607. *The Chemistry of Triterpenes and Related Compounds. Part XLIII.* The Constituents of the Bark of* Platanus x hybrida *Brot. and the Structure of Platanic Acid.*

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Betulin, betulinaldehyde and its acetate, sitosterol, betulinic and betulonic acid, platanic acid, and a new diketo-acid, $C_{29}H_{44}O_4$, have been isolated from the bark of the plane tree, *Platanus x hybrida* Brot.

Platanic acid is a nortriterpene and has been identified as 3β-hydroxy-20-oxo-30-norlupan-28-oic acid. The new acid is the corresponding 3-oxo-derivative.

The bark of the London plane tree, *Platanus x hybrida* Brot., is supposed to contain a compound which causes inflammation of the eye and an examination of its constituents was therefore carried out by Dávid [1] in 1946. He isolated a main constituent and named it " platanol." Two years later the bark of the same species was examined by Bruckner *et al.*[2] who found the main constituent to be betulinic acid which seemed to be identical with " platanol " and also with a compound named " platanin " [3] isolated from the bark of *P. orientalis* L. Later betulinic acid was isolated [4] from the wood of *P. vulgaris* Spach. and also shown [5] to be the main constituent of the heartwood and bark of *P. occidentalis* L. from which latter species sitosterol and stearic acid were isolated.

Almost simultaneously with the start of the present investigation Thomas and Müller [6] reported a reinvestigation of the bark of *P. occidentalis*. Besides betulinic acid they isolated as their methyl esters docosanoic acid and a new hydroxy-keto-acid which they named platanic acid. From the neutral fraction β-sitosterol and betulinaldehyde acetate were obtained. The investigation now reported is a re-examination of the bark of *Platanus x hybrida* Brot.

The bark was extracted with methanol, and the extract partly evaporated, whereupon betulinic acid crystallised. The ether-soluble portion of the remaining material was separated into neutral and acidic fractions. From the acidic fraction methyl betulinate, methyl betulonate which previously had not been isolated from a natural source, a new diketo-ester, $C_{30}H_{46}O_4$, and methyl platanate were obtained. Oxidation of methyl platanate with chromic acid gave the new diketo-ester.

From the neutral fraction, betulinaldehyde, its acetate, betulin, and sitosterol were obtained. The acetate of betulinaldehyde melted over a wide range, 173—180°, and repeated recrystallisations from different solvents did not give a sharper melting point. This observation is similar to that reported [6] for the same compound when isolated from

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[1] Dávid, *Magyar Gyógyszerésztud. Társaság Értsitöje*, 1944, **20**, 8; *Chem. Abs.*, 1946, **40**, 7306.
[2] Bruckner, jun., Kovács, and Koczka, *J.*, 1948, 948.
[3] Jaretzky, *Arch. Pharm.*, 1944, **282**, 75.
[4] Pachéco and Mentzer, *Compt. rend.*, 1954, **238**, 1160.
[5] Yagishita and Iseda, *Nippon Nogei-kagaku Kaishi*, 1955, **29**, 964; *Chem. Abs.*, 1958, **52**, 20431.
[6] Thomas and Müller, *Chem. and Ind.*, 1961, 1794.

P. occidentalis, although the compound previously [7] had been reported to melt sharply at 199—200°. The acetate was, however, characterised by its infrared spectrum ($\nu_{max.}$ 1240 cm.$^{-1}$) and by hydrolysis to betulinaldehyde with physical constants identical with those previously recorded.[6,8] Reduction of the aldehyde with lithium aluminium hydride gave betulin.

Thomas and Müller [6] reported that methyl platanate was a hydroxy-keto-ester, $C_{31}H_{50}O_4$, forming a monoacetate. It gave a negative tetranitromethane test, indicative of a saturated pentacyclic structure. After publication of their communication Thomas and Müller obtained through Professor C. Djerassi mass spectra of methyl platanate and its acetate, details of which they kindly communicated to us. The spectra indicated that platanic acid is a C_{29} rather than a C_{30} compound, and agreed with the formula $C_{29}H_{46}O_4$. Methyl platanate exhibits no light absorption in the 2000 Å region and hence has no double bond. This confirms the view that platanic acid is a pentacyclic nortriterpene. Its hydroxy-group must be secondary in view of the oxidation of methyl platanate to a diketo-ester. The differences in molecular rotations between methyl platanate and its acetate ($\Delta[M]_D$ +58°) and between it and the diketo-ester ($\Delta[M]_D$ +148°) are very similar to the corresponding figures for the methyl betulinate series ($\Delta[M]_D$ on acetylation +64°; $\Delta[M]_D$ on oxidation +124°),[9] suggesting that platanic acid has a 3β-hydroxyl group in a normal triterpene ring A.

Methyl platanate, its acetate, and its oxidation product exhibit a characteristic strong infrared band at about 1352 cm.$^{-1}$, indicative of the presence of an acetyl group. Confirmation of this conclusion was provided by a signal at τ 7·84 in the nuclear magnetic resonance (n.m.r.) spectra of these compounds. The intensity of the signal corresponded to one methyl group, and the chemical shift is characteristic [10] of that of a methyl group adjacent to a carbonyl group.

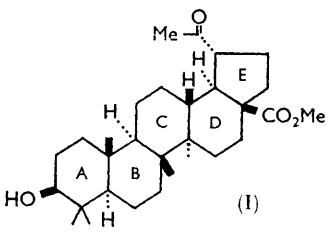
The mass spectrum of methyl platanate also provides evidence for the presence of $CH_3{\cdot}CO$. Peaks at *m/e* 429 (M-43) and *m/e* 43 can be attributed to the cleavage of an acetyl group.

The n.m.r. spectra of methyl platanate and its oxidation product are very similar to those of methyl betulinate and methyl betulonate. Not only do the signals of the various methyl groups (see Table, p. 3273) agree with one another, but also the complete signal patterns due to the ring-protons show striking similarities. The main differences between the two series of compounds are (i) the methyl signal at τ *ca.* 8·34 found with betulinic acid derivatives is shifted to τ 7·84 in the platanic acid series, and (ii) the platanic acid derivatives exhibit no signals corresponding to the characteristic doublet (τ 5·4) due to the side-chain olefinic protons of the betulinic acid derivatives. As it can be shown [11] from both an empirical and a theoretical point of view that a carbon–carbon double bond and a carbon–oxygen double bond show similarities in their diamagnetic anisotropies, the close similarities of the two groups of spectra suggest that methyl platanate is methyl 3β-hydroxy-20-oxo-30-norlupan-28-oate (I).

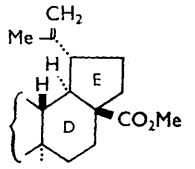
Ruzicka *et al.*[12,13] have prepared the methyl ester of this acid and some of its derivatives although the methods used do not exclude the possibility of admixture with the corresponding 19-epimer. The physical constants reported for the methyl ester (m. p. 253°, $[\alpha]_D$ −45°) and its acetate (m. p. 235°, $[\alpha]_D$ −16°) agree to some extent with those of methyl platanate (m. p. 250—251°, $[\alpha]_D$ −30°) and its acetate (m. p. 204—205°, $[\alpha]_D$ −16°).

[7] Ruzicka and Brenner, *Helv. Chim. Acta*, 1939, **22**, 1523.
[8] Ruzicka and Rey, *Helv. Chim. Acta*, 1941, **24**, 529.
[9] From Simonsen and Ross, " The Terpenes," Cambridge Univ. Press, 1957, Vols. IV and V.
[10] Jackman, " Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 57.
[11] Pople, Paper presented at " The Faraday Society General Discussion on High Resolution Nuclear Magnetic Resonance," Oxford, 1962. *Discuss. Faraday Soc.*, in the press.
[12] Ruzicka and Lamberton, *Helv. Chim. Acta*, 1940, **23**, 1338.
[13] Ruzicka and Rey, *Helv. Chim. Acta*, 1943, **26**, 2143.

In order to establish the identity of methyl platanate, methyl betulinate (II) was ozonised under mild conditions to avoid epimerisation at position 19; the main product (40%) (I) was identical with methyl platanate. Surprisingly, considerable amounts (30%) of the corresponding 3-oxo-derivative were also isolated from the reaction mixture.



(I)



(II)

Experimental

M. p.s were determined on a Kofler block. Rotations were determined for $CHCl_3$ solutions at room temperature. The alumina used for chromatography was of activity (I—II) which had been deactivated with 10% of 10% aqueous acetic acid. Light petroleum refers to the fraction with b. p. 40—60°. The n.m.r. spectra were obtained with *ca.* 10—15% $CHCl_3$ solutions on an " AEI " instrument operating at 60 Mc./sec. with tetramethylsilane as internal standard. The chemical shifts are given in τ units.[14]

Gas–liquid chromatography (g.l.c.) was carried out with a Pye Argon Chromatograph. The packing was 1% silicone-coated material prepared according to Horning's method.[15]

Extraction of the Bark of Platanus x hybrida *Brot.*—The fragmented bark (4·5 kg.) was continuously extracted with methanol for a week. When the extract was concentrated to 3 l. and cooled, a solid was obtained which, after 4 recrystallisations from methanol, afforded betulinic acid (47 g.). Evaporation of the combined mother-liquors gave a resinous solid (145·2 g.) which was leached with cold ether (3 × 2 l.) with vigorous stirring and then with boiling ether (3 × 1 l.) to give an ether-insoluble fraction (55·6 g.) which was not investigated further. The ether solutions were concentrated to 3 l. and extracted with 10% aqueous sodium hydroxide (3 × 0·75 l.). During the first two extractions sodium salts came out of solution and were separated. The ethereal phase afforded a neutral fraction (A) (23·5 g.) which partly crystallised. The aqueous phases were acidified, and then ether-extraction afforded an amorphous acid fraction (B) (8·5 g.). The insoluble sodium salts were suspended in water, and the mixture was acidified and extracted with ether. The extract was filtered through a column of " Florisil " (300 g.) and then evaporated to give crystals (25·7 g.) which were methylated with ethereal diazomethane. The product was recrystallised several times from methanol and then from benzene, to give methyl betulinate (14·9 g.), m. p. and mixed m. p. 222—224°, $[\alpha]_D$ +5° (*c* 0·7). The mother-liquors were evaporated and the residue (10·6 g.) was combined with the methylated acid fraction (B).

Investigation of the Neutral Fraction (A).—Part of this fraction (6 g.) in ether (1 l.) was filtered through a " Florisil " column (20 g.). The evaporated filtrate was made into a slurry in light petroleum (50 c.c.) and adsorbed on alumina (300 g.). Elution with the solvents indicated gave the following fractions: (1—4) Light petroleum (L.P.) (400 c.c.), 0·421 g.; (5—6) L.P.–benzene (1 : 1) (200 c.c.), 1·405 g.; (7—9) L.P.–benzene (1 : 1) (300 c.c.), 0·76 g.; (9—10) benzene (200 c.c.), 0·091 g.; (11—12) benzene (200 c.c.), 0·674 g.; (13—14) benzene (200 c.c.), 0·188 g.; (15—18) benzene–ether (20 : 1) (400 c.c.), 0·656 g.; (19—20) benzene–ether (10 : 1) (200 c.c.), 0·152 g.; (21—24) benzene–ether (10 : 1) (400 c.c.), 0·563 g.; (25) ether (1 l.), 1·062 g. Fractions (1—4) were mixtures of long-chain hydrocarbons, alcohols, and esters.

Fractions (5—6) were crystalline. Recrystallisation from methanol gave 3β-acetoxylup-20(29)-en-28-one (betulinaldehyde acetate [7]), m. p. 173—180° (evacuated tube; decomp.), $[\alpha]_D$ +30° (*c* 1·31), $\nu_{max.}$ (in CCl_4) 2700, 1735 (ε corresponding to 2 C=O), 1640, 1240, and 890 $cm.^{-1}$. This compound decomposed on gas–liquid chromatography. Hydrolysis gave 3β-hydroxylup-20(29)-en-28-one (betulinaldehyde [5,8]) as needles [from light petroleum (b. p. 60—80°), and

[14] Tiers, *J. Phys. Chem.*, 1958, **62**, 1151.
[15] Horning, personal communication.

sublimation], m. p. 190—193° (evacuated tube), $[\alpha]_D$ +19° (*c* 1·06), $\nu_{max.}$ (in CCl_4), 3600, 2700, 1735, 1640, and 890 cm.$^{-1}$. Reduction of the hydroxy-aldehyde (0·10 g.) in dioxan (25 c.c.) with an excess of lithium aluminium hydride gave betulin, m. p. and mixed m. p. 254—256°.

Fractions (7—10) were mixtures. Fractions (11—12) were recrystallised from light petroleum (b. p. 60—80°) and sublimed, to give 3β-hydroxylup-20(29)-en-28-one (betulinaldehyde) as needles, m. p. and mixed m. p. 190—192°. Fractions (13—14) were shown (g.l.c.) to be mixtures of mainly betulinaldehyde (*ca.* 60%) and sitosterol. Fractions (15—18) were crystallised from benzene to give sitosterol, m. p. 137—138°. Fractions (19—20) were mainly sitosterol. Fractions (21—24) were recrystallised from ethanol and sublimed, to give betulin as needles, m. p. and mixed m. p. 254—256·5°.

Investigation of the Acidic Fraction B.—This fraction was filtered in ether through a " Florisil " column (30 g.), and the filtrate treated with ethereal diazomethane. The methylated product was combined with the residue from the mother-liquors from the crystallisation of methyl betulinate (see above). The combined material (13·7 g.) was adsorbed from benzene on alumina (550 g.). The solvents indicated eluted the following fractions: (i) L.P. (800 c.c.), 1·27 g.; (ii) L.P.–benzene (1 : 1) (400 c.c.), 1·38 g.; (iii) L.P.–benzene (1 : 1) (1800 c.c.), 4·92 g.; (iv) benzene (1200 c.c.), 0·87 g.; (v) benzene (2 l.), 1·65 g.; (vi) ether (2 l.) and ethanol–ether (1 : 20) (2 l.), 2·01 g.

Fraction (i) was a waxy mixture of long-chain fatty esters. Fraction (ii) crystallised from methanol, to give methyl 3-oxolup-20(29)-en-28-oate (methyl betulonate) as needles, m. p. 164—165°, $[\alpha]_D$ +31° (*c* 1·03). Fraction (iii) was crystalline and shown (g.l.c.) to consist mainly of methyl betulinate (90%) and a minor component (5%). Recrystallisation from benzene gave needles of methyl betulinate, m. p. and mixed m. p. 224—225°. Repeated chromatography of the product (1·15 g.) from the evaporated mother-liquors afforded further amounts of methyl betulinate and a mixture which on recrystallisation from methanol gave methyl 3,20-dioxo-30-norlupan-28-oate, m. p. and mixed m. p. with a sample prepared from methyl platanate (see below), 160—162°.

Fractions (iv) and (vi) were not investigated in detail. Fraction (v) was shown (g.l.c.) to consist mainly of one component. Recrystallisation from benzene gave methyl platanate as needles, m. p. and mixed m. p. with a sample from *P. occidentalis* kindly supplied by Dr. Müller, 250—251°, $[\alpha]_D$ −30° (*c* 1·05). The methyl group shifts of the n.m.r. spectrum of the compound are given in the Table. Acetylation afforded the acetate as needles (from light petroleum–benzene), m. p. 204—206°, $[\alpha]_D$ −16° (*c* 0·99). Thomas and Müller [6] report, m. p. 206—207°, $[\alpha]_D$ −13·1°.

Lithium Bromide Halolysis [16] *of Methyl Platanate.*—Anhydrous lithium bromide (0·5 g.) and the ester (0·20 g.) in collidine (10 c.c.) were heated under nitrogen under reflux for 8 hr. The resulting acidic product (0·16 g.) was recrystallised from ethanol and sublimed under a high vacuum at 180°, to give *platanic acid* as needles, m. p. 285—287°, $[\alpha]_D$ −51° (*c* 1·00) (Found: C, 72·95; H, 10·05. $C_{29}H_{46}O_4$ requires C, 75·95; H, 10·1%), $\nu_{max.}$ (in Nujol) 3480, 3200, 1710, and 1695 cm.$^{-1}$.

Oxidation of Methyl Platanate.—The ester (0·10 g.) in acetone (25 c.c.) was oxidised with 8N-chromic acid, to give *methyl 3,20-dioxo-30-norlupan-28-oate* as needles (from methanol), m. p. 162—164°, $[\alpha]_D$ +2° (*c* 1·04) (Found: C, 75·9; H, 9·55. $C_{30}H_{46}O_4$ requires C, 76·55; H, 9·85%), $\nu_{max.}$ (in CCl_4) 1728 and 1710 cm.$^{-1}$ (the two bands together have an intensity corresponding to 3 carbonyl groups). The methyl group shifts of the n.m.r. spectrum of the diketo-ester are given in the Table.

Ozonisation of Methyl Betulinate.—The ester (3·30 g.) in ethyl acetate (50 c.c.) and methanol (10 c.c.) was treated with 6% ozonised oxygen at −70° until the solution became slightly bluish. The excess of ozone was removed with nitrogen. Water (100 c.c.) was added and the solvents were removed on the water-bath (1 hr.). The cooled mixture was extracted with ether and afforded a neutral solid (3·21 g.), gas–liquid chromatography of which indicated the presence of three main components. The solid was adsorbed from benzene–light petroleum (1 : 1) (20 c.c.) on alumina (100 g.). The solvents indicated eluted the following fractions: (1—2) L.P.–benzene (1 : 1) (100 c.c.), 0·79 g.; (3—4) L.P.–benzene (1 : 1) (100 c.c.), 0·45 g.; (5—6) L.P.–benzene (1 : 1) (100 c.c.), 0·18 g.; (7—10) benzene (200 c.c.), 0·72 g.

[16] Elsinger, Schreiber, and Eschenmoser, *Helv. Chim. Acta*, 1960, **43**, 113; Taschner and Libesek, *Roczniki Chem.*, 1956, **30**, 323.

Fractions (3—4) were crystallised from methanol to give methyl 3,20-dioxo-30-norlupan-28-oate as needles, m. p. and mixed m. p. 162—164°. Fractions (7—10) were crystallised from benzene, to give methyl 3β-hydroxy-20-oxo-30-norlupan-28-oate (methyl platanate), m. p. and mixed m. p. 250—251°.

Retention Times.—The relative retention times on gas–liquid chromatography of the compounds isolated are as follows and, where the compounds are known, were identical with those of authentic samples: Ester series: methyl betulinate (taken as 1), methyl betulonate (0·90), methyl 3,20-dioxo-30-norlupan-28-oate (1·30), and methyl platanate (1·45). Other neutral compounds: sitosterol (taken as 1), betulinaldehyde (1·70), and betulin (2·23).

Compound	τ values of Me groups					
	C-23, C-24, C-25			C-26	C-27	C-30
Me platanate	9·23	9·19	9·00—9·02	9·11	9·00—9·02	7·84
Me 3,20-dioxo-30-norlupan-28-oate	9·07	8·98	8·93	9·07	8·98	7·83
Me betulinate *	9·23	9·19	9·03	9·07	9·03	8·33
Me betulonate	9·03—9·04	9·00	8·94	9·08	9·03—9·04	8·34

* Interpretation according to Lehn and Ourisson.[17]

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[17] Lehn and Ourisson, *Bull. Soc. chim. France*, 1962, 1137.