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STUDIES ON INDIAN MEDICINAL PLANTS—XVIII.*

THE NON-ALKALOIDAL CONSTITUENTS FROM THE SEEDS OF *ALANGIUM LAMARCKII* THW.†

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Abstract—Betulinic acid, betulinaldehyde, betulin and lupeol, showing a remarkable biogenetic sequence, along with hydroxylactone A of betulinic acid and β-sitosterol have been isolated from the seed kernels of *Alangium lamarckii* Thw. (Alangiaceae). The so-called "sterol" previously reported from other laboratories from the same source is presumably identical with betulinic acid. The hydroxylactone B of the triterpene acid isolated in the neutral part during chromatographic separation is probably an artefact. It has, however, been shown to have equatorial hydroxyl function. The preparation of hitherto unreported 3-desoxy betulonic acid (III), m.p. 270–271°, $[\alpha]_D+20°$, is also reported.

INTRODUCTION

THE isolation of the so-called ipecac alkaloids from the seeds of *Alangium lamarckii* Thw. (syn. *A. salviifolium* (L.f.) Wang. ssp. *hexapetalum* (Lam. Wang) belonging to the family Alangiaceae has already been reported.[1] During the course of their isolation, we have had the occasion to examine the non-alkaloidal constituents of the seeds of the same plant as well.

Two groups of workers[2,3] almost simultaneously reported the isolation of probably the same compound described as a "sterol", alangol, m.p. 296° or alengol, m.p. 302–307° from the seed kernels of *A. lamarckii*. While Bhargava and Dutta[2] put forward an unlikely formula $C_{42}H_{84}O_7$, Lakshminarasimhaiah *et al.*[3] assigned the more rational $C_{30}H_{48-50}O$ composition to it. Both the groups described a monoacetate, m.p. 265°, obtained by the treatment of the compound directly with acetic anhydride[3] or with the same reagent in pyridine.[2] The latter group of workers[3] also reported a diacetate, m.p. 330–334° (decomp.) when acetylation was effected by passing gaseous hydrogen chloride through the glacial acetic acid solution of the compound. Salgar and Merchant[4] also recently claimed apparently the same compound, m.p. 288–289° as a sterol from the fruits of the same

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[1] H. BUDZIKIEWICZ, S. C. PAKRASHI and H. VORBRÜGGEN, *Tetrahedron* **20**, 399 (1964).

[2] P. N. BHARGAVA and S. B. DUTTA, *Proc. Indian Acad. Sci.* **16 A**, 328 (1942).

[3] A. LAKSHMINARASIMHAIAH, B. L. MANJUNATH and B. S. NAGARAJ, *J. Mysore Univ.* **3 B**, 113 (1942).

[4] S. S. SALGAR and J. R. MERCHANT, *Current Sci. (India)* **33**, 744 (1964).

plant. Following the isolation procedure adopted by previous workers,[2,3] we could however obtain, in good yield, a compound, m.p. 306°, $[\alpha]_D$ +6·25°, in the acidic fraction of the hydrolysate of the light petroleum extract of the seed kernels. With the Liebermann–Burchard reagent, it gave a violet colour characteristic of a triterpene rather than a sterol. From the neutral part, a number of hitherto unreported compounds were isolated that form the subject matter of this communication.

RESULTS AND DISCUSSION

The elementary analysis of the triterpene acid corresponded to the molecular formula, $C_{30}H_{48}O_3$ (Mol. wt., 456 by mass spectrometry; direct inlet system). The infra-red spectrum of the compound in nujol showed a strong >C=O peak at 1689 cm^{-1} (5·92 μ) suggestive of the presence of an acid or enolised β-diketone. The bands at 1639 and 883 cm^{-1} (6·1 and 11·3 μ) indicated the presence of a methylene group that was confirmed by the signals at $\delta = 4{\cdot}52$ and 4·70 in the NMR spectrum of the compound which also exhibited peaks at $\delta = 1{\cdot}57$ for the methyl of an isopropylene group and at $\delta = 11{\cdot}4$ for an acidic low-field proton.

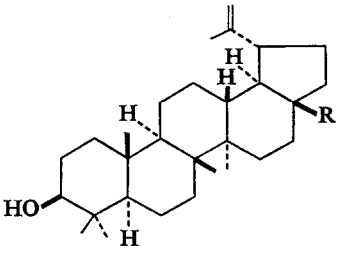
The acetylation of the compound proved to be interesting. With acetic anhydride in pyridine at 100° it afforded a monoacetate, $C_{32}H_{50}O_4$, m.p. 291–292°, $[\alpha]_D$ +25°, and a stable mixed anhydride, $C_{34}H_{52}O_5$, m.p. 204°. The formation of the latter is characteristic of a sterically hindered quaternary carboxyl group in some triterpene acids, also corroborated by the typical anhydride bands at 1792 and 1701 cm^{-1} (5·53 and 5·88 μ) in the i.r. spectrum and its cleavage by methanol under reflux for an hour. The loss of 42 mass units corresponding to an expulsion of a ketene in its mass spectrum would also be satisfactorily explained on the basis of the anhydride formation. Thus all the oxygen atoms in the compound are accounted for. When, however, the acetylation was carried out in glacial acetic acid:sulphuric acid :water (50:1:1) at room temperature, in addition to the expected monoacetate, another compound, m.p. >330°, was obtained in fair yield (30 per cent). The latter exhibited bands at 1754 cm^{-1} (5·7 μ) corresponding to a lactone >C=O, 1724 and 1250 cm^{-1} (5·8 and 8 μ) for an acetate and the band for OH was absent. It was therefore inferred to be an acetyl lactone. The diacetate of alengol reported by earlier workers[3] is also believed to be an acetyl lactone. The suspected identity of alangol (=alengol) with betulinic acid (I)[5] was finally proved by the direct comparison of the methyl ester and the ester acetate of the respective compounds. Further confirmation of this identity was secured through the oxidation of the compound with Jones' reagent,[6] the physical constants of the resulting ketone were in good agreement with those of betulonic acid (II).[7] The keto-acid on Wolff–Kishner reduction yielded the hitherto unreported *3-desoxy betulonic acid* (III), m.p. 270–271°, $[\alpha]_D$ +20°, although the methyl ester is already known.[7]

Robertson *et al.*[8] observed that betulinic acid on treatment with hydrogen bromide in acetic acid, or betulinic acid acetate when heated with formic acid, affords an acetyl lactone that on hydrolysis gives a hydroxylactone A, $C_{32}H_{50}O_4$, m.p. >320°, $[\alpha]_D$ +75·2°. On the other hand, betulinic acid or its methyl ester when treated with formic acid furnishes a formate lactone which when hydrolysed yields a different compound, hydroxylactone B, m.p. >330°, $[\alpha]_D$ +59·05°. The mechanism of the lactone formation in presence of acid has been explained by Davy *et al.*[9] Both the hydroxylactones, however, gave the same ketolactone, $C_{30}H_{46}O_3$,

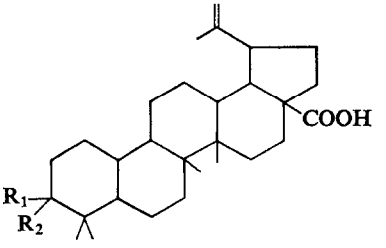
[5] J. SIMONSEN and W. C. J. ROSS, *The Terpenes*, Vol. 5, p. 316, Cambridge University Press, Cambridge (1957).
[6] R. G. CURTIS, I. HEILBRON, E. R. H. JONES and G. F. WOODS, *J. Chem. Soc.* 461 (1953).
[7] J. SIMONSEN and W. C. J. ROSS, *The Terpenes* Vol. 4, p. 297, Cambridge University Press, Cambridge (1957).
[8] A. ROBERTSON, G. SOLIMAN and E. C. OWEN, *J. Chem. Soc.* 1267 (1939).
[9] G. S. DAVY, T. G. HALSALL and E. R. H. JONES, *Chem. & Ind.* (London) 233 (1951).

m.p. 337–338°, $[\alpha]_D$ +88°, on chromic acid oxidation. This showed that the hydroxylactones are C_3 epimers but the orientation of the OH group in them was not elucidated.

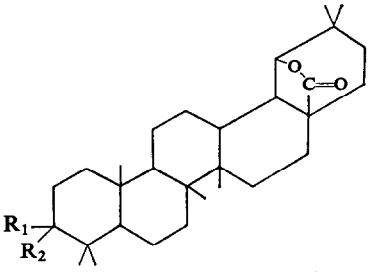
The acetyl lactone (IV) obtained in the present investigation, on saponification furnished a hydroxylactone (V), m.p. >320°, $[\alpha]_D$ +57°. The same compound was also obtained by sodium borohydride or sodium-alcohol reduction of the keto-lactone (VI) prepared from betulonic acid on treatment with glacial acetic acid in aqueous sulphuric acid. This hydroxylactone was proved to be identical in all respects with the hydroxylactone B prepared according to the recorded procedure.[8] Therefore, the OH group at C_3 centre in the latter must be equatorial and that in hydroxylactone A, axial.[10]



I, R=COOH, Betulinic acid
VII, R=CH_2OH, Betulin
VIII, R=CHO, Betulinaldehyde
IX, R=CH_3, Lupeol



II, $R_1=R_2=O$, Betulonic acid
III, $R_1=R_2=H$, 3-desoxy betulonic acid



IV, $R_1=H$, $R_2=CH_3COO$, Acetyl lactone
V, $R_1=H$, $R_2=OH$, Hydroxylactone
VI, $R_1=R_2=O$, Ketolactone

In view of the reported[11] co-occurrence of betulinic acid with the isomeric oleanolic acid in many cases and the difficulty in their separation we made a deliberate search for the latter in the acidic fraction following the method of Djerassi and Lippman.[12] Its presence could not, however, be detected.

In the neutral fraction, we encountered β-sitosterol, betulin (VII), m.p. 254–256°, $[\alpha]_D$ +21° and another compound, m.p. 190°, $[\alpha]_D$ +19° which formed a precipitate with 2,4-DNP. It gave a monoacetate, m.p. 199–200°, $[\alpha]_D$ +28°. The melting point of the acetate was exactly the same as that reported by Ruzicka and Brenner[13] for betulinaldehyde

[10] S. C. Pakrashi and T. B. Samanta, *Proc. 54th Indian Sci. Congr.* (pt. III), 170 (1967).
[11] C. Djerassi, L. H. Liu, E. Farkas, A. E. Lippman, A. J. Lemin, L. E. Geller, R. M. McDonald and B. J. Taylor, *J. Am. Chem. Soc.* **77**, 1200 (1955), footnote No. 8.
[12] C. Djerassi and A. E. Lippman, *J. Am. Chem. Soc.* **76**, 5780 (1954).
[13] L. Ruzicka and M. Brenner, *Helv. Chim. Acta.* **22**, 1523 (1939).

(VIII).[14] Although Aplin *et al.*[15] recently reported much lower range of melting point (173–180°) for the same acetate, the i.r. spectrum and other characteristics together with co-occurrence of betulinic acid and betulin left little doubt as to the identity of our compound with betulinaldehyde. This aldehyde has so far been isolated from only a very few plant sources, viz. the bark of the plane tree, *Platanus occidentalis* L.[16] and *P. x hybrida* Brot.[15] An authentic specimen was not available and the identity could not be rigorously established chemically due to paucity of material.

Finally, chromatographic fractionation of the neutral part separated a high melting compound, the i.r. spectrum of which and that of its acetate were superimposable on those of hydroxylactone B and its acetate mentioned above. Most probably the compound was an artefact which might have been formed by the influence of acid used in the hydrolysis process adopted in the separation of betulinic acid. To ascertain this, the mother liquor of the original light petroleum extract (without acid hydrolysis) after the separation of betulinic acid was subjected to TLC over silica gel G using benzene:ether (6:4) solvent system. Hydroxylactone B could not be found, but, besides the other compounds already mentioned, hydroxylactone A and lupeol could easily be identified. Only one spot (R_f ·836) remains yet to be identified.

Evidently, betulinic acid occurs in the free form. The isolation of long sought for lupeol completes the biogenetic sequence: betulinic acid ⇌ betulinaldehyde ⇌ betulin ⇌ lupeol, rarely encountered in a plant source. Again, the presence of hydroxylactone A in the original extract and of hydroxylactone B instead in the neutral part after acid hydrolysis points to the possibility of epimerization at the C_3-centre either during acid treatment or chromatography over alumina.

Incidentally, along with betulinic acid and other products, Djerassi and Lippman[12] isolated a lactone (i.r. band at 5·66 μ), m.p. 331–334°, $[\alpha]_D$ +57·7°, in trace amounts from the Cactus species, *Lemaireocereus hystrix*. They presumed it to be an isomer of thurberogenin.[17] From the mode of isolation, the physical constants and in the context of our present observation, it appears likely that the compound in fact could be hydroxylactone B of betulinic acid also obtained as an artefact.

EXPERIMENTAL

Air-dried and milled seed kernels of *Alangium lamarckii* (2·25 kg) were successively extracted with light petroleum, C_6H_6 and $CHCl_3$ in a Soxhlet for 20 hr each. The residue obtained from light petroleum extract was refluxed in methanolic (250 ml) HCl (100 ml, conc.) for 4 hr, CH_3OH distilled off under reduced pressure and the residue poured on to iced water. The precipitated brown mass was filtered, washed and dried. The dry material (20 g) was dissolved in $CHCl_3$ and extracted with 3 per cent KOH. The precipitated K-salt was filtered, washed free from alkali and dried (Fraction A, 3·5 g). The organic layer containing the neutral components was washed, dried and distilled (Fraction B, 16 g).

Treatment of Fraction A: Isolation of Betulinic Acid (I)

Fraction A was suspended in 2 N HCl and extracted with ether. The ethereal extract was washed, dried and concentrated. The residue (2·0 g) was charcoalized in MeOH–$CHCl_3$ and on repeated crystallizations from MeOH yielded fine needles of betulinic acid, m.p. 305–306°, $[\alpha]_D$ +6·25° (c=0·37 in abs. EtOH.); Lit.,[5] m.p. 316–318°, $[\alpha]_D$ = +7·8° (Found: C, 78·04; H, 10·36; Calc. for $C_{30}H_{48}O_3$: C, 78·89; H, 10·59 per cent). The C_6H_6 and $CHCl_3$ extracts on similar treatment as in the case of light petroleum extract gave only betulinic acid in 2·8 g and 2·2 g respective yields, total yield of betulinic acid being 0·43 per cent.

[14] L. RUZICKA and E. REY, *Helv. Chim. Acta.* **24**, 529 (1941).
[15] R. T. APLIN, T. G. HALSALL and T. NORIN, *J. Chem. Soc.* 3269 (1963).
[16] A. F. THOMAS and J. M. MULLER, *Chem. & Ind. (London)* 1794 (1961).
[17] C. DJERASSI, L. E. GELLER and A. J. LEMIN, *J. Am. Chem. Soc.* **75**, 2254 (1953); C. DJERASSI, E. FARKAS, L. H. LIU and G. H. THOMAS, *ibid.* **77**, 5330 (1955) for structure.

Sodium-Alcohol Reduction of VI

To a mixture of ketolactone (0·1 g) and abs. EtOH (20 ml), Na (1·4 g) was gradually added and then refluxed till all the metal dissolved. It was cooled, poured on to iced water, extracted with $CHCl_3$, washed with water, dried over Na_2SO_4, solvent distilled and the residue chromatographed over neutral alumina (Brockmann, Activity I). The material eluted with 50–90 per cent benzene in light petroleum and crystallized from C_6H_6 in needles in 64 per cent yield was proved to be identical with hydroxylactone B of betulinic acid by TLC on silica gel G using C_6H_6:$CHCl_3$ (3:1) as the solvent system, also confirmed by i.r. comparison. A trace material was eluted in later part of chromatography in 30–60 per cent $CHCl_3$ in C_6H_6. The i.r. spectrum of the compound, presumably a triol, showed complete absence of $>C=O$ band in the 6 μ region. It was directly converted to acetate which awaits further characterization.

Wolff-Kishner Reduction of Betulonic Acid to III

Crude betulonic acid (0·25 g) was refluxed for 6 hr under purified N_2 in diethylene glycol (35 ml) with 95 per cent hydrazine (5 ml) and *n*-butanol (5 ml), cooled to 100° and KOH (0·9 g) was added cautiously. The reaction mixture was then distilled during 1 hr until the temp. inside the flask reached 222° and kept at this temp. for 11 hr. After cooling and diluting with water (100 ml), the mixture was extracted with ether (25 ml × 4), the combined ether phase was washed with water, dried ($MgSO_4$) and evaporated. The colourless residue (0·2 g) did not show any starting ketone on TLC (C_6H_6:EtOAc, 5:1). Recrystallization from acetonitrile (80 ml) gave crystals (0·19 g), m.p. 264–270°. A further recrystallization gave the analytical sample of 3-desoxybetulonic acid, m.p. 270–271°, $[\alpha]_D+20°$ (c=0·7). (Found: C, 81·42; H, 10·80. $C_{30}H_{48}O_2$ requires: C, 81·76; H, 10·98 per cent).

Isolation of Betulinaldehyde (VIII)

The neutral fraction B (16 g) was chromatographed over a column of acid washed alumina (100 g) light petroleum (1 l.) eluate gave heavy viscous oil (12 g) subsequent elution with C_6H_6 (10 per cent) in pet. ether (500 ml) yielded an oily material (0·62 g) which was converted to acetate by heating with Ac_2O (10 ml) and pyridine (5 ml) in the usual way. The crude acetate was sublimed *in vacuo* and the sublimate (0·2 g) crystallized from C_6H_6-acetone in white flakes, m.p. 199–200°, $[\alpha]_D+28°$, λ_{max}^{nujol}, 5·76, 6·1, 8·06 and 11·3 μ.

The pure acetate was hydrolyzed with 5 per cent alc. KOH for 1 hr and the product obtained after usual work up on vacuum sublimation and crystallization from C_6H_6-acetone afforded white flakes, m.p. 190°; $[\alpha]_D+19°$; λ_{max}^{nujol} 2·9, 5·88, 6·1 and 11·3 μ. It gave a precipitate with 2:4-DNP reagent.

Isolation of β-Sitosterol, Hydroxylactone B (V) *and Betulin* (VII)

Fractions eluted with 30 per cent C_6H_6 in light petroleum (1 l.) yielded a white solid (0·55 g), m.p. 136–137°, $[\alpha]_D-35°$; benzoate, m.p. 145° $[\alpha]_D-16°$. The compound was identified as β-sitosterol by direct comparison with authentic specimens.

The original chromatography was continued with C_6H_6-light petroleum (1:1). The eluted substance (0·93 g) when crystallized several times from $CHCl_3$–MeOH proved to be the hydroxylactone B of betulinic acid by i.r. comparison.

Finally, C_6H_6 and C_6H_6–$CHCl_3$ (500 ml) eluates (0·9 g) when crystallized from C_6H_6–MeOH several times afforded betulin, m.p. 254–256°, $[\alpha]_D+21°$; diacetate, m.p. 224–227°; dibenzoate, m.p. 178–179°, $[\alpha]_D+37°$.

Detection of Hydroxylactone A of Betulinic Acid and Lupeol (IX)

Air-dried and milled seed kernels of *A. lamarckii* (2 kg) were extracted with light petroleum (b.p. 60–80°). The extract was concentrated, cooled, filtered, the residue washed with light petroleum and then with a few drops of methanol. The solid mass (2·5 g) was charcoalized thrice and repeatedly crystallized from MeOH furnished betulinic acid (0·5 g). The TLC analysis of mother-liquor on silica gel G using ether:benzene (4:6) as solvent system showed the presence of lupeol, hydroxylactone A of betulinic acid along with betulin, betulinaldehyde, β-sitosterol and betulinic acid.

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Methyl Betulinate

Crude betulinic acid (0·23 g) was suspended in ether (50 ml) and stirred with a magnetic stirrer for 20 hr in the dark at room temp. with an excess of ethereal CH_2N_2. After destroying the excess of CH_2N_2 with HOAC the mixture was filtered from some sticky, colourless material and the filtrate evaporated. The slightly yellowish crystalline residue (0·32 g) was dissolved in CH_2Cl_2 and filtered over 20 g neutral alumina (A II). The eluate (100 ml) was evaporated and recrystallized from MeOH-CH_2Cl_2 to furnish pure methyl ester, m.p. 221–222° that remained undepressed on admixture with an authentic sample of methyl betulinate. The i.r. spectrum in $CHCl_3$ was superimposable.

Methylester Acetate of Betulinic Acid

Prepared in the usual way from methyl betulinate (0·08 g) with Ac_2O and pyridine and crystallized from CH_2Cl_2-MeOH, m.p. 193–195°. The crystals (0·06 g) were dissolved in C_6H_{12}-C_6H_6 (1:1) and filtered over a column of neutral alumina (12 g). The first eluant (100 ml) on evaporation and crystallization from CH_2Cl_2-MeOH gave pure acetate, m.p. 201–202°, that remained undepressed on admixture with an authentic methyl acetyl betulinate.

Preparation of Acetate and Acetate mixed Anhydride of Betulinic Acid

Crude betulinic acid (0·27 g) was heated with Ac_2O (10 ml) and pyridine (5 ml) for 1·5 hr on a steam-bath, the solvents removed at 8·1 mm with repeated addition of toluene. The residue on recrystallization from acetone-MeOH furnished strong needles of the acetate mixed anhydride, m.p. 204°, $[\alpha]_D$+11·1° (c=1·17). (Found: C, 75·82; H, 9·78, Calc. for $C_{34}H_{52}O_5$: C, 75·51; H, 9·69 per cent). The mother liquors afforded on crystallization pure monoacetate as plates, m.p. 291–292°, $[\alpha]_D$+25° (c=0·81). (Found: C, 77·15; H, 9·68, Calc. for $C_{32}H_{50}O_4$: C, 77·06; H, 10·11 per cent).

In a later preparation, the crude acetate (1·4 g) after evaporation of the solvents was refluxed with MeOH (200 ml) for 1 hr to destroy the anhydride, pure monoacetate was obtained immediately on recrystallization.

Lactonization of Betulinic Acid

Betulinic acid (1 g) was added to 50 ml of a mixture of glacial HOAc–H_2SO_4–H_2O (50:1:1) and kept at room temp. for 7 days. The resulting mass was poured on to ice water and the precipitate obtained was washed and dried. The product (1 g) upon crystallization from $CHCl_3$–MeOH provided shining crystals (0·35 g) of lactone acetate, m.p. >330°, λ_{max}^{nujol}, 5·60, 5·70, 8·0, 8·65, 8·93, 9·35, 9·80, 10·32, 10·5 and 10·8 μ. The crystals were dissolved in C_6H_6 (50 ml) and refluxed with 5 per cent alc. KOH (50 ml) for ½ hr on a water-bath. The resultant product was worked up as usual and upon crystallization from $CHCl_3$ gave fine white needles (0·30 g), m.p. >330°, $[\alpha]_D$+57° (c=0·1); λ_{max}^{nujol} 2·9, 5·7, 7·1, 7·6, 8·7, 8·95, 9·4, 9·6, 9·75, 9·95, 10·35, 10·55, 10·85 and 11·0 μ which proved to be the hydroxylactone B of betulinic acid by direct comparison with a sample prepared according to Robertson *et al.*[8] through formate lactone.

Betulonic Acid (II)

Crude betulinic acid (0·68 g) was dissolved in acetone (400 ml), cooled in ice (flask temp. 7–8°) and an excess of Jones' reagent[6] added during 10 min under vigorous magnetic stirring which was continued for further 15 min. The excess oxidant was destroyed by isopropanol, finely powdered $NaHCO_3$ was added and mixture stirred for 5 min filtered over anh. $MgSO_4$. Evaporation of the filtrate gave colourless crystals (0·66 g), m.p. 246–247°, which on TLC (C_6H_6 : EtOAc; 3:1) did not show any trace of starting material. Recrystallization from acetone-cyclohexane and acetonitrile gave pure betulonic acid, m.p. 261–264°, $[\alpha]_D$+37·4° (c=0·83), O.R.D. $[\alpha]_{589}$=+25°, $[\alpha]_{314}$=+543°, $[\alpha]_{268}$=−61° Lit.[7] m.p. 252°, $[\alpha]_D$+31° (Found: C, 79·00, H, 10·12, Calc. for $C_{30}H_{46}O_3$: C, 79·24; H, 10·20 per cent).

Lactone of Betulonic Acid (VI)

Betulonic acid (0·24 g) was lactonized in exactly the same way described above and the crude substance (0·22 g) was crystallized several times from $CHCl_3$–MeOH to give white needles, m.p. 315°.

Sodium Borohydride Reduction of VI

To a cooled solution of ketolactone (0·23 g) in MeOH (250 ml) $NaBH_4$ (1·2 g) was gradually added with stirring. The mixture was left overnight at room temp. and was then refluxed for ½ hr on a steam bath, cooled, poured on to iced water, Conc. HCl was added dropwise until slightly acidic and again kept on a steam bath for 1 hr. The white substance was then extracted with $CHCl_3$. The organic layer was washed, dried and concentrated. The residue (0·2 g) was crystallized from $CHCl_3$–MeOH in white needles, m.p. 320°, $[\alpha]_D$+56°. The i.r. spectrum was superimposable on that of hydroxylactone B.