STUDIES ON DELPHINIUM DENUDATUM WALL

Examination of the Petroleum Ether Extractive of the Roots

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In addition to β-Sitosterol and its glucoside, an unsaturated hydrocarbon, $C_{32}H_{58}$, b.p. 200°/0.1 mm. n_D^{34} 1.4800 with no rotation and a monohydric unsaturated alcohol, $C_{16}H_{30}O$. b.p. 156-60°/0.1 mm., n_D^{34} 1.4646, with rotation nil, have been isolated from the petroleum ether extractive of the roots of *Delphinium denudatum* Wall.

Delphinium denudatum Wall (N.O-Ranunculaceae) commonly known as Jadwar, is an annual generally occurring in the Panjab and the Western temperate Himalayan regions.[1] Its root is bitter and is said to act as a stimulant, alterative and tonic.[2] It is reported to be used in tooth ache.[3] As no previous chemical investigation of the plant had been reported the work was undertaken at these laboratories with the object of isolating and examining the alkaloids present. During the course of these studies, isolation of two alkaloids named denudatine and denudatidine from the roots were reported by two Indian authors.[4]

In the present work, the roots of the plant were obtained from the local market, crushed and first extracted with petroleum ether to remove the fatty matter as a preliminary to extraction of the bases with ethanol. On evaporation of the petroleum ether solution, an oily mass was obtained. This paper deals with the examination of this oil.

The oil was first saponified by the usual process and the unsaponifiable matter separated. It was a waxy solid, m.p. 110-114°C. which was crystallised from ethanol (95%) to give the crude sterol, m.p. 126-27°C. The above processes of separation and the subsequent ones are schematically represented in the Chart. The mother liquor (I) on evaporation gave a brown liquid which was separated by chromatography to give the unsaturated alcohol provisionally formulated as $C_{16}H_{30}O$.

The crystalline matter, m.p. 126-27°C. on recrystallisation from ethyl acetate gave sitosterol-β-D-glucoside and the residue from the mother liquor (II) so obtained was crystallised from ethanol to give crystals m.p. 133-34°C. which were separated by chromatography to give β-sitosterol. The mother liquor obtained (III) has given the unsaturated hydrocarbon which has been given the provisional formula, $C_{32}H_{58}$.

Both the hydrocarbon and the alcohol were separated by chromatographic processes and their purity checked by thin layer chromatography using two different solvents for development.

The unsaturation in both these compounds was indicated by their U.V. and I.R. spectra and confirmed by the bromine and permanganate tests.

Owing to shortage of material for further examinations of the hydrocarbon and the alcohol, further work towards the characterisation of these compounds could not be continued.

Experimental

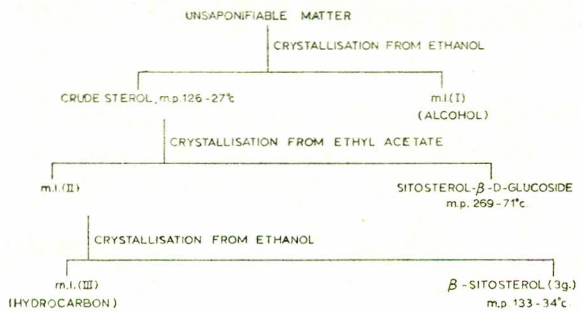
All melting points, unless otherwise mentioned, are taken on the metal block and are uncorrected. All microanalyses, besides the molecular weight determinations, have been carried out by A. Bernhardt, Microanalytical Laboratorium im Max-Planck-Institut fur Kohlenforschung, Mulheim (Ruhr), West Germany.

Extract of the Roots.—Air dried roots (18 kg.) were crushed into small pieces, dried for one day in the air and then exhaustively extracted by steeping in petroleum ether (60-80°C.) at room temperature (30°C.). On removal of the solvent, a brown oily mass (92 g.) was obtained.

Isolation of Sitosterol-β-D-Glucoside.—The above oil (82 g.) was saponified with N/2 alcoholic potassium hydroxide for one hour and after usual processing (reference British Pharmacopeia) the unsaponifiable matter (13.13 g., 16 percent of the extractive), m.p. 110-14°C., was separated. On crystallisation from ethanol, crystals, m.p. 126-127°C. were obtained which on recystallisation from ethyl acetate yielded a small quantity of a colourless crystalline substance melting at 269-271°C. It was almost insoluble in all the common organic solvents except in dimethylformamide and pyridine. After two successive recrystallisations from dimethylformamide and washing with chloroform a colourless sample, m.p. 293-294°C.

(Kofler), was obtained. The mixed m.p. with an authentic sample of sitosterol-β-D-glucoside was undepressed. The infra-red spectrum was quite identical with that of the authentic sample.

CHART



Isolation of β-Sitosterol.—From the mother liquor (II) of the sitosterol-β-D-glucoside the solvent was removed and the residue purified by four crystallisations from ethanol. The final crystallisate (3 g.) m p. 133-34°C. was subjected to further purification by adsorption analysis on a column (42×2.5 cm.) of Alumina Woelm almost neutral (Activity grade I) (100 g.). Elutions were done with mixtures of chloroform and petroleum ether, progressively increasing the quantities of the former (0.5:99.5; 10:90 and 12:88). Fractions of 50 ml. were collected throughout. The residues f om these fractions after preliminary examinations were divided into three groups: group I m.p. 44-45°C. II and III 134-36°C., 139.5-140°C., (Kofler block) respectively. The latter two were combined and recrystallised thrice from ethanol when a colourless crystalline compound, m.p. 137-38°C., $[\alpha]_D^{27.5}$-36° was obtained. The homogeniety of the sterol was checked by thin layer chromatography (Fig. 1) on silica gel G (E. Merck) developing with two agents ethanol (95%) and hexane: ethyl acetate (85:15) and detecting the spots with iodine vapours.[5] (Lit., m.p. 136-37°C;[6] $[\alpha]_D^{24}$-36.7° (in chloroform). The compound gave positive Liebermann-Burchard test.

Acetylation of β-Sitosterol.—The above sterol (300 mg.) was refluxed with acetic anhydride (6 ml.) and pyridine (6 ml.) for one hour. The solution was then poured into water and the acetate sucked off (298 mg.) After three recry-

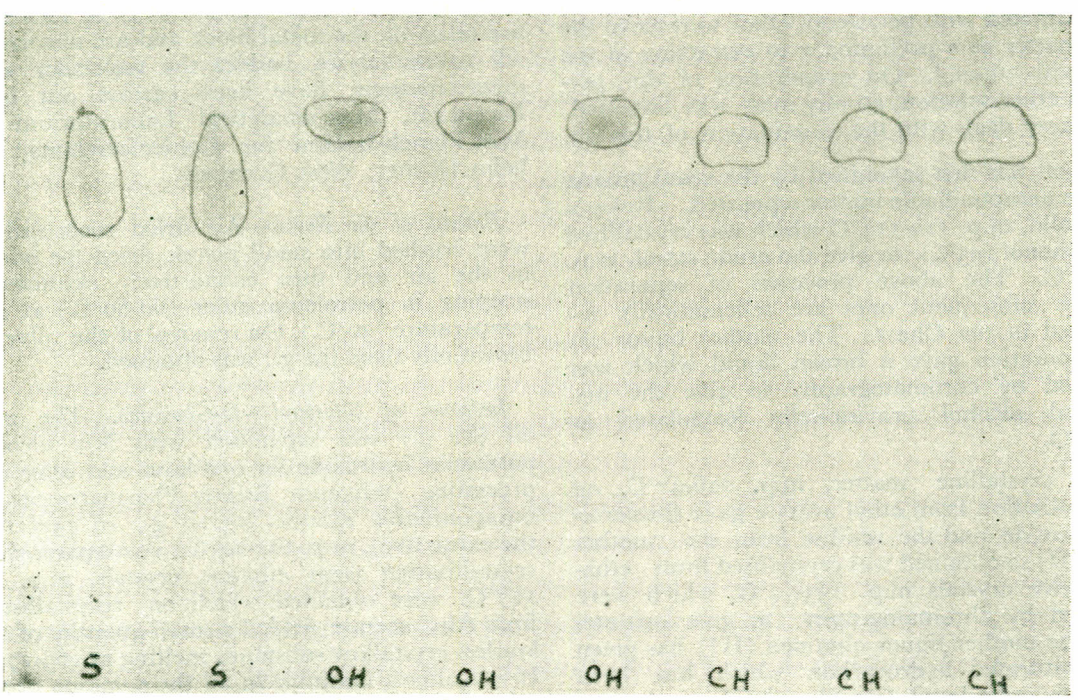


Fig. 1.—Thin layer chromatography on silica gel G. developed by ethanol (95%) and detected by iodine vapours. S—β-sitosteral; CH=Hydrocarbon; OH=Alcohol.

stallisations from alcohol crystals, m.p. 127-128°C., $[\alpha]_D^{24}$ -38°, (c, 0.5 in chloroform) were obtained. (Lit., m p. 126-127°C; $[\alpha]_D^{31}$ -39.5° (in chloroform))[6]. (Found: C, 81.99; H, 11.46 Calculated for $C_{31}H_{52}O_2$; C, 81.58; H, 11.40%).

Benzoylation of β-Sitosterol.—Sterol (200 mg.) was refluxed with benzoyl chloride (0.8 ml.) and pyridine (5 ml.) for one hour and a half. The solution was then poured into water and extracted with ether. The ethereal solution was washed with HCl (1 N) and sodium hydrogen carbonate solution (5%) and finally with water. After three recrystallisations from ethanol the analytical sample, m.p. 144-144.5°C., $[\alpha]_D^{23}$ -13.8° (c, 1 in chloroform) was obtained (Lit., m.p. 142-43°C., $[\alpha]_D^{20}$ -14°).[8] (Found: C, 83.66; H, 10.51. Calculated for $C_{36}H_{54}O_2$: C, 83.39; H, 10.41%).

Isolation of Hydrocarbon.—The semi-solid brown residue (2 g.) from the combined mother liquors (III) obtained after the fractional crystalisation of the sterol was submitted to adsorption analysis on a column (30×2.5 cm.) of alumina (80 g.), Brockman grade II and III (E. Merck). Elution was begun with petroleum ether, fractions of 50 ml. being collected. The following eluates were obtained: petroleum ether (100 ml.), hydrocarbon; petroleum ether (400 ml.), negligible oily matter; petroleum ether-benzene (1:1) (200 ml.), negligible oil; benzene (600 ml.) β-sitosterol (0.46 g.).

The light yellow hydrocarbon fraction was subjected to distillation *in vacuo*. A distillate boiling in the range of 200-204°/0.1—0.4 mm. was separated which was distilled thrice when a viscous oil, b.p. 200°/0.1 mm. with constant refractive index $[n]_D^{34}$ 1.4800 was obtained. The homogeniety of the hydrocarbon was checked by thin layer chromatography[9] (Fig. 1) on silica gel G (E. Merck), developing with two different solvents, ethanol (95%) and methanol, and detecting by iodine vapours.[5] It exhibited no optical rotation. (Found: C, 86.72; H, 13.14%, mol. wt. 468, $C_{32}H_{58}$ requires C, 86.80; H, 13.20%; Mol. wt. 443).

Its infra-red spectrum showed peaks at 2950, 1620, 1450, 1360 and 720 cm^{-1}. The compound had λ_{max}. 228, 256 mμ (ε=9600, 2600) in the ultra-voilet spectrum, which suggested the presence of unsaturation in the compound confirmed by ready absorption of bromine and decolourisation of potassium permanganate solution. It gave negative Liebermann-Burchard and Salkowsky tests.

Isolation of Alcohol.—The mother liquor (I) of the preliminary crystallisation of the β-sitosterol was freed from solvent *in vacuo* and the residue (2.87 g.) was passed through a column (38×2.5 cm.) of alumina, Brockmann of the activity grade II and III (E. Merck) (100 g.). Elution was started with petroleum ether and fractions of 100 ml. were collected. The first 600 ml. of eluates furnished an oil, subsequent elution with petroleum ether-benzene (1:1, 700 ml.) gave the alcohol.

The alcohol obtained above was subjected to distillation *in vacuo*. After the fifth distillation at 156-60°C./0.1 mm. a sample with constant refractive index $[n]_D^{34}$ 1.4646 was obtained. The optical rotation was nil. The homogeniety of the substance was checked by thin layer chromatography[9] (Fig. 1) on silica gel G (E. Merck) developing with two solvents methanol and ethanol (95%) and detecting with iodine vapours.[5] (Found: C, 80.58; H, 12.80; O, 6.68; mol. wt. 284. $C_{16}H_{30}O$ requires C, 80.58; H, 12.68; O, 6.71%; mol. wt. 238). λ_{max} 204, 256 mμ (ε=12341 and 2870) suggested the presence of unsaturation in the alcohol which was confirmed by ready absorption of bromine and decolourisation of potassium permanganate solution. Its infra-red spectra showed peaks at 3350, 2950, 1620, 1460, 1360 and 1000 cm^{-1}. It gave negative Liebermann-Burchard and Salkowsky tests.

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