

CHEMICAL EXAMINATION OF THE LEAVES OF *DIOSPYROS MELANOXYLON* ROXB.

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Diospyros melanoxyton Roxb. (Syn. *D. tupru*), a medium-sized deciduous tree, is widely distributed in the Indian Peninsula extending northwards up to Bihar and Central India. From the Madhya Pradesh and Andhra Pradesh forests large quantities of the leaves of this tree are collected every year for use in the *beedi* (leaf-covered cigarette) manufacture; they are employed for wrapping on account of their flavour, flexibility and resistance to decay.^{1, 2} However, although the tree occurs plentifully in the forests of the Mysore State and a good bit of the *beedi* industry is located in the State, yet the leaves from the local forests are not preferred for the wrappage, since they appear to be lacking in some of the required properties. During the course of our attempts to process the Mysore leaves in order to render them suitable for the *beedi* wrappage, they were subjected to a systematic chemical examination and the results are reported in this article. Although the leaves are reported to be possessing carminative and diuretic properties and are used in ophthalmic ailments,³ they do not appear to have been chemically examined before. Recently, the isolation of lupeol and betulin has been reported from the bark and sapwood of the plant.⁴

The powdered leaves were exhaustively extracted with petroleum ether, acetone and alcohol in succession. The petroleum ether extract furnished ceryl alcohol, lupeol, betulin, β -sitosterol and a new triterpene alcohol, $C_{30}H_{50}O$, m.p. 212–13°. The characterisation of these compounds was done by a study of their elementary analysis, colour reactions, optical rotation and melting points. The acetone extract could be separated into a new triterpene carboxylic acid, m.p. 272–74° provisionally named diospyric acid, along with a considerable amount of amorphous tannins. The acid contained a hydroxyl group, and formed a monoacetate, a methyl ester and a methyl ester acetate. From the alcoholic extract separated a colourless crystalline compound, which, from a study of its properties, was identified

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as sequoyitol, a monomethyl ether of meso-inositol. The occurrence of sequoyitol in nature was reported for the first time by Sherrard and Kurth from Californian redwood^{6,6} and later by Riggs from *Macrozamia riedles*.⁷ The detection of sequoyitol in the leaves of *D. melanoxylon* appears to be the first case of its occurrence in the plants belonging to the N.O. *Ebenaceae*.

EXPERIMENTAL

The powdered air-dried leaves (4 kg.) were exhaustively extracted with petroleum ether (b.p. 40–60°), acetone and alcohol (95%) in succession by cold percolation and each extract was worked out separately.

Petroleum ether extract.—The combined dark green extract (5×8 l.) was concentrated to about 300 ml. and kept at room temperature for a few days, when a crystalline solid (12.84 g.) was obtained. This was filtered and washed with a little petroleum ether. The filtrate on further concentration and keeping deposited some more of the crystalline substance (1.5 g.). Both the fractions (14.34 g.) were combined and suspended in petroleum ether and chromatographed over alumina column (300 g.). Petroleum ether, benzene, sulphuric ether, chloroform and their mixtures were used as eluants. The first 4 elutions were done with petroleum ether, which extracted a crystalline solid (No. 1) melting at 73–76°, in a yield of 1.40 g. The subsequent 24 elutions were done with mixtures of benzene and petroleum ether (1:1–9 by vol.), when 6.04 g. of a solid (No. 2) melting between 206–11° was obtained. Further 10 elutions with mixtures of ether and benzene (1:1–3 by vol.) isolated 2.24 g. of a solid (No. 3), m.p. 244–50°, while extraction with pure ether (4 elutions) and finally with chloroform and ether (1:3 by vol.; 4 elutions) yielded 1.68 g. of a fourth crystalline solid (No. 4) melting between 128 and 131°.

Substance No. 1.—On repeated crystallisations from acetone it gave colourless plates, m.p. 78–79° (Found: C, 81.54; H, 14.32; $C_{26}H_{54}O$ requires C, 81.67; H, 14.14%). The m.p. of the compound was not depressed when mixed with an authentic sample of ceryl alcohol. On boiling with acetic anhydride and fused sodium acetate, the compound furnished a monoacetate, m.p. 63–64° agreeing with that of ceryl acetate.

Substance No. 2.—It was found to be a mixture. The infra-red spectrum did not show any characteristic peaks for the presence of ester or carbonyl groupings. The product was acetylated with acetic anhydride and pyridine in the usual manner and the acetate was separated into ether-soluble (A) and ether-insoluble (B) fractions.

Ether-soluble acetate fraction (A).—On crystallisation from acetone and methanol it yielded colourless needles, m.p. 214–15°, $[\alpha]_D + 28^\circ$ (CHCl_3 ; c 1%) (Found: C, 82.15; H, 11.37; $\text{C}_{32}\text{H}_{52}\text{O}_2$ requires C, 82.05; H, 11.11%). It gave positive Liebermann-Burchard colour reaction and purple colour with thionyl chloride. The mixed melting point with an authentic sample of lupeol acetate was undepressed.

The acetate was hydrolysed with 5% alcoholic potassium hydroxide. The deacetylated product crystallised from methanol as colourless needles, m.p. 212–13°, $[\alpha]_D + 45^\circ$ (CHCl_3 ; c 1%). It showed unsaturation with tetranitromethane and gave positive tests for triterpene (Found: C, 84.42; H, 11.82; $\text{C}_{30}\text{H}_{50}\text{O}$ requires C, 84.50; H, 11.73%). The compound showed no depression in melting point on admixture with an authentic sample of lupeol.

Ether-insoluble acetate fraction (B).—It crystallised from methanol as colourless needles, m.p. 275–76°, $[\alpha]_D - 5^\circ$ (CHCl_3 ; c 1%). It gave a yellow colour with tetranitro methane and responded to tests for triterpene (Found: C, 82.29; H, 11.42; $\text{C}_{32}\text{H}_{52}\text{O}_2$ requires C, 82.05; H, 11.11%). On deacetylation, it yielded a triterpene alcohol, m.p. 212–13°. This analysed for $\text{C}_{30}\text{H}_{50}\text{O}$. The detailed study of this compound is under progress.

Substance No. 3.—On repeated crystallisations from acetone it yielded colourless shining needles, m.p. 252–54°, $[\alpha]_D + 21.8^\circ$ (CHCl_3). Liebermann-Burchard colour reaction was positive. A pink colour changing to purple was obtained with thionyl chloride (Found: C, 81.32; H, 11.44; $\text{C}_{30}\text{H}_{50}\text{O}_2$ requires C, 81.45; H, 11.31%). Mixed m.p. of the compound with an authentic sample of betulin was undepressed. The compound afforded a diacetate with acetic anhydride and pyridine. The diacetate crystallised from methanol as colourless needles, m.p. 214–15° (Found: C, 77.74; H, 10.46; $\text{C}_{34}\text{H}_{54}\text{O}_4$ requires C, 77.57; H, 10.26%).

Substance No. 4.—On crystallisation from ethanol it furnished colourless needles, m.p. 136–37°, $[\alpha]_D - 34.5^\circ$. It gave a positive L.-B. colour reaction (pink—violet—blue—green) (Found: C, 83.77; H, 12.48; $\text{C}_{29}\text{H}_{50}\text{O}$ requires C, 84.05; H, 12.08%). It formed an acetate, m.p. 127–28°, $[\alpha]_D - 38^\circ$ (CHCl_3 ; c 1%). The compound was identified as β -sitosterol by mixed m.p. and infra-red spectrum.

Acetone Extract

Isolation of diospyric acid.—The leaves were subsequently extracted with acetone (4×8 l.) in batches. The combined extract was concentrated

to 500 ml. and kept at room temperature for two days. A crystalline solid was deposited which was purified by several crystallisations from acetone and ethanol using animal charcoal. The pure product was obtained as colourless shining needles, m.p. 272–74°, $[\alpha]_D + 66^\circ$ (pyridine; c 1%). It was readily soluble in dilute sodium hydroxide solution, indicating its acid character and was precipitated unchanged on acidification with mineral acids. It gave positive L.-B. colour reaction and with thionyl chloride a pink colour changing to purple was observed. The compound showed prominent infra-red bands at 3450, 3030, 1724, 1695, 1462, 1381, 1130 and 1036 cm^{-1} (in nujol) (Found: C, 78.64; H, 10.64; $\text{C}_{30}\text{H}_{48}\text{O}_3$ requires C, 78.94; H, 10.52%).

Diospyric acid acetate.—The above acid (500 mg.) was refluxed with acetic anhydride in presence of pyridine at 130° for five hours. With usual working and chromatography over neutral alumina using ether as eluant 380 mg. of the acetate was obtained. The latter crystallised from methanol as colourless needles melting at 190–92°, $[\alpha]_D + 68^\circ$ (CHCl_3 ; c 1%) (Found: C, 77.18; H, 10.22; $\text{C}_{32}\text{H}_{50}\text{O}_4$ requires C, 77.11; H, 10.04%).

Methyl diospyrate.—The acid (500 mg.) was dissolved in ether and treated with an excess of ethereal solution of diazomethane. The reaction flask was kept at 0° for 24 hours. The excess of the reagent was destroyed with a drop of acetic acid. The methyl ester crystallised from dilute methanol as colourless silky needles (440 mg.), m.p. 117°. The ester resisted hydrolysis even on prolonged boiling with alcoholic potassium hydroxide (Found: C, 78.88; H, 10.92; $\text{C}_{31}\text{H}_{50}\text{O}_3$ requires C, 79.15; H, 10.64%).

Methyl diospyrate acetate.—The methyl diospyrate (200 mg.) on acetylation with acetic anhydride and pyridine yielded methyl diospyrate acetate. It was crystallised from methanol as colourless needles (170 mg.), m.p. 242–44°, $[\alpha]_D + 48.5^\circ$ (CHCl_3 ; c 1%) (Found: C, 77.24; H, 10.28; $\text{C}_{33}\text{H}_{52}\text{O}_4$ requires C, 77.34; H, 10.15%).

Alcoholic Extract

The acetone-extracted leaves were then exhausted with alcohol (6×8 l.). The total extract was concentrated under reduced pressure to 500 ml. and kept at room temperature for a week. A crystalline solid was deposited, which was filtered and washed with alcohol to remove the adhering colouring matter. The residue on repeated crystallisations from 70% alcohol was obtained as colourless crystals (2.8 g.) melting at 237–38°. It was soluble in water, insoluble in ethanol, ether and ethyl acetate, and sparingly soluble in hot dilute alcohol. It was optically inactive. It was sweet in taste but

did not give Molisch's test or any colour with aqueous ferric chloride solution (Found: C, 43.44; H, 6.98; OCH_3 , 15.62; $\text{C}_6\text{H}_{11}\text{O}_5\text{OCH}_3$ requires C, 43.30; H, 7.21; OCH_3 , 15.98%). On acetylation with acetic anhydride and fused sodium acetate the compound furnished an acetate which crystallised from methanol as long needles melting at 198–200° (Found: C, 50.66; H, 6.10; $\text{C}_{17}\text{H}_{24}\text{O}_{11}$ requires C, 50.49; H, 5.94%).

The compound (500 mg.) was refluxed with 5 ml. of hydriodic acid (A.R.) for two hours at 130°. The reaction mixture was cooled to room temperature and diluted with 50 ml. of acetone. The acetone removed the excess of hydriodic acid and the residue on several crystallisations from methanol yielded colourless crystals, m.p. 224–25° (Found: C, 39.84; H, 6.74; $\text{C}_6\text{H}_{12}\text{O}_8$ requires C, 40.0; H, 6.66%). The melting point of the demethylated product remained undepressed on admixture with an authentic sample of mesoinositol. On heating with acetic anhydride and fused sodium acetate, it formed a crystalline acetate, m.p. 214–15° (mesoinositol hexaacetate, m.p. 215°).

Aqueous Extract

The aqueous extract of the organic solvent-exhausted leaves yielded potassium chloride to an extent of 0.8%.

SUMMARY

The leaves of *Diospyros melanoxylon* Roxb. growing in Mysore, have been shown to contain ceryl alcohol, lupeol, betulin, β -sitosterol, sequoyitol, a new triterpene alcohol, m.p. 212–13°, and a new triterpene carboxylic acid named diospyric acid, m.p. 272–74°.

Note (Correction added at the proof stage.)—When this paper is under print, a note has appeared from Row *et al.* (Row, L. R., Rao, C. S. and Ramaiah, T. S., *Curr. Sci.*, 1964, 33, 367), wherein it is stated that lupeol and betulin occur along with betulinic acid in the ebony of *D. melanoxylon* also.

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