product of methanolysis of the $O_5$ acetate.

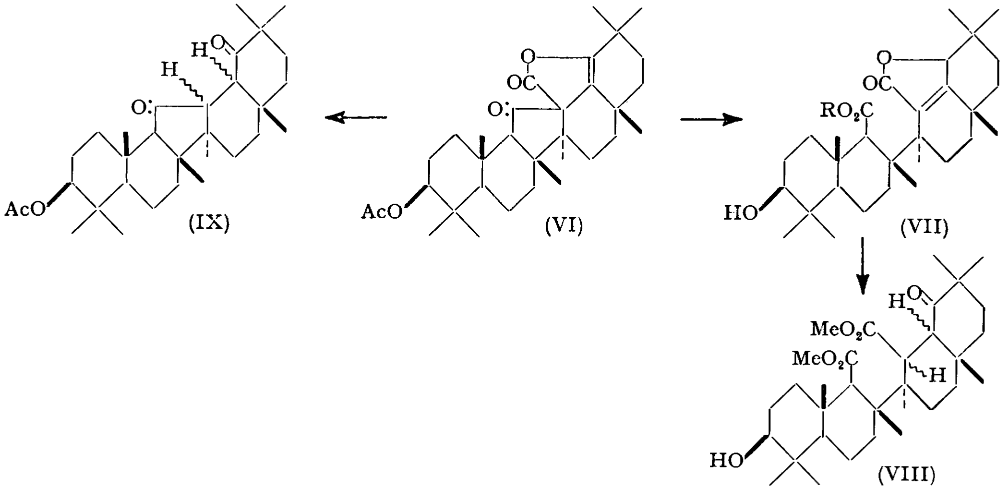
The presence of an αβ-unsaturated lactone grouping in the methyl ester, $C_{31}H_{48}O_5$, was confirmed by vigorous alkaline hydrolysis which gave an amorphous acid product, methylation of which yielded a crystalline dimethyl ester, $C_{32}H_{52}O_6$, in which all the oxygen functions have been characterised. Acetylation of the dimethyl ester yields a dimethyl ester acetate, $C_{34}H_{54}O_7$. The dimethyl ester and its acetate contain an isolated carbonyl group the presence of which was established by low-intensity absorption at 2800 Å. This behaviour of the methyl ester, $C_{31}H_{48}O_5$, with alkali is characteristic of αβ-unsaturated lactones (Paist, Blout, Uhle, and Elderfield, *J. Org. Chem.*, 1941, **6**, 273) and establishes the relation depicted below.



Methyl ester, $C_{31}H_{48}O_5$ Dimethyl ester, $C_{32}H_{52}O_6$

These considerations lead us to the view that (VII; R = Me) is the formula for the methyl ester, $C_{31}H_{48}O_5$, and consequently, the acid, $C_{30}H_{46}O_5$, is (VII; R = H) and the dimethyl ester, $C_{32}H_{52}O_6$, is (VIII). Accordingly the $O_5$ acetate is represented as a βγ-unsaturated lactone (VI) and support for this formula was forthcoming from the infra-red absorption spectrum of the related alcohol, $C_{30}H_{44}O_4$ (Mower *et al.*, *loc. cit.*), which was examined by Dr. A. R. H. Cole, whom we thank. In carbon tetrachloride the alcohol shows two carbonyl bonds at 1745 (5-membered ring ketone) and at 1781 cm.$^{-1}$ (βγ-unsaturated γ-lactone) together with an ethylenic band at 1692 cm.$^{-1}$.

The acetate, $C_{31}H_{48}O_4$, obtained from the $O_5$ acetate (VI) by treatment with *aqueous* alkali (followed by acetylation) is considered to be the diketone (IX), the formation of which has involved rupture of the βγ-lactone group in (VI) followed by decarboxylation. Some support for this formula is to be found in the optical properties of the acetate, $C_{31}H_{48}O_4$, and of the corresponding alcohol, $C_{29}H_{46}O_3$. In the ultra-violet region, the acetate shows only low-intensity carbonyl absorption at 2900 Å. The infra-red absorption spectrum of the alcohol, $C_{29}H_{46}O_3$, for which we are greatly indebted to Dr. G. D. Meakins, shows two well-defined peaks in the carbonyl region, at 1725 and 1706 cm.$^{-1}$. Although the peak at 1725 cm.$^{-1}$ is decidedly low for a carbonyl group in a 5-membered ring, the presence of two isolated carbonyl groups appears well established.



## EXPERIMENTAL

Rotations were measured in chloroform at 15—22° and ultra-violet absorption spectra were determined in ethanol solution.

*Treatment of the* $O_5$ *Acetate with Aqueous-methanolic Alkali.*—The $O_5$ acetate (1·1 g.) was heated under reflux for 3 hr. with a solution of potassium hydroxide (5 g.) in water (10 c.c.) and methanol (90 c.c.). The mixture was diluted with water and extracted with ether, to yield a neutral fraction. The aqueous alkaline phase was acidified and extracted with ether, to give an acid fraction. The neutral fraction, which did not crystallise, was acetylated with pyridine and acetic anhydride at 100°. The acetate, $C_{31}H_{48}O_4$, separated from methanol–acetone as needles (660 mg.), m. p. 259·5—260·5°, $[\alpha]_D$ +158° (*c*, 0·9). It does not give a colour with tetranitromethane (Found: C, 76·6; H, 9·85. $C_{31}H_{48}O_4$ requires C, 76·8; H, 10·0%). Hydrolysis of the acetate by refluxing 5% ethanolic potassium hydroxide for 3 hr. gave the *alcohol*, $C_{29}H_{46}O_3$, which separated from aqueous methanol as needles, m. p. 102—103°, $[\alpha]_D$ +147° (*c*, 1·5) (Found: C, 76·3; H, 10·7. $C_{29}H_{46}O_3,CH_3{\cdot}OH$ requires C, 75·9; H, 10·6%). Acetylation of the alcohol (acetic anhydride–pyridine at 100°) gave the parent acetate, $C_{31}H_{48}O_4$, which separated from methanol–acetone as needles, m. p. and mixed m. p. 259·5—260·5°, $[\alpha]_D$ +154° (*c*, 1·0).

The acid fraction was crystallised from acetone–light petroleum, to give the *acid*, $C_{30}H_{46}O_5$ (50 mg.), m. p. 272—274° (279—281° *in vacuo*), $[\alpha]_D$ −36° (*c*, 1·3) (Found: C, 74·2; H, 9·6. $C_{30}H_{46}O_5$ requires C, 74·0; H, 9·5%). Light absorption: Max. at 2260 Å (ε 12,700).

*Treatment of the* $O_5$ *Acetate with Methanolic Potassium Hydroxide.*—The $O_5$ acetate (1·78 g.) was heated under reflux for 2 hr. with potassium hydroxide (1·7 g.) in absolute methanol (34 c.c.). The mixture was diluted with water and extracted with ether to give a neutral fraction; acidification of the aqueous alkaline phase with dilute hydrochloric acid followed by extraction with ether gave an acid fraction. The neutral fraction was digested with aqueous methanol, and the crystalline solid recrystallised from the same solvent, to yield a *methyl ester* as cubes (765 mg.), m. p. 234—236°, $[\alpha]_D$ −46° (*c*, 1·6) (Found: C, 74·6; H, 9·6; OMe, 6·0. $C_{30}H_{45}O_4{\cdot}OCH_3$ requires C, 74·7; H, 9·7; OMe, 6·2%). Light absorption: Max. at 2250 Å (ε 15,500). The methyl ester does not give a colour with tetranitromethane but it slowly gives a positive Légal test.

The *methyl ester acetate* was obtained from the methyl ester by using pyridine and acetic anhydride at 100°. It separated from aqueous methanol as long prisms, m. p. 192—194°, $[\alpha]_D$ −33° (*c*, 1·2) (Found: C, 73·3; H, 9·3. $C_{33}H_{50}O_6$ requires C, 73·0; H, 9·3%). Light absorption: Max. at 2240 Å (ε 15,700).

The first mother-liquor from the methyl ester was evaporated and the amorphous residue acetylated (acetic anhydride–pyridine at 100°). The product crystallised from methanol–acetone, to yield the acetate, $C_{31}H_{48}O_4$, as needles, m. p. 253—256·5°, undepressed in m. p. when mixed with the specimen described above.

The acid fraction was crystallised from acetone–light petroleum, to give the acid, $C_{30}H_{46}O_5$, as needles (40 mg.), $[\alpha]_D$ −37°, m. p. and mixed m. p. 272—273° (Found: C, 74·05; H, 9·6%). Light absorption: Max. at 2260 Å (ε 12,720). Esterification of the acid with diazomethane gave the methyl ester, $C_{31}H_{48}O_5$, m. p. 231—232·5°, undepressed when mixed with the specimen described above; it shows a maximum at 2250 Å (ε 15,000).

*Treatment of the Methyl Ester,* $C_{31}H_{48}O_5$ *with Alkali.*—A solution of the methyl ester (0·25 g.) in methanolic potassium hydroxide (20%; 40 c.c.) was kept in an autoclave at 200° for 8 hr. The cold mixture was diluted with water (no precipitation), the solution acidified with dilute hydrochloric acid, and the precipitated acid isolated by means of ether. The acid separated from methanol–acetone as an amorphous solid, m. p. 332—333° (decomp.), $[\alpha]_D$ −28° (*c*, 1·25 in pyridine) (Found: C, 70·8, 70·8; H, 9·6, 9·7; OMe, 4·3. $C_{30}H_{48}O_6$ requires C, 71·4; H, 9·6. $C_{30}H_{47}O_5{\cdot}OCH_3$ requires C, 71·8; H, 9·7; OMe, 6·0%). The amorphous acid does not give a colour with tetranitromethane. Light absorption: Max. at 2800 Å (ε 390).

The *dimethyl ester,* prepared from the amorphous acid by treatment with ethereal diazomethane, separated from acetone–*n*-hexane as hexagonal plates, m. p. 228°, $[\alpha]_D$ −35° (*c*, 1·0) [Found: C, 72·3; H, 10·0; OMe, 11·1. $C_{30}H_{46}O_4(OCH_3)_2$ requires C, 72·1; H, 9·8; OMe, 11·6%]. The dimethyl ester does not give a colour with tetranitromethane.

Acetylation of the dimethyl ester by treatment overnight with acetic anhydride in pyridine gave the *dimethyl ester acetate,* prisms, m. p. 202·5—203·5°, $[\alpha]_D$ −28·5° (*c*, 1·3), from *n*-hexane (Found: C, 71·0, 71·2, 70·8; H, 9·8, 9·8, 9·5. $C_{34}H_{54}O_7$ requires C, 71·0; H, 9·5%). Light absorption: Max. at 2800 Å (ε 340).

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