The Chemistry of Dammar Resin.

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By the use of column and reversed-phase paper chromatography a thorough analysis of dammar resin has been made. The " β-resene " has been shown to be a polymeric material, while the remainder of the resin is principally a complex mixture of neutral and acidic triterpenes, some of which have been isolated and partly characterised. The main neutral triterpenes are: dammadienone, $C_{30}H_{48}O$; dammadienol $C_{30}H_{50}O$; two isomeric hydroxydammarenones, $C_{30}H_{50}O_2$, and their related diols, $C_{30}H_{52}O_2$; and a third ketol, hydroxyhopanone, $C_{30}H_{50}O_2$. In addition to dammarolic acid, previously described, three further acids have been isolated one of which has been shown to be ursonic acid: the other two are dammarenolic acid, $C_{30}H_{50}O_3$, and dammarenonic acid, the latter obtained only in small yield as its methyl ester, $C_{31}H_{50}O_3$.

DAMMAR is the general name given to a group of natural resins originating from trees of the *Dipterocarpaceae* family which occur in Malaya, Indonesia, and the East Indies generally. The material in this investigation (obtained from Messrs. M. Hamburger and Sons Ltd., London) is described as " Pale Bold Indonesia Dammar." This variety is known also as " Dammar Mata Kuching " or " Cats Eye Dammar " and is said (Barry, " Natural Varnish Resins," London, 1932; Howes, " Vegetable Gums and Resins," Waltham, Mass., 1949) to originate from various species of *Hopea* notably *H. micrantha,* Hook. This material gives a paper chromatogram indistinguishable from that given by a sample of dammar from the Museum, Royal Botanic Gardens, Kew, bearing the label " Dammar Mata Kuching, from *Hopea* sp. Malaya, T. H. Barry 1932." It was moreover similarly indistinguishable (Mills and Werner, *J. Oil Colour Chemists' Assoc.*, 1954, **37**, 131) from Penak dammar, the second main variety of dammar, which originates from *Balanocarpus heimii*, King (Chengal tree), occurring mainly in Malaya. It seems therefore that dammars of different botanical origin are probably closely similar. For an account of the origin of the semi-fossil dammars such as Daging, Batu, Sengai, and Hitam see Barry (*op. cit.*). These resins contain higher proportions of polymerised material and oxidation products than the dammars of recent origin (Mills and Werner, *loc. cit.*).

References to early researches on dammar are given by Tschirch (Tschirch and Stock, " Die Harze," 3rd edn., Berlin, 1933, Vol. II, p. 395). The first systematic work was carried out by Tschirch and Glimann (*Arch. Pharm.*, 1896, 587). They isolated an acid which they named dammarolic acid. The neutral portion of the resin was divided into ethanol-soluble and ethanol-insoluble fractions, which were designated " α- " and " β-resene " respectively. From the " β-resene " Zinke and Unterkreuter (*Monatsh.*, 1918, **39**, 865) obtained a material $C_{30}H_{48}$. These results were confirmed by Parijs (*Pharm. Weekblad*, 1934, **71**, 354). Mladenović and Barković (*Monatsh.*, 1940, **73**, 206, 214) isolated an acid which they also called dammarolic acid though it differed from the material described by Tschirch. Barković and Vukčević-Kovačević (*Arhiv Kem.*, 1946, **18**, 66; *Chem. Abs.*, 1948, **42**, 2950) isolated two lactones from the products of the degradative oxidation of the neutral " α-resene " fraction; and Haas (*Pharm. Weekblad*, 1949, **84**, 505) obtained an acid which was almost certainly an impure sample of the dammarolic acid of Mladenović and Barković (*loc. cit.*). As will appear in the sequel dammar is a mixture of greater complexity than is suggested by the foregoing review.

The resin was first extracted with methanol and the methanol-soluble material chromatographed on alumina. Elution was carried out with the usual range of solvents, and fractions of equal volumes of eluant were examined by paper chromatography using a reversed-phase system devised for this purpose which employs odourless kerosene as the stationary phase and aqueous propan-2-ol as the mobile phase (Mills and Werner, *Nature*, 1952, **169**, 1064 and *loc. cit.*; cf. Rawlins and Werner, *Endeavour*, 1954, **51**, 140). The fractions were then worked up by crystallisation or further chromatography according as they were apparently homogeneous or mixtures. The principal neutral constituents isolated are listed in Table 1.

TABLE 1. *Neutral constituents of dammar.*

	Approx. R_F	Formula	M. p.	$[\alpha]_D$
β-Resenes				
Ether-soluble	0·15	—	160—210°	−25°
Ether-insoluble	0·15	—	210—240	−33
Dammadienone	0·25	($C_{30}H_{48}O$)	(72—75)	(+81)
Dammadienol	0·35	$C_{30}H_{50}O$	136—138	+47
Hydroxydammarenone I	0·62	$C_{30}H_{50}O_2$	145—147	+60
Hydroxydammarenone II	0·62	$C_{30}H_{50}O_2$	134—136	+66
Hydroxyhopanone	0·66	$C_{30}H_{50}O_2$	252—256	+64
Dammarenediol I	0·82	$C_{30}H_{52}O_2$	142—144	+27
Dammarenediol II	0·82	$C_{30}H_{52}O_2$	131—133	+33

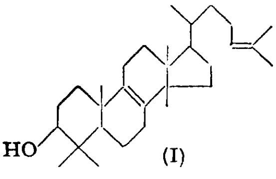
The " β-resene " has been re-examined by the methods of Zinke and Unterkreuter (*loc. cit.*), and similar materials were obtained. Both of these, however, contained oxygen, and determination of their molecular weights [by an isopiestic method (Wright, *Analyst*, 1948, **73**, 387) kindly carried out by Imperial Chemical Industries Limited, Paints Division, Slough] showed these to be higher than those corresponding to C_{30} compounds. Chromatography of the " β-resene " on alumina yielded no crystallisable material, but a continuous series of fractions of increasing molecular weight. It is concluded that the " β-resene " is a mixture of polymers of low molecular weight.

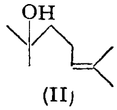
The alcohol dammadienol was isolated as its acetate by repeated crystallisation of a crude acetate which was itself apparently homogeneous on a paper chromatogram. The related ketone, dammadienone, was also obtained, with difficulty and in small yield, from an apparently homogeneous resinous fraction of the resin. Perbenzoic acid titration of dammadienol showed the presence of two double bonds and consequently if the empirical formulæ indicated are correct the compounds must be tetracyclic. The infrared spectra of the alcohol and ketone showed bands at 3075, 1640, and 890 cm.$^{-1}$, indicating the presence of a vinylidene group. Dammadienone gives a positive Zimmermann reaction and its infrared spectrum shows a band at 1711 cm.$^{-1}$ due to a six-membered ring ketone group. Barton and de Mayo (*J.*, 1954, 887) have shown that in the triterpene series this reaction is specific for compounds having a 3-oxo-group which must therefore be present in dammadienone. The method of molecular-rotation differences (Klyne, *J.*, 1954, 1979) indicates that the hydroxyl group of dammadienol has the more stable β-configuration (Δ_1 + 80°).

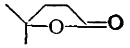
The two isomeric ketols, hydroxydammarenone I and II, were separated by chromatography and fractional crystallisation. On reduction with sodium and alcohol they yielded dammarenediol I and II which were also isolated directly from the resin. Perbenzoic acid titration indicated the presence of one double bond and consequently if the formulæ indicated are correct the compounds are tetracyclic. For reasons similar to those given above hydroxydammarenone I and II have a 3-oxo-group and dammarenediol I and II a 3β-hydroxy-group (diol I, Δ_1 + 31°; diol II, Δ_1 + 24°). The latter is further indicated by the mode of formation of the diols (Barton, *Experientia*, 1950, **6**, 316). Hydroxydammarenone I and II do not readily yield acetates but the presence of a hydroxyl group is shown by their infrared spectra (bands at 3620 cm.$^{-1}$).

Evidence for the tertiary nature of this hydroxyl group and of a tetracyclic formulation for these compounds was provided by their oxidation with chromium trioxide. In both cases acetone was obtained (as its 2 : 4-dinitrophenylhydrazone). Hydroxydammarenone I also yielded a crystalline compound, m. p. 218—220°, $C_{27}H_{42}O_3$, which gave no colour with tetranitromethane. Hydroxydammarenone II gave a similarly saturated compound, m. p. 183—185°, having the same formula. Both compounds had the same R_F value, gave similar colour reactions on the paper chromatogram, gave similar Zimmermann reactions, and had closely similar infrared spectra which showed bands at 1711 and 1785 cm.$^{-1}$ indicative of a six-membered ring ketone and a saturated γ-lactone grouping respectively.

Both ketols must therefore contain an *iso*propylidene group. The hydroxyl group must be involved in the lactone formation and consequently, if a substituted side chain of lanosterol type, as in (I), is assumed, either ketol could have partial formula (II) and the two lactones partial formula (III).


(I)


(II)


(III)

The saturated nature of these two lactones and the comparatively low ultraviolet absorption of the hydroxydammarenone I and II in the 2100—2250-Å region indicate that there is no endocyclic tetrasubstituted double bond characteristic of tetracyclic triterpenes of the euphadienol and lanosterol series. Since all naturally occurring tetracyclic triterpenes of known structure contain this system, it was suggested by Professor D. H. R. Barton (personal communication) that these compounds might contain a *cyclo*propane ring as in *cyclo*artenol (Barton, *J.*, 1951, 1444). However, an attempted isomerisation of the keto-lactone I by treatment with hydrogen chloride in chloroform solution gave back starting material. It seems possible that the keto-lactone I may be identical with the lactone L_I, m. p. 216°, isolated by Barković and Vukčević-Kovačević (*loc. cit.*) on oxidation of dammar, despite the fact that they reported their compound as giving a yellow colour with tetranitromethane. Their analytical data agree almost as well with the formula $C_{27}H_{42}O_3$ as with their formula $C_{25}H_{40}O_3$.

A third ketol, hydroxyhopanone, is eluted from alumina together with hydroxydammarenone I and II from which, however, it is easily separated on account of its much lower solubility. For reasons similar to those given above this compound contains a 3-oxo-group and a tertiary or hindered secondary hydroxy-group. Though isomeric with the above ketols it differs from them in being saturated to tetranitromethane and must therefore be pentacyclic.

One further neutral compound, possibly not pure, was also isolated. This has been only cursorily examined and details are given in the Experimental section.

TABLE 2. *Acidic constituents of dammar.*

Acid	Approx. R_F	Formula	M. p.	$[\alpha]_D$
Dammarolic	0·96	$C_{30}H_{48-50}O_6$	315° *	+49°
Dammarenolic	0·86	$C_{30}H_{50}O_3$	138—142	+43
Ursonic	0·74	$C_{30}H_{46}O_3$	270—275 *	+80
Me dammarenonate	0·44	$C_{30}H_{50}O_3$	120—121·5	+62

* With decomp.

The acids were isolated principally by chromatography of the mixture of methyl esters, but ursonic acid was also obtained from the fractions of the main chromatographic separation lying between those containing dammadienone and dammadienol. It is highly unlikely that the acid itself could have been eluted at this stage, and it is believed to have been formed from a labile constituent which was detected on the paper chromatograms of these fractions but has not so far been isolated. Nevertheless the acid is believed to be an original constituent of dammar since it was also isolated from the ether–methanol fractions of the same separation where it might be expected to be eluted. Furthermore its methyl ester was obtained in the separation of the esters of the mixed acids. The melting point and rotation of the methyl ester were in good agreement with the recorded values for methyl ursonate and the melting point was undepressed on admixture with an authentic specimen (kindly supplied by Mr. K. H. Overton). This identification was confirmed by conversion of the ester into uvaol and methyl ursolate acetate.

The acid, m. p. *ca.* 315°, obtained in the present work from the ether-insoluble portion of the acid fraction, appears to be identical with the dammarolic acid, m. p. 325°, of Mladenović and Barković (*loc. cit.*). The acetate and acetate methyl ester were prepared. Both had wide melting ranges and gave unsatisfactory analyses. The methyl ester, which was crystalline and of sharp melting point, gave a yellow colour with tetranitromethane, though Mladenović and Barković state that the acid is saturated.

Dammarenolic acid appears to be the major acidic constituent of the resin. It was isolated as its methyl ester. This did not yield an acetate but the presence of a hydroxyl group was shown by an infrared band at 3620 cm.$^{-1}$. Perbenzoic acid titration showed the presence of two double bonds, one of which is present in a vinylidene group (infrared spectrum: bands at 3070, 1640, and 895 cm.$^{-1}$). If the suggested molecular formulæ for the acid and its derivatives are correct a tricyclic formulation must be assumed for them. Oxidation of the ester yielded acetone and a trisnor-γ-lactone, which suggests the presence of a side chain similar to that in the hydroxydammarenones. Reduction of the ester with lithium aluminium hydride yielded a diol characterised as its monoacetate.

Methyl dammarenonate, the ester of an acid which has not itself been prepared, was also obtained by chromatography of the mixed esters. The ultraviolet absorption was characteristic of an $\alpha\beta$-unsaturated ester and the infrared spectrum showed a strong band at 1707 cm.$^{-1}$ due to six-membered ring ketone and conjugated ester groups. The ester gave a positive Zimmermann reaction.

EXPERIMENTAL

Rotations were determined in $CHCl_3$ at room temperature. Alumina used for chromatography was Peter Spence neutral type " H " partially deactivated by being spread out in the air overnight. Light petroleum refers to the fraction of boiling range 60—80°.

Paper Chromatography.—R_F values are liable to considerable variation and must be regarded as relative only. Those given were determined under comparable conditions on paper impregnated with odourless kerosene, the developing solvent being the lower phase of the system propan-2-ol–water–odourless kerosene (7 : 3 : 1 by vol.). In the Halphen–Hicks reaction (Hicks, *J. Ind. Eng. Chem.*, 1911, **3**, 86) the dried papers are sprayed with a solution of phenol in carbon tetrachloride and suspended in bromine vapour for a short while. Not all triterpenes give a positive reaction in this test and the modified Noller reaction (Noller *et al.*, *J. Amer. Chem. Soc.*, 1942, **64**, 3047) is more general. In this the papers are suspended in a jar full of the vapour from a mixture of thionyl chloride with about 20% of stannic chloride. The colours produced are usually intensified and sometimes undergo characteristic changes if after ½ minute's exposure the strips are removed for about ½ min. and then replaced for a further short period. The reaction with antimony trichloride, described by Hashimoto (*Experientia*, 1953, **9**, 194), appears to be less sensitive than the above reactions.

β-Resene.—The coarsely ground resin (1 kg.) was digested with hot methanol (4 l.) until a uniform suspension was obtained. The cooled solution was filtered from the insoluble β-resene (*ca.* 290 g.) and the " dewaxed " dammar recovered by pouring the solution into a large volume of brine and filtering off the resin. The β-resene was purified by precipitation three times from chloroform (100 g. in 100 ml.) with methanol (400 ml.). It was divided into ether-soluble and ether-insoluble fractions by the methods of Zincke and Unterkreuter (*loc. cit.*). The ether-soluble material had m. p. 160—210°, $[\alpha]_D$ $-25°$ (c 1·59) (Found: C, 85·7; H, 11·55%; M, 3220, 3200). The ether-insoluble material had m. p. 210—240°, $[\alpha]_D$ $-33°$ (c 1·55) (Found: C, 85·4; H, 11·55%; M, 4700, 5120).

The β-resene mixture (5 g.) was absorbed from light petroleum on alumina (30 × 3·8 cm.), and elution with gradually increasing concentrations of benzene in light petroleum, followed by chloroform in benzene, and 38 100-ml. fractions were collected. Molecular weights increased with the number of the fraction, *e.g.*,

Number of fraction	3	13	18	23	37
Mol. wt.	1830	2500	2600	3000	4040

Main Chromatographic Separation.—" Dewaxed " dammar (120 g.) was warmed with light petroleum (200 ml.) until a uniform suspension was obtained which was then diluted to 1 l. with light petroleum. A sticky precipitate, comprising the bulk of the acidic constituents, was allowed to settle. The supernatant solution was run during ½ hr. on to alumina (85 × 4·8 cm., *ca.* 1300 g.), and washed in with several small volumes of light petroleum. The following eluting solvents were then passed : (i) benzene in light petroleum increasing in concentration by increments of 5% of benzene every 500 ml. of eluant from 0 to 50% of benzene and by increments of 10% of benzene every 500 ml. of eluant from 60 to 100% of benzene; (ii) ether in benzene increasing in concentration by increments of 10% of ether every 250 ml. of eluant from 10% to 100% of ether; (iii) methanol in ether increasing in concentration by increments of 10% of methanol every 250 ml. of eluant from 10% to 100% of methanol. Fractions of 250 ml. were collected, about 56 in all. Paper chromatograms of every other fraction were run, one drop (20 μl.) being used. The composition being thus disclosed the fractions were divided into batches showing maximum separation of constituents and evaporated, and the material worked up as follows :

Fractions 5—6. These contained residual β-resene (R_F 0·15) and some essential oil, and were not examined further.

Fractions 11—15 (*ca.* 3·5 g.) Although the resinous material from these fractions gave a single spot on the paper chromatogram it did not crystallise directly from the usual solvents (Found : C, 84·0; H, 11·35. Calc. for $C_{30}H_{48}O$: C, 84·85; H, 11·4%). It readily yielded, by the usual methods, a crystalline oxime mixture, m. p. 200—210° (from aqueous ethanol) (Found : C, 81·8; H, 11·35; N, 3·15. Calc. for $C_{30}H_{49}ON$: C, 81·95; H, 11·25; N, 3·2%), and a 2 : 4-dinitrophenylhydrazone mixture, m. p. 140—155° (Found : C, 71·35; H, 8·85; N, 9·45. Calc. for $C_{36}H_{52}O_4N_4$: C, 71·5; H, 8·65; N, 9·25%). The ketone mixture (2 g.) was absorbed from light petroleum (25 ml.) on alumina (20 × 1·8 cm.). Benzene–light petroleum (1 : 19) eluted the material in 5 100-ml. fractions. The first did not crystallise. The material from the second and third fraction was dissolved in hot ethanol, and the solution cooled to room temperature, decanted from gum, and set aside overnight. A small quantity of waxy solid which separated was treated as above with methanol, giving nodules which, on recrystallisation twice from methanol, gave leaflets (20 mg.), m. p. 72—75°, $[\alpha]_D$ +81° (*c* 0·78), R_F 0·25. Colour reactions : Noller, mauvish; Halphen–Hicks, orange pink. The compound gave a yellow colour with tetranitromethane and a positive Zimmermann reaction.

Fractions 16—23 (*ca.* 9 g.). These fractions, when examined shortly after running from the column, showed a single spot of R_F value 0·42 which gave a blue Noller reaction and no colour in the Halphen–Hicks test. It was not possible to isolate the material corresponding to this spot, since in a few days or possibly during working up the substance it is almost completely converted into ursonic acid, R_F 0·74, a substantial amount of which is present in the fractions after only one day. In one experiment the fractions were treated as follows :

After 4 days the solutions were evaporated and divided into acidic (*ca.* 7 g.) and neutral (*ca.* 2 g.) fractions by extraction with aqueous alkali. Rechromatography of the neutral fraction failed to yield crystalline material even though once again some of the fractions initially contained only the original substance of R_F 0·42. The acid fraction, crystallised from aqueous dioxan, gave impure ursonic acid (5 g.), m. p. 250—268° (decomp.). The material was dissolved in ethanol, a large volume being necessary owing to the presence of a poorly soluble neutral *compound* which separated from the dilute solution as prisms (350 mg.), m. p. *ca.* 300° (decomp. with prior sintering) (after recrystallisation from ethanol–ethyl acetate), $[\alpha]_D$ +66° (*c* 0·74) (Found : C, 76·5; H, 9·95. $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%). This was probably present in the aqueous alkaline extract, which was very cloudy, on account of its very low solubility in ether. It was unchanged on treatment with diazomethane, and gave no colour in the Halphen–Hicks or the Noller reaction or with tetranitromethane. It gave a positive Zimmermann reaction. The infrared spectrum determined in Nujol showed bands at 3500, 1733, and 1703 cm.$^{-1}$. Concentration of the ethanol solution gave ursonic acid, a portion of which, crystallised several times from methanol, had m. p. 270—275° (decomp.), $[\alpha]_D$ +80° (*c* 0·9) (Found : C, 78·95; H, 10·25. Calc. for $C_{30}H_{46}O_3$: C, 79·25; H, 10·2%), R_F 0·74. Colour reactions : Noller, pale orange, through mauve to bright blue; Halphen–Hicks, negative. The bulk of the acid was methylated with diazomethane and crystallised from ethanol, to give methyl ursonate as large plates, m. p. and mixed m. p. 192—194°, $[\alpha]_D$ +84° (*c*, 1·23) (Found : C, 79·1; H, 10·45. Calc. for $C_{31}H_{48}O_3$: C, 79·45; H, 10·3%), R_F 0·46. Colour reactions : Noller, pale orange turning to mauve (slow, long exposure necessary); Halphen–Hicks, negative. Reduction of the ester with lithium aluminium hydride, followed by chromatography and crystallisatiom from methanol, yielded uvaol, as long needles, m. p. 225—227°, $[\alpha]_D$ +63° (*c*, 1·34) (Found : C, 81·4; H, 11·35. Calc. for $C_{30}H_{50}O_2$: C, 81·4; H, 11·4%), R_F 0·8. Colour reactions : Noller, mauve; Halphen–Hicks, negative. Reduction of the ester with sodium borohydride, acetylation of the product with pyridine–acetic anhydride, chromatography, and crystallisation from methanol yielded methyl ursolate acetate, m. p. and mixed m. p. 245—247°, $[\alpha]_D$ +55° (*c* 1·22) (Found: C, 77·35; H, 10·4. Calc. for $C_{33}H_{52}O_4$: C, 77·3; H, 10·25%), R_F 0·33. Colour reactions: Noller, pink; Halphen–Hicks, weak blue.

Fractions 25—34 (*ca.* 12 g.). These fractions consist largely of a mixture of alcohols (R_F 0·35) and ketols (R_F 0·62). Two other spots are also present on the paper chromatogram but the materials they represent have not been isolated. The material was treated with pyridine–acetic anhydride overnight and the products, isolated in the usual way, were chromatographed on alumina (30 × 3·8 cm.). Light petroleum eluted the alcohol acetate mixture (R_F 0·19), and the unchanged ketols were subsequently eluted by gradually increasing concentrations of benzene in light petroleum. The acetate mixture, crystallised from aqueous ethanol, had m. p. 100—130° (4·5 g.). It was rechromatographed on alumina. The earlier material eluted, which carried with it a small amount of β-resene, was rejected, and the remaining material was crystallised many times from ethanol to yield *dammadienyl acetate* as flat needles (300 mg.), m. p. 151—153°, $[\alpha]_D$ +60° (*c* 0·68) (Found: C, 81·95; H, 11·35. $C_{32}H_{52}O_2$ requires C, 82·0; H, 11·2%), R_F 0·19. Colour reactions: Noller, ill-defined greyish-mauve; Halphen–Hicks, orange-brown → pink. The material gave a yellow colour with tetranitromethane. Hydrolysis of dammadienyl acetate for 6 hr. with 10% ethanolic potassium hydroxide followed by chromatographic purification and crystallisation from methanol yielded *dammadienol* as needles, m. p. 136—138°, $[\alpha]_D$ +47° (*c* 0·6) (Found: C, 84·3; H, 11·9. $C_{30}H_{50}O$ requires C, 84·45; H, 11·8%), R_F 0·35. Colour reactions were as for the acetate. The infrared spectrum, determined in CS_2, showed bands at 3620, 3070, 1640, and 895 cm.$^{-1}$.

The ketol fractions were combined and crystallised from light petroleum. This is desirable as it removes any β-resene, which tends to be present in all the fractions in small quantities and is not removed by crystallisation from polar solvents such as methanol.

The ketol mixture (m. p. 110—130°) was dissolved in methanol and allowed to crystallise overnight. On white nodular material clusters of large plates rested. These were picked out and had m. p. 128—133°. Upon recrystallisatiom three times from methanol they gave *hydroxydammarenone II* as plates (300 mg.), m. p. 134—136°, $[\alpha]_D$ +66° (*c* 1·18) (Found: C, 81·55; H, 11·6. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%), R_F 0·63. Colour reactions: Noller, greenish; Halphen–Hicks, orange-brown → pink. The material gave a yellow colour with tetranitromethane and a positive Zimmermann reaction. The infrared spectrum in CS_2 showed bands at 3620 and 1711 cm.$^{-1}$. Light absorption in EtOH at 210, 215, 220, and 225 mμ had ε 1670, 660, 185, and 45 respectively; $\lambda_{max.}$ 290 mμ (ε 36). The remaining nodular material and a further quantity recovered from the mother-liquor (total 4 g.) were adsorbed from light petroleum on alumina (30 × 3·8 cm.), and eluted with increasing concentrations of benzene in light petroleum (30 × 100 ml.). The eluted materials crystallised from aqueous methanol. The first two fractions yielded hydroxydammarenone II, m. p. 127—132°, raised to the m. p. of the above material by crystallisation. The remaining fractions yielded mixtures, m. p. 118—125°, which were combined and wastefully crystallised six times from methanol, to yield *hydroxydammarenone I* as needles (300 mg.), m. p. 145—147° (depressed to 120—130° on admixture with hydroxydammarenone II), $[\alpha]_D$ +60° (*c* 1·34) (Found: C, 81·25; H, 11·5%), R_F and colour reaction as for hydroxydammarenone II. The infrared spectrum in CS_2 also showed bands at 3620 and 1711 cm.$^{-1}$ and was almost identical with that of hydroxydammarenone II.

Fractions 35—41 (13 g.). The material was dissolved in ether (100 ml.) and set aside overnight. *Hydroxyhopanone* separated as fine needles (500 mg.), m. p. 246—250°, raised by several crystallisations from ethanol to 252—256° (slight charring), $[\alpha]_D$ +64° (*c* 1·68) (Found: C, 81·35; H, 11·55. $C_{30}H_{50}O_2$ requires C, 81·4; H, 11·4%), R_F 0·66. (When run alone on a paper chromatogram this material tends to crystallise at the origin, causing streaking.) Colour reactions: Noller, brownish; Halphen–Hicks, brownish-yellow. The material showed no colour with tetranitromethane, and gave a positive Zimmermann reaction. The infrared spectrum in CS_2 showed bands at 3610 and 1706 cm.$^{-1}$.

On treatment with pyridine–acetic anhydride overnight at room temperature this compound was recovered unchanged (m. p., mixed m. p., R_F). Treatment with cold ethanolic 2 : 4-dinitrophenylhydrazine sulphate containing an excess of sulphuric acid yielded the 2 : 4-*dinitrophenylhydrazone* of the dehydration product (or products), m. p. 241—243° (decomp.) (after chromatographic purification and crystallisation from ethanol–benzene) (Found: C, 71·2; H, 8·85. $C_{36}H_{52}O_4N_4$ requires C, 71·5; H, 8·65%). The *oxime*, crystallised from ethanol–chloroform, had m. p. 295—298° (decomp.) (Found: C, 78·25; H, 11·35; N, 3·2. $C_{30}H_{51}O_2N$ requires C, 78·7; 11·25; N, 3·05%).

The material remaining in the ether solution from the above crystallisation consisted largely of a mixture of hydroxydammarenone I and II which after further chromatographic purification and five wasteful crystallisations from methanol yielded hydroxydammarenone I (800 mg.).

Fractions 43—46 (5 g.). The material from these fractions was adsorbed from benzene on alumina (25 × 3·8 cm.). Elution was first with benzene and then with 5 and 10% of ether in benzene (20 × 100 ml.). The fractions 2—7 gave material which on crystallisation twice from aqueous methanol and several times from nitromethane gave *dammarenediol II* as needles (500 mg.), m. p. 131—133°, $[\alpha]_D$ +33° (*c* 1·19) (Found : C, 81·0; H, 11·9. $C_{30}H_{52}O_2$ requires C, 81·0; H, 11·8%). The *monoacetate* was prepared by pyridine–acetic anhydride overnight and after chromatography and crystallisation from methanol was obtained as plates, m. p. 135—137°, $[\alpha]_D$ +37° (*c* 0·92) (Found : C, 79·2; H, 11·5. $C_{32}H_{54}O_3$ requires C, 78·95; H, 11·2%). The material from fractions 10—20, recrystallised from aqueous methanol and from nitromethane, gave *dammarenediol I* as needles (400 mg.), m. p. 142—144°, $[\alpha]_D$ +27° (*c* 1·23) (Found : C, 80·6; H, 11·9%). The *monoacetate*, prepared and purified as described above, was obtained as needles, m. p. 149—150° (clearing 151°), $[\alpha]_D$ +32° (*c* 0·87) (Found : C, 79·0; H, 11·3%). The R_F of both diols was 0·82. Colour reactions : Halphen–Hicks, orange-pink; Noller, mauvish-grey. The acetates had R_F 0·5 and gave similar colour reactions.

Fractions 51 *and* 52 (2 g.). These fractions showed on a paper chromatogram a spot having the same R_F and giving the same colour reactions as ursonic acid. The material was divided into acidic and neutral fractions. The acidic fraction was esterified with diazomethane, and the product adsorbed from benzene on alumina (20 × 1·8 cm.). Elution with the same solvent yielded an ester which, crystallised several times from methanol, gave in small yield methyl ursonate as stout needles (100 mg.), m. p. and mixed m. p. 192—195°, $[\alpha]_D$ +86° (*c* 0·5). The neutral fraction was not investigated.

Isolation of Further Acids.—The light petroleum-insoluble portion of the resin was dissolved in ether and extracted with 5% aqueous potassium hydroxide, then several times with water. The aqueous extracts were made just acid by 2N-hydrochloric acid; the precipitated acids were washed with water, dried (SiO_2 gel), and refluxed with ether (10 g. in 100 ml.). On cooling, the solution deposited a small amount of insoluble material which was recovered by filtration or centrifuging. This material, dissolved in ethanol, was decolorised with charcoal, and after crystallising several times from acetone yielded dammarolic acid as a microcrystalline powder, m. p. 315° (decomp.; sintering slightly at 250° and charring at 280°), $[\alpha]_D$ +49° (*c* 1·58) (Found : C, 71·3; H, 10·0. Calc. for $C_{30}H_{48}O_6$: C, 71·4; H, 9·6. Calc. for $C_{30}H_{50}O_6$: C, 71·1; H, 9·95%), R_F 0·96. Colour reactions : Noller, pale orange → mauve → blue; Halphen–Hicks, negative. The material gave a very pale yellow colour with tetranitromethane. The methyl ester (diazomethane) formed needles, m. p. 222—224°, $[\alpha]_D$ +52° (*c* 1·24), from methanol (Found : C, 72·4; H, 10·0. Calc. for $C_{31}H_{50}O_6$: C, 71·8; H, 9·7. Calc. for $C_{31}H_{52}O_6$: C, 71·5; H, 10·05%. The figures given are the least unsatisfactory of several analyses). The acetate, prepared in the usual way, had m. p. 160—175°, $[\alpha]_D$ +37° (*c* 1·19) (Found : C, 70·5; H, 9·15%). The *acetate methyl ester* had m. p. 145—150° (from methanol). The m. p. was lowered to 125—140° on drying at 100° for a short while (Found : C, 71·35; H, 9·6%).

The acids remaining in the ether solution were methylated with diazomethane, and the resulting resinous mixture of esters (20 g.) was absorbed from light petroleum on alumina (30 × 3·8 cm.), and elution was carried out with light petroleum (1·5 l.), followed by benzene in light petroleum increasing in concentration by increments of 5% of benzene every 100 ml. of eluate up to 50% of benzene and by increments of 10% of benzene from 60 to 100% of benzene. Fractions of 100 ml. were collected and their composition determined by paper chromatography.

The column was rather overloaded and fractions 4 and 5 contained an unresolved mixture which was therefore re-chromatographed as described below.

Fractions 10—25, individually crystallised from aqueous methanol, yielded similar materials (total 2·5 g.), m. p. 86—90° which, combined and recrystallised from aqueous methanol and methanol, yielded *methyl dammarenolate* as needles, m. p. 89—92° not raised by further crystallisation, $[\alpha]_D$ +41° (*c* 1·45) (Found : C, 78·7; H, 11·25. $C_{31}H_{52}O_3$ requires C, 78·75; H, 11·1%), R_F 0·54. Colour reactions : Noller, pale orange → greyish-mauve; Halphen–Hicks, orange-brown → pink. The material gave a yellow colour with tetranitromethane. The infra-red spectrum in CS_2 showed bands at 3620, 1746, 3070, 1640, 895 cm.$^{-1}$. Treating the ester with pyridine–acetic anhydride overnight at 20° or for 40 min. on the steam-bath gave only starting material (m. p., mixed m. p., R_F). Hydrolysis of the ester for 48 hr. at 20° with 10% ethanolic potassium hydroxide yielded *dammarenolic acid*, needles, m. p. 138—142° (from aqueous methanol), $[\alpha]_D$ +43° (*c* 0·98) (Found : C, 78·45; H, 11·15. $C_{30}H_{50}O_3$ requires C, 78·5; H, 11·0%), R_F 0·86. Colour reactions as for the methyl ester.

The material from fractions 4 and 5 was re-adsorbed from light petroleum on alumina (25 × 3·8 cm.). Elution with increasing concentrations of benzene in light petroleum as above and crystallisation from methanol yielded, in order of elution: (*a*) *Methyl dammarenonate*, long needles, m. p. 120—121·5° (from methanol) (70 mg.), $[\alpha]_D$ +62° (*c* 0·78) (Found: C, 78·75; H, 10·45. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%), R_F 0·44. Colour reactions: Noller, yellow $\longrightarrow$ brownish; Halphen–Hicks, yellow-brown. The material gave a yellow colour with tetranitromethane and a positive Zimmermann reaction. The infrared spectrum in CS_2 showed a broad and intense band at 1707 cm.$^{-1}$. Light absorption in ethanol: $\lambda_{max.}$ 214 mμ (ε 16,000). (*b*) Methyl ursonate, rectangular plates, m. p. and mixed m. p. 191—194° (from ethanol) (200 mg.), $[\alpha]_D$ +85° (*c* 1·03) (Found: C, 79·25; H, 10·4%). (*c*) A further 1 g. of methyl dammarenolate, m. p. 86—90° raised by further crystallisation to the m. p. of the material previously described.

Oxidation of Dammadienol.—The alcohol (150 mg.) in benzene (1 ml.) and "AnalaR" acetic acid (5 ml.) was treated with chromium trioxide (100 mg.) in a few drops of water, and set aside overnight. The products were isolated in the usual way. The acid fraction (40 mg.) showed on a paper chromatogram a single spot of high R_F, and colour reactions: Noller, mauve; Halphen–Hicks, negative. It was not examined further. The neutral fraction was adsorbed from light petroleum on alumina (10 × 1·2 cm.). Benzene–light petroleum (1 : 19) eluted a product which, crystallised twice from methanol, gave *dammadienone* as leaflets (45 mg.), m. p. 79—80°, mixed m. p. with the material (m. p. 72—75°) isolated direct from the resin 72—76°, $[\alpha]_D$ +90° (*c* 0·56) (Found: C, 84·55; H, 11·55. $C_{30}H_{48}O$ requires C, 84·85; H, 11·4%). The R_F and colour reactions were identical with those of the material (m. p. 72—75°) isolated from the resin. The infrared spectrum in CS_2 showed bands at 1711, 3070, 1640, and 895 cm.$^{-1}$.

Oxidation of Hydroxydammarenone I.—The ketol I (0·5 g.) in benzene (2·5 ml.) and "AnalaR" acetic acid (15 ml.) was treated with chromium trioxide (0·4 g.) in a few drops of water, and set aside overnight at 20°. The solution was poured into dilute aqueous sodium metabisulphite and steam-distilled. To the distillate (50 ml.) was added a solution of 2 : 4-dinitrophenylhydrazine (250 mg.) in dilute hydrochloric acid. The mixture was extracted with benzene and the product adsorbed from benzene on alumina (20 × 1·8 cm.). Benzene detached and eluted a band, which on evaporation and crystallisation from methanol gave acetone 2 : 4-dinitrophenylhydrazone (130 mg.), m. p. and mixed m. p. 125—127°. A blank experiment, identical with the above apart from the omission of the hydroxydammarenone I, yielded no acetone 2 : 4-dinitrophenylhydrazone. The residue from the steam-distillation was worked up in the usual way and adsorbed from benzene on alumina (20 × 1·8 cm.). Benzene–ether (9 : 1; 250 ml.) eluted a product, *keto-lactone I*, which crystallised from methanol as needles (260 mg.), m. p. 211—217°, raised by several further crystallisations to 218—220°, $[\alpha]_D$ +53° (*c* 1·11) (Found: C, 77·8, 78·05; H, 10·25, 10·2. $C_{27}H_{42}O_3$ requires C, 78·2; H, 10·2%), R_F 0·9. Colour reactions: Noller, yellow $\longrightarrow$ mauve; Halphen–Hicks, negative. The compound gave no colour with tetranitromethane and a positive Zimmermann reaction. The infrared spectrum in CS_2 showed bands at 1711 and 1785 cm.$^{-1}$.

Oxidation of Dammarenediol II.—Dammarenediol II (300 mg.) in benzene (1·5 ml.) and "AnalaR" acetic acid (10 ml.) was treated with chromium trioxide (400 mg.) in a few drops of water and set aside overnight at 20°. The procedure was then as in the preceding section, acetone 2 : 4-dinitrophenylhydrazone (60 mg.) being obtained, having m. p. and mixed m. p. 125—127°. The residue from the steam-distillation was worked up in the usual way and adsorbed from benzene on alumina (15 × 1·8 cm.). Benzene–ether (9 : 1; 200 ml.) eluted a product, *keto-lactone II*, which crystallised from aqueous methanol as platelets (150 mg.), m. p. 178—182° raised by crystallisation from methanol to m. p. 183—185° $[\alpha]_D$ +69° (*c* 1·06) (Found: C, 77·7; H, 10·45. $C_{27}H_{42}O_3$ requires C, 78·2; H, 10·2%). The R_F, Noller, and Halphen–Hicks colours, and the tetranitromethane and Zimmermann reactions were as for the keto-lactone I. The infrared spectrum in CS_2 showed bands at 1711 and 1785 cm.$^{-1}$.

Oxidation of Hydroxydammarenone II.—The ketol II (200 mg.) in benzene (1 ml.) and "AnalaR" acetic acid (7·5 ml.) was treated with chromium trioxide (150 mg.) and the solution kept overnight. The product was isolated, chromatographed, and crystallised as in the preceding section, to yield the keto-lactone II, m. p. and mixed m. p. 183—185°.

Oxidation of Methyl Dammarenolate.—Methyl dammarenolate (500 mg.) in benzene (2·5 ml.) and "AnalaR" acetic acid (15 ml.) was treated with chromium trioxide (400 mg.) in a few drops of water, and the solution kept overnight at 20°. The procedure was as in the oxidation of hydroxydammarenone I, acetone 2 : 4-dinitrophenylhydrazone (110 mg.) being obtained (m. p.

and mixed m. p. 126—128°). The residue from the steam-distillation was adsorbed from benzene–light petroleum (1 : 1) on alumina (20 × 1·8 cm.). Elution with the same solvent (750 ml.) yielded a few mg. of material which crystallised from aqueous methanol as needles, m. p. 114—120°, R_F 0·45. This material was not further examined. Elution with benzene (500 ml.) yielded the main product, a ***methyl ester lactone***, which crystallised from aqueous methanol as needles, m. p. 121—126°, raised to m. p. 125—128° by further crystallisation from methanol, $[\alpha]_D$ +43° (*c* 0·7) (Found : C, 75·85; H, 10·15. $C_{28}H_{44}O_4$ requires C, 75·65; H, 10·0%), R_F 0·93. Colour reactions : Noller, pink or mauve; Halphen–Hicks, negative. The compound gave a pale yellow colour with tetranitromethane and a negative Zimmermann reaction. The infrared spectrum in CS_2 showed bands at 1785, 1746, 3060, 1638, and 895 cm.$^{-1}$.

Reduction of Hydroxydammarenone I.—The ketol I (150 mg.) was dissolved in ethanol (35 ml.) and sodium (3 g.) added during ½ hr. The product was isolated in the usual way and adsorbed from benzene on alumina (10 × 1·8 cm.). Benzene–ether (19 : 1; 500 ml.) eluted a product which was crystallised from aqueous methanol and then from nitromethane to give *dammarenediol I* as needles (110 mg.), m. p. 142—144°, undepressed on admixture with the sample isolated from the resin, $[\alpha]_D$ +27° (*c* 1·16). The R_F and colour reactions were also identical.

Reduction of Hydroxydammarenone II.—The ketol II (200 mg.) was reduced in ethanol (50 ml.) with sodium (4 g.), and the product isolated and chromatographed as above. Crystallisation from aqueous methanol and then from nitromethane yielded *dammarenediol II* (150 mg)., m. p. 130—132° (undepressed on admixture with the sample isolated from the resin), $[\alpha]_D$ +34° (*c* 1·09).

Attempted Isomerisation of Keto-lactone I.—A stream of dry hydrogen chloride was passed for 3 hr. through a solution of the keto-lactone I (70 mg.) in chloroform (20 ml.). The solvent was removed *in vacuo*. The residue, crystallised from methanol, gave unchanged material (50 mg.), m. p. and mixed m. p. 215—218°.

Lithium Aluminium Hydride Reduction of Methyl Dammarenolate.—Methyl dammarenolate (250 mg.) was refluxed for 3 hr. with lithium aluminium hydride (150 mg.) in dry ether (50 ml.). Water and 2N-sulphuric acid were added and the product was isolated with ether. The material did not crystallise readily and was therefore acetylated overnight with pyridine–acetic anhydride; the product, chromatographed over alumina in benzene and crystallised from methanol, was a *diol monoacetate*, needles (200 mg.), m. p. 124—127°, $[\alpha]_D$ +45° (*c* 0·97) (Found : 78·85; H, 11·3. $C_{32}H_{54}O_3$ requires C, 78·95; H, 11·2%), R_F 0·55. Colour reactions : Noller, mauvish ; Halphen–Hicks, orange-brown to pink.

Perbenzoic Acid Titrations.—Titration in chloroform at 0° gave the results tabulated.

	Acid added (mols.)	Acid consumed (mols.)		
		1 day	2 days	4 days
Dammadienol	5·1	2·13	2·28	2·31
Dammarenediol II monoacetate	4·4	1·07	1·05	1·05
Me dammarenolate	4·4	1·93	2·05	2·03
Me ursonate	4·1	0·05	—	0·0

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