CHEMICAL EXAMINATION OF THE LEAVES OF IXORA PARVIFLORA VAHL. (RUBIACEAE)

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(Received August 6, 1966; revised July 31, 1967)

From the leaves of *Ixora parviflora* Vahl., two pure, crystalline alcohols, m.p. 246-48° and 136-37°, a crystalline non-reducing sugar, m.p., 162.5-63°, and a reducing sugar have been isolated. Of the two alcohols, the one with m.p. 246-48° has been found to possess the molecular formula $C_{30}H_{50}O_2$ and is proved to be a diol as it gave a diacetyl and dibenzoyl derivative. This compound has been tentatively designated as "ixorol." The other alcohol, m.p. 136-37°, has been identified as β-sitosterol. The non-reducing and reducing sugars have been identified as D-mannitol and D-glucose respectively. D-Mannitol has been found to be present in the leaves to the extent of 0.1%.

Ixora parviflora Vahl. (English: *Torch tree:* Hindi: *Kotagandhal*) is an evergreen shrub or small tree commonly found in the Chittagong hill tracts. The different parts of this tree are used in the indigenous system of medicine.[1,2] No chemical work has hitherto been done on this plant. The results of a chemical examination of the leaves of this plant are recorded in the present communication.

Fresh leaves of *I. parviflora* were extracted with ethanol and the residue, obtained on removal of solvent from the extract, was partitioned between light petroleum and water. Chromatographic purification of the light petroleum soluble fraction led to the isolation of two pure, crystalline compounds. One of these had m.p. 246–48° and analysed for $C_{30}H_{50}O_2$. Its IR spectrum (Fig. 1) revealed the presence of hydroxyl function only in the molecule. The compound responded to the Liebermann-Burchard test and gave a crystalline diacetate, $C_{34}H_{54}O_4$, m.p. 218-19° as well as a dibenzoate, $C_{44}H_{58}O_4$, m.p. 176-77°. The IR spectra of the acetate as well as the benzoate did not show any hydroxyl absorption. We have tentatively designated this compound as "ixorol." The other compound which had m.p. 136-37° and $[\alpha]_D^{33}$ −34.6°, also showed, from its IR spectrum the presence of hydroxyl function only in the molecule. It gave a positive Liebermann-Burchard test and formed a crystalline acetate, m.p. 129-30°, $[\alpha]_D^{33}$ −4.6°. The compound was identified as β-sitosterol by mixed m.p. and comparison of IR spectra. Its acetate also showed no depression in melting point on admixture with an authentic sample and had identical IR spectrum as that of β-sitosterol acetate.

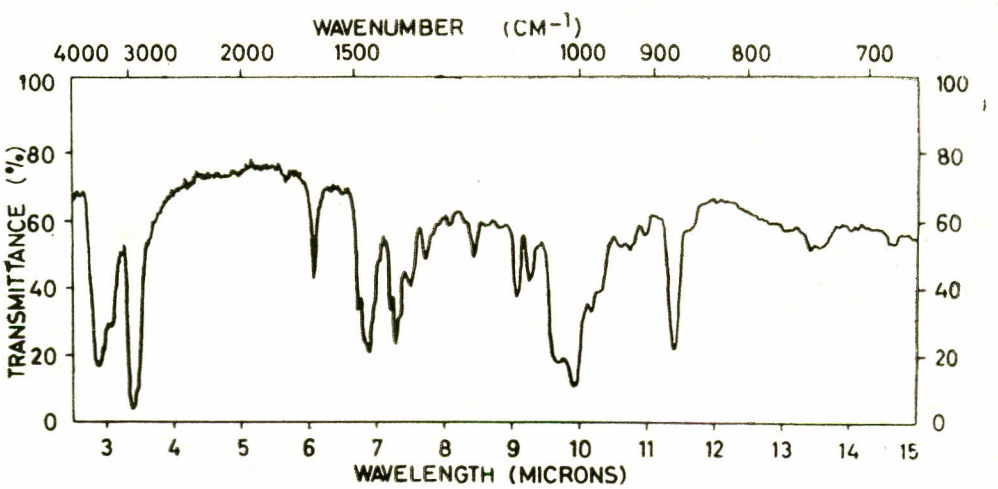

Fig. 1.—IR spectrum of ixorol in KBr.

The water-soluble fraction of the residue obtained on removal of solvent from the ethanolic extract, was found to contain a substance (21.8% of the aqueous extractives) which crystallised from ethanol in small, white feathery needles, m.p. 162.5-163°. Its sweet taste, solubility, optical activity and non-reducing action on Fehling's solution indicated it to be D-mannitol.[3] The identity was established by mixed m.p. determination. Further confirmation of the compound being D-mannitol was obtained from the preparation of its crystalline hexaacetate, m.p. and m.m.p. 122-23°.

Besides mannitol, glucose was found to be present in the water-soluble fraction when examined by paper chromatography. D-Glucose was isolated as its phenylhydrazone derivative, m.p. 204° (decomp), m.m.p. 202° (decomp).

Experimental

Analyses were done by the Microanalytical Section of these Laboratories. M. ps are uncorrected. Light petroleum (b.p. 66-69°) was used.

Extraction.—Fresh leaves (5 kg) of the plant were soaked in ethanol (10 l.) for about a week and then the extract was completely freed from solvent. A thick, dark-brown syrup was obtained as residue which was partitioned between light petroleum and water. The aqueous fraction which was acidic, on complete removal of solvent, yielded a thick, dark-brown syrup (22.9 g) whereas the light petroleum fraction gave a dark-brown oily residue (31.4 g). The resinous mass (6 g) which did not dissolve in either of the solvents was not investigated.

Isolation of Ixorol and β-Sitosterol.—5 g of the light petroleum soluble fraction was chromatographed on a column of activated neutral alumina using light petroleum, light petroleum–benzene, benzene and benzene–chloroform successively for elution. The later fractions of the light petroleum eluate yielded crude β-sitosterol, m.p. 132-35°. On further purification by chromatography and repeated crystallisations, pure β-sitosterol was obtained in the form of colorless needles, m.p. and m.m.p. 136-137°, $[\alpha]_D^{33}$—34.6° (c=0.433; $CHCl_3$), yield 0.5 g. The sterol produced a violet coloration with Liebermann-Burchard reagent. Acetylation with acetic anhydride and pyridine at room temperature yielded β-sitosterol acetate which crystallised from methanol in colourless shining leaflets, m.p. and m.m.p. 129-30°, $[\alpha]_D^{33}$—42.6° (c=0.234; $CHCl_3$). The IR spectra of the sterol as well as its acetate were found to be identical with those of authentic β-sitosterol and β-sitosterol acetate respectively.

Further elution of the alumina column with light petroleum–benzene (9:1, 3:1, 1:1 and 1:3), benzene and benzene–chloroform (3:1 and 1:1) gave fractions which yielded solid substances, melting within the range 218–22°. These were combined and repeatedly crystallised from methanol while fine, colourless needles of ixorol, m.p. 246-48°, $[\alpha]_D^{32}$+17.6° (c=1.08; $CHCl_3$), were obtained (yield 1.4 g). Further crystallisations did not raise the m.p. of the compound. (Found: C, 81.75 and H, 11.06%. Calc. for $C_{30}H_{50}O_2$: C, 81.39 and H, 11.38%). With the Liebermann-Burchard reagent, ixorol produced a light purple coloration which gradually turned into violet. Its IR spectrum in KBr (Fig. 1) showed hydroxyl absorptions at 3629 $cm.^{-1}$ and 3268 cm^{-1}.

Ixorol Acetate.—Ixorol was treated with acetic anhydride and pyridine, and the mixture left for two days at room temperature. It was then worked up in the usual manner. The acetate crystallised from methanol in colourless, shining needles, m.p. 217.5–19°. (Found: C, 77.98; H, 9.95%. Calc. for $C_{34}H_{54}O_4$: C, 77.52; H, 10.33%). IR spectrum of the acetate in KBr showed strong absorption peaks at 1733 cm^{-1} and 1242 cm^{-1}, and no hydroxyl absorption was present.

Ixorol Benzoate.—Benzoyl chloride was added to a solution of ixorol in pyridine. The mixture was left at room temperature for 2 days and then worked up in the usual manner. The benzoate crystallised from methanol in small, white needles, m.p. 176-77°. (Found: C, 81.01; H, 8.92%. Calc. for $C_{44}H_{58}O_4$: C, 81.19 ; H, 8.98%).

D-*Mannitol and* D-*Glucose.*—The aqueous extractive (22.9 g) was dissolved in water and the solution treated with lead acetate solution and the precipitate filtered off. H_2S was then passed through the filtrate in order to remove the excess lead salt, filtered, and the pale straw-coloured filtrate freed from solvent under reduced pressure. The residual light brown syrup was extracted with boiling ethanol and the ethanolic extract, on concentration, gave white, feathery needles of D-mannitol, m.p. and m.m.p. 162.5-63° (4.992 g, i.e. 0.1% on the basis of the weight of fresh leaves). D-Mannitol was acetylated with acetic anhydride and pyridine and the hexaacetate crystallised from ethanol in colourless, stout, hexagonal prisms, m.p. and m.m.p. 122-23°.

The substance insoluble in boiling ethanol strongly reduced Fehling's solution and, on exa-

mination by paper chromatography (ascending method) using n-butanol–pyridine–water (10:3:3) as solvent mixture and aniline oxalate as the developer, it yielded one spot (R_f 0.26) on the chromatogram that was due to glucose. D-Glucose was isolated as its phenylhydrazone derivative, m.p. 204° (decomp with shrinkage at 199°), m.m.p. 202° (decomp with shrinkage at 199.5°).

References

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2. K.M. Nadkarni, *Indian Materia Medica*, third edition, vol. I, p. 699.
3. H.T. Clarke, *A Handbook of Organic Analysis* (Edward Arnold Ltd., London), fourth edition, p. 119.