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Betulic Acid and Betulin, the Triterpenoid Constituents of *Dillenia Indica* Linn.

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The triterpenic components occurring in the trunk-bark of *Dillenia indica* Linn. have been shown to be betulic acid, $C_{30}H_{48}O_3$, and betulin, $C_{30}H_{50}O_2$, respectively. The ultraviolet, infrared, and high resolution proton magnetic resonance spectra of the acid and its derivatives have been studied besides various degradative experiments. Its identity with betulic acid has been subsequently established from a comparison of the X-ray diffraction pattern of its methyl ester with that of an authentic sample of methyl betulate. The betulic acid isolated from *D. indica* on reduction with LAH produces its congener, betulin, in quantitative yield.

Dillenia indica L. (Fam. *Dilleniaceae*), popularly known as 'Chalta' in Bengal, is a tree native to India. Although its use in different ailments is described in Ayurvedic medicine, the systematic study of its chemistry has not been reported so far. The present communication describes the isolation and properties of its triterpenoid constituents which have been shown to be betulic acid and betulin. A preliminary investigation of the chloroform extract of the trunk-bark revealed the presence of an acidic and a neutral component. The composition of the acid ($C_{30}H_{48}O_3$) and the alcohol ($C_{30}H_{50}O_2$) and the characteristic colour reactions suggested their triterpenoid character.

The triterpenic acid was isolated from its chloroform extract as water-insoluble, white potassium salt which on treatment with mineral acids released the free acid. It failed to crystallise from usual solvents. Attempts to purify this compound by chromatography over deactivated alumina met with failure as the substance was tenaciously retained in the Tswett column. The acid was subsequently purified through its acetate or its methyl ester. Both the derivatives crystallise well from various solvents.

Acetyl compound of the acid crystallised from a mixture of methanol and ether (1:1) in needles, m.p. 282-84° (decomp.) and it was highly soluble in chloroform and ether but sparingly so in methanol or ethanol. It showed peak absorptions in the IR spectrum at 5.75μ, 5.88μ (acetate and carboxy carbonyls), 6.08μ (unsaturation), 7.3μ (-C.Me), 8.1 μ (acetate stretching, Type A band[1]). Acetyl compound smoothly regenerated the original acid on saponification.

Methyl ester of the triterpenic acid, prepared by the action of diazomethane, was purified by chromatography over alumina. The compound crystallised in needles, m.p. 220-22°; peak absorptions in the UV region at 205 mμ (ε, 14,750), indicative of a double bond and in the IR spectrum at 2.72 μ (hydroxyl), 5.82 μ (ester carbonyl), 6.08 μ (unsaturation), 7.3 μ (-C.Me), and 11.2μ (exocyclic methylene group). The ester could not be readily saponified to the free acid. Treatment with 10% methanolic KOH solution for 10 hours at reflux temperature resulted in partial saponification, 90% of the original ester

1. Herling and Dobsinor, *J. Amer. Chem. Soc.*, 1951, 73, 3215.

being recovered unchanged. But saponification with diethylene glycollic KOH for 2 to 3 hours at 150-60° reformed the original acid, m.p. 296-98° (decomp.) in 90% yield. UV absorption: λ_{max} at 205 mμ (ϵ, 391). Light absorption in the infrared at 2.90μ (hydroxyl), 5.90μ (quaternary carboxy carbonyl), 6.08μ (unsaturation), 7.3μ (-C.Me) and 11.2μ (exocyclic methylene group); chemical evidence of the latter was available from ozonolysis. Formaldehyde was produced during ozonolysis and it was characterised as its D. N. P., m.p. 220°.

Methyl ester of the triterpenic acid on reduction with LAH in a mixture of tetrahydrofuran-ether (1:2) furnished a diol, $C_{30}H_{50}O_2$, which crystallised from a mixture of methanol-chloroform (1:1) in shining flakes, m.p. 246-48°. It is free from methoxyl. The diol contains a secondary and a primary alcoholic group.

On hydrogenation in presence of the Adams catalyst, the diol (m.p. 248°) afforded a dihydro derivative, m.p. 280-82°. The infrared spectrum of the reduction product lacks the peak absorption at 11.2 μ which evidently points out the saturation of the exocyclic methylene group during hydrogenation.

The dihydromethyl ester (m.p. 230-32°), obtained from the methyl ester by catalytic hydrogenation, underwent retropinacoline rearrangement with phosphorus pentachloride and gave a product, m.p. 123-25°. On ozonolysis it formed acetone, identified as its D.N. P., m.p. 122°. This evidently points out that in the triterpenic acid of *D. indica*, the ring A had the same configuration as that of lupeol, with which further relation could be established by several degradative studies. The methyl ester on reduction with LAH, followed by chromic acid oxidation and Wolff-Kishner reduction, produced a hydrocarbon $C_{30}H_{50}$, m.p. 163°. It was proved to be identical with α-lupene by following the usual procedure.

From the elemental analysis of the triterpene acid, its ozonolysis, its conversion to α-lupene, and from the studies of the retropinacoline rearrangement of its dihydromethyl ester, the compound appears to be betulic acid. But it was difficult to assess the identity of the triterpene compound with betulic acid from a comparison of their properties because the literature on betulic acid, so far published, is extremely confusing. Different investigators have recorded different physical constants and different properties[2] (Table I). The N. M. R. spectrum of the methyl ester of the triterpenic acid has therefore been examined in deuterochloroform with a trace of trifluoroacetic acid as the solvent, employing a 60 megacycle instrument for studying the nature of the compound from the characteristic chemical shifts (Fig. 1).

The positions of the peaks are shown in ppm (parts per million) referenced relative to the signals from tetramethylsilane as the standard.

From the N. M. R. proton count by electronic integration, the presence of 50 protons in the methyl ester of the acid was established and the spectrum exhibited several characteristic peaks, interpretation of which is given as follows.

2. Kawaguti and Kim, *J. Pharm. Soc. Japan*, 1940, **60**, 343; Barton and Jones, *J. Chem. Soc.*, 1944, 659; White *et al.*, *ibid.*, 1952, 4065; Takemoto and Yahagi, *J. Pharm. Soc. Japan*, 1955, **75**, 765; Stabursvik, *Acta Chim. Scand.*, 1955, **7**, 446; Djerassi *et al.*, *J. Amer. Chem. Soc.*, 1955, **77**, 1200; Ribas *et al.*, *Anales real Soc. espan. Y quim.*, 1957, **53B**, 369; Vystreil and Cermy, *Czech. Chem. Comm.*, 1959, **24**, 3279.

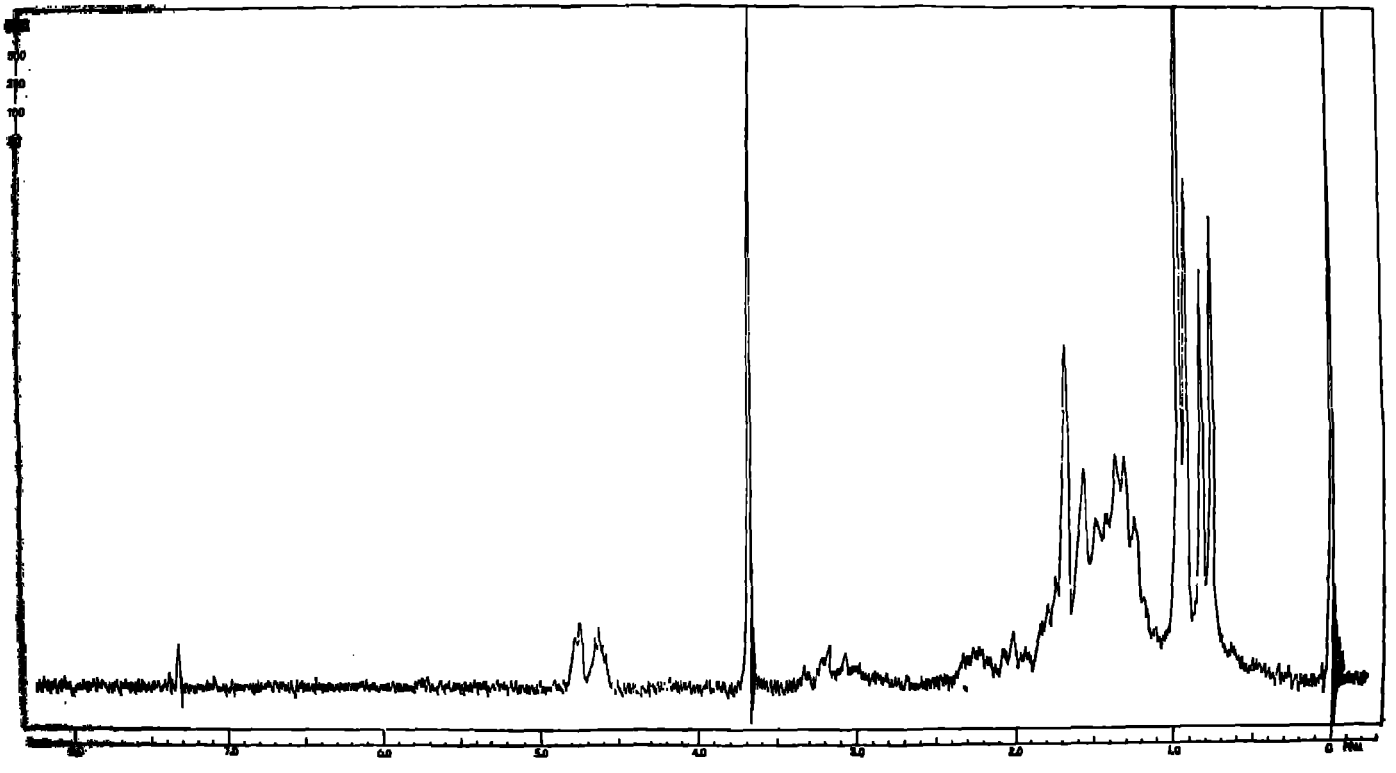
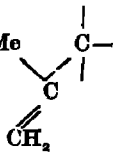


FIG. 1. N.M.R. of methyl betulate.

(*a*) Olefinic proton signals for $=CH_2$:—Two doublets in the region, 4.6—4.8 ppm.
(*b*) An intense signal at 3.75 ppm for -COOMe.
(*c*) A singlet for -CHOH at 2.0 ppm.
(*d*) A sharp peak for Me occurring as



at 1.75 ppm.

(*e*) A tall singlet corresponding to 5 Me groups at 1.0 ppm.

An authentic sample of methyl betulate was, however, available through the courtesy of Dr. R. P. Rastogi, C. D. R. I., Lucknow. X-ray diffraction pattern (Fig. 2) of methyl ester of the triterpenic acid and methyl betulate was the same and superimposable.

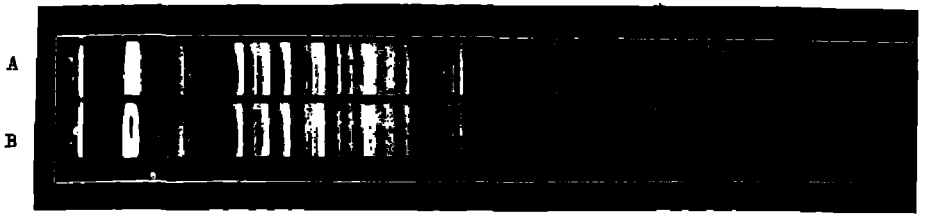
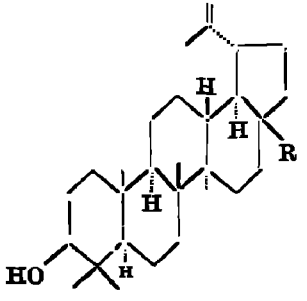


FIG. 2. X-ray diffraction pattern.
A—Methyl ester of triterpenic acid from *D. indica*, L.
B—Methyl betulate.

The triterpenic acid of *D. indica* L. was thus shown to be betulic acid (I), also known as betulinic acid[3].

The triterpene alcohol, m.p. 248°, the congener of betulic acid, was obtained pure and crystalline by chromatography of the chloroform extract of the plant material (over deactivated alumina), pretreated with alkali to remove betulic acid.

The alcohol showed no depression in melting point when admixed with the diol from betulic acid[2]. Their UV and IR absorption spectra were superimposable, thus establishing their identities. The simultaneous occurrence of betulic acid and its alcohol, betulin (I: R=CH_2OH) appears to be interesting from the viewpoint of biogenesis.



(I: R= –COOH or CH_2OH)

3. White, "Reviews of Pure and Applied Chemistry", the Royal Aust. Chem. Inst., 1956, 6, No. 4, 216.

TABLE I

Betulic acid.		Acetylbetulic acid.		Methyl ester.	Methyl ester acetate.	Dihydromethyl ester,	Betulin.		Betulin diacetate,		
M. P.	$[\alpha]_D$.	M.P.	$[\alpha]_D$.	M.P. $[\alpha]_D$	M.P. $[\alpha]_D$	M.P.	M.P.	$[\alpha]_D$.	M.P.	$[\alpha]_D$.	
296-98°(d)	14±2°	282-84°(d),	+20°	220-22° +1.4° (Keto-ester, m.p. 164-66°)	198-200° +45°	230-32°	248°	+21.7°	222-24°	+38.8°	From *D. indica* L.
>300°(d)	..	290° (d)	..	224-25° ..							Authenticsample (from Dr. R. P. Rastogi)
299-303° (for betulinic acid)	..	..	..	221-23° +5°	201-204° +19°	..	251-53°	+13°	..	..	Djerassi *et al.*[2]
315° (for betulinic acid)	..	290-91° ..		222° ..	201-202° ..	239-40° ..	..	..	..	..	Rivas *et al.*[3]
		(Keto-ester, m.p. 167°)		223-25° +4.7°			245-49°	+21.6°	216-17°	+28.7°	Gentiolone identified as betulic acid. Vystrieil *et al.*[2]
320-21°	+12°	295°	+24° ..	224-26° +5°	200-202° +17°	..	261°	+20°	223°	+22°	White[3]

*EXPERIMENTAL

Isolation of Betulic Acid.—Sun-dried powdered trunk-bark (2.0 kg.) of *D. indica* (collected during September to December) was defatted with petroleum ether (b.p. 40-60°) for 24 hours. The defatted bark powder was then soxhletted with chloroform for 48 hours. The yellowish brown chloroform solution (3 litres) was shaken with 1 litre of 5% aqueous KOH solution when an insoluble white potassium salt separated. Free acid was released from the potassium salt and the acid liberated was taken in ether. The ether solution on evaporation yielded crude betulic acid (15 g.). It could not be crystallised from ethanol, chloroform, acetone, or acetic acid. Purification of the acid was accomplished through its methyl ester.

Methyl Betulate.—The crude acid (15 g.) in ether (1.5 litres) and methanol (150ml) was treated with excess of diazomethane (in ether) and kept overnight. The excess of diazomethane was removed with glacial acetic acid and the solution was treated with alkali (10%). The ether solution was processed in the usual way and concentrated when crystalline methyl betulate (13.0 g.) separated. It was filtered, dissolved in chloroform, and chromatographed over deactivated alumina. The column was eluted with a mixture of benzene-ether (4:1). The eluates were collected in 25 fractions (100 ml each.). First fifteen fractions were combined, m.p. 217-21°, evaporated, and crystallised repeatedly from ether-methanol mixture (2:1) to yield shining colorless needles of the methyl ester, m.p. 220-22°, $[\alpha]_D^{28}+1.4$ (5.56% in chloroform). (Found in a sample dried over P_2O_5 : C, 78.91; H, 10.80; OMe, 6.40. $C_{31}H_{50}O_3$ requires C, 79.14; H, 10.63; OMe, 6.59%).

Regeneration of Betulic Acid from the Methyl Ester.—The ester (1.0 g.) was dissolved in diethylene glycol (40 ml) containing KOH (1g). The mixture was heated at 150-60° for 2½ to 3 hours. It was cooled, poured into ice-cooled water (500 ml), and extracted with ether to remove any unchanged ester. The aqueous portion was acidified and the acid obtained was dissolved in ether. The latter on concentration yielded a gelatinous mass which was filtered and dried (0.7 g.). The betulic acid, thus obtained, melted at 296-98° (decomp.); $[\alpha]_D^{28}+14\pm2°$ (15% in pyridine). It did not produce any characteristic coloration with tetranitromethane, but turned pink with the Liebermann-Burchard reagent. (Found in a sample dried over P_2O_5: C, 78.23; H, 10.38; H^+, 0.41; C.Me, 3.8. $C_{30}H_{48}O_3$ requires C, 78.72; H, 10.52; H^+, 0.43; C.Me, 3.3%).

Acetylbetulic Acid.—Betulic acid (0.45 g.) in pyridine (25 ml) was mixed with acetic anhydride (10 ml) and left for 12 hours. It was then poured into ice-cold water. The precipitate was taken up in ether and crystallised from a mixture of ether-methanol (1:1) when colorless needles were obtained. m.p. 282-84° (decomp.); $[\alpha]_D^{28}+20°$ (1.0% in chloroform). It gave no characteristic coloration with tetranitromethane. (Found: C, 76.33; H, 9.50; H^+, 0.23; COMe, 8.96. $C_{32}H_{50}O_4$ requires C, 77.06; H, 10.04; H^+, 0.20; COMe, 8.63%).

Saponification of Acetylbetulic Acid.—The acetyl compound was gently boiled with methanolic KOH (0.5*N*). The reaction product on acidification liberated betulic acid, m.p. 296-98° (decomp.); $[\alpha]_D^{28}+14.6°$ (1% pyndine). Mixed m.p. with an authentic sample of betulic acid remained unchanged, their IR and UV spectra being identical. (Found: C, 78.61; H, 10.32. $C_{30}H_{48}O_3$ requires C, 78.72; H, 10.52%).

*The melting points recorded in this paper are uncorrected. Ultraviolet absorption spectra have been studied in spectral ethanol and optical rotation at 589 mμ.

Acetylmethyl betulate was prepared from methyl betulate, following the same procedure as described above. It was, however, obtained as a gummy mass from the reaction products which on chromatography over alumina and elution with a mixture of ether-benzene (1:1) yielded acetylmethyl betulate. The latter crystallised from ether-methanol mixture (1:1) in needles, m.p. 198-200°; $[\alpha]_D^{25}+45°$ (8.55% in chloroform). (Found : C, 76.97; H, 10.18; OMe, 5.90. $C_{33}H_{52}O_4$ requires C, 77.32; H, 10.15; OMe, 6.04%).

Ketomethyl betulate was prepared from the methyl ester by oxidation with chromic acid (according to the method of Poos *et al.*[4]). The compound crystallised from ether and methanol in rods, m.p. 164-66°. The ester gave positive Zimmermann reaction. (Found : C, 79.04 ; H, 9.65. $C_{31}H_{48}O_3$ requires C, 79.48; H, 10.25%). It yielded an oxime, m.p. 224-26°. (Found: C, 76.84; H, 9.92; N, 2.21. $C_{31}H_{49}O_3N$ requires C, 77.01; H, 10.14; N, 2.09%).

Dihydromethyl Betulate.—To the methyl ester (1g.), dissolved in glacial acetic acid (100 ml), PtO_2 (0.30 g.) was added. The mixture was stirred in an atmosphere of hydrogen when the ester showed an uptake of one mole of hydrogen. The acetic acid solution was filtered from the catalyst, diluted with water, and the precipitate separating was dissolved in chloroform. The latter on concentration and chromatography over deactivated alumina, using a mixture of ether-chloroform (1:1) as the elutes, yielded dihydromethyl betulate in shining crystals (0.90 g.), m.p. 230-32°. (Found: C, 78.72; H, 10.93. $C_{31}H_{52}O_3$ requires C, 78.81; H, 11.1%). Acetylation with pyridine-acetic anhydride mixture at room temperature afforded crystalline acetyldihydromethyl betulate, m.p. 230-32°. (Found: C, 76.86; H, 10.32; COMe, 7.4. $C_{33}H_{54}O_4$ requires C, 77.04; H, 10.50 ; COMe, 7.58%).

Preparation of Betulin from Methyl Betulate.—A solution of the methyl ester (1g.), dissolved in tetrahydrofuran (25 ml), was added dropwise to a mixture of LAH (1g.) in ether (100 ml) and the mixture was refluxed for 3 hours. It was cooled and excess LAH was decomposed by adding a saturated solution (aqueous) of sodium sulphate. The solution was filtered and the residue was repeatedly washed with ether. The ethereal extract of the solution was worked up as usual and on concentration deposited shining flakes. These were recrystallised from methanol-chloroform mixture (1:1) in flakes, m.p. 246-48°, yield 0.68 g.; $[\alpha]^{30}_D+21.7°$ (2.07% in $CHCl_3$). It gave no characteristic coloration with tetrantiromethane. (Found: C, 81.17; H, 11.80; OMe, Nil. $C_{30}H_{50}O_2$ requires C, 81.44; H, 11.30%).

Betulin Diacetate.—Betulin (0.5 g.), dissolved in acetic anhydride (20 ml) containing 15 drops of pyridine, was refluxed for 2 hours. The reaction mixture was kept overnight and the shining crystals separating were filtered and crystallised from a mixture of ether-methanol (1:1) when glistening colorless needles of betulin diacetate (0.35 g.) were obtained; m.p. 218-20°; $[\alpha]^{30}_D+38.8°$ (3.35% in chloroform). (Found: C, 76.98; H, 9.96; COMe, 16.05. $C_{34}H_{54}O_4$ requires C, 77.56; H, 10.27; COMe, 16.35%).

Dihydrobetulin was prepared by following the same method as adopted for the dihydro ester (*vide supra*). It crystallised in needles from a mixture of methanol and chloroform (1:1), m.p. 280-82°. (Found : C, 81.17; H, 12.00. $C_{30}H_{52}O_2$ requires C, 81.10; H, 11.71%). During hydrogenation in glacial acetic acid, the dihydro derivative underwent acetylation. The product was subsequently deacetylated with methanolic alkali. Dihydrobetulin, thus

4. *J. Amer. Chem. Soc.*, 1953, 75, 422.

obtained, crystallised from methanol-chloroform mixture (1:1) in colorless needles m.p. 280-82°.

Isolation of Betulin.—The chloroform extract of the powdered trunk-bark of *D. indica* L, left after removal of betulic acid by alkali treatment, was chromatographed over deactivated alumina. The column was first washed with 150 ml of benzene when a negligible amount of a yellow oily eluate was obtained. The oil developed a bluish green coloration in the Liebermann-Burchard reaction. It could not be further characterised. The column was then eluted with a mixture of benzene-ether (1:1) (200 ml) (fractions of 20 ml were collected). The 6th, 7th, and 8th fractions on evaporation at room temperature furnished needles, m.p. 244-46° (0.06 g.). It crystallised from methanol-chloroform mixture (1:1) in glistening needles, m.p. 246-48° (0.05 g.). The melting point was not depressed when admixed with the diol from methyl betulate and their color reactions (pink with Liebermann-Burchard reagent) and infrared spectra were identical. (Found : C, 81.21; H, 11.10. $C_{30}H_{50}O_2$ requires C, 81.44; H, 11.30%). The earlier fractions of the eluates (2nd, 3rd, and 4th) yielded only an amorphous material, m.p. 170-90°. It produced, greenish blue coloration with the Liebermann-Burchard reagent. The amorphous material on acetylation gave crystalline acetyl derivative, m.p. 130-32°. Further characterisation of this substance was not possible due to paucity of material.

Betulin Diacetate.—On acetylation (*vide supra*), betulin yielded a crystalline diacetate, m.p. 222-24°.

Retropinacoline Rearrangement of Dihydromethyl Betulate.—A mixture of dihydromethyl ester (1g.), dry petroleum ether (25 ml; b.p. 60-80°), and PCl_5 (0.7g.) was kept stirred when a clear solution was obtained in 15 to 20 minutes. Stirring was continued for half an hour and the solution was repeatedly washed with warm water until the petroleum ether layer showed absence of phosphorus compound (molybdate test). The petroleum ether layer was then dried and chromatographed over alumina (40 g.). The column was eluted with the same solvent. The eluates (200 ml) were concentrated and crystallised from a mixture of petroleum ether-methanol (2:1) when colorless needles, m.p. 123-25°, were obtained. It gave a light yellow coloration with tetranitromethane. (Found : C, 81.62; H, 11.29. $C_{31}H_{50}O_2$ requires C, 81.91; H, 11.12%).

Ozonolysis of Retropinacoline Rearrangement Product of Dihydromethyl Betulate.—The retropinacoline rearranged product (0.25 g.) on ozonolysis at 20° in glacial acetic acid yielded acetone which was characterised as its 2,4 dinitrophenylhydrazone, m.p. 122,° undepressed on admixture with 2,4-dinitrophenylhydrazone of acetone.

Ozonolysis of the Methyl Ester.—Methyl betulate on ozonolysis in glacial acetic acid at 20° produced formaldehyde. It was characterised as its 2,4-D.N.P., m.p. 220°.

Oxidation of Betulin by Chromic Acid and Conversion of the Oxidation Product to α-Lupene by Wolff-Kishner Reduction.—To a solution of chromic acid (1g.) in pyridine (35ml) was added a solution of betulin (0.5g.) in the same solvent, (10ml), the temperature of the mixture being kept at 0°. The mixture was kept at room temperature for 2 hours, then diluted with water, and extracted with ether. It was washed with alkali. The ether residue was directly subjected to the Wolff-Kishner reduction. The residue was dissolved in ether-ethanol (1:1) mixture (10 ml). Diethylene glycol (15 ml) and hydrazine hydrate (4 ml) were added to it. The mixture was refluxed for 30 minutes. Powdered KOH (1.5 g.) was then

added to the mixture and the refluxing was continued for another 30 minutes; the condenser was removed and the temperature was allowed to rise to 200°. After refluxing for 2 to 3 hours, the solution was poured into water and extracted with ether, which on evaporation furnished a gummy mass. The latter was dissolved in petroleum ether (b.p. 40-60°) and chromatographed over alumina. On elution with petroleum ether and removal of the solvent, ∝-lupene (0.01 g.) was obtained. It crystallised from petroleum ether when colorless needles (0.008 g.), m.p. 163°, were obtained. The substance showed no depression in m.p. when admixed with an authentic sample of ∝-lupene. (Found: C, 87.52; H, 11.98. $C_{30}H_{50}$ requires C, 87.80; H, 12.19%).

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