The Chemistry of Extractives from Hardwoods. Part XVIII. The Constitution of Arjunolic Acid, a Triterpene from* Terminalia arjuna.

By F. E. King, T. J. King, and J. M. Ross.

[Reprint Order No. 5561.]

Arjunolic acid, the principal extraneous constituent of the timber kumbuk (*Terminalia arjuna*), is a triterpene acid, $C_{30}H_{48}O_5$. It contains a readily lactonised olefinic bond and three alcoholic substituents, of which two constitute an α-glycol system. Pyrolysis of the acid in presence of copper caused the liberation of formaldehyde, a degradative test believed to be typical of 3 : 23-hydroxylated triterpenes, *e.g.*, bassic acid and hederagenin.

The tentative structure thus suggested for the new triterpene, *i.e.*, 2 : 3 : 23-trihydroxyolean-12-en-28-oic acid (II), was verified by degradation to a diosphenol and formaldehyde by alkaline hydrolysis of the ketone obtained through the oxidation of 18-*iso*arjunolic lactone diacetate. The diosphenol is identical with a known derivative of hederagenin (Ruzicka, Jeger, and Norymberski, *Helv. Chim. Acta*, 1944, **27**, 1185).

The ornamental tree *Terminalia arjuna* Bedd. (*T. glabra* W. and A.), found in India, Ceylon, and Burma, yields a rich purple-brown heavy wood of fine texture superficially resembling American black walnut (*Juglans nigra*). Small quantities are available in Gt. Britain and are marketed as kumbuk. Solvent extraction of this dense and difficultly powdered wood has led to the isolation of arjunolic acid, a new trihydroxytriterpene carboxylic acid.

Preliminary treatment with boiling light petroleum removed a small oily fraction containing oleanolic acid (I) and " β-sitosterol." Arjunolic acid, the principal extraneous component of the wood (2½%), was obtained in the subsequent ether-extraction; owing to its low solubility prolonged treatment was necessary, the crude acid separating from the boiling solvent as a white crystalline powder. From the portion remaining in the ethereal solution traces of ellagic acid were isolated.

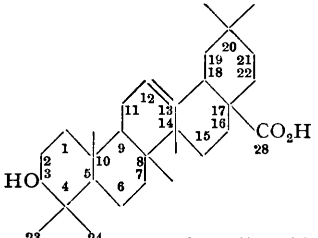
---

* Part XVII, *J.*, 1954, 1399.

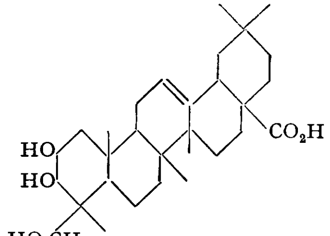
Arjunolic acid has the molecular formula $C_{30}H_{48}O_5$ and responds to the usual qualitative tests for triterpenes. Experiments described in this memoir unequivocally establish the constitution of the acid as 2 : 3 : 23-trihydroxyolean-12-en-28-oic acid (II), thus superseding the putative structure already published (*Chem.* and *Ind.*, 1953, 1325).

The triterpene alkali salts are sparingly soluble in water, but suspensions of the acid in ethanol dissolve on the addition of aqueous sodium hydroxide; moreover, the carboxyl function is readily discerned by the formation with diazomethane or methyl sulphate–alkali, but not with methanolic hydrochloric acid, of a crystalline (mono)methyl ester. As with other triterpene acids, drastic conditions of hydrolysis are necessary to recover the acid from its ester. The crystalline ethyl and *p*-nitrobenzyl esters also served to characterise the acid. The hydroxylic character of the remaining three oxygen atoms was shown by the preparation of arjunolic acid triacetate and of methyl arjunolate triacetate which, however, were not obtained crystalline.

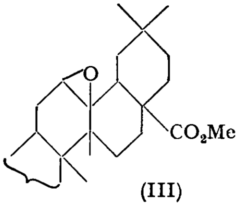
Arjunolic acid resisted catalytic hydrogenation over platinum oxide, but was shown to be unsaturated by means of tetranitromethane. The degree of unsaturation was determined by oxidation of the acid or of its derivatives with perphthalic acid and analysis of the oxidation products. Methyl arjunolate, for example, afforded a simple epoxide (III), m. p. 174—175°, which, being without action on tetranitromethane, contained no olefinic bond. Methyl arjunolate thus resembles methyl oleanolate (acetate) (Picard and Spring, *J.*, 1940, 1387) and differs from the corresponding α-amyrin derivatives which under comparable conditions are inert to peroxidation. Like the oleanolate oxide (Picard and Spring, *loc. cit.*), methyl arjunolate oxide is isomerised by mineral acid to a dihydro-oxo-compound (IV), m. p. 191—192°, more easily obtained, however, from methyl arjunolate triacetate by the action of hot hydrogen peroxide–acetic acid followed by alkaline hydrolysis.



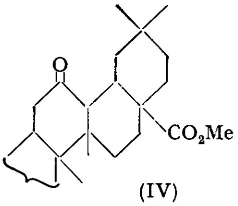
(I) Oleanolic acid



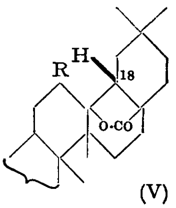
(II) Arjunolic acid



(III)



(IV)



(V)

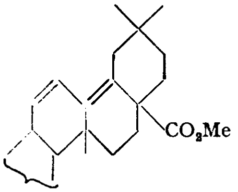
The products (III) and (IV) are well differentiated by their infra-red absorption spectra. Thus, methyl arjunolate and methyl epoxyarjunolate in carbon tetrachloride solutions exhibit a single carbonyl band, at 1724 and 1723 cm.$^{-1}$ respectively, due to the methoxy-carbonyl group. On the other hand, the spectrum of methyl dihydro-oxoarjunolate (determined in Nujol suspension owing to the low solubility in carbon tetrachloride) has two carbonyl bands, at 1683 and 1722 cm.$^{-1}$, corresponding respectively to saturated keto- and ester-carbonyl groups. We are indebted to Dr. G. D. Meakins, University of Manchester, for these and other infra-red absorption determinations recorded in this paper, which were undertaken through the kindness of Professor E. R. H. Jones, F.R.S.

The position of the double bond with respect to the carboxyl group was deduced from the peroxidation of arjunolic acid to a hydroxy-lactone (V; R = OH) and from the ready formation of a highly crystalline bromo-lactone (V; R = Br) of which a triacetyl derivative was prepared. Easy lactonisation is characteristic of carboxylic acids of the β-amyrin series having a double bond γδ to the carboxyl group (see, for example, Jeger, *Fortschr. Chem. Org. Naturstoffe*, 1950, **7**), and it was accordingly inferred that arjunolic acid is unsaturated at the 12-position as in oleanolic acid.

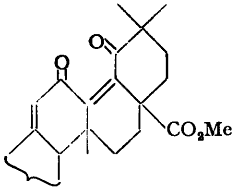
With boiling methanolic potassium hydroxide the bromo-lactone was converted into a saturated neutral compound, $C_{31}H_{50}O_6$, m. p. 250—252°, which like the epoxide of m. p. 174—175° was isomerised by mineral acid to the dihydro-oxoarjunolate (IV). An isomeric neutral product of higher melting point than the normal epoxide has been observed in the methanolic alkaline hydrolysis of oleanolic bromo-lactone (Kitisato, *Acta Phytochem. Tokyo*, 1933, **7**, 169; *Chem. Centr.*, 1934, I, 2765), but its structure does not appear to have been ascertained. Light absorption measurements on the compound of m. p. 250—252° disclose a carbonyl band at 1724 cm.$^{-1}$ corresponding to the ester-carbonyl band of methyl arjunolate and of methyl epoxyarjunolate and differing from the carbonyl absorption (1779 cm.$^{-1}$) of the bromo-lactone. The absence of a true carbonyl group is confirmed by the ultra-violet absorption spectrum which lacks the weak carbonyl peak at *ca.* 280 mμ. From these data it is evident that the intermediate of m. p. 250—252° is a stereoisomeride of methyl epoxyarjunolate, m. p. 174—175°, and it is concluded that a similar relation exists between the analogous derivatives of oleanolic acid.

The production of isomeric epoxide esters is to be expected from mechanistic considerations. In the hydrolysis of arjunolic bromo-lactone, the formation of the methoxycarbonyl group, which results from the attack of methoxide ion on the lactone •CO–O• bond, will leave a negatively charged oxygen at $C_{(13)}$. The configuration of this oxygen atom, which is axial and therefore β, will not be affected by the ensuing elimination of Br^- and will consequently remain unchanged in the resulting oxide ring. Conversely, it can be deduced that the preparation of epoxides by peroxidation of the 12 : 13-double bond will afford products in which the added oxygen has the opposite (α-)configuration. This follows from the configuration assumed by the 12-hydroxyl group in the hydroxyoleanolic acid obtained in this way from oleanolic acid, which Barton and Holness (*J.*, 1952, 78) regard as axially and therefore α-bonded. Since it may be accepted that this substituent is derived from the rearrangement of an initially formed epoxy-acid, a process not involving the $C_{(12)}$–O bond, the epoxide ring also will have the α-configuration, *i.e.*, it is the converse of that originating from the bromo-lactone.

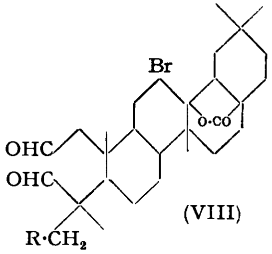
As is usual in the fission of lactones with alcoholic alkali, the use of potassium hydroxide in ethanol instead of the methanolic reagent resulted in the formation of an acidic product from the bromo-lactone (V; R = Br), which with diazomethane yielded methyl dihydro-12-oxoarjunolate (IV).



(VI)



(VII)



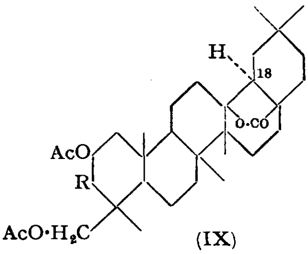
(VIII)

A further resemblance between the new triterpene and oleanolic acid was observed in the reaction of methyl arjunolate triacetate with selenium dioxide. Treatment with 0·5 mol. of the reagent in boiling acetic acid and de-acetylation of the product afforded a crystalline diene (VI) having the characteristic triple ultra-violet absorption bands ($\lambda_{max.}$ 243·5, 251, and 260·5 mμ) of a triterpene 11 : 13(18)-diene (Ruzicka and Jeger, *Helv. Chim. Acta*, 1942, **25**, 775; Barton and Holness, *loc. cit.*). The oxidation corresponds to that of methyl oleanolate acetate (Ruzicka, Grob, and van der Sluys-Veer, *Helv. Chim. Acta*, 1939, **22**, 788) and the change is accompanied by the typical large negative shift in optical rotatory power. More vigorous oxidation of the oleanolate with larger quantities of selenium dioxide gave a 10 : 13(18)-diene-12 : 19-dione with strong absorption in the ultra-violet ($\lambda_{max.}$ 275 mμ, ε 12,500) (*idem, loc. cit.*; Ruzicka, Jeger, and Winter, *ibid.*, 1943, **26**, 265) and, although non-crystalline, the material similarly obtained from methyl arjunolate triacetate was estimated from its light absorption ($\lambda_{max.}$ 275 mμ, ε 6800) to contain approximately 50% of the comparable diene-dione (VII).

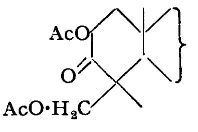
The evidence of these preliminary experiments suggested that arjunolic acid was a dihydroxy-derivative of oleanolic acid. Indications as to the disposition of the hydroxyl groups was afforded by the quantitative oxidation of methyl arjunolate with sodium periodate, the disappearance of 1 mol. of the reagent establishing the existence of a 1 : 2-glycol unit. Arjunolic acid was likewise readily oxidised by lead tetra-acetate but without the formation of any purifiable product. On the other hand, arjunolic bromo-lactone with this reagent yielded a crystalline material of which the elementary composition was in agreement with its representation as a dicarbonyl compound, presumably (VIII; R = H), but the 2 : 4-dinitrophenylhydrazone was amorphous. The action of zinc dust and acetic acid on the supposed dialdehyde (VIII) effected debromination and cleavage of the lactone ring, and a crystalline acid was obtained which was unsaturated (tetranitromethane) and gave an amorphous 2 : 4-dinitrophenylhydrazone.

Important information concerning the situation of the hydroxyl substituents was acquired from experiments on the pyrolysis of arjunolic acid. When heated with copper catalysts at 270—290° the acid gave small but consistent amounts of formaldehyde, identified by means of the dimedone derivative. A similar decomposition yielding formaldehyde has been observed with other triterpenes, *e.g.*, bassic acid and hederagenin, and it is regarded as typical of the 3 : 23-diol system in the triterpene series (Tsuda and Kitagawa, *Ber.*, 1938, **71**, 1604). If accepted, this evidence, together with that afforded by the periodate and tetra-acetate oxidations, at once establishes the orientation of the three hydroxyl groups in arjunolic acid. An attempt was made to confirm the presence of neighbouring hydroxyl substituents, *e.g.*, as in the case of the 3 : 23-diol grouping in hederagenin, by the preparation of an *iso*propylidene derivative, but the product could not be obtained crystalline.

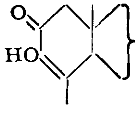
The foregoing deductions were confirmed beyond doubt by developments which arose from a study of the lactonisation of arjunolic acid. Under conditions devised by Winterstein and Wiegand (*Z. physiol. Chem.*, 1931, **199**, 46), namely, treatment at room temperature with a mixture of hydrobromic and acetic acids, a lactone triacetate was obtained. To this derivative the 18-*iso*(α-)structure (IX; R = OAc) is ascribed since it is known that similar reagents effect the translation of the D–E ring junction in oleanolic acid from the *cis* to *trans* (Barton and Holness, *loc. cit.*). The very high yield of acidic material remaining when arjunolic acid was treated with cold hydrochloric and acetic acids, conditions in which acids of the 18-*iso*(α-)series undergo almost total lactonisation, excluded the possibility that the original triterpene already possessed the *iso*-configuration. The *iso*lactone triacetate was easily deacetylated with alkalis, and the resulting trihydroxy-lactone was characterised by a crystalline tribenzoate.



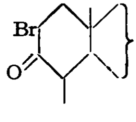
(IX)



(X)



(XI)



(XII)

With boiling hydrochloric and acetic acids (Winterstein and Stein, *Z. physiol. Chem.*, 1931, **200**, 64) a new derivative was obtained which proved to be a lactone diacetate, later shown to be (IX; R = OH). Oxidation of the diacetate with chromic anhydride–acetic acid gave 3-oxo-18-*iso*(α-)arjunolic lactone 2 : 23-diacetate (X) which was then subjected to alkaline hydrolysis. Analyses of the crystalline product, m. p. *ca.* 320°, were anomalous and implied that in addition to the removal of acetyl groups a net loss of CH_4O had occurred. Moreover, re-acetylation of the compound, m. p. 320°, afforded a new derivative, m. p. 274—275°, analysis of which confirmed the decreased molecular weight. The nature of the hydrolysis product was indicated by the relative ultra-violet absorptions of the compound ($\lambda_{max.}$ 281 mμ, ε 12,000) and its acetate ($\lambda_{max.}$ 246 mμ, ε 11,800) which indicate a diosphenol structure, *i.e.*, (XI), and in agreement with this the compound of m. p. 320° had a ferric reaction. The behaviour of the oxoarjunolic lactone diacetate is therefore reminiscent of that of icterogenin (Barton and de Mayo, *J.*, 1954, 887) which under the influence of alkali sustains loss of the methylol grouping at $C_{(4)}$, and from the cautious hydrolysis of the oxoarjunolic lactone diacetate (X) formaldehyde was subsequently isolated as its dimedone derivative.

The production of the diosphenol (XI) from (X) requires the removal also of two hydrogen atoms, either by atmospheric oxidation or, since the compound is obtained (27%) even in the total absence of air, by a disproportionation. An analogy for the reaction is, in fact, found in the spontaneous oxidation of 2-bromohedragone lactone (XII) during alkaline hydrolysis, a method whereby the diosphenol (XI) was originally prepared (Ruzicka, Jeger, and Norymberski, *loc. cit.*). A small amount of the derivative (XI) was also obtained by direct oxidation of the 18-*iso*arjunolic lactone with chromic acid, elimination of the 23-carbon atom presumably occurring as carbon dioxide through the decarboxylation of an intermediate β-keto-acid. This is comparable with the oxidation of methyl asiatate to a diosphenol (Polonsky, *Compt. rend.*, 1951, **233**, 93) and is a result to be expected from the very close resemblance of the two triterpenes.

The identification of the diosphenol (XI) as that earlier obtained from hederagenin also determines the detailed stereochemical structure of arjunolic acid with, of course, the exception of the spatial relation of the three hydroxyl groups. The fluency of reaction exhibited by these substituents, both in the formation and hydrolysis of derivatives, implies that the secondary 2- and 3-hydroxyl groups are *trans*-equatorial and therefore respectively α and β. Further evidence as to the configuration of these and of the 23-hydroxymethyl group is being sought, *e.g.*, by partial synthesis of the triterpene.

EXPERIMENTAL

Except where otherwise stated, specimens for analysis were dried for 2 hr. at 120°/0·1 mm. Ethanol solutions were used for ultra-violet light absorption measurements, and chloroform for the optical rotations.

Solvent Extraction of Terminalia arjuna *Heartwood.*—Continuous extraction of the powdered wood (4 kg.) with boiling light petroleum (b. p. 60—80°) for 18 hr. afforded a pale yellow solution containing suspended gelatinous solid (2 g.) which was collected and crystallised from methanol (norite). After purification as the potassium salt (cf. Dodge, *J. Amer. Chem. Soc.*, 1918, **40**, 1917) and further repeated crystallisation (from methanol), oleanolic acid (35 mg.) was obtained as needles, m. p. 306—308° (lit., 310°) (Found, in a specimen dried at 150°/0·1 mm.: C, 78·7; H, 10·7. Calc. for $C_{30}H_{48}O_3$: C, 78·9; H, 10·6%); the methyl ester, prepared from methyl sulphate–methanolic alkali, had m. p. 203° (Found: C, 78·6; H, 11·0; OMe, 6·5. Calc. for $C_{31}H_{50}O_3$: C, 79·1; H, 10·7; OMe, 6·6%), and the acetate m. p. 262° (Found: C, 77·1; H, 10·1; OAc, 9·7. Calc. for $C_{32}H_{50}O_4$: C, 77·1; H, 10·1; OAc, 8·6%).

The orange-red oil (10·3 g., 0·3%) obtained by evaporation of the clear petroleum solution slowly deposited solid which yielded "β-sitosterol" (0·1 g.), m. p. 136°, after numerous crystallisations from methanol; chromatography of the oil in benzene on alumina and elution with ether–methanol (1 : 1) isolated a further quantity, m. p. 137—138°, which was characterised by the acetate, m. p. 125—126° (Found: C, 81·6; H, 10·7; OAc, 10·0. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·4; OAc, 9·4%), and benzoate, m. p. 145—146° (Found: C, 82·8; H, 10·4. Calc. for $C_{36}H_{54}O_2$: C, 83·3; H, 10·5%).

Further extraction with boiling ether for 48 hr. produced a colourless crystalline deposit (104 g., 2·6%) consisting of arjunolic acid, m. p. 315—325°. The yellow supernatant liquid was removed and evaporated, and the residue heated with hot acetic acid (15 c.c.); the remaining solid then crystallised from pyridine in minute pale yellow needles, soluble in aqueous sodium hydroxide to a deep yellow solution, and giving a blue-black precipitate with ferric chloride and other reactions of ellagic acid, *e.g.*, Griessmeyer and Alvarez tests (*Abstr.*, 1907, **92**, ii, 143).

Arjunolic Acid (II).—The product, m. p. 315—325°, was first subjected to Soxhlet extraction with acetone and thereafter recrystallised by dissolving it in a large volume of this solvent (1 g. in 175 c.c.), the solution then being concentrated tenfold. Pure *arjunolic acid* formed minute plates, m. p. 337—340°, $[\alpha]_D^{19}$ +63·5° (*c*, 0·51 in EtOH) (Found: C, 74·2, 73·9; H, 9·9, 9·6. $C_{30}H_{48}O_5$ requires C, 73·7; H, 9·9%). The acid is readily soluble in acetic acid, dioxan, pyridine, and 2-ethoxyethanol, but slightly or very slightly soluble in other organic solvents. It is not dissolved by aqueous carbonates or, apparently, by aqueous sodium hydroxide owing to the sparing solubility of its sodium salt, best obtained as long silky needles by treatment of an alcoholic solution of the acid with the concentrated aqueous alkali. Its solution in sulphuric acid was yellow changing to red; Liebermann–Burchardt reaction, pink to brown; Tschugaieff reaction, orange, red, red-brown. A solution of the acid in tetranitromethane was faintly yellow.

Arjunolic acid was esterified (yield 90%) when suspended in methanol and treated with ethereal diazomethane, and by the action of methyl sulphate on the acid in methanolic sodium or potassium hydroxide. *Methyl arjunolate* crystallised from methanol in needles, m. p. *ca.* 209—211°; absorbed from benzene solution on alumina, eluted with ether–methanol (3 : 1), and dried at 120°/0·1 mm., it had m. p. 215—216° followed by resolidification and final m. p. 248—250°. After prolonged drying (10 hr.) at 200°, it had m. p. 248—250° with slight previous sintering, $[\alpha]_D^{20}$ +68° (*c*, 1·65) (Found, in a specimen briefly dried at 225°/20 mm.: C, 73·7; H, 10·2; OMe, 6·4. $C_{31}H_{50}O_5$ requires C, 74·1; H, 10·0; OMe, 6·2%). Refluxing in 10% methanolic potassium hydroxide for 3 hr. resulted in almost quantitative recovery of the ester, m. p. 215—216°.

The *ethyl* ester, prepared from a solution of arjunolic acid in aqueous-ethanolic sodium hydroxide and ethyl sulphate, crystallised from aqueous ethanol in needles, m. p. 202—203°. The product appeared to be solvated and was prepared for analysis by brief drying above the m. p. (Found: C, 74·0; H, 9·9. $C_{32}H_{52}O_5$ requires C, 74·4; H, 10·1%), $[\alpha]_D^{20}$ +58° (*c*, 1·58).

Refluxing the acid with *p*-nitrobenzyl bromide in methanolic potassium hydroxide for 1 hr. gave the p-*nitrobenzyl* ester which crystallised from aqueous ethanol in minute leaflets, m. p. 236—238°, $[\alpha]_D^{19}$ +34° (*c*, 0·91) (Found, in a specimen dried at 150°/0·1 mm.: C, 71·6; H, 8·4; N, 2·2. $C_{37}H_{53}O_7N$ requires C, 71·2; H, 8·6; N, 2·25%).

The *acid triacetate* was obtained from arjunolic acid by treatment with acetic anhydride–pyridine or –perchloric acid at room temperature or with acetic anhydride–sodium acetate (heating), but it formed a colourless amorphous powder which did not crystallise (Found: C, 70·0; H, 9·0; OAc, 18·0. $C_{36}H_{54}O_8$ requires C, 70·3; H, 8·9; 3OAc, 21·0%). Esterification of the triacetate, or acetylation of methyl arjunolate, gave the amorphous *methyl arjunolate triacetate* (Found: C, 69·9; H, 9·0. $C_{37}H_{56}O_8$ requires C, 70·7; H, 9·0%).

Methyl 12 : 13-*Epoxyarjunolate* (III).—Methyl arjunolate (0·1064 g.) in dioxan (5 c.c.) was mixed with monoperphthalic acid in ether (5 c.c.; 0·57N), and the solution made up to 25 c.c. with ether and set aside at 0°. Samples (5 c.c.) were removed after 24, 44, and 145 hr., iodometric titrations indicating a consumption of 0·34, 0·69, and 1·09 mols. respectively of the per-acid.

Methyl arjunolate (3·24 g.) in ether–dioxan (3 : 1; 200 c.c.) was then treated with ethereal monoperphthalic acid (35 c.c.; 0·57N), and after 14 days at 0—2° the mixture was washed with N/4-sodium hydroxide and dried (K_2CO_3). Evaporation of the solvent and crystallisation from methanol gave *methyl* 12 : 13-*epoxyarjunolate* (3·0 g.) in rosettes of needles, m. p. 174—175°, $[\alpha]_D^{19}$ +36° (*c*, 1·92) (Found: C, 71·7; H, 9·5; OMe, 6·0. $C_{31}H_{50}O_6$ requires C, 71·8; H, 9·7; OMe, 6·0%).

Methyl 12 : 13-*Epoxyarjunolate Triacetate*.—Methyl arjunolate triacetate (1·1 g.), dissolved in ether (20 c.c.), was treated with monoperphthalic acid (50 c.c.; 0·3N) at 0—2° for 12 days. By working up of the product in the usual way, a colourless amorphous *epoxide* (1·12 g.) was obtained (Found: C, 69·0; H, 8·9. $C_{37}H_{56}O_9$ requires C, 68·9; H, 8·7%).

Methyl Dihydro-12-*oxoarjunolate* (IV).—Methyl arjunolate triacetate (1·0 g.) in acetic acid (30 c.c.) was treated on a steam-bath with aqueous hydrogen peroxide (2 c.c.; 30%), added in ½ hr. After being further heated for 2 hr., the solution was poured into water, and the amorphous product collected and hydrolysed with boiling methanolic potassium hydroxide. Working up in the usual way gave *methyl dihydro*-12-*oxoarjunolate* (0·6 g.) which crystallised from aqueous methanol in long flattened needles, m. p. 191—192°, $[\alpha]_D^{20}$ +30° (*e*, 1·45) (Found: C, 71·7; H, 9·6; OMe, 5·7. $C_{31}H_{50}O_6$ requires C, 71·8; H, 9·7; OMe, 6·0%).

The dihydro-oxoarjunolate was also obtained when methyl epoxyarjunolate or its acetate was heated for 2—6 hr. on a steam-bath in acetic acid containing a small amount of concentrated hydrochloric acid, the amorphous acetate being hydrolysed with alkali. However, amorphous material produced during the rearrangement of the epoxide was wasteful to remove and the hydrogen peroxide–acetic acid method is preferable.

12-*Hydroxyarjunolic Lactone* (V; R = OH).—A dioxan solution of arjunolic acid (0·1036 g.), and monoperphthalic acid in ether (5 c.c.; 0·49N), was made up to 25 c.c.; titres after 24, 44, and 145 hr. were equivalent to 0·18, 0·48, and 1·08 mols. of per-acid consumed. Oxidation on a larger scale (3·3 g.) gave, after washing with 0·1N-sodium hydroxide, 12-*hydroxyarjunolic lactone* (2·2 g.) as leaflets (from aqueous methanol), m. p. 278—280°, $[\alpha]_D^{20}$ +28·5° (*c*, 0·76) (Found: C, 71·7; H, 9·6. $C_{30}H_{48}O_6$ requires C, 71·4; H, 9·6%).

The *triacetate* was similarly obtained from arjunolic acid triacetate. After removal of acidic material, it separated from a concentrated ethereal solution, and when recrystallised from aqueous methanol formed stout prisms, m. p. 179—180°, $[\alpha]_D^{19}$ +20° (*c*, 1·20) (Found: C, 68·0; H, 8·4. $C_{36}H_{54}O_9$ requires C, 68·5; H, 8·6%).

Arjunolic Bromo-lactone (V; R = Br).—A solution of arjunolic acid (5 g.) and sodium acetate (10 g.) in acetic acid (100 c.c.; 90%) was stirred during the dropwise addition of 4% bromine–acetic acid (100 c.c.), and after 1 hr. the mixture was poured into water (800 c.c.) containing sodium thiosulphate (10 g.). The precipitate was recrystallised from methanol (norite), the *bromo-lactone* separating in prisms (3·5 g.), m. p. 240—244° (decomp.), $[\alpha]_D^{19}$ +55° (*c*, 1·24) (Found: C, 63·6; H, 8·2; Br, 14·6. $C_{30}H_{47}O_5Br$ requires C, 63·5; H, 8·3; Br, 14·1%). With boiling acetic anhydride–pyridine for ½ hr. it gave the *bromo-lactone triacetate* which crystallised from methanol in large irregular prisms, m. p. 182—183°, $[\alpha]_D^{20}$ +54° (*c*, 2·46) (Found: C, 62·1; H, 7·8. $C_{36}H_{53}O_8Br$ requires C, 62·3; H, 7·7%).

When the bromo-lactone (0·5 g.) in acetic acid (15 c.c.) was treated with zinc dust (3·5 g.) on a steam-bath for 3 hr., arjunolic acid was regenerated, and after precipitation from the filtered solution with water and crystallisation from methanol it had m. p. and mixed m. p. 336—338° (Found: C, 74·0; H, 9·6%). The acid was also obtained by reduction with sodium amalgam in boiling ethanol.

Hydrolysis of Arjunolic Bromo-lactone.—(i) The bromo-lactone (1·1 g.) was heated under reflux with 10% methanolic potassium hydroxide during 3 hr. *Methyl* iso*epoxyarjunolate* was precipitated by pouring the solution into water, and it crystallised from aqueous methanol as long thin rods or needles (0·8 g.), m. p. 250—252° with extensive sintering at 240°. Drying at room temperature gave a *hydrate* (Found: C, 69·5; H, 9·8. $C_{31}H_{50}O_6,H_2O$ requires C, 69·4; H, 9·7%); the anhydrous material was obtained after drying at 150°/0·1 mm. and had $[\alpha]_D^{20}$ +48° (*c*, 1·48) (Found: C, 71·5; H, 9·8; OMe, 6·0. $C_{31}H_{50}O_6$ requires C, 71·8; H, 9·7; OMe, 6·0%).

Treatment of this compound in chloroform solution at room temperature with a stream of hydrogen chloride during ½ hr. resulted in a quantitative conversion into methyl dihydro-12-oxo-arjunolate, m. p. and mixed m. p. 191—192°.

(ii) Hydrolysis of the bromo-lactone (0·5 g.) with ethanolic potassium hydroxide gave an amorphous material almost completely soluble in hot 0·1N-sodium hydroxide containing 10% of methanol. The product obtained by acidification of the alkaline solution could not be crystallised, but on treatment with diazomethane in ether formed methyl dihydro-12-oxo-arjunolate (0·24 g.), m. p. and mixed m. p. 191—192°.

Methyl 11 : 13(18)-*Dehydroarjunolate* (VI).—Methyl arjunolate triacetate (1·4 g.) was heated with pure selenium dioxide (0·125 g.) in boiling acetic acid (50 c.c.) for 17 hr. The product, precipitated when the mixture was poured into water, was heated under reflux for 2 hr. with 5% methanolic potassium hydroxide. *Methyl* 11 : 13(18)-*dehydroarjunolate* was obtained by addition of water, and it crystallised from methanol in colourless laths, m. p. 250—251°, $[\alpha]_D^{20}$ −153° (*c*, 1·49) (Found, in a specimen dried at 150°/0·1 mm.: C, 74·6; H, 9·6. $C_{31}H_{48}O_5$ requires C, 74·6; H, 9·7%). Light absorption: max. 243·5, 251, and 260 mμ; ε 21,400, 24,500, and 15,000. Barton and Holness (*loc. cit.*) record for methyl dehydro-oleanolate acetate, max., 242, 252, and 261 mμ and ε 25,500, 29,100, and 18,900 respectively.

Methyl arjunolate triacetate was also oxidised with excess of selenium dioxide during 17 hr., and the product fractionated on a column of alumina. Ether–benzene (1 : 1) eluted an amorphous acetate which when hydrolysed yielded a further small amount of the diene. Hydrolysis of the fraction eluted by ether gave a straw-coloured gum with light absorption max. at 275 mμ (ε 6800).

Oxidation of Methyl Arjunolate with Sodium Periodate.—An alcoholic solution of methyl arjunolate (0·205 g.) was treated with aqueous sodium periodate (10 c.c.; 0·092N), alcohol being added to make up 50 c.c. The mixture was kept at 18° screened from light, and portions (10 c.c.) were removed after ½, 1, 3½, and 20 hr. Estimation of excess of periodate with standard sodium arsenite in the usual way showed the oxidation respectively of 40, 70, 86, and 97% of a 1 : 2-glycol unit.

Oxidations with Lead Tetra-acetate.—(i) *Arjunolic acid.* Warm acetic acid solutions of lead tetra-acetate (11 g. in 200 c.c.) and of arjunolic acid (10 g. in 100 c.c.) were mixed (34°) and set aside at room temperature for 18 hr. Excess of tetra-acetate was destroyed with ethylene glycol and the mixture poured into water (1·5 l.). The precipitate was collected and dissolved in ether (300 c.c.), and the solution extracted with 1% aqueous sodium hydroxide.

The *acid* thus isolated was an amorphous solid (5·4 g.), m. p. 223—225° (Found : C, 74·5; H, 9·5. $C_{30}H_{46}O_5$ requires C, 74·1; H, 9·5%), which was unaffected by heating in boiling xylene. The residue undissolved by the alkali was a powder (2·36 g.) which could not be crystallised.

(ii) *Arjunolic bromo-lactone.* Arjunolic bromo-lactone (4·45 g.) was oxidised with lead tetra-acetate as for arjunolic acid, and the acidic fraction (0·2 g.) extracted from an ethereal solution of the product with excess of 0·1% sodium hydroxide. The neutral *aldehydo-lactone* crystallised from methanol in leaflets (3·75 g.), m. p. 211—213° raised by recrystallisation to m. p. 222°, $[\alpha]_D^{20}$ +93° (*c*, 1·10) (Found : C, 63·8; H, 8·3; Br, 13·6. $C_{30}H_{45}O_5Br$ requires C, 63·7; H, 8·0; Br, 14·2%). It yielded an amorphous yellow precipitate with 2 : 4-dinitrophenylhydrazine sulphate in ethanol.

A solution of the compound, m. p. 222° (1·05 g.), in acetic acid (30 c.c.) was heated on a steam-bath with zinc dust (7·0 g.). A stream of nitrogen passing through the reaction vessel remained carbon dioxide-free throughout the 2½ hours' heating. The solid precipitated from the filtered solution was dissolved in ether which was then extracted with 1% aqueous sodium hydroxide. The *acid* thus isolated (0·77 g.) crystallised from aqueous methanol in needles, m. p. 251° (decomp.) after sintering at 235°, $[\alpha]_D^{20}$ +152° (*c*, 0·76) (Found : C, 74·3; H, 9·4. $C_{30}H_{46}O_5$ requires C, 74·0; H, 9·5%), and gave an amorphous yellow derivative with methanolic 2 : 4-dinitrophenylhydrazine sulphate.

Pyrolysis of Arjunolic Acid.—A mixture of arjunolic acid (1 g.) and finely divided copper (5 g.) was heated in a metal-bath at 270—290° for 1 hr., and the evolved gases were passed into saturated aqueous dimedone. After a further 1 hr. the precipitate (60 mg.) was collected and when crystallised from methanol it had m. p. 188—189° alone or mixed with formaldehyde dimedone derivative.

18-iso(α-)*Arjunolic Lactone.*—Arjunolic acid (5 g.) was dissolved in hydrogen bromide–acetic acid (50 c.c. of 50%) and after 48 hr. at room temperature the brown solution was poured into water. The solid which separated crystallised from methanol in well-defined plates (4·8 g.) consisting of *triacetyl*-18-iso(α-)*arjunolic lactone*, m. p. 265—266°, $[\alpha]_D^{19}$ +12° (*c*, 3·15) (Found : C, 70·2; H, 8·9. $C_{36}H_{54}O_8$ requires C, 70·3; H, 8·9%). Hydrolysis for ½ hr. at 100° or for 1 hr. at room temperature with varying quantities of aqueous-alcoholic alkali invariably gave 18-iso(α-)*arjunolic lactone* which originally crystallised from aqueous methanol in felted needles but later spontaneously changed to the more stable form of compact leaflets, m. p. *ca.* 350° (decomp.), $[\alpha]_D^{20}$ +6° (c, 1·05) (Found : C, 73·8; H, 10·0. $C_{30}H_{48}O_5$ requires C, 73·7; H, 9·9%). With benzoyl chloride–pyridine overnight at room temperature the lactone afforded a *tribenzoate* which crystallised from methanol in needles, m. p. 284—285°, $[\alpha]_D^{19}$ +61° (*c*, 0·93) (Found : C, 76·8; H, 7·2. $C_{51}H_{60}O_8$ requires C, 76·5; H, 7·5%). The lactone and its acetate gave no perceptible colour with tetranitromethane.

18-iso(α-)*Arjunolic Lactone Diacetate* (IX; R = OH).—A mixture of arjunolic acid (5 g.), concentrated hydrochloric acid (35 c.c.), and acetic acid (75 c.c.) was heated under reflux for 17—24 hr. The solid obtained by pouring the solution into water was collected, dried, and triturated with ether (20 c.c.). The crystalline material remaining undissolved gave 18-iso-(α-)*arjunolic lactone diacetate* (yield normally 15—20% but on one occasion 60%) when crystallised from methanol as leaflets, m. p. 285—286°, $[\alpha]_D^{20}$ −3·5° (*c*, 1·26) (Found : C, 71·2; H, 9·1; Ac, 14·7. $C_{34}H_{52}O_7$ requires C, 71·3; H, 9·1; Ac, 15%). The diacetate was colourless in tetranitromethane.

The lactone diacetate was obtained by the action of boiling hydrochloric acid–acetic acid (1 : 2) (*a*) on 18-*iso*(α-)arjunolic lactone for 2 hr. (yield 10—20%), m. p. 280—283°, and (*b*) on the lactone triacetate for 15 min. (yield 10%), m. p. 279—282°. Partial hydrolysis of the triacetate with alkali, even with only 1 equiv. of alkali, invariably gave the trihydroxy-lactone. Conversely, acetylation of the trihydroxy-lactone with acetic anhydride (2 mols.)–pyridine afforded the triacetate as the sole purifiable product.

Diacetyloxo-18-iso*arjunolic Lactone* (X).—The arjunolic lactone diacetate (0·8 g.) in acetic acid (50 c.c.), mixed with chromium trioxide (0·16 g.), was set aside at room temperature overnight. The excess of chromic anhydride was then decomposed with methanol, and the solution poured into water. The resulting *diacetyloxo*-18-iso*arjunolic lactone* (0·66 g.) crystallised from methanol in long leaflets, m. p. 261°, $[\alpha]_D^{19}$ +40° (*c*, 1·48) (Found : C, 71·7; H, 8·9. $C_{34}H_{50}O_7$ requires C, 71·6; H, 8·8%).

3 : 13-*Dihydroxy*-2-*oxo*-23-*norolean*-3-*en*-28-*oic Lactone* (XI).—(i) Diacetyloxo-18-*iso*arjunolic lactone (0·5 g.) was hydrolysed with boiling methanolic potassium hydroxide (20 c.c.; 10%). The product obtained by pouring the solution into dilute hydrochloric acid was crystallised from a large volume of ethanol and then from chloroform–methanol, forming leaflets (60 mg.), m. p. *ca.* 320° (decomp.), $[\alpha]_D^{19}$ +77° (*c*, 1·05) (Found : C, 76·9; H, 9·0. Calc. for $C_{29}H_{42}O_4$: C, 76·6; H, 9·2%). Light absorption in dioxan : max. 281 mμ; ε 12,000. The diosphenol formed a pale cream potassium salt, and in ethanol with a trace of ferric chloride gave a violet colour, becoming green with excess of the reagent. Ruzicka, Jeger, and Norymberski (*loc. cit.*) record for the substance prepared from hederagenin, m. p. (variable) 325—326°, $[\alpha]_D$ +73·4° to +88·7°; $\lambda_{max.}$ (in dioxan) 280 mμ, ε 11,000, violet ferric colour. A specimen from this source was without effect on the m. p.

With cold pyridine–acetic anhydride the acetate was obtained, leaflets (from methanol), m. p. and mixed m. p. 274—275°, $[\alpha]_D^{19}$ +86° (*c*, 0·84) (Found : C, 74·9; H, 8·9. Calc. for $C_{31}H_{44}O_5$: C, 75·0; H, 8·9%). Light absorption : max. 246 mμ, ε 11,800. Ruzicka *et al.* (*loc. cit.*) record m. p. 272—273°, $[\alpha]_D$ +87°; $\lambda_{max.}$ 246 mμ, ε 12,000.

(ii) Diacetyloxo-18-*iso*arjunolic lactone (0·7 g.) in ethanol (100 c.c.) was mixed with N-sodium hydroxide (5 c.c.) and set aside in a closed flask under nitrogen. Next day the solution was acidified with aqueous sulphuric acid and diluted to 150 c.c. The precipitate was collected and when crystallised from chloroform–methanol gave the diosphenol (0·14 g.), m. p. 318—320°. The filtrate was evaporated to dryness and the distillate mixed with saturated aqueous dimedone (20 c.c.); after 1 hr. the mixture was concentrated, whereupon the formaldehyde derivative (50 mg.), m. p. and mixed m. p. 189—190°, was obtained.

(iii) 18-*iso*Arjunolic lactone (0·5 g.) in acetic acid (25 c.c.) was treated with chromic oxide (0·27 g.) and kept at room temperature overnight. The solution was then heated to 100° for ½ hr. and poured into water, and the solid was collected. When crystallised first from a large volume of ethanol and then chloroform–methanol it afforded the diosphenol (90 mg.) as leaflets, m. p. 318—320° (decomp.).

One of the authors (J. M. R.) thanks the Department of Scientific and Industrial Research for a Maintenance Allowance.

THE UNIVERSITY, NOTTINGHAM. [*Received, July* 16*th*, 1954.]