599. *The Chemistry of the Triterpenes. Part IX.* Elucidation of the Betulin–Oleanolic Acid Relationship.*

By G. S. Davy, T. G. Halsall, and E. R. H. Jones.

The inter-relation of the lupeol and the β-amyrin series of triterpenes has again been demonstrated. Betulonic acid is converted into a saturated keto-lactone by isomerisation with acidic reagents. Reduction of this lactone with lithium aluminium hydride gives a triol, acetylation of which is accompanied by dehydration giving the diacetate of moradiol. The relation of this diol to germanicol and oleanolic acid has been elucidated by Barton and Brooks (*J.*, 1951, 257), and hence a relation between betulin and oleanolic acid is established. It is therefore confirmed that β-amyrin, germanicol, and lupeol are identical in rings A, B, C, and D, except for the presence of a double bond in ring C in β-amyrin (cf. Ames, Halsall, and Jones, *J.*, 1951, 450); further, it can now be concluded that the primary alcohol group of betulin and the carboxyl group of oleanolic acid occupy the same position relative to their pentacyclic ring systems.

Conversion of the carboxyl groups of betulinic acid and oleanolic acid into acetyl groups, and isomerisations of the resulting products under acidic conditions, are described.

A review of the lactonisation of betulinic acid indicated some anomalies which have been investigated and resolved. A preliminary account of part of this work has already been published (Davy, Halsall, and Jones, *Chem. and Ind.*, 1950, 732).

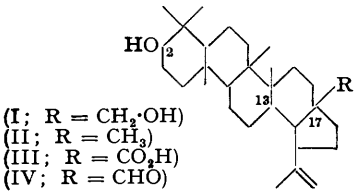
In continuation of our investigations (cf. Ames, Halsall, and Jones, *J.*, 1951, 450) on the inter-relation of the major groups of triterpenes and the members thereof, it was desirable to establish the relation between betulin (probably I), a triterpene of the lupeol group, and oleanolic acid (V), a member of the β-amyrin group of established structure. There would then follow a proof that the primary alcohol group of betulin occupies the same position relative to rings A, B, C, and D as the carboxyl group of oleanolic acid. Conversion of the primary alcohol group of betulin into a methyl group yields lupeol (Ruzicka and Brenner, *Helv. Chim. Acta*, 1939, **22**, 1532). Hence betulin differs from lupeol only in having one of the six methyl groups in the now established structure (Ames, Halsall and Jones, *loc. cit.*) replaced by a $-CH_2 \cdot OH$ group. Some evidence for the position of the $-CH_2 \cdot OH$ group relative to the *iso*propenyl side chain has already been provided by Ruzicka and Rey (*Helv. Chim. Acta*, 1943, **26**, 2143) who showed that the acetate of the betulin degradation product, bisnorlupanoldicarboxylic acid, formed an anhydride. This evidence, on the basis of the known structure of lupeol, suggests that the primary alcohol group of betulin is at $C_{(17)}$. Final proof of this is provided by the results described in this paper.

In establishing the inter-relation of lupeol (II) and β-amyrin (VI), both substances were converted *via* the corresponding ketones, β-amyrenone and lupenone, or the corresponding hydrocarbons, into δ-amyrene (VIII), or a derivative thereof, by isomerisation with acidic reagents. Similar isomerisation of betulin, provided that the hydroxyl groups remained intact and unreactive throughout the isomerisation, would yield δ-erythrodiol (IX), which could be obtained either by isomerisation of erythrodiol (VII) or by reduction of δ-oleanolic acid (Barton and Brooks, *loc. cit.*). However, treatment of betulin with formic acid leads to the production of the saturated cyclic ether, *allo*betulin (see following paper). Because of this, and the general unsuitability of triterpene alcohols for such isomerisation reactions (cf. Ames, Halsall, and Jones, *loc. cit.*), no attempt was made to isomerise betulin itself. Instead, attention was turned to betulinic acid (III), the acid obtained by the oxidation of the primary alcohol group of betulin, and to the corresponding ketone, betulonic acid. Robertson, Soliman, and Owen (*J.*, 1939, 1267) have already reported that betulinic acid is converted by hydrogen bromide in acetic acid into a saturated hydroxy-lactone, designated "lactone-A."

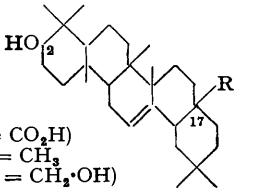
With sulphuric acid in acetic acid–benzene, methyl betulonate similarly gave a saturated keto-lactone, also obtained by chromic acid oxidation of "lactone-A." Treatment of the keto-lactone with lithium aluminium hydride resulted in the reductive scission of the lactone giving

* Part VIII, *J.*, 1951, 458.

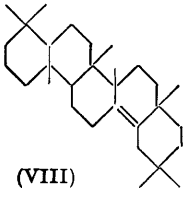
a triol, the $C_{(2)}$-keto-group also being reduced. One effect of this fission is thus to convert the lactonic carbonyl group, *i.e.*, the carboxyl group of the original betulonic acid, into a primary alcohol group ($-O-CO- \longrightarrow -OH\ HO\cdot CH_2-$). Acetylation of the triol with acetic anhydride in pyridine gave a diacetate. Since betulin also forms a diacetate under these conditions,



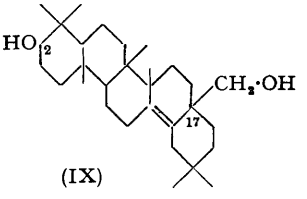
(I; R = $CH_2\cdot OH$)
(II; R = CH_3)
(III; R = CO_2H)
(IV; R = CHO)



(V; R = CO_2H)
(VI; R = CH_3)
(VII; R = $CH_2\cdot OH$)

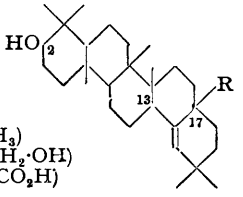


(VIII)

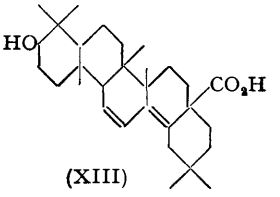


(IX)

the two acetyl groups may be allocated to the secondary hydroxyl group at $C_{(2)}$ and the primary hydroxyl group, the new hydroxyl group resulting from the lactone fission being unreactive. Acetylation with acetic anhydride and pyridine under reflux gave a mixture of the diacetate and a triacetate. Acetic anhydride with boron trifluoride as catalyst also gave the triacetate, together with the diacetate of a mono-unsaturated diol formed apparently by dehydration involving the unreactive hydroxyl group. This diacetate proved to be that of moradiol (XI),



(X; R = CH_3)
(XI; R = $CH_2\cdot OH$)
(XII; R = CO_2H)



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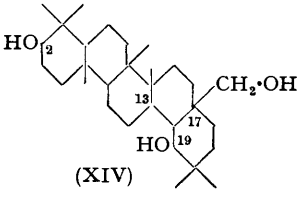

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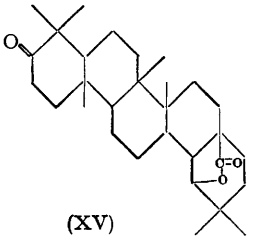
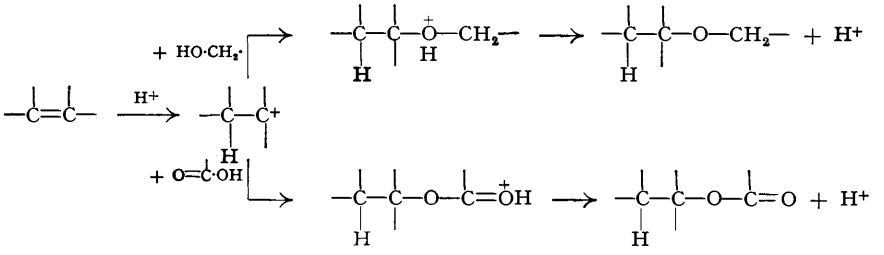


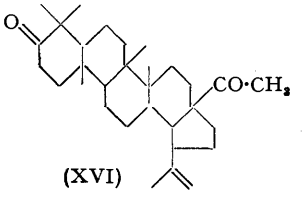
(XV)

belonging to the germanicol (X) group of triterpenes (Barton and Brooks, *loc. cit.*). The identity of the deacetylation product with an authentic sample of moradiol, kindly provided by Dr. D. H. R. Barton, was confirmed by a mixed melting-point determination. Barton and Brooks (*loc. cit.*) obtained moradiol by lithium aluminium hydride reduction of morolic acid which was most elegantly and completely demonstrated, partly by conversion into dehydro-oleanolic acid (XIII), to have structure (XII). The carboxyl group of oleanolic acid is therefore located at the same position ($C_{(17)}$) relative to the ring system as the primary alcohol group of moradiol. This group in the moradiol obtained from the lactone is derived from the carboxyl group of betulinic acid and hence the primary alcohol group of betulin. Consequently this group and the carboxyl group of oleanolic acid (V) occupy the same position relative to the pentacyclic system, and further confirmation of the common carbon skeleton and stereochemistry of rings A, B, and C in β-amyrin (VI), lupeol (II), and germanicol (X) is provided. The identity of the stereochemical configuration at $C_{(13)}$ in lupeol (II) and germanicol (X) is also proved.

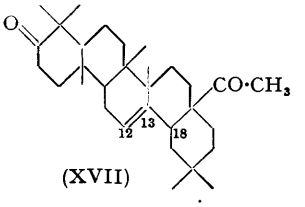
If it is assumed that no rearrangement occurs on dehydration of the triol, it can be assigned structure (XIV), proof of which and its implications are discussed in the following paper. The proof is unnecessary for the logical development of the arguments presented in this paper. Structure (XIV) for the triol leads to (XV) for the keto-lactone.



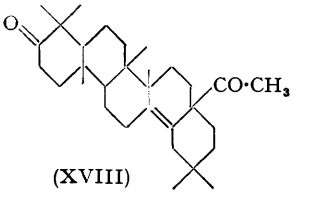
The formation of the saturated ether from betulin, and of the saturated lactone from betulonic acid, can be regarded as being interaction of the primary alcohol and carboxyl groups respectively with a carbonium ion formed under the acidic conditions. Inspection of these formulæ indicated that in order to proceed from the betulin to the δ-amyrin series by acidic isomerisation the primary alcohol group of betulin would have to be modified in some way other than by conversion into a carboxyl group or a derivative. Transformation of the carboxyl group of betulonic acid into an acetyl group and isomerisation of the resulting homolupenedione (XVI) appeared to offer a way out of this difficulty. For comparative



(XVI)



(XVII)



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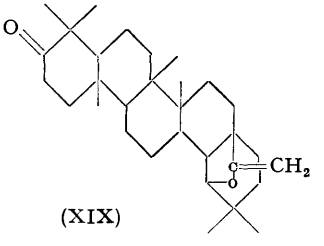
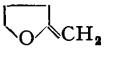


(XIX)



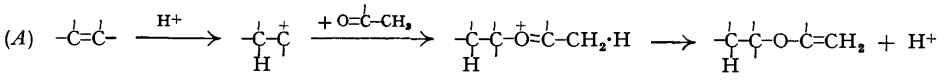
(XX)

purposes the corresponding homo-dione, homo-oleanenedione (XVII), was prepared by standard reactions. This dione was isomerised by sulphuric acid in acetic acid to the corresponding δ-compound, δ-homo-oleanenedione (XVIII). The absorption spectrum of this compound showed the presence of the two keto-groups and confirmed that the double bond had moved from the 12 : 13- to the 13 : 18-position (the use of ultra-violet light absorption data for the determination of double bond environment will be dealt with in forthcoming publications from these laboratories).

The homolupenedione was prepared in two ways. The first method used was that employed for the preparation of homo-oleanenedione. In the second the acetate of betulinaldehyde (IV) was treated with methyl-lithium, giving homolupenediol, oxidised to the dione (XVI).

The homolupenedione was then treated with 20% sulphuric acid in acetic acid. The same product was obtained whether the reaction solution was worked up after a shorter or longer time. The absorption spectrum of the isomerisation product indicated the presence of *only one* keto-group but also a double bond. Unsaturation was also indicated by a yellow colour with tetranitromethane. These data are only compatible with the formation of a cyclic vinyl ether which, in view of the structure put forward for the keto-lactone (XV), was formulated as (XIX) (cf. *A*). Support for this formulation was forthcoming from the absorption spectrum of tetrahydro-2-methylenefuran (XX) (Eglinton, Jones, and Whiting, forthcoming publication).

The spectrum corresponded exactly with that of the isomerisation product. Final confirmation of structure (XIX) was provided by the ozonolysis of the isomerisation product, the keto-lactone (XV) being obtained.



In addition to studying the action of hydrogen bromide in acetic acid on betulinic acid and its acetate, Robertson *et al.* (*loc. cit.*) also treated the acetate with formic acid, " lactone A " acetate being again obtained. They reported, however, that a different lactone, " lactone B," was obtained by similar treatment both of methyl betulinate and of the free acid. Further investigation of the reactions carried out by Robertson *et al.* have now shown that the two lactones must be identical, since on oxidation with chromic acid they give the same keto-lactone. " Lactone-A " is not easy to purify and is very high melting. It is therefore not suitable for comparison purposes. The corresponding keto-" lactone-A " is easier to purify and characterise.

Experimental.

(All m. p.s were determined on a Kofler block and are corrected. Rotations were determined in chloroform.)

The betulin was obtained from birch bark by Ruzicka and Isler's method (*Helv. Chim. Acta*, 1936, **19**, 506).

The oleanolic acid was obtained from spent cloves by the following method.

Partly dried spent cloves (1·5 kg.) were refluxed with ethanol (6 l.) for 2 hours. The warm extract was filtered and concentrated to one-third of its volume, excess of water was added, and the precipitated material filtered off and dried. The crude acid was then suspended in benzene (300 c.c.) and brought into solution by heat. The hot liquid was shaken with sodium hydroxide solution (250 c.c.; 30%). The clear liquor was run off and discarded and the suspension of sodium salt separated from the benzene layer. The suspension was then acidified and the acid collected and dried. The acid (20 g.) obtained after two crystallisations was suspended in hot ethanol, and sodium hydroxide solution was added slowly with shaking until the acid was just in solution. On cooling, the sodium salt which separated in shining needles was collected and washed well with aqueous ethanol. The salt was dissolved in hot ethanol, and the solution acidified with acetic acid, and cooled, whereupon oleanolic acid (12 g.), m. p. 285—300°, separated.

Methyl δ-Oleanonate.—Methyl oleanonate (1 g.) in benzene (10 c.c.) was treated with a mixture of acetic acid (80 c.c.) and sulphuric acid (16 c.c.; *d* 1·84) with shaking. After 14 days at 20°, dilution with water and isolation with benzene yielded a product (1·09 g.), which was dissolved in benzene (15 c.c.) and chromatographed on a column of alumina (60 g.; activity I). Elution with benzene–ether (6 : 1) (120 c.c.) gave a fraction (880 mg.) which crystallised from chloroform–methanol as needles (460 mg.), m. p. 168—170°. Two further recrystallisations from the same solvent gave *methyl δ-oleanonate*, m. p. 172—173°, $[\alpha]_D^{20}$ −46° (*c*, 1·20), −44° (*c*, 0·76) (Found : C, 79·25; H, 10·3. $C_{31}H_{48}O_3$ requires C, 79·4; H, 10·3%). Light absorption in chloroform–methanol (1 : 1) : Max. 2850—2940 Å; ε = 36. Further elution with benzene–ether (5 : 1) (80 c.c.) gave a fraction (150 mg.), crystallisation of which gave oleanonic lactone, m. p. 354—356°, $[\alpha]_D^{20}$ +37° (*c*, 1·11).

Homo-oleanonyl Acetate.—Acetyloleanoloyl chloride (5 g.) in benzene (100 c.c.) was added slowly to methylmagnesium iodide prepared from magnesium (4 g.) and methyl iodide (16 g.) in ether (60 c.c.). After 10 minutes, the temperature was raised and the ether displaced. The benzene solution was then refluxed for 1½ hours. The neutral fraction (3·3 g.) obtained on working up the solution was dissolved in benzene (30 c.c.) and chromatographed on a column of alumina (80 g.; activity I—II). The fraction eluted by benzene–ether (1 : 1) was acetylated in pyridine with acetic anhydride. The acetylated product (3·3 g.) was dissolved in *n*-pentane (40 c.c.), adsorbed on a column of alumina (70 g.; activity I—II), and eluted with *n*-pentane–benzene (4 : 1) (100 c.c., 390 c.c., and 290 c.c.) and benzene (250 c.c.). Fractions of 1·53 g. (m. p. 184—191°), 0·75 g., 0·144 g. (m. p. 208—215°), and 0·72 g. (m. p. 214—217°) were obtained. Crystallisation of fractions 3 and 4 from chloroform–methanol gave flat needles (770 mg.), m. p. 214—217°. Further recrystallisation gave *homo-oleanonyl acetate*, m. p. 215—217°, $[\alpha]_D^{20}$ +66° (*c*, 0·80) (Found : C, 80·05; H, 10·7. $C_{33}H_{52}O_3$ requires C, 79·85; H, 10·55%). Light absorption in chloroform : Max., 2810—2890 Å; ε = 62.

Homo-oleanenedione.—Homo-oleanonyl acetate (700 mg.) was refluxed with alcoholic potassium hydroxide (30 c.c.; 2%) for 1 hour. The crude homo-oleanonol, isolated by dilution with water and extraction with ether, was dissolved in acetone (70 c.c.), and a solution of chromic acid (200 mg.) in water (5 c.c.) and sulphuric acid (0·2 c.c.) added slowly with shaking during 15 minutes. Dilution with water and isolation with ether yielded a product which was chromatographed in benzene–pentane (30 c.c.; 1 : 1) on alumina (40 g.; activity I—II). Elution with benzene (200 c.c.) gave a fraction (380 mg.), two crystallisations of which from methanol gave *homo-oleanenedione*, m. p. 216—222°, $[\alpha]_D^{20}$ +90·5° (*c*, 0·72) (Found : C, 82·05; H, 10·5. $C_{31}H_{48}O_2$ requires C, 82·25; H, 10·7%). Light absorption in chloroform : Max., 2880—2920 Å; ε = 123.

δ-Homo-oleanenedione.—Homo-oleanenedione (250 mg.) was dissolved in benzene (2·5 c.c.), and a

mixture of acetic acid (20 c.c.) and sulphuric acid (4 c.c.; *d* 1·84) added with shaking. After 13 days at 20° dilition with water and isolation with benzene and ether yielded a product which was adsorbed from benzene–pentane (20 c.c.; 1 : 1) on a column of alumina (30 g.; activity I—II). Elution with benzene (120 c.c.) gave a fraction (88 mg.) crystallising from methanol as needles, m. p. 188—198°. Two further recrystallisations from chloroform–methanol gave δ-*homo-oleanenedione*, m. p. 195—198·5°, $[\alpha]_D^{20}$ +22·5° (*c*, 0·40) (Found : C, 82·2; H, 10·9%). Light absorption in ethanol : Inflexion, 2840—2920 Å; ε = 105.

Homolupenonyl Acetate.—Acetylbetulinic acid (7 g.) was dissolved in chloroform (40 c.c.), and thionyl chloride (20 c.c.) was added. After being kept at 20° overnight the solution was added to excess of ether and washed repeatedly with dilute sodium hydroxide (*ca.* 5%) and then with water. The residue (6·6 g.), obtained after drying and evaporation of the ethereal solution, was dissolved in benzene (100 c.c.) and added slowly to methylmagnesium iodide prepared from magnesium (4 g.) and methyl iodide (16 g.) in ether (60 c.c.). After 10 minutes at 20° the temperature was raised and the ether displaced; the benzene solution was then refluxed for 2 hours. The neutral fraction (4·42 g.) obtained on working up the solution was adsorbed from benzene (30 c.c.) on a column of alumina (50 g.; activity I—II). Elution with benzene–ether (1 : 1; 270 c.c.) gave a fraction (2·41 g.) which was acetylated in pyridine with acetic anhydride. The acetylated product (2·94 g.) was dissolved in *n*-pentane (40 c.c.), adsorbed on neutral alumina (60 g.; activity I—II), and eluted with *n*-pentane (100 c.c.) and then *n*-pentane–benzene (1 : 1) (3 × 100 c.c.).

Crystallisation of the first *n*-pentane–benzene (1 : 1) fraction (1·00 g.), m. p. 222—230·5°, from chloroform–methanol gave flakes (507 mg.), m. p. 222—230·5°. Three recrystallisations from the same solvent gave *homolupenonyl acetate*, m. p. 235·5—238°, $[\alpha]_D^{20}$ +24° (*c*, 1·27) (Found : C, 79·9; H, 10·3. $C_{33}H_{52}O_3$ requires C, 79·85; H, 10·55%). Light absorption in alcohol–chloroform : Max., 2920—2950 Å; ε = 44.

Homolupenedione.—Homolupenonyl acetate (389 mg.) in benzene (5 c.c.) was boiled under reflux with alcoholic potassium hydroxide (20 c.c.; 2%) for 1 hour. After the addition of excess of water the product was isolated by extraction with benzene–ether (1 : 1). The crude homolupenonol (341 mg.) was dissolved in chloroform (15 c.c.), and a solution of chromic acid (150 mg.) in acetic acid (10 c.c.)–acetone (20 c.c.)–water (2 c.c.) was added slowly with shaking. After 30 minutes at 20°, dilution with water and isolation with ether yielded a product which was adsorbed from benzene (30 c.c.) on a column of alumina (30 g.; activity I—II). Elution with benzene (200 c.c.) gave a fraction (239 mg.) which crystallised from chloroform–methanol as shining needles (146 mg.), m. p. 191—193·5°. Recrystallisation from chloroform–methanol gave *homolupenedione*, m. p. 193—195°, $[\alpha]_D^{20}$ +48° (*c*, 1·30) (Found : C, 82·15; H, 10·35. $C_{31}H_{48}O_2$ requires C, 82·25; H, 10·7%). Light absorption in ethanol–chloroform; Max. 2910—2930 Å, ε = 63. A further quantity of the diketone (50 mg.) was obtained by oxidation of the fraction (102 mg.) eluted by ethanol–ether (100 c.c.).

Homolupenediol.—To a boiling solution (50 c.c.) of methyl-lithium prepared from lithium (3·4 g.), methyl iodide (42 g.), ether (50 c.c.), and benzene (150 c.c.), acetylbetulinaldehyde (1·0 g.) in benzene (50 c.c.) was slowly added. After refluxing for 16 hours, the cooled solution was poured into dilute hydrochloric acid, and the product isolated by ether-extraction. Treatment of the residue, which had separated during the reaction, with dilute hydrochloric acid and ether gave a further quantity of product. The product (1·33 g.) was adsorbed from benzene (30 c.c.) on a column of alumina (50 g.; activity I). Elution with ether–ethanol (160 c.c.) gave a fraction (1·1 g.) two crystallisations of which from ethanol gave *homolupenediol* as prisms (430 mg.), m. p. 252—262° (decomp.), $[\alpha]_D^{20}$ +15° (*c*, 1·36) (Found : C, 81·45; H, 11·65. $C_{31}H_{52}O_2$ requires C, 81·5; H, 11·45%). Acetylation with acetic anhydride–pyridine gave *homolupenediol diacetate* as needles, m. p. 235—237·5°, $[\alpha]_D^{20}$ +36° (*c*, 0·57) (Found : C, 77·8; H, 10·4. $C_{35}H_{56}O_6$ requires C, 77·75; H, 10·4%).

Oxidation of Homolupenediol.—A solution of homolupenediol (420 mg.) in benzene (10 c.c.) (prepared by warming) was treated with acetone (30 c.c.) and cooled to 20°. Chromic acid (400 mg.) in dilute sulphuric acid (2 c.c.) was added. The precipitate which formed was dissolved by the addition of chloroform (15 c.c.). Chromic acid (200 mg.) and acetone (10 c.c.) were then added. After 30 minutes at 20°, dilution with water and isolation with ether yielded a product (390 mg.) which was adsorbed from benzene (20 c.c.) on a column of alumina (30 g.; activity I). Elution with benzene (3 × 130 c.c.), benzene–ether (1 : 1) (100 c.c.), and ether–ethanol (100 c.c.) gave five fractions : (i) 40 mg., (ii) 100 mg., m. p. 189—194°, (iii) 30 mg., m. p. 191—194°, (iv) 107 mg., m. p. 192—194° and (v) 35 mg. Fractions (ii), (iii), and (iv) were crystallised separately from chloroform–methanol. Fraction (ii) : light absorption in chloroform : Max., 2930—2970 Å, ε = 63; $[\alpha]_D^{20}$ +48·5° (*c*. 1·34). Fraction (iv) : light absorption in chloroform : Max., 2920—2980 Å, ε = 70·5; $[\alpha]_D^{20}$ +44·5° (*c*, 1·16). A mixed m. p. of fraction (ii) with fraction (iv) or with homolupenedione prepared from homolupenonyl acetate gave no depression.

Isomerisation of Homolupenedione.—Homolupenedione (300 mg.) was dissolved in benzene (3 c.c.), and a mixture of acetic acid (24 c.c.) and sulphuric acid (4·8 c.c.; *d* 1·84) was added with shaking. After 2½ days at 20° one-third of the solution was worked up. The product (110 mg.) was dissolved in benzene (5 c.c.) and adsorbed on a column of alumina (6 g.; activity I). Elution with benzene–ether (1 : 1; 25 c.c.) gave a fraction (65 mg.) which crystallised from methanol as needles, m. p. 220—228°, $[\alpha]_D^{20}$ +66° (*c*, 0·51). Two further recrystallisations gave crystals which melted at 228—235°, followed, at 236°, by the growth of crystals melting at 280° (decomp.). After 14 days the remainder of the solution was worked up. The product (200 mg.) was adsorbed on alumina (6 g.; activity I—II), and the fraction (165 mg.) eluted with benzene–ether (1 : 1; 50 c.c.) crystallised from methanol as needles, m. p. 220—224°, with needles growing at 230° and remelting at 265—275° (decomp.), $[\alpha]_D^{20}$ +61° (*c*, 0·73). A further recrystallisation gave material of m. p. 229—236°. There was no depression

of the m. p. on admixture with the crystals obtained after 2½ days (Found: C, 82·75; H, 10·85. $C_{31}H_{48}O_3$ requires C, 82·25; H, 10·7%). Light absorption in ethanol–chloroform: Max., 2840—2910 Å; $\varepsilon = 38$. Light absorption in ethanol: 2110 ($\varepsilon = 7600$), 2160 ($\varepsilon = 6900$), 2200 ($\varepsilon = 5280$), and 2230 Å ($\varepsilon = 3040$). The crystals gave a yellow colour with tetranitromethane in chloroform.

Ozonolysis of the Product from the Isomerisation of Homolupenedione.—The isomerisation product (103 mg.) was dissolved in chloroform (10 c.c.), and a stream of ozonised oxygen (16 mols.) passed through the solution at 20° for 15 minutes. The solution was evaporated to dryness, and methanol (about 10 c.c.) added. The crystalline residue obtained after evaporation of the methanol was dissolved in benzene (10 c.c.) and adsorbed on to a column of alumina (10 g.; activity I—II). Elution with benzene–ether (2 : 1) (2 × 25 c.c.), after initial elution with benzene (25 c.c.) and benzene–ether (2 : 1) (25 c.c.), gave two fractions: 35 mg., m. p. 312—316°, and 18 mg., m. p. 305—308°. The former fraction was recrystallised three times from chloroform–methanol, to give betulonic lactone as needles, m. p. 334—336°, undepressed with an authentic specimen (see below), $[\alpha]_D^{20}$ +92° (*c*, 0·17).

Action of Sulphuric Acid–Acetic Acid on Methyl Betulonate.—Methyl betulonate (1 g.) was dissolved in benzene (10 c.c.) and a mixture of acetic acid (80 c.c.) and sulphuric acid (16 g.; *d* 1·84) was added with shaking. After 15 days at 20°, dilution with water and isolation with benzene yielded a product which was dissolved in benzene (40 c.c.) and adsorbed on a column of neutral alumina (60 g.; activity I). Elution with benzene (110 c.c.; 80 c.c.) and benzene–ether (6 : 1) (2 × 70 c.c.) gave four fractions: (i) 20 mg., (ii) 120 mg., m. p. 315—320°, (iii) 620 mg., m. p. 318—328° (decomp.), and (iv) 150 mg., m. p. 318—320° (decomp.). Fractions (ii), (iii), and (iv) were crystallised from chloroform–methanol. The combined crystallised fractions (402 mg.) were recrystallised from chloroform–methanol, giving *betulonic lactone* as flat needles, m. p. 333—335°, $[\alpha]_D^{20}$ +88° (*c*, 1·11), +82° (*c*, 2·23) (Found: C, 79·45; H, 10·5. $C_{30}H_{46}O_3$ requires C, 79·25; H, 10·2%).

Action of Formic Acid on Acetylbetulinic Acid.—Acetylbetulinic acid (900 mg.) was boiled under reflux with formic acid (50 c.c.; *d* 1·20) for 5 hours. The mixture was diluted with water, and the product isolated by benzene-extraction. Washing with alkali left a neutral fraction (400 mg.), which was adsorbed from benzene on a column of alumina (40 g.; activity I—II). Elution with ether (120 c.c.) gave a fraction (84 mg.) which crystallised from chloroform–methanol as flat needles, melting above 350°, $[\alpha]_D^{20}$ +57° (*c*, 0·67). This fraction (80 mg.) was dissolved in benzene (10 c.c.), and the solution was boiled under reflux for 1 hour with ethanol (10 c.c.) and potassium hydroxide (1 g.). The product, isolated by ether-extraction, was dissolved in chloroform (5 c.c.) at 30° and a solution of chromic acid (500 mg.) in acetic acid (20 c.c.) was added. After 20 minutes, the product was isolated by dilution with water and extraction with benzene. The product (74 mg.) was dissolved in benzene and adsorbed on a column of alumina (6 g.; activity I—II). Elution with benzene–ether (25 c.c.; 1 : 1) gave a fraction (30 mg.), crystallisation of which from chloroform–methanol gave betulonic lactone as short needles, m. p. 336—337° undepressed when admixed with a specimen prepared from methyl betulonate, $[\alpha]_D^{20}$ +82° (*c*, 0·56).

Action of Formic Acid on Betulinic Acid.—Betulinic acid (2·4 g.) was boiled under reflux with formic acid (70 c.c.; *d* 1·2) for 4 hours. The mixture was diluted with water, and the product was isolated by benzene-extraction. The neutral fraction (420 mg.) was boiled under reflux with benzene (20 c.c.), ethanol (20 c.c.), and potassium hydroxide (5 g.) for 30 minutes. The product (350 mg.), isolated by dilution with water and extraction with ether, was dissolved in chloroform (25 c.c.), chromic acid (2·5 g.) in acetic acid (100 c.c.) was added slowly, with shaking, and the mixture kept at 20° for 30 minutes. The product, isolated by benzene-extraction, was dissolved in benzene and adsorbed on a column of alumina (30 g.; activity I—II). Elution with benzene–ether (80 c.c.; 1 : 1) gave a fraction (110 mg.) which was crystallised from chloroform–methanol, to give flat needles, m. p. 327—328·5°. Two recrystallisations from the same solvent gave betulonic acid lactone, m. p. 337—338° undepressed when admixed with a specimen prepared from methyl betulonate, $[\alpha]_D^{20}$ +78° (*c*, 0·75).

Reductive Fission of Betulonic Lactone by Lithium Aluminium Hydride.—Betulonic lactone (1 g.) was dissolved in tetrahydrofuran (100 c.c.) under reflux and freshly powdered lithium aluminium hydride (500 mg.) added to the cooled solution. After the initial reaction the mixture was refluxed for 30 minutes. Excess of lithium aluminium hydride was then decomposed by careful addition of water. After the addition of excess of dilute hydrochloric acid the product (1·14 g.) was isolated with ether and crystallised from acetone–chloroform–methanol, to give flat needles (824 mg.), m. p. 247—255°. Three further recrystallisations from methanol gave the triol as flat needles, m. p. 259—267°. Further purification was effected by hydrolysis of the triol diacetate (see below). The diacetate (50 mg.) was boiled under reflux with alcoholic potassium hydroxide (10 c.c.; 2%) for 1 hour. Dilution with water and extraction with ether gave the *triol* which crystallised from methanol as needles, m. p. 296—298°, $[\alpha]_D^{20}$ +24° (*c*, 0·69) (Found: C, 77·8; H, 11·45. $C_{30}H_{52}O_3$ requires C, 78·2; H, 11·4%).

Acetylation of the Triol.—(a) *Use of acetic anhydride–pyridine at* 100°. The triol (756 mg.) was heated on the steam-bath with acetic anhydride (40 c.c.) and pyridine (20 c.c.) for 2½ hours. The warm solution was then poured into excess of water, and the product (1·0 g.), isolated with benzene, was chromatographed in benzene–pentane (30 c.c.; 1 : 1) on alumina (40 g.; activity I—II). After elution with benzene–*n*-pentane (1 : 1) (520 c.c.), and benzene (280 c.c.), elution with benzene (640 c.c.) and benzene–ether (1 : 1) (140 c.c.) gave a series of fractions melting at *ca.* 240° after crystallisation from methanol. The combined crystallised fractions (500 mg.) were recrystallised from chloroform–methanol, giving the *triol diacetate* as short needles, m. p. 240—242·5°, $[\alpha]_D^{20}$ +22° (*c*, 0·78) (Found: C, 74·65; H, 10·75. $C_{34}H_{56}O_5$ requires C, 74·95; H, 10·4%).

(b) *Use of boiling acetic anhydride–pyridine.* The triol (104 mg.) was refluxed with acetic anhydride (25 c.c.) and pyridine (5 c.c.) for 100 minutes. Dilution of the slightly cooled solution with water and isolation with benzene yielded a product (121 mg.) which was chromatographed in pentane (20 c.c.) on

alumina (10 g.; activity I—II). After elution with *n*-pentane and *n*-pentane–benzene, elution with benzene (75 c.c.) gave a fraction (61 mg.), m. p. 213—214°. After further elution with benzene (25 c.c.), a fraction (27 mg.), m. p. 242—244°, was obtained with benzene–ether (1 : 1) (25 c.c.). Crystallisation of the fraction melting at 213—214° from methanol gave the triol triacetate as prisms, m. p. 213—214°, undepressed on admixture with a specimen of the triacetate prepared by method (c). Crystallisation of the fraction melting at 242—244° from methanol gave the triol diacetate, m. p. 242—244° undepressed on admixture with a specimen prepared by method (a).

(c) *Use of boron trifluoride catalyst.* The triol (497 mg.) was suspended in acetic anhydride (120 c.c.) and boron trifluoride–acetic acid complex (4 c.c.) added dropwise with shaking. After 18 hours at 20°, dilution with water and isolation with benzene–ether (1 : 1) yielded a gum (766 mg.) which was adsorbed from benzene–*n*-pentane (30 c.c.; 1 : 2) on a column of alumina (50 g.; activity I–II). After elution with *n*-pentane–benzene (2 : 1) (100 c.c.), elution with *n*-pentane–benzene (1 : 1) (200 c.c.; 100 c.c.) and benzene (150 c.c.; 100 c.c.) gave fractions : (i) 130 mg., m. p. 277—277·5°, (ii) trace, (iii) 220 mg., m. p. 212—214·5°, and (iv) 29 mg. Crystallisation of fraction (i) from chloroform–methanol gave flakes, m. p. 277—277·5° (50 mg.). Recrystallisation from the same solvent gave moradiol diacetate, m. p. 278—278·5°, $[\alpha]_D^{20}$ +27° (*c*, 0·44) (Found : C, 77·6; H, 10·25. Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3%). A mixed m. p. with an authentic specimen of moradiol diacetate, m. p. 277·5—278°, $[\alpha]_D^{20}$ +22° (*c*, 1·75), gave no depression. Crystallisation of fraction (iii) from methanol gave prisms, m. p. 212—214·5°. Further recrystallisations from methanol gave the *triol triacetate*, m. p. 213—216° (Found : C, 74·0; H, 9·55. $C_{36}H_{58}O_6$ requires C, 73·7; H, 9·95%).

Hydrolysis of Moradiol Diacetate using Lithium Aluminium Hydride.—Moradiol diacetate (26 mg.) was dissolved in benzene (5 c.c.) and a solution of lithium aluminium hydride in ether (5 c.c.; approx. 0·9%) was added. After five minutes' refluxing, water was cautiously added dropwise, followed by excess of dilute sulphuric acid. Extraction with ether gave a product which crystallised from chloroform–methanol as flat needles (14 mg.), m. p. 201—209°. Two recrystallisations from methanol gave moradiol as flat needles, m. p. 221—223° undepressed when admixed with an authentic specimen, $[\alpha]_D^{20}$ −17° (*c*, 0·47). The authentic specimen had m. p. 221—223·5°, $[\alpha]_D^{20}$ −9° (*c*, 0·5).

Thanks are offered to Mr. E. S. Morton and Mr. H. Swift for microanalyses and to Miss E. Fuller for the ultra-violet absorption spectra. The authors thank Messrs. Whiffen & Sons Ltd. for generously supplying the spent cloves used for the preparation of oleanolic acid. One of the authors (G. S. D.) is indebted to the University of Manchester for a post-graduate scholarship.

THE UNIVERSITY, MANCHESTER, 13. [*Received, July 5th,* 1951.]