130. *The Chemistry of Triterpenes and Related Compounds. Part XXXVIII.* The Acidic Constituents of Dammar Resin.*

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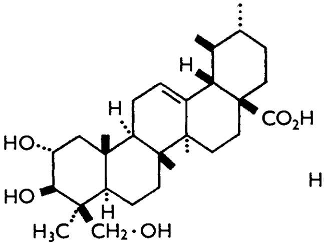
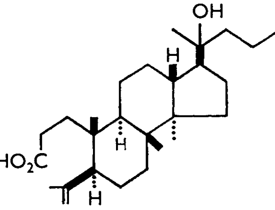
The acidic constituents of dammar resin have been further investigated. The so-called dammarolic acid has been shown to be asiatic acid (I). Two new triterpene acids which do not contain a hydroxyl or keto-group have been isolated as their methyl esters. Some reactions of the known constituent dammarenolic acid are described.

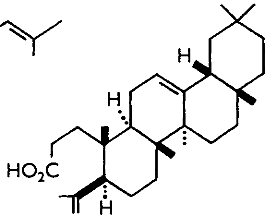
Recently we have been concerned[1] with hydroxyhopanone, a neutral constituent of dammar resin, and have also been interested in the acidic constituents of the resin. Mills and Werner[2] have already investigated the acids in some detail and isolated ursonic, dammarolic, dammarenolic, and dammarenonic acid as their methyl esters. Only the first of these was an acid of known structure. The crude acids from dammar resin have now been further investigated, Mills and Werner's procedure being followed. While the work was in progress the structure of dammarenolic acid was elucidated[3] as (II), and only a few additional experiments on the acid are described later in this paper.



(I)



(II)



(III)

Dammarolic acid, m. p. 325—326°, was first isolated from the resin by Mladenovic and Barkovic[4] who considered it to be a saturated monobasic acid, $C_{30}H_{50}O_6$, with four hydroxyl groups, of which one was primary and one secondary. Mills and Werner,[2] by repeated recrystallisation of the ether-insoluble acidic fraction of the resin, obtained an acid, m. p. *ca.* 315°, which appeared identical with dammarolic acid. It was characterised as its methyl ester, m. p. 222—224°, $[\alpha]_D$ +52°, which gave a yellow colour with tetranitromethane and gave analytical results in agreement with $C_{31}H_{50-52}O_6$.

In our investigation the ether-insoluble portion of the acidic fraction of dammar resin was methylated with diazomethane, and the resulting methyl esters were separated by chromatography on alumina. The following compounds were isolated, in order of elution: methyl ursonate, " compound A " (m. p. 147—149°, $[\alpha]_D$ +48°), methyl dammarenolate, " compound B " (m. p. 234—236°, $[\alpha]_D$ +117°), and methyl dammarolate (m. p. 223—225°, $[\alpha]_D$ +50°).

Methyl dammarolate proved to be difficult to analyse (cf. Mills and Werner[2]), but the analytical data indicated the formula $C_{31}H_{50}O_5$ rather than $C_{31}H_{50-52}O_6$ as previously suggested. Its ultraviolet spectrum showed " end-absorption " typical of a trisubstituted double bond. Boiling ethylene glycol had to be used as the solvent for the hydrolysis of the ester as it was recovered unchanged from boiling methanolic potassium hydroxide.

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* Part XXXVII, *J.*, 1960, 1715.

[1] Dunstan, Fazakerley, Halsall, and Jones, *Croat. Chim. Acta*, 1957, **29**, 173; Fazakerley, Halsall, and Jones, *J.*, 1959, 1877; Baddeley, Halsall, and Jones, *J.*, 1960, 1715.

[2] Mills and Werner, *J.*, 1955, 3132.

[3] Arigoni, Barton, Bernasconi, Djerassi, Mills, and Wolff, *Proc. Chem. Soc.*, 1959, 306; *J.*, 1960, 1900.

[4] Mladenovič and Barkovič, *Monatsh.*, 1940, **73**, 306.

The ester was shown to contain a 1,2-glycol group by its reaction with lead tetra-acetate. From these results and a comparison of the constants of the ester and the corresponding acid with those of methyl asiatate and asiatic acid [5] (I) it appeared likely that the two compounds were identical. This identity was confirmed by a mixed melting point determination between methyl dammarolate and methyl asiatate (kindly supplied by Professor E. Lederer), no depression being observed. The infrared spectra (in "Nujol") of the two samples were identical. The so-called dammarolic acid is therefore asiatic acid.

	Acid		Methyl ester	
	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$
Dammarolic acid	*ca.* 317° (decomp.)	+48°	223—225°	+50°
Asiatic acid [5]	310	+53	225	+52

"Compound A" and "compound B," isolated in low yield, are of interest as there is no spectroscopic evidence in either case for a hydroxyl or six-membered-ring keto-group so characteristic of triterpenes. The absence of these functional groups suggests that a normal triterpene ring A cannot be present in these compounds, and that the parent acids may be transformation products of normal triterpenes with ring A, at least, modified. The infrared spectra of these compounds had no band at 890 cm.$^{-1}$, so ring A cannot have been modified exactly as in dammarenolic acid [3] (II) and nyctanthic acid [6] (III).

"Compound A" gives analytical values indicative of $C_{32}H_{48-50}O_6$ or $C_{33}H_{50-52}O_6$. Its infrared spectrum with a broad band at 1727 cm^{-1} suggests that it is a dimethyl ester. Measurements of the apparent integrated absorption intensity of this band by the method of Jones, Ramsey, Keir, and Dobriner [7] are in agreement with the presence of two carbonyl groups. A strong band at 1140 cm.$^{-1}$ indicates [8] that the remaining two oxygen atoms may be present in ether linkages. The ultraviolet spectrum of "compound A" is very similar to that of methyl ursonate. "Compound A" was recovered unchanged after 24 hr. in 10% methanolic potassium hydroxide.

"Compound B" gives analytical values indicative of $C_{32}H_{50-52}O_6$ and appears to be an unsaturated dimethyl ester lactone. The infrared spectrum (in CCl_4), with bands at 1745, 1427, and 1060 cm.$^{-1}$, agrees [9] with the presence of a δ-lactone grouping. When "compound B" was treated with methanolic potassium hydroxide at room temperature for two days the product isolated after acidification contained no lactone ring. The apparent integrated absorption intensity of the carbonyl bands of the infrared spectrum of "compound B" indicated [7] the presence of three carbonyl groups, accounted for by the δ-lactone group and two methoxycarbonyl groups (band at 1730 cm.$^{-1}$). The ultraviolet absorption spectrum of "compound B" was similar to that of methyl ursonate and methyl asiatate. These results suggest that the parent acids corresponding to "compound A" and "compound B" may be derived from α-amyrin derivatives, ring A having been modified and further oxidation having occurred.

Methyl tetrahydrodammarenolate [3] (IV) was dehydrated and the product ozonised: formaldehyde and isohexanal were isolated as their 2,4-dinitrophenylhydrazones, indicating the side chain (V). The C_8 nature of the side chain was confirmed by the pyrolytic procedure of Cox, High, and Jones.[10] Treatment of the tetrahydro-ester with phenylmagnesium bromide gave a crystalline alcohol (VI) which was dehydrated to a substituted α-phenylstyrene with maximal absorption at 2525 Å (ε 17,400), indicating the chromophore $Ph_2C{=}CHR$ and hence of the grouping $-CH_2{\cdot}CO_2H$ in dammarenolic acid. Compounds

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[5] Boiteau, Buzas, Lederer, and Polonsky, *Nature*, 1949, **163**, 258; Polonsky, *Bull. Soc. chim. France*, 1953, 173; Djerassi, Thomas, Livingston, and Thompson, *J. Amer. Chem. Soc.*, 1957, **79**, 5292.

[6] Whitham, *Proc. Chem. Soc.*, 1959, 271; *J.*, 1960, 2016.

[7] Jones, Ramsey, Keir, and Dobriner, *J. Amer. Chem. Soc.*, 1952, **74**, 80.

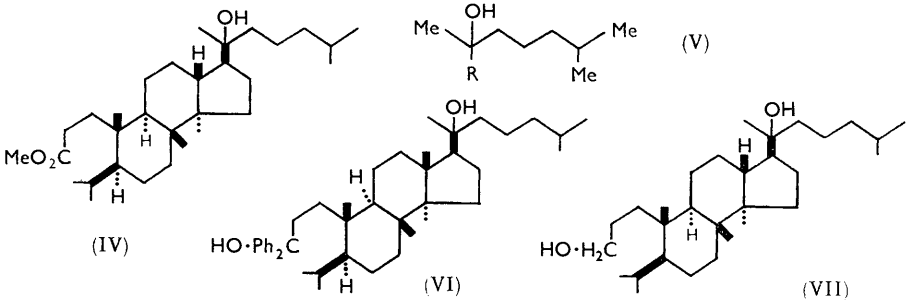
[8] Bellamy, "The Infra-red Spectra of Complex Molecules," Methuen & Co., London, 1958, p. 116.

[9] Jones and Gallagher, *J. Amer. Chem. Soc.*, 1959, **81**, 5242.

[10] Cox, High, and Jones, *Proc. Chem. Soc.*, 1958, 234.

with the grouping $Ph_2C{=}CRR'$ absorb at 2450—2480 (cf. ref. 11) and $Ph_2C{=}CHR$ at 2515—2530 Å (cf. ref. 12). Reduction of methyl tetrahydrodammarenolate (IV) with lithium aluminium hydride gave the corresponding diol (VII), characterised as its monoacetate.

Incidentally, a polymorphic form of dammarenolic acid was obtained.



(IV) (V) (VI) (VII)

EXPERIMENTAL

M. p.s were determined on a Kofler block and are corrected. Rotations were determined for chloroform solutions at room temperature. Light petroleum refers to the fraction with b. p. 60—80°. The alumina used for chromatography was Peter Spence's grade " H." Activity II refers to grade " H " alumina deactivated with 5% of 10% acetic acid, and activity III to grade " H " alumina deactivated with 10% of 10% acetic acid.

Examination of the " Ether-insoluble " Acids from Dammar Resin.—The crude acids (200 g.) obtained from dammar resin were powdered and heated under reflux with ether (2 l.). The resulting milky suspension was centrifuged, and the clear ethereal solution decanted from the gelatinous residue. The residue was dried to give an amorphous pale yellow solid (22·8 g.) which was heated under reflux in ethanol (300 c.c.). The resulting yellow solution was filtered and a portion of the filtrate (250 c.c.) was diluted with ethanol (250 c.c.) and cooled to below 5°. The cooled solution was treated overnight with an excess of ethereal diazomethane, to give a mixture of methyl esters (14 g.) which was adsorbed from benzene on alumina (activity III) (1 kg.). The alumina was eluted with benzene (2 l.), then with benzene–ether, the ether increasing in concentration by increments of 10% for every 500 c.c. of eluant, next ether alone, and finally ether containing 5% of methanol. Fractions of 500 c.c. volume were collected.

Fractions 1—4 were combined to give a pale yellow glass (2·4 g.) which was rechromatographed as described below. Fraction 5 was recrystallised from methanol, to give *compound B* (150 mg.) as spars, m. p. 228—233°, raised by further recrystallisation to 234—236°, $[\alpha]_D$ +117° (*c* 0·93) (Found: C, 72·45; H, 9·15. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5. $C_{32}H_{52}O_6$ requires C, 72·1; H, 9·8%), $\nu_{max.}$ (in CCl_4), 1745, 1730, 1427, 1220, 1181, 1138, and 1060 cm.$^{-1}$, (in CS_2) 818 and 805 cm.$^{-1}$ (in CCl_4; spectrum determined on a " Unicam " S.P. 100 infrared spectrophotometer) 1750 (ε_a 982) and 1727 cm.$^{-1}$ (ε_a 826), $\Delta\nu_{\frac{1}{2}}{}^a$ = 38 cm.$^{-1}$, $B = 8{\cdot}9 \times 10^4$ mole^{-1} l. cm.$^{-2}$.*

Fractions 6—19 yielded small amounts of resins which were not further investigated. Fraction 20 and subsequent fractions were eluted with 5% methanol in ether. Recrystallisations of these fractions from aqueous methanol yielded methyl asiatate (dammarolate) as needles (1·5 g.), m. p. 223—225°, $[\alpha]_D$ +50° (*c* 1·31) (Found: C, 73·0, 72·7; H, 9·8, 9·9; O, 16·75. Calc. for $C_{31}H_{50}O_5,\frac{1}{2}MeOH$: C, 72·9; H, 10·1; O, 17·0%), $\nu_{max.}$ (in " Nujol ") 3448 and 1725 cm.$^{-1}$. A sample recrystallised from benzene was analysed (Found: C, 74·0; H, 10·0. Calc. for $C_{31}H_{50}O_5$: C, 74·05; H, 10·05%). The m. p.s and mixed m. p. of the methyl ester and of authentic methyl asiatate, determined simultaneously in an oil-bath, were all 215—217°. The infrared spectra of the methyl ester and methyl asiatate were identical.

Chromatography of fractions 1—4. These fractions were adsorbed from light petroleum on

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* ε_a = apparent molar extinction coefficient. $\Delta\nu_{\frac{1}{2}}{}^a$ = apparent half band width. $B = \frac{M}{cl}\int_{\nu_{\text{I}}}^{\nu_{\text{II}}} \ln\frac{T_0}{T}\, d\nu$ where M = molecular weight, c = concn. in g./l., and l = cell width in cm.[7]

[11] Cocker and Halsall, *J.*, 1956, 4262; Turnbull, Vasistha, Wilson, and Woodger, *J.*, 1957, 569; Shoppee and Stephenson, *J.*, 1954, 2230.

[12] Cocker and Halsall, *J.*, 1956, 4262; Davy, Jones, and Halsall, *Rec. Trav. chim.*, 1950, 368.

alumina (activity III) (250 g.). The column was eluted with light petroleum (750 c.c.), followed by benzene–light petroleum, the benzene increasing in concentration by increments of 10% every 250 c.c., and finally benzene. Fractions of 250 c.c. were collected. Subfractions 1—7 were negligible. Subfraction 8 (325 mg.) gave methyl ursonate as plates (from ethanol), m. p. 191—194°, $[\alpha]_D$ +85° (*c* 1·23).

Subfraction 9 (440 mg.) crystallised from ethanol, to give "*compound A*" as platelets, m. p. 147—149°, $[\alpha]_D$ +48° (*c* 1·15) (Found: C, 72·85, 73·2; H, 9·65, 9·55. $C_{32}H_{50}O_6$ requires C, 72·4; H, 9·5. $C_{32}H_{48}O_6$ requires C, 72·7; H, 9·15. $C_{33}H_{50}O_6$ requires C, 73·05; H, 9·3. $C_{33}H_{52}O_6$ requires C, 72·75; H, 9·6%), $\nu_{max.}$ (in CS_2) 1727, 1190, and 1140 cm.$^{-1}$, $\nu_{max.}$ (in CCl_4; spectrum determined on a "Unicam" S.P. 100 infrared spectrophotometer) 1727 (ε_a 990), shoulder 1739 (ε_a 635), $\Delta\nu_{\frac{1}{2}}{}^a = 25$ cm.$^{-1}$, $B = 5{\cdot}0 \times 10^4$ mole^{-1} l. cm.$^{-2}$.

Subfraction 10 could not be recrystallised. Subfractions 11—12 (750 mg.) were recrystallised from aqueous methanol, to give methyl dammarenolate as needles, m. p. 88—92°, $[\alpha]_D$ +44° (*c* 1·32). Subfractions 14—15 gave small amounts of "compound B," m. p. 212—218°.

Hydrolysis of Methyl Asiatate (*Methyl Dammarolate*).—The ester (100 mg.) in ethylene glycol (10 c.c.) was heated under reflux for 20 hr. with potassium hydroxide (750 mg.). After acidification with hydrochloric acid and dilution with water, extraction with chloroform yielded a product which was crystallised from acetone to give asiatic acid, m. p. *ca.* 318° (decomp.), $[\alpha]_D$ (in EtOH) +48° (*c* 0·56).

	ε at stated λ			
	2220 Å	2150 Å	2100 Å	2050 Å
Methyl ursonate	1080	2540	4550	6150
Methyl asiatate	600	1730	3080	4050
"Compound A"	810	2130	4080	5800
"Compound B"	725	1960	3710	4910

Action of Lead Tetra-acetate on Methyl Asiatate (*Methyl Dammarolate*).—The ester (4·44 mg.) was treated with a standard solution (2 c.c.) of lead tetra-acetate in acetic acid at 20° for 16 hr. After this time 0·82 mol. of lead tetra-acetate had been consumed.

Hydrolysis of "Compound B."—"Compound B" (28 mg.) was kept at 20° in 10% methanolic potassium hydroxide (15 c.c.) for 48 hr. After acidification and dilution with water, ethereal extraction afforded an amorphous solid, $\nu_{max.}$ 3610, 1727, 1715 (sh), 1433, 1143, and 1109 cm.$^{-1}$.

Dehydration of Methyl Tetrahydrodammarenolate and Ozonolysis of the Products.—Methyl tetrahydrodammarenolate[3] (V) (500 mg.), m. p. 105—106°, $[\alpha]_D$ +29°, was treated in pyridine (10 c.c.) with phosphorus oxychloride and the mixture kept for 4 hr. Dilution with water and extraction with ether afforded an oil (470 mg.), $\nu_{max.}$ (liquid film) 1736, 1650, 1167, and 870 (broad) cm.$^{-1}$, giving a yellow colour with tetranitromethane. The oil in acetic acid (40 c.c.) was treated with 6% ozonised oxygen for 25 min. Zinc dust (500 mg.) and water (50 c.c.) were added and the mixture steam-distilled. The distillate (60 c.c.) was neutralised to phenolphthalein with 40% sodium hydroxide solution, and then 2,4-dinitrophenylhydrazine (300 mg.) in 2N-hydrochloric acid was added. The resulting 2,4-dinitrophenylhydrazones were extracted with benzene and the extract dried ($MgSO_4$) and filtered through alumina (activity II) (150 g.) to remove 2,4-dinitrophenylhydrazine. The filtrate was evaporated and the residue (90 mg.) was chromatographed on bentonite (24 g.)–kieselguhr (6 g.). Elution with benzene afforded isohexanal 2,4-dinitrophenylhydrazone as yellow plates (25%) (from methanol), m. p. and mixed m. p. 96—97°, and formaldehyde 2,4-dinitrophenylhydrazone as yellow needles (20%) (from methanol), m. p. and mixed m. p. 164—166°.

Preparation of a Diphenyl-alcohol (VI) *from Methyl Tetrahydrodammarenolate.*—Methyl tetrahydrodammarenolate (500 mg.) in ether (5 c.c.) was treated with ~1·7M-ethereal phenylmagnesium bromide (3 c.c.). The mixture was heated under reflux for 1·5 hr. and kept for 24 hr. After dilution with ammonium chloride solution the product was isolated with ether. Crystallisation from slightly aqueous methanol or light petroleum gave the *alcohol* (VI) as plates, m. p. 151—153°, $[\alpha]_D$ +24° (*c* 0·94) (Found: C, 83·55; H, 10·45. $C_{42}H_{64}O_2$ requires C, 83·95; H, 10·7%), $\nu_{max.}$ (in CS_2) 3497, 3003, 750, and 698 cm.$^{-1}$, $\lambda_{max.}$ 2590 Å (ε 610).

Dehydration of the Alcohol (VI).—The alcohol (200 mg.) was heated under reflux in acetic acid (5 c.c.) containing acetic anhydride (1 c.c.) for 4 hr. The solvent was removed and the resulting oil was filtered through alumina (activity I), to give an oil (150 mg.), $\nu_{max.}$ 1590, 775, 763, and 702 cm.$^{-1}$, $\lambda_{max.}$ 2525 Å (ε 17,400).

Reduction of Methyl Tetrahydrodammarenolate with Lithium Aluminium Hydride.—The methyl ester (500 mg.) in ether (100 c.c.) was heated under reflux with lithium aluminium hydride (300 mg.) for 3 hr. The excess of hydride was destroyed with ethyl acetate, and the ethereal phase was washed with 2N-sulphuric acid. Evaporation of the ethereal solution afforded a solid which crystallised from 10% aqueous methanol to give the *diol* (VII) as plates, m. p. 125—126°, $[\alpha]_D$ +32° (*c* 1·07) (Found: C, 80·45; H, 12·4. $C_{30}H_{56}O_2$ requires C, 80·3; H, 12·6%), $\nu_{max.}$ (in CS_2) 3534 and 1050 cm.$^{-1}$. Acetic anhydride–pyridine afforded a monoacetate, needles, m. p. 123—126°, $[\alpha]_D$ +33° (*c* 1·36), $\nu_{max.}$ (in CS_2) 3534, 1734, 1232, and 1030 cm.$^{-1}$.

Side-chain Determination[10] *with Methyl Tetrahydrodammarenolate.*—The methyl ester (120 mg.) together with two drops of benzenesulphonyl chloride was heated at 400° for 2 hr. The pyrolysed mixture was distilled at 200° for $\frac{1}{2}$ hr. and the distillate was dissolved in ether and examined by vapour-phase chromatography. It was found to contain 2-methylheptane and 2-methylheptene as its major constituents.

Hydrolysis of Methyl Dammarenolate.—The methyl ester (250 mg.) in 10% ethanolic potassium hydroxide was kept at 20° for 48 hr. After acidification ether-extraction afforded an oil which was chromatographed on silica gel (25 g.). Elution with ether–benzene (1 : 4) gave a fraction which on crystallisation from aqueous methanol or light petroleum gave dammarenolic acid in a new polymorphic *form* as needles, m. p. 95—100°, $[\alpha]_D$ +39° (*c* 1·04) (Found: C, 78·7; H, 11·0. $C_{30}H_{50}O_3$ requires C, 78·55; H, 11·0%), $\nu_{max.}$ (in CS_2) 3500, 3030, 1704, 1016, and 890 cm.$^{-1}$. When the polymorph was dissolved in aqueous methanol, and the solution seeded with a sample of dammarenolic acid, m. p. 135—140°, $[\alpha]_D$ +42° (kindly supplied by Dr. J. S. Mills and Professor C. Djerassi), needles of m. p. 138—141° were obtained. The infrared spectra (CS_2) of the two polymorphs were identical.

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