

564. *The Chemistry of Triterpenes and Related Compounds.*
*Part XXVIII.** β -Boswellic Acid.

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The varying rotations given in the literature for β -boswellic acid and its derivatives have been shown to be due to the presence of the corresponding 9(11)-dehydro-compounds as impurity. The configurations of the 3-hydroxyl and 4-carboxyl groups have been proved to be α and β respectively, and the acid is therefore the ursane analogue of α -boswellic acid.

β -BOSWELIC ACID was recently needed as a source of nor- β -boswellenone for use as a model compound for the study of certain reactions of lupene-I. Although the acid was thought to be the ursane analogue (I) † of α -boswellic acid (3 α -hydroxyolean-12-en-24-oic acid)⁵ (II) no satisfactory proof of the configuration of the hydroxyl and carboxyl groups existed. In addition there were serious discrepancies in the literature in the optical rotations quoted for β -boswellic acid and its derivatives.⁶

β -Boswellic acid is, as its acetate, one of the principal constituents of frankincense (olibanum). It was first obtained in a fairly pure state by Winterstein and Stein⁷ along with the isomeric α -boswellic acid, and was characterised by them as a monohydroxy-acid, C₃₀H₄₈O₃. By mild oxidation with chromium trioxide, Simpson and Williams⁸ obtained a neutral ketone, nor- β -boswellenone, C₂₉H₄₆O, showing the acid to be a β -hydroxy-acid. They also showed that it had an inert double bond. β -Boswellic acid was later converted into α -amyrin (III), by the Wolff-Kishner reduction of its aldehyde,⁹ leading to its formulation¹⁰ as (IV).

It was noted by Winterstein and Stein⁷ that the rotation of β -boswellic acid varied from one preparation to another and they suggested the presence of an isomer of high dextrorotation to account for this. Simpson and Williams⁸ also noted the variable rotation of β -boswellic acid, and showed that it depended to some extent on the method of preparation of the acid. In the method usually employed,⁷ cold acetic anhydride treatment of the barium salt of crude boswellic acid acetate (obtained by precipitation from the ether extract of olibanum) affords the mixed anhydride of *O*-acetylboswellic and acetic acid. Decomposition of this anhydride and crystallisation from methanol affords β -boswellic acid acetate of low rotation. If, however, the crude barium salt is boiled with acetic anhydride a higher yield of β -boswellic acid acetate is obtained, but the rotation of this material is approximately double that of the acid prepared by the first method.

It has now been shown that the high rotations of some samples of β -boswellic acid are due to the presence, as an impurity, of the corresponding 9 : 11-dehydro-derivative (V) with the homoannular diene chromophore. The ultraviolet spectra of samples of β -boswellic acid and its derivatives showed some absorption at 2810 Å with extinction values such that if they were plotted against rotation, a straight line was obtained for any given derivative (cf. Table, p. 2908). All dienes of this type in the oleanane and ursane series show maximum absorption at 2800—2820 Å (ϵ 11,000—12,000) and high dextrorotations.¹¹

* Part XXVII, *J.*, 1954, 4471.

† Ring ϵ of the ursane ring system is not included since no completely convincing evidence is as yet available to decide between the various structures which have recently been proposed.^{1, 2, 3, 4}

¹ Corey and Ursprung, *Chem. and Ind.*, 1954, 1387.

² Beaton, Spring, Stevenson, and Strachan, *J.*, 1955, 2610.

³ Meisels, Rugg, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1955, **38**, 1298.

⁴ Meakins, *Chem. and Ind.*, 1955, 1353.

⁵ Vogel, Jeger, and Ruzicka, *Helv. Chim. Acta*, 1951, **34**, 2323.

⁶ See Elsevier's "Encyclopaedia of Organic Chemistry," 1940, Vol. XIV, pp. 559—561; and 1953, Supplement, p. 1087 S.

⁷ Winterstein and Stein, *Z. physiol. Chem.*, 1932, **208**, 9.

⁸ Simpson and Williams, *J.*, 1938, 686, 1712.

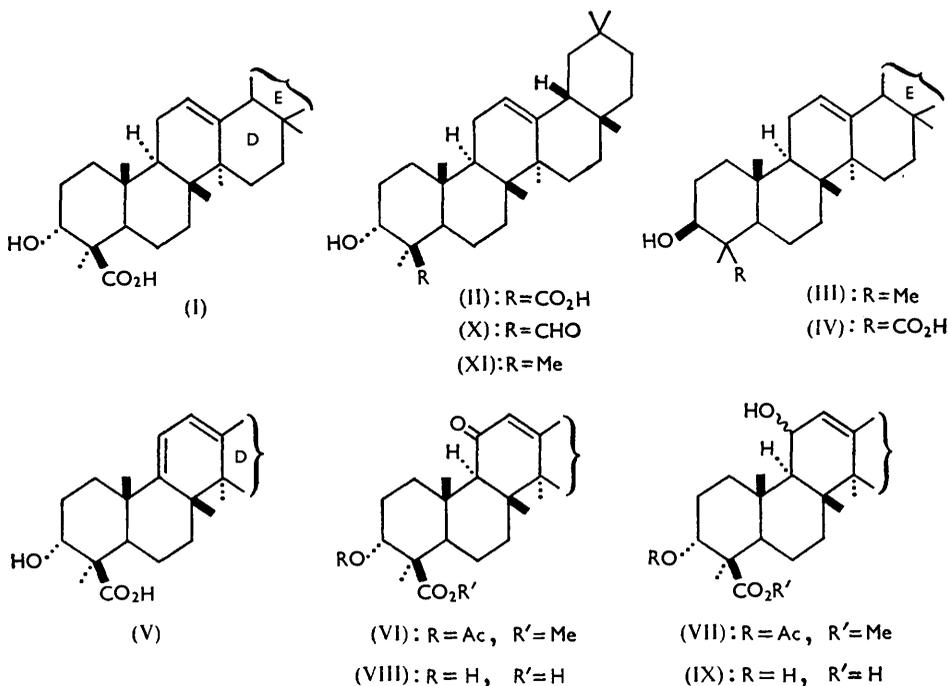
⁹ Ruzicka and Wirz, *Helv. Chim. Acta*, 1939, **22**, 948.

¹⁰ Jeger, *Fortschr. Chem. org. Naturstoffe*, 1950, **7**, 59.

¹¹ Cf. ref. 6, pp. 952 S, and 1071 S.

It has not been possible to separate pure β -boswellic acid or any pure derivatives from the diene contaminant by chromatography or repeated crystallisation. Most of the diene impurity was eliminated by treatment of β -boswellic acid with lithium in ammonia in the presence of ethanol, a product containing *ca.* 0.5% of diene impurity being thus obtained. The β -boswellic acid and its derivatives prepared in this manner were used for the experiments described later in this paper; their rotations were lower than any described in the literature. A second reduction lowered the diene content to 0.05%.

The presence of the diene impurity suggests that the compound of high dextrorotation isolated by Trost¹² from frankincense and called γ -boswellic acid may well be mainly the 9:11-dehydro-derivative of β -boswellic acid. It also explains the observation by Simpson and Williams⁸ that a methyl β -boswellate acetate of low rotation (*i.e.*, containing little diene impurity) could be obtained by hydrogenolysis of the 11-oxo-derivative (VI) which had been prepared by chromic acid oxidation of methyl β -boswellate acetate of high rotation (*i.e.*, of high diene content). Under the conditions of oxidation the diene impurity also gives rise to the 11-oxo-derivative (VI). Hydrolysis of the acetate of low rotation prepared in this manner gave, however, a sample of methyl β -boswellate of high dextrorotation, suggesting that the initial hydrogenolysis product contained some of the 11-hydroxy-derivative (VII), an allylic alcohol. This probably dehydrates to the homoannular diene system during the hydrolysis or the subsequent working up of the product. Support for



this suggestion is provided by the observation by Barton and Holness¹³ that hydrogenolysis of methyl 3 β -acetoxy-12-oxo-olean-9(11)-en-28-oate followed by chromatography of the product afforded, as well as the desired methyl 3 β -acetoxyolean-9(11)-en-28-oate, the corresponding diene. This may also have arisen from an allylic alcohol formed as a by-product of the hydrogenolysis. Finally the observation by Simpson and Williams⁸ that the rotation of the resulting nor- β -boswellenone was more or less independent of the rotation of the β -boswellic acid from which it was prepared is explained since any initial dehydro-derivative will be oxidised during the reaction.

¹² Trost, *Ann. Chim. appl.*, 1937, 27, 178.

¹³ Barton and Holness, *J.*, 1952, 78.

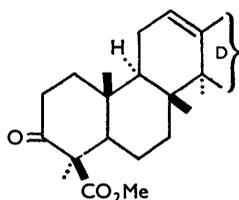
Since β -boswellic acid obtained from olibanum contains the corresponding 9(11):12-diene (V) as impurity it was of interest to discover whether the diene occurs as such, or as a precursor, in olibanum.

The barium salt of crude boswellic acid acetate (obtained as above) showed only maximum absorption at 2500 Å (ϵ ca. 2200), indicating the presence of a compound such as 11-oxo- β -boswellic acid (VIII). No shoulder was evident in the 2700—2900 Å region, indicating the absence of the homoannular diene (V). After the barium salt had been heated in ethanolic hydrochloric acid the ultraviolet spectrum had a shoulder at ca. 2800 Å (ϵ 400) indicative of the presence of about 3% of diene. These results show that the diene (V) is not present in olibanum, but that it contains the allylic alcohol (IX) or its acetate which is dehydrated under acidic conditions. It is now clear why, in the preparation of β -boswellic acid acetate, only a small amount of diene is found if the barium salt is treated with cold acetic anhydride whereas with boiling acetic anhydride the product contains much diene. So far it has not been possible to isolate the diene-precursor (IX).

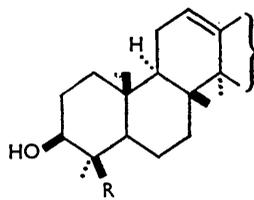
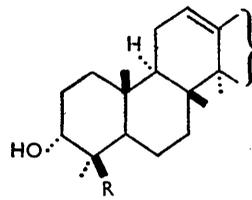
The second problem concerning β -boswellic acid was the configurations of the hydroxyl and the carboxyl group. Ruzicka and Wirz¹⁴ have shown that Wolff-Kishner reduction of the aldehyde (X) obtained from α -boswellic acid (II) of the oleanane group gave mainly *epi*- β -amyrin (olean-12-en-3 α -ol) (XI) together with a small amount of β -amyrin. Since *epi*- β -amyrin was the main product, and since β -amyrin was not epimerised under the Wolff-Kishner reduction conditions, the Swiss workers concluded that the hydroxyl group of α -boswellic acid was in the 3 α (*epi*)-configuration.¹⁵ On the other hand reduction of β -boswellic acid by a similar route indicated that its hydroxyl group was in the 3 β -configuration since α -amyrin (urs-12-en-3 β -ol) was the only product.⁹ However, this conclusion is open to criticism since epimerisation may have taken place during the reduction.

The carboxyl group of α -boswellic acid was shown to have the β -configuration by Vogel, Jeger, and Ruzicka⁵ by comparison of the molecular rotations of a pair of C₆₀-epimers derived from α -boswellic acid (II) and hederagenin, with the molecular rotations of a similar pair of diterpene epimers of known configuration. Further evidence in favour of the carboxyl group's having the 4 β (axial)-configuration in α -boswellic acid, and also in β -boswellic acid may be deduced from the resistance of esters of these acids to hydrolysis.¹⁶

The evidence so far discussed shows that α -boswellic acid has a 3 α -hydroxyl group and a 4 β -carboxyl group but that in β -boswellic acid, whilst the 4-carboxyl group is still



(XII)

(XIII): R = CO₂Me(XV): R = CH₂·OH(XIV): R = CO₂Me(XVI): R = CH₂·OH

β -orientated, the 3-hydroxyl group also has the β -configuration. However this conclusion is at variance with molecular-rotation evidence. In a triterpene the change in molecular rotation on acetylation of a 3 α -hydroxyl group is usually large and negative (cf. Klyne and Stokes¹⁷). For a 3 β -hydroxyl group the change is usually small but positive. When pure β -boswellic acid is converted into its acetate the ΔM_D value is -175° , indicative of a 3 α -hydroxyl group. Klyne and Stokes¹⁷ have also come to this conclusion.

Chemical evidence for the 3 α -configuration has been obtained from the reduction of methyl β -boswellonate (XII) with sodium borohydride and with lithium aluminium hydride.

¹⁴ Ruzicka and Wirz, *Helv. Chim. Acta*, 1940, **23**, 132; 1941, **24**, 248.

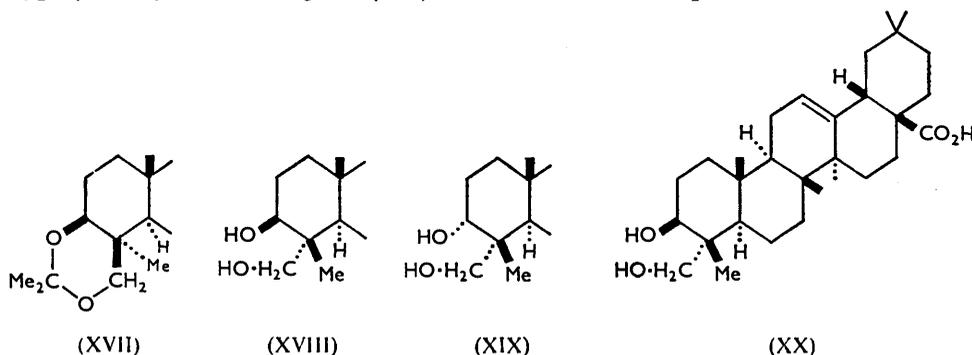
¹⁵ Bischof, Jeger, and Ruzicka, *ibid.*, 1949, **32**, 1911.

¹⁶ Bilham, Kon, and Ross, *J.*, 1942, **35**.

¹⁷ Klyne and Stokes, *J.*, 1954, 1979.

With the former reagent a hydroxy-ester (XIII) isomeric with methyl β -boswellate (XIV) was obtained. The hydroxyl group in (XIII) must be equatorial (3β) since, so far as is known, borohydride reduction of a keto-group in a six-membered ring always leads to an equatorial hydroxyl group and only occurs if the ketone is not sterically hindered.^{18,19} Reduction of the esters (XII) and (XIII) with lithium aluminium hydride afforded the diol (XV), which likewise must possess a 3β -hydroxyl group. On the other hand, reduction of β -boswellic acid with lithium aluminium hydride gave a different diol (XVI), containing the original α -hydroxyl group of β -boswellic acid. Support for the structures assigned to (XIII), (XIV), (XV), and (XVI) may be adduced from the molecular rotations of the pairs of epimeric esters (XIII) and (XIV), and diols (XV) and (XVI), the 3β -hydroxy-compounds (XIII) and (XV) being more dextrorotatory than the 3α -isomers (XIV) and (XVI) respectively. Klyne and Stokes¹⁷ have pointed out that triterpenoid 3β -hydroxyl groups make a positive contribution to the rotation of the molecule where 3α -hydroxyl groups make a negative contribution.

Chemical evidence has also been obtained which confirms the β -configuration of the 4-carboxyl group of β -boswellic acid. Treatment of the diol (XV) with acetone containing 1% of sulphuric acid afforded a cyclic *isopropylidene* derivative (XVII) in *ca.* 50% yield. On hydrolysis of this with acid in the presence of 2 : 4-dinitrophenylhydrazine sulphate, the diol (XV) was recovered along with acetone 2 : 4-dinitrophenylhydrazone. By contrast similar treatment of the diol (XVI) resulted in the recovery of starting material unchanged. Of the four possible diols of this type, (XV), (XVI), (XVIII), and (XIX), all would be expected to form condensation products except (XVI) where the two axial groups (hydroxyl and hydroxymethyl) are not suitably disposed. No diol of type (XIX) is known, but a diol of type (XVIII), *i.e.*, hederagenin (XX), forms a condensation product.²⁰



Barton and Jones,²¹ writing long before anything was known about the stereochemistry of ring A of the boswellic acids, suggested that hydrogen bonding took place between the hydroxyl and carboxyl groups of the boswellic acids and that such hydrogen bonding might be responsible for the (then) apparently anomalous molecular-rotation differences found with these acids and their derivatives. It is now clear that the 3α - and 4β -substituents, being both axial and antiparallel, could not take part in hydrogen bonding and that it is the 3α -configuration of the hydroxyl groups which is responsible for the anomalous rotation differences found with these acids, as compared with the differences observed with other triterpenes of the α - and the β -amyryn group.

EXPERIMENTAL

Rotations were determined in CHCl_3 at room temperature unless otherwise stated. M. p.s were determined on a Kofler block. The alumina used for chromatography, unless otherwise stated, had an activity of I—II. Light petroleum refers to the fraction with b. p. 40—60°. Ultraviolet spectra were determined in EtOH.

¹⁸ Halsall, Hodges, and Jones, *J.*, 1953, 3019.

¹⁹ Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.

²⁰ Jacobs, *J. Biol. Chem.*, 1925, **63**, 631.

²¹ Barton and Jones, *J.*, 1944, 659.

Isolation of β -Boswellic Acid (essentially the method of Winterstein and Stein?).—Olibanum (1 kg.) was divided into two portions and each was shaken with ether (1 l.) for 14 hr. After filtration the ethereal extract was again divided into two portions and each was treated with barium hydroxide (30 g.) suspended in a saturated solution of barium hydroxide (750 c.c.). The barium salt of the crude boswellic acid acetate was immediately precipitated, filtered off, washed with ether, and dried to a pale yellow solid (330 g.). The barium salt was then boiled for 4 hr. with acetic anhydride (500 g.) containing a small amount of pyridine. On cooling, the mixed anhydride of crude boswellic acid acetate and acetic acid crystallised and was separated. The mixed anhydride was dissolved in chloroform and heated under reflux with methanol for 1 hr. The product, crystallised from chloroform-methanol, gave crude β -boswellic acid acetate as rhombs (53 g.), m. p. 262—269°. Further crystallisation from chloroform-methanol afforded a mixture of β -boswellic acid acetate (3 α -acetoxyurs-12-en-24-oic acid) and ca. 30% of 3 α -acetoxyursa-9(11):12-dien-24-oic acid. This mixture had m. p. 273—276°, $[\alpha]_D +149^\circ$ (*c*, 2.1), and an absorption max. at 2810 Å (ϵ 3420). From different preparations of β -boswellic acid acetate samples containing varying quantities of the diene as impurity were obtained. Samples of methyl β -boswellate acetate, contaminated with the corresponding dehydro-compound, were obtained by methylation of ethereal solutions of free acid with diazomethane. The constants of a number of diene-containing samples of β -boswellic acid acetate and of the methyl ester are given in the annexed Tables.

Constants of mixtures of β -boswellic acid acetate with the corresponding 9(11):12-diene.

$[\alpha]_D$	+63°	+70.5°	+101°	+132°	+149°
ϵ at 2810 Å	60	375	1330	2700	3420
Diene (%)	0.5	3.3	11.9	24	30.5
M. p.	275—278°	270—274°	270—274°	285—289°	273—276°

Constants of mixtures of methyl β -boswellate acetate with the corresponding 9(11):12-diene.

$[\alpha]_D$	+62°	+76.5°	+81°	+102°	+105°	+107.5°	+112°
ϵ at 2810 Å	60	504	706	1760	1915	2000	2180
Diene (%) ...	0.5	4.5	6	16	17	18	19
M. p.	191—193°	190—193°	191—193°	184—185°	186—189°	186—187°	183—185°

Treatment of a Mixture of β -Boswellic Acid Acetate and the Corresponding 9(11):12-Diene with Lithium in Liquid Ammonia in the Presence of Ethanol.—The mixture (ϵ 3420 at 2810 Å, corresponding to 30% diene content) (500 mg.) in ether (25 c.c.), dioxan (25 c.c.), and ammonia (ca. 50 c.c.) was treated with lithium (3 g.). After the mixture had been stirred for 1 hr., all the lithium had dissolved; ethanol was added dropwise until the solution became colourless. The solution was then added to water with caution, acidified with hydrochloric acid, and extracted with ether. The product (450 mg.) was reacylated with acetic anhydride-pyridine to give β -boswellic acid acetate (3 α -acetoxyurs-12-en-24-oic acid), m. p. 275—278° (from methanol), $[\alpha]_D +63^\circ$ (*c*, 1.88), $\epsilon =$ ca. 60 at 2810 Å (0.5% diene). After a second reduction with lithium and ammonia as above the product showed slight light absorption at 2810 Å (ϵ 5—10) indicating that the last traces of diene are not easily removed. Except where otherwise stated β -boswellic acid acetate obtained after a single reduction with lithium and ammonia was used for the experiments described below.

Hydrolysis of β -boswellic acid acetate with 10% methanolic potassium hydroxide afforded β -boswellic acid (3 α -hydroxyurs-12-en-24-oic acid) as rhombs, m. p. 212—215°, resolidifying as prisms, m. p. 228—232°, $[\alpha]_D +107^\circ$ (*c*, 0.75). Methylation of β -boswellic acid acetate with ethereal diazomethane gave methyl β -boswellate acetate (methyl 3 α -acetoxyurs-12-en-24-oate) as prisms (from methanol), m. p. 191—193°, $[\alpha]_D +62^\circ$ (*c*, 0.88) (Found: C, 77.4; H, 10.15. Calc. for $C_{33}H_{52}O_4$: C, 77.3; H, 10.2%). Hydrolysis of methyl β -boswellate acetate with 10% methanolic potassium hydroxide afforded methyl β -boswellate (methyl 3 α -hydroxyurs-12-en-24-oate) as prisms (from aqueous methanol), m. p. 195—196°, $[\alpha]_D +111^\circ$ (*c*, 1.6). Oxidation of methyl β -boswellate in acetone with chromic acid (2 mols.) in sulphuric acid gave methyl β -boswellonate (methyl 3-oxours-12-en-24-oate) as needles (from methanol), m. p. 160—162°, $[\alpha]_D +102^\circ$ (*c*, 0.62).

Reduction of β -Boswellic Acid with Lithium Aluminium Hydride.— β -Boswellic acid (125 mg.) in ether (50 c.c.) was treated with lithium aluminium hydride (50 mg.) at 20° overnight. After decomposition of the reagent with water, ethereal extraction afforded a product (110 mg.) which gave urs-12-ene-3 α :24-diol (XVI) as leaflets (from aqueous methanol), m. p. 183—185°, $[\alpha]_D$

+73° (*c*, 1.9). It was not possible to obtain satisfactory analytical figures for this compound and it was hence characterised as the diacetate. Acetylation with acetic anhydride in pyridine at 100° for 3 hr. gave the *diacetate* as plates (from aqueous methanol), *m. p.* 161—163°, $[\alpha]_D + 34^\circ$ (*c*, 0.5) (Found: C, 77.4; H, 10.4. $C_{34}H_{54}O_4$ requires C, 77.5; H, 10.3%).

Reduction of Methyl β -Boswellonate (Methyl 3-Oxours-12-en-24-oate) with Lithium Aluminium Hydride.—Methyl β -boswellonate (200 mg.) in ether (50 c.c.) was treated with lithium aluminium hydride (100 mg.) at 20° overnight. After decomposition of the reagent with water, ether extraction afforded a product (290 mg.) which gave *urs-12-ene-3 β :24-diol* (XV) as plates (from methanol), *m. p.* 224—226°, $[\alpha]_D + 85^\circ$ (*c*, 1.2) (Found: C, 81.35; H, 11.15. $C_{30}H_{50}O_2$ requires C, 81.4; H, 11.4%). The diol was also obtained (together with 24-norurs-12-en-3-one, *m. p.* 190—192°) on treatment of methyl β -boswellonate with lithium in ammonia in the presence of ethanol. It crystallised from methanol as plates, *m. p.* 224—226°, undepressed on admixture with the lithium aluminium hydride reduction product, $[\alpha]_D + 88.5^\circ$ (*c*, 1.72).

Reduction of Methyl β -Boswellonate with Sodium Borohydride.—Methyl β -boswellonate (300 mg.) in dioxan (50 c.c.) was treated with a solution of sodium borohydride (200 mg.) in water (10 c.c.) at 20° overnight. After addition of water, isolation with ether yielded a product (300 mg.) which gave *methyl 3 β -hydroxyurs-12-en-24-oate* (XIII) as needles (from aqueous methanol), *m. p.* 155—157°, $[\alpha]_D + 122^\circ$ (*c*, 0.75), $[\alpha]_D + 124^\circ$ (*c*, 0.82) in acetone, $[\alpha]_D + 121^\circ$ (*c*, 0.78) in dioxan (Found: C, 78.85; H, 10.5. $C_{31}H_{50}O_3$ requires C, 79.1; H, 10.7%). Acetylation of the product gave *methyl 3 β -acetoxyurs-12-en-24-oate* as crystals (from chloroform-methanol), *m. p.* 182—185°, $[\alpha]_D + 104^\circ$ (*c*, 0.37) (Found: C, 76.85; H, 10.2. $C_{33}H_{52}O_4$ requires C, 77.3; H, 10.2%).

Reduction of Methyl 3 β -Hydroxyurs-12-en-24-oate (XIII) with Lithium Aluminium Hydride.—Methyl 3 β -hydroxyurs-12-en-24-oate (XIII) (50 mg.) in ether (50 c.c.) was treated with lithium aluminium hydride (100 mg.) at 20° overnight. The product (50 mg.) was worked up in the usual manner to give *urs-12-ene-3 β :24-diol* (XV) as plates (from methanol), *m. p.* and mixed *m. p.* 223—225°, $[\alpha]_D + 84^\circ$ (*c*, 0.25).

Treatment of Urs-12-ene-3 β :24-diol (XV) with 1% Sulphuric Acid in Acetone.—(a) *Urs-12-ene-3 β :24-diol* (XV) (300 mg.) in dry acetone (100 c.c.) containing sulphuric acid (1 c.c.) was kept at 20° overnight. After addition of sodium carbonate, the solution was poured into sodium carbonate solution. Ether-extraction yielded a product which was adsorbed from benzene on alumina (50 g.). Elution with benzene (200 c.c.) afforded a product (130 mg.) which gave the *3 β :24-isopropylidenedioxyurs-12-ene* as leaflets (from methanol), *m. p.* 160—162°, $[\alpha]_D + 87^\circ$ (*c*, 0.9) (Found: C, 81.95; H, 11.2. $C_{33}H_{54}O_2$ requires C, 82.1; H, 11.25%).

(b) *Urs-12-ene-3 β :24-diol* (300 mg.) was treated with 1% sulphuric acid in acetone as above and the product and sulphuric acid were then adsorbed from the mixture on alumina (100 g.). Elution with benzene (200 c.c.) afforded a product (150 mg.) which gave the *isopropylidene* derivative as leaflets (from aqueous methanol), *m. p.* 161—163°, $[\alpha]_D + 87^\circ$ (*c*, 0.78). Further elution afforded unchanged diol (150 mg.) as plates (from methanol), *m. p.* 222—225° undepressed on admixture with the starting material, $[\alpha]_D + 120.5^\circ$ (*c*, 0.46).

When *urs-12-ene-3 α :24-diol* (XVI) was treated with 1% sulphuric acid in acetone, and the product isolated as above [methods (a) and (b)], only unchanged starting material was obtained.

Hydrolysis of 3 β :24-isopropylidenedioxyurs-12-ene.—This derivative (40 mg.) in methanol (25 c.c.), distilled from Brady's reagent, was heated under reflux with 2:4-dinitrophenylhydrazine (50 mg.) and sulphuric acid (0.5 c.c.) for 5 min. and then kept for 1 hr. After dilution with water, extraction with benzene yielded a product which was adsorbed from benzene on alumina (50 g.). Elution with benzene-ether (19:1; 1 l.) afforded crystals (15 mg.; theor., 19 mg.) which gave acetone 2:4-dinitrophenylhydrazone as needles (from aqueous methanol), *m. p.* and mixed *m. p.* 120—127°.

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