ALKALOIDS OF THE ROOTS OF *DESMODIUM GANGETICUM*

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Previously, the occurrence of several simple indolic bases in the aerial portions of *Desmodium gangeticum* D.C. (Leguminosae: Papilionaceae) was reported by us.[1] Since the roots of this species find use in the ayurvedic system of medicine,[2] we have now investigated their alkaloidal constituents. The present communication describes the isolation and identification of seven alkaloids representing three structural types, viz. carboxylated and decarboxylated tryptamines, and β-phenylethylamine. The bases identified are: *N*,*N*-dimethyltryptamine, its N_b-oxide, hypaphorine, hordenine, candicine, *N*-methyltyramine, and β-phenylethylamine. In addition, the presence of an apparently new β-phenylethylamine alkaloid was also detected. Preliminary findings indicated that the last-mentioned alkaloid is related to halostachine, a β-hydroxy-β-phenylethylamine alkaloid occurring with tryptamines in the Chenopodiaceae.[3]

While co-occurrence of β-phenylethylamines and simple indolic bases has been known in the genus *Acacia*[4] (Leguminosae: Mimosae), the quaternary β-phenylethylamine base, candicine, appears to have been isolated only from the Argentine cactus.[3] Further, the previous results[1] indicated that the elaboration of alkaloids by *Desmodium gangeticum* had not been restricted to the "proto alkaloids" (alkamines)[5] only, since this species also contained two β-carboline alkaloids in its aerial portions. This alkaloid versatility of *D. gangeticum* obviously suggests it to be a phylogenetically complex species in the subfamily Papilionaceae, different from those[6] producing either the carboxylated or decarboxylated tryptamines or the β-phenylethylamines.

Some of the curative properties ascribed[2] to the root extracts of *D. gangeticum* seem to be due to the major alkaloid, hordenine, which is known to increase the flow of urine and is a remedy for diarrhoea and dysentery.[7] Pharmacological evaluation of the species is currently being done in this laboratory.

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[1] Banerjee, P. K., and Ghosal, S., *Aust. J. Chem.*, 1969, **22**, 275.

[2] Chopra, R. N., Nayar, S. L., and Chopra, I. C., "Glossary of Indian Medicinal Plants." p. 94. (C.S.I.R.: New Delhi 1956.)

[3] Reti, L., in "The Alkaloids." (Eds. R. H. F. Manske and H. L. Holmes.) Vol. III, p. 322. (Academic Press: New York 1953.)

[4] Johns, S. R., Lamberton, J. A., and Sioumis, A. A., *Aust. J. Chem.*, 1966, **19**, 1539.

[5] Hegnauer, R. in "Comparative Phytochemistry." (Ed. T. Swain.) p. 211. (Academic Press: New York 1966.)

[6] Dutta, S. K., Ph.D. Dissertation, Banaras Hindu University, 1969.

[7] Barger, G., and Dale, H. H., *J. Physiol.*, 1910, **41**, 19.

Experimental

All melting points were determined on a Kofler block in open capillary and are uncorrected. The u.v. and i.r. spectra were taken in ethanol and Nujol respectively. Descending paper chromatography was on Whatman No. 1 paper, and three solvent systems were used, viz. n-butyl acetate–acetic acid–n-butanol–water (85 : 40 : 15 : 22, solvent 1), n-butanol–acetic acid–water (4 : 1 : 2, solvent 2), and ethanol–ammonia (4 : 1, solvent 3). Besides Dragendorff and Ehrlich reagents, a new staining reagent, α-nitroso-β-naphthol–nitrous acid, was used for the detection of the alkaloids. The colour test was found to be positive with the tyramines and only 5–7 γ concentration was required to produce a reddish purple colour. The corresponding *p*-methoxy compounds did not give any coloration with this reagent. The reagent was previously used by Udenfriend *et al.*[8] for detecting arylhydroxyindoles. The colour test also served, in this investigation, to distinguish *p*-hydroxy-β-phenylethylamines from hydroxytryptamines since the reddish purple colour produced by the former turned yellow on keeping while the violet colour produced by the latter bases remained stable for a long time. The different behaviour of the two types of amines on the carotid blood pressure of dogs also served to identify them. Thus, while β-phenylethylamines (being catechol amine liberators) raised the blood pressure, the tryptamines lowered it through histamine liberation.[9]

Two essential properties of the mixture of alkaloids, the relative basicities and the differential solubilities, were utilized for their isolation and purification. Thus, *N*,*N*-dimethyltryptamine N_b-oxide, although in a pure condition it was insoluble in petroleum, had appreciable solubility in the solution of fat in petroleum. The mixture of bases obtained from the defatted roots were extracted at three pH levels (4, 7, and 9) with chloroform to separate weak, moderately strong, and strong chloroform-soluble alkaloids. The water-soluble bases were separated through the differential precipitation of their reineckates in alkaline and acidic conditions.

Petroleum-soluble Bases

Dried and milled roots of *D. gangeticum* (1·6 kg) were defatted with petroleum (60–80°). The petroleum extract was concentrated (200 ml) and then extracted with aqueous citric acid (2N, 200 ml). The acidic solution afforded a brown basic gum (0·4 g) consisting of a mixture of two alkaloids, R_F 0·82 and 0·89 (solvent 1), which were identified as β-phenylethylamine and *N*,*N*-dimethyltryptamine N_b-oxide in the following way.

β-Phenylethylamine.—The brown basic gum was triturated with light petroleum (40–60°) at room temperature. The petroleum-soluble fraction (0·28 g) afforded β-phenylethylamine as a light brown oil, R_F 0·82 (solvent 1); yellow picrate from aqueous alcohol, m.p. 171–174°; mixed m.p. with the authentic picrate, m.p. 171–173°, remained undepressed (Found: N, 16·0. Calc. for $C_8H_{11}N,C_6H_3N_3O_7$: N, 16·0%).

N,N-Dimethyltryptamine N_b*-oxide.*—The petroleum-insoluble fraction (0·12 g), R_F 0·89 and 0·58, on co-chromatography with the marker showed single spots having the same R_F values (solvents 1 and 3 respectively); λ_{max} 224, 277, and 292 mμ; yellow picrate from ethanol, m.p. and mixed m.p. 176–178°.

Chloroform-soluble Acetates (Alkaloids from pH Level 4)

The chloroform-soluble acetates, obtained as a brown amorphous solid (0·9 g), were taken up in a small volume of alcohol (10 ml) and upon cooling *N*-methyltyramine (0·5 g) separated.

N-Methyltyramine.—It crystallized from aqueous alcohol as prisms, m.p. 130–131° (lit.[8] 130–131°); R_F 0·59 (solvent 2); λ_{max} 222–224, 230sh, 275–277, and 300–305 mμ; yellow picrate from alcohol, m.p. and mixed m.p. 147–149° (Found: N, 14·5. Calc. for $C_9H_{13}NO,C_6H_3N_3O_7$: N, 14·7%).

The alcoholic mother-liquor, after the separation of *N*-methyltyramine, was concentrated and the residue was chromatographed over alumina. The chloroform eluates from the column

[8] Udenfriend, S., Weisbach, H., and Clark, C. T., *J. biol. Chem.*, 1955, **215**, 337.

[9] Ghosal, S., Dutta, S. K., Sanyal, A. K., and Bhattacharya, S. K., *J. mednl Chem.*, 1969, **12**, 480.

gave *N*,*N*-dimethyltryptamine (0·38 g), a thick brown oil, R_F 0·58 (solvent 1); λ_{max} 222, 277, 287, and 294 mμ; yellow picrate from alcohol, m.p. and mixed m.p. 166–168°. The chloroform–methanol eluates afforded *N*,*N*-dimethyltryptamine N_b-oxide (0·02 g) and was identified as shown above.

Chloroform-soluble Moderately Strong Bases (Alkaloids from pH Level 7)

A light purple, basic gum (0·95 g) showed two spots on paper, R_F 0·28 and 0·50 (solvent 1), due to an unidentified β-phenylethylamine and hordenine, respectively. These were separated by column chromatography. The chloroform–benzene eluates afforded a brown liquid (0·018 g), R_F 0·28 (solvent 1); Dragendorff, orange; Ehrlich and α-nitroso-β-naphthol–nitrous acid, negative; λ_{max} 233 and 295–300 mμ; *m/e* 163(M^+), 162 (strong), 119, 103, 78, 77.

Hordenine.—The chloroform and the chloroform–methanol (95 : 5) eluates afforded hordenine (0·82 g); R_F 0·50 (solvent 1); crystallized from benzene–petroleum as needles, m.p. 117–118° (lit.[3] 117–118°); u.v. λ_{max} 222–224, 277, and 300–305 mμ; i.r. λ_{max} 2·8 (OH), 3·53 (NMe), and 6·2 μ (substituted benzene); *m/e* 165(M^+), 121, 107, 58 (strongest); picrate, m.p. 137–139° (lit.[3] 139–140°); methiodide, m.p. 229–230° (lit.[3] 229–230°) (Found: C, 43·1; H, 5·5; N, 4·5. Calc. for $C_{11}H_{18}INO$: C, 42·9; H, 5·8; N, 4·5%).

Chloroform-soluble Strong Bases (Alkaloids from pH Level 9)

The amorphous solid (0·55 g) obtained from the above fraction showed two spots on paper chromatograms, R_F 0·36 and 0·50 (solvent 1), due to candicine and hordenine respectively. The crude base was treated with alcoholic potassium iodide whereupon candicine iodide separated.

Candicine.—The iodide crystallized from alcohol as needles, m.p. 229–230° (lit.[3] 229–230°) (Found: N, 4·4. Calc. for $C_{11}H_{18}INO$: N, 4·5%).

Water-soluble Bases

The aqueous alkaline mother-liquor, after the separation of the above bases, was treated with a saturated aqueous solution of ammonium reineckate; choline reineckate separated out. The mother-liquor was acidified and ammonium reineckate solution was again added to ensure precipitation of the residual reineckate complex. Hypaphorine was regenerated from the pink complex by passing its acetone solution over De-Acidite FF (pH 8).[1]

Hypaphorine.—The alkaloid crystallized from alcohol as needles, m.p. and mixed m.p. 262°; R_F 0·55 (solvent 2); u.v. λ_{max} 224, 275, and 292 mμ; i.r. λ_{max} 6·3 (COO^-, betaine); superimposable i.r. spectrum with authentic hypaphorine;[6] hydrochloride from alcohol, m.p. 232°.

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