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STRUCTURE OF IPOMINE, A NEW ALKALOID FROM *IPOMOEA MURICATA* JACQ

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Abstract—Studies on the basic fraction from *Ipomoea muricata* Jacq. seeds, grown in Senegal, resulted in the isolation of two hexahydroindolizine alkaloids, the previously described ipalbidine and a new alkaloid, ipomine, $C_{30}H_{35}NO_8$, the structure of which is established as 1-β-ipalbidinyl-4-*p*-coumaroyl-D-glucopyranoside.

Misra and Tewari[1] in their investigation of the seeds of *Ipomoea muricata* Jacq. grown in India, showed the presence of phytosterols, fatty acids, caffeic acid and the glucoside muricatine, but no alkaloidal material was indicated. Our studies of the same species grown in Senegal, revealed the presence of two alkaloidal components. A minor product $C_{15}H_{19}NO$, m.p. 144° obtained in 0.001% yield, was found to be identical with ipalbidine, a hexahydroindolizine alkaloid previously isolated from *Ipomoea laba* L. seeds and identified by Heacock *et al.*[2] The other major product, obtained in 0.02% yield is a new ester alkaloid and is designated ipomine. The structure of ipomine, m.p. 139° $C_{30}H_{35}NO_8$, $[\alpha]_D$ +46.6° is established as 1 - β - ipalbidinyl - 4 - *p* - coumaroyl - D - glucopyranoside **1** on the basis of the following evidence.

Ipomine shows a positive test for a phenol ($FeCl_3$), λ_{max} (EtOH), 232 (ε14,320) and 315 (ε13,870), shifted on adding alkali to 240 (ε13,420) and 370 nm (ε18,520). This large bathochromic shift is typical of *p*-hydroxy cinnamic acid esters,[3] which is further supported by the IR, ν_{max} (film), 3400 (OH), 1740, 1250 cm^{-1} (ester). Ipomine is a glycosidic derivative, since it exhibits a positive Molisch's test. Acid hydrolysis of ipomine affords the alkaloid ipalbidine, D-glucose and *p*-coumaric acid. Enzymatic hydrolysis with emulsin yields ipalbidine. This reaction proves that ipomine is a β-D-glucoside with D-glucose attached from its C_1-position by a β-linkage to the phenolic group of ipalbidine and not to the carboxylic group of the *p*-coumaric acid. Methylation of ipomine with methyl sulphate and potassium carbonate in acetone followed by hydrolysis with 2N HCl for 0.5 h affords 2,3,6 - tri - *O* - methyl - D - glucopyranose. The carboxyl group of *p*-coumaric acid is linked therefore to the D-glucose residue through the C_4-position.

The ^1H and ^{13}C NMR spectra of ipomine, measured in d_6-DMSO, are consistent with the proposed structure. In the proton spectrum an AB pair of doublets (J 16 Hz) at δ6.36 (1 proton) and 7.59 (1 proton) and an AA'BB' multiplet (J_{AB} 9.0 Hz) at δ6.80 (2 protons) and 7.52 (2 protons) are in accord with a *p*-hydroxycinnamic ester group and a 4 proton singlet at δ7.00 can be assigned to the *p*-substituted phenyl group protons. Broad signals in the region 3.5–5.0 ppm are consistent with the presence of a sugar moiety and a singlet at δ1.46 can be assigned to the C-methyl protons. The ^{13}C NMR spectrum resolves all carbon signals which have been assigned as indicated on the structure **1**.

EXPERIMENTAL

M.ps were measured with a Kofler hot stage apparatus and are uncorrected. IR spectra were taken in KBr films on a Perkin Elmer spectrophotometer model IR 4. Proton NMR spectrum was recorded on a Varian HA 100 spectrometer and ^{13}C NMR spectra on a varian CFT 20. Chemical shifts are reported relative to TMS (δ0.0).

Isolation of ipomine. Impomoea muricata Jacq., dry powdered seeds (1.12 kg) were moistened with Na_2CO_3 soln (2N, 800 ml) and continuously extracted with EtOAc (4 l.) for 25 h. The ex-

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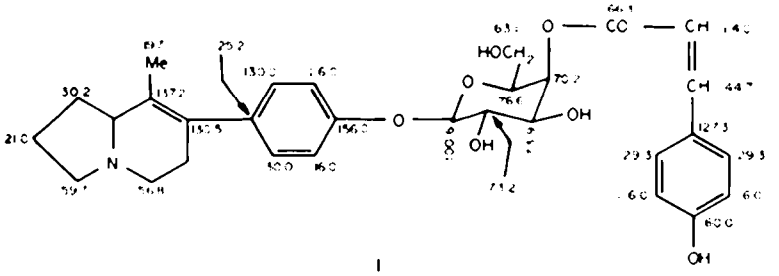

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tract was evaporated to give a dark yellow syrup (227 g), which was dissolved in 1% aq tartaric ether acid–ether mixture (1:1) total (3 l.). The ether layer was separated and the aqueous layer containing the basic fraction made alkaline with Na_2CO_3 to pH 10, and extracted with EtOAc (3 × 1.5 l.). Evaporation of this extract gave the alkaloidal material as a yellow oily residue (7 g). TLC [silica gel G, $CHCl_3$–MeOH (17:3), Dragendorff's reagent] revealed two components, minor R_f 0.65 and major, R_f 0.25. The alkaloid mixture (7 g) was chromatographed on alumina column (grade V, 250 g). Elution with $CHCl_3$–MeOH (98.5:1.5) yielded 10 mg, R_f 0.65 as a colourless plates (from EtOAc). Elution with $CHCl_3$–MeOH (95:5) gave yellow glassy material (250 mg), R_f 0.25, which on trituration with ether gave an amorphous solid which was purified by prep. TLC [silica gel G, $CHCl_3$–MeOH (17:3)].

The material of R_f 0.65 obtained as colourless plates, m.p. 144–46° (from EtOAc), reported[2] m.p. for ipalbidine 147–148° (Found: C, 76.5; H, 8.2; N, 5.7. $C_{31}H_{39}NO$ requires: C, 78.6; H, 8.3; N, 6.1%). It gave an orange colour with $FeCl_3$, λ_{max} (EtOH), 235 (ε10,010) and 280 nm (ε1810) shifted on adding alkali to 260 (ε11,120) and 290 nm (shoulder), reported[2] λ_{max} for ipalbidine, 236 (ε10,040) and 278 nm (ε1730) shifted on adding alkali to 248 (ε24,300) and 295 nm (shoulder), ν_{max} (film), 3400 cm^{-1} (OH). The MS of the isolated alkaloid showed the same peaks as those reported for ipalbidine,[2] 229 [M]; 214 [M-CH_3]; 160 [M-C_4H_7N]; 145 [M-(C_4H_7N + CH_3)]; 70 [M-$C_{11}H_{11}O$].

The compound of R_f 0.25, amorphous solid, m.p. 139–43° (from ether), $[\alpha]_D$ +46.4° (c, 0.55) designated as ipomine (Found: C, 62.3; H, 6.8; N, 2.3. $C_{30}H_{51}NO_8 \cdot 2H_2O$ requires: C, 62.8; H, 6.8; N, 2.4%), λ_{max} (EtOH), 232 (ε14,328) 315 nm (ε13,870) shifted on addition of alkali 240 (ε13,420) and 370 nm (ε18,520), ν_{max}, 3500 (OH), 1740 cm^{-1} (ester). MS showed M^+ at *m/e* 537 in addition to the same peaks exhibited by ipalbidine. Ipomine gave a picrate, m.p. 138–41° (from MeOH) and an orange colour with FeCl, soln.

Acid hydrolysis of ipomine. Ipomine (80 mg) was dissolved in EtOH (30 ml), 36% HCl was added (2 ml) to make the resulting soln 2N and refluxed for 0.5 h. Extraction with EtOAc after addition of NH_4OH to pH 10 resulted in oily material, purified by prep TLC [silica gel G, $CHCl_3$–MeOH (17:3)] to give 12 mg of plates (from EtOAc), m.p. 143–45°, R_f 0.65, identified as ipalbidine. The hydrolysate was processed for the sugar identification, which was found to be D-glucose [Whatman No. 1, BuOH–AcOH–H_2O (4:1:5), diphenylamine reagent], R_f 0.18.

Emulsin hydrolysis of ipomine. Ipomine (15 mg) was dissolved in a small amount of hot 50% EtOH and diluted with phosphate buffer (pH 4.6) to 10 ml. Emulsin (50 mg) and toluene (2 drops) were added and the mixture was allowed to stand at 20–25° for 48 h. Extraction with EtOAc resulted in the detection of ipalbidine, R_f 0.65 (same UV spectrum).

Isolation of p-coumaric acid. The experiment of the mineral acid hydrolysis of ipomine was repeated as above using 50 mg. After extraction of the aglycone ipalbidine, the hydrolysate was acidified to pH 4, evaporated to dryness, homogeneously mixed with alumina, 2 g, and chromatographed on alumina column [grade V, 18 g]. Elution with $CHCl_3$–MeOH (97:3) gave lustrous plates, 8 mg (from EtOH), m.p. and m.m.p. with authentic sample of *p*-coumaric acid 210–212°. λ_{max} (EtOH) 228 (ε12,590), 314 nm (ε25,180) shifted on addition of alkali to 240 (ε8,380) and 339 nm (ε26,220), ν_{max}, 3450 (OH), 1700 and 1250 cm^{-1} (COOH). The IR spectrum is identical in every respect with the *p*-coumaric acid.

Methylation of ipomine. To ipomine (30 mg) in dry acetone (10 ml) was added methyl sulphate (10 ml), K_2CO_3 (200 mg) and the mixture refluxed for 1 h, filtered and the filterate evaporated to dryness. Subsequent hydrolysis with 2N HCl in EtOH for 0.5 h and extraction with pet. ether gave a yellow residue which was purified by prep TLC [silica gel G, benzene–EtOAc (9:1)] to give needles, (from pet. ether), 3 mg, m.p. 120°, no depression upon admixture with an authentic sample of 2,3,6-tri-*O*-methyl-D-glucopyranoside.

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