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ALKALOIDS OF *MUCUNA PRURIENS* CHEMISTRY AND PHARMACOLOGY

By S. Ghosal, S. Singh and S. K. Bhattacharya

Introduction

Mucuna pruriens DC. (*Papilionaceae*) which grows abundantly in India, finds use (Kirtikar and Basu, 1933) in the indigenous system of medicine for a variety of purposes, viz., as an uterine stimulant, in dysentery, as a nerve tonic, diuretic, and aphrodisiac.

Majumdar and Mehta (1944) previously reported the presence of alkaloids in the seeds of this species but could not characterize them. Later, Bowden et al. (1954) recorded evidence for the presence of serotonin in the trichomes of its pods.

The incomplete phytochemical investigation and the reported uses of the plant extracts in the indigenous system of medicine prompted the present authors to undertake a detailed phytochemical and pharmacological evaluation of this species. Various individual parts of the plant, e. g., the seeds, pods, trichomes of pods, leaves, stems, and roots, were investigated separately for their alkaloid contents. The isolation, characterization, and pharmacological evaluation of the alkaloids constitute the subject of the present paper.

Experimental

All melting points were determined on Kofler block in open capillary and are uncorrected. The ultraviolet spectra were taken in aldehyde free ethanol in a Carl Zeiss spectrophotometer, model VSU 1 and the infrared spectra were taken in Nujol in a Hilger & Watts Infrascan machine. Descending paper chromatography was done on Whatman paper No. 1 using n-butyl acetate-acetic acid-n-butanol-water (85 : 40 : 15 : 22) as the developer. Three spraying reagents, viz., Dragendorff, Ehrlich, and α-nitroso-β-naphthol-nitrous acid (Ghosal and Banerjee, 1969), were used. The general procedure for the separation and identification of the individual bases involved column chromatography over Brockmann neutral alumina, preparative paper chromatography on Whatman 3 MM paper, and preparation of picrate and reineckate where possible. The following general procedure, as described under isolation of alkaloids from the leaves, was followed for the extraction of alkaloids from the other parts except the trichomes of pods where a method essentially similar to that of Bowden (loc. cit.) was employed.

Isolation of Alkaloids from the Leaves of Mucuna pruriens

Green leaves (2 kg, wet wt.) were macerated in a waring blender with a mixture of chloroform (3 l) and ammonia (15N, 50 ml). The mixture was corked and kept at room temperature, with occasional shaking, for one week. It was filtered and the solvent was removed under reduced

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pressure. The viscous brown slurry (150 g) was poured into aqueous acetic acid (4%, 100 ml) with stirring, and the mixture was kept at room temperature overnight. The clarified solution was extracted with chloroform to remove weaker bases as chloroform-soluble acetates.

Chloroform-soluble Acetates

The chloroform-soluble acetates, obtained as a brown amorphous solid (1.2 g), was chromatographed over Brockmann neutral alumina (1.4 × 18 cm). Petroleum (40–60°), benzene, chloroform, methanol and mixtures thereof were used as eluents. The course of separation of the alkaloids was followed through paper chromatography.

N,N-Dimethylterypamine: The chloroform eluates from the column afforded a thik brown oil (0.08 g), R_F, 0.58; Dragendorff, orange; Ehrlich, purple changing to blue; α-nitroso-β-naphtol-nitrous acid, brown; u. v.: λ_{max} 225, 288, and 294–96 mμ; yellow picrate from alcohol, m. p. and m. m. p. 168–170°.

Anal. Calcd. for $C_{12}H_{16}N_2$, $C_6H_3N_3O_7$: N, 16.78. Found: N, 16.88.

N,N-Dimethyltryptamine-N_b-oxide: The chloroform-methanol eluates (99 : 1) afforded a brown basic gum (0.06 g), R_F, 0.88; Dragendorff, brownish-orange; Ehrlich, cherry red; u. v.: λ_{max} 224–26, 272, and 292 mμ; yellow picrate from alcohol, m. p. and m. m. p. with authentic sample, 178–80°.

Reduction of the compound with zinc dust and acetic acid gave N,N-dimethyltryptamine, isolated as its picrate, m. p. 166–68°.

Chloroform-soluble Strong Bases

The pH of the aqueous solution, after separation of the chloroform-soluble acetates, was brought to 9 with ammonia and the liberated bases were taken in chloroform (three 50 ml portions). The chloroform extract was washed with water, dried (anhydrous Na_2SO_4), and the solvent was removed at reduced pressure to give a brown basic gum (2.7 g).

Chromatography of the basic gum: A portion of the basic gum (1.2 g) was taken in benzene and was chromatographed on Brockmann neutral alumina (1.4 × 18 cm).

5-Methoxy-N,N-dimethyltryptamine: The petroleum-benzene (3 : 1) eluates, on evaporation, afforded a brown amorphous material (0.05 g) which showed a single spot at R_F, 0.58; Dragendorff, orange; Ehrlich, blue; α-nitroso-β-naphthol-nitrous acid, dull violet; u. v.: λ_{max} 224, 277, 292, and 305 mμ; i. r.: λ_{max} 2.88 (NH), 3.55 (NMe), 6.15 (OMe), 6.18 μ(C=C); the picrate crystallized from alcohol as orange needles, m. p. 168–70°. M. m. p. with an authentic sample, m. p. 172°, remained undepressed.

Unidentified β-carboline: The benzene eluates afforded a β-carboline base, R_F, 0.29; Dragendorff, orange; Ehrlich, negative; Fröhde, bluish-purple; blue-violet flurescence under u. v. lamp on papers (characteristic of β-carbolines); u. v.: λ_{max} 239, 245, 270, 292, and 340–42 mμ; i. r.: λ_{max} 2.92 (OH), 6.08 (C=N), 6.15 (C=C), and 13.3 μ (4 adjacent aromatic protons); the picrate crystallized from alcohol as yellow flakes, m. p. 256–58°. M. m. p. with harman picrate, m. p. 256–58° showed depression by about 10°.

Anal. (for base-picrate): Found: N, 15.87.

The chloroform eluates of the column afforded a second crop (0.048 g) of N,N-dimethyltryptamine.

Bufotenine: The methanol washings of the column gave a brown basic gum (0.22 g) which showed two spots, R_F, 0.27 and 0.33 on paper chromatograms. They were separated by preparative paper chromatography on Whatman 3 MM paper. The component, R_F, 0.27 showed u. v.: λ_{max} 224, 272, 277, 292, and 305–10 mμ; the base picrate crystallized from alcohol as orange-yellow needles, m. p. 175–77°: M. m. p. with authentic bufotenine picrate, m. p. 174–77°, remained undepressed.

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Unidentified 5-oxy-indole-3-alkylamine: The component having R_F, 0.33 was also a 5-oxy-indole-3-alkylamine; Dragendorff, orange; Ehrlich, blue; α-nitroso-β-naphthol-nitrous acid, violet; u. v.: λ_{max} 222–226, 277, 288–92, 305–10 mμ. Attempted preparation of picrate with this component failed.

Water-soluble Bases

The aqueous mother liquor, after separation of the chloroform soluble tertiary bases, was treated with a saturated aqueous solution of ammonium reineckate. The precipitated reineckate complex was washed with water, dried (2.4 g), m. p. 268–70° (dec.), and its acetone solution was passed through a column of De-Acidite FF (pH, 8) (Ghosal and Banerjee, 1969).

Choline: The regenerated hygroscopic base was identified as choline by co-chromatography with authentic sample, R_F, 0.29. The base picrate crystallized from alcohol as orange needles, m. p. 240–42°. M. m. p. with choline picrate, m. p. 241°, remained undepressed.

The mother liquor, after the separation of choline reineckate, was cooled, acidified (pH, ~ 1) with sulphuric acid, and some more ammonium reineckate solution was added to it.

Unidentified indole-3-alkylamine: The light pink reineckate complex (0.92 g), m. p. 290–95° (dec.), was decomposed by passing its acetone solution over De-Acidite FF. The regenerated base showed only one spot on papergrams, R_F, 0.36; Dragendorff, orange; Ehrlich, faint purple; α-nitroso-β-naphthol-nitrous acid, dull violet; u. v.: λ_{max} 220, 280, and 305 (sh) mμ. No crystalline derivative of this compound could be prepared.

Isolation of serotonin from the trichomes of pods: The golden yellow trichomes of pods (ca. 50 g) were extracted with alcohol without prior defatting at room temperature for 4 weeks. The alcoholic extract was concentrated under reduced pressure. The alcoholic concentrate showed only one spot, R_F, 0.42 (Whatman 3 MM), which corresponded to that of authentic serotonin. Preparative paper chromatography was done on the same paper. A band around R_F, 0.42 was cut out and it was eluted with alcohol. The eluate was concentrated under reduced pressure. The concentrate was divided into two portions. One was subjected to the usual pharmacological screening for serotonin (smooth muscle preparations) and with the other portion the following tests were performed. Colour reaction: Dragendorff, brownish-orange; Ehrlich, purple changing to blue; α-nitroso-β-naphthol-nitrous acid, violet; Jepson and Stevens (1953) test for N_b-unsubstituted tryptamines, positive; u. v.: λ_{max} 224–26, 288–94, and 305 (sh) mμ. The picrate crystallized from aqueous alcohol as orange-yellow needles, m. p. 191–93°. M. m. p. with authentic serotonin picrate, m. p. 190–92°, remained undepressed.

Pharmacology

A detailed pharmacological screening was made with the indolic bases from *Mucuna pruriens* DC. The results of these investigations, done on cardio-vascular system, central nervous system, smooth and skeletal muscles, are briefly described below.

Smooth and Skeletal Muscles: The indolic bases produced, in varying degrees, non-specific spasmolytic actions on smooth muscle preparations against acetylcholine-, histamine-, serotonin-, and oxytocin-induced spasms. 5-Methoxy-N, N-dimethyltryptamine and the unidentified 5-oxy-indole-3-alkylamines produced neuromuscular blocking activity of the d-tubocurarine type (Ghosal et al., 1969 a). The water-soluble indole-3-alkylamine produced a blocking which was

intensified by washing the tissue (frog rectus abdominis muscle). The unidentified β-carboline produced only potentiation of the acetylcholine response on frog rectus abdominis muscle indicating thereby that the 6-position of the β-carboline is devoid of any oxygen function (Ghosal et al., 1969 b).

Blood Pressure: The alkaloids produced a rise in dog's carotid blood pressure in small doses. Subsequent doses caused tachyphylaxis which was later followed by hypotensive response and again tachyphylaxis. The hypotensive response was found to be histamine-release-mediated (Ghosal et al., 1969 a). In pentobarbitone anaesthetised dogs, administration of 5-methoxy-N, N-dimethyltryptamine and the unidentified 5-oxy-indole-3-alkylamines caused severe respiratory depression, broncho spasm, and acute fall in blood pressure leading to death in higher concentrations. When respiration was maintained in these cases artificially, the drugs produced a pressor response which showed tachyphylaxis on repeated doses. The pressor response was blocked by priscoline and was absent in reserpinised dogs and is, presumably, catechol-amine-release-mediated.

Frog Heart: The alkaloids had no perceptible effects on frog heart up to a dose of 200 μg/ml.

5-Methoxy-N, N-dimethyltryptamine and the other two 5-oxy-indole-3-alkylamines produced hyperactivity in albino rats.

Results and Discussion

Individual parts of *Mucuna pruriens* were investigated for their alkaloid contents. While the trichomes of the pods contained only serotonin, the pods, seeds (both green and mature dry), leaves, and roots afforded several indole-3-alkylamines (except serotonin), viz., N,N-dimethyltryptamine and its N_b-oxide, bufotenine, 5-methoxy-N,N-dimethyltryptamine, and two unidentified 5-oxy-indole-3-alkylamines. In addition, an unidentified β-carboline and choline were also obtained from the various parts of the plant.

Interestingly, in other plant families, such as in the *Urticaceae, Ranunculaceae, Malvaceae,* also where serotonin is reported to occur in certain parts of the plants, it occurs alone (Singh, 1969). Conversely, certain species belonging to other families such as *Desmodium pulchellum* Benth ex Baker (*Papilionaceae*) (Ghosal and Mukherjee, 1966), *Desmodium gangeticum* DC. (*Papilionaceae*) (Ghosal and Banerjee, 1969), *Arundo donax* L. (*Gramineae*) (Dutta and Ghosal, 1967), and several *Acacia* species (*Mimosae*) (Ghosal et al., 1969), which liberally elaborate indole-3-alkylamines do not bear serotonin at all. These determinations, especially the phenomenon that serotonin elaboration by *Mucuna pruriens* takes place only at an advanced stage of the plants' growth, support a previous hypothesis by Ghosal and Mukherjee (1966) that serotonin is not

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the common component enroute to the 5-oxy-indole-3-alkylamines in plants. The common intermediate in the metabolism of tryptophan in plants is, presumably, N,N-dimethyltryptamine. There are ample chemical and biochemical analogies (Ghosal et al., 1969 b; Agurell and Nilson, 1968) for this contention.

The results of pharmacological screening with the total extracts and the individual alkaloids indicated that the curative properties ascribed to the plant extracts are owing, at least in part, to the alkaloids isolated. Thus, it is possible that the basis of the plant extract's use in the indigenous system of medicine as an uterine stimulant lies in the smooth muscle stimulant action of some of the contained indole-3-alkylamines. The promotion of aphrodisiac action is consistent with the presence of 5-methoxy-N,N-dimethyltryptamine in the various parts, the compound being a well known hallucinogenic agent (Ahlborg et al., 1968).

Acknowledgement

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Summary

Four indole-3-alkylamines, viz., N,N-dimethyltryptamine, its N_b-oxide, bufotenine, and 5-methoxy-N,N-dimethyltryptamine along with two uncharacterized 5-oxy-indole-3-alkylamines and a β-carboline were isolated from the various parts, except the trichomes of pods, of *Mucuna pruriens* DC. The trichomes of pods afforded only serotonin. Besides these, choline was found in all parts of the plant.

The above determinations and the existing evidences in the literature would indicate that serotonin is not the common substance in the metabolism of tryptophan enroute to the 5-oxy-indole-3-alkyl amines elaborated by *Mucuna pruriens*. On the other hand, ample chemical and biochemical analogies are drawn to support a hypothesis for the metabolism of tryptophan where N,N-dimethyltryptamine was envisaged as this common substance.

A detailed pharmacological screening of the total extracts and the individual alkaloids was done on cardio-vascular system, central nervous system, and smooth and skeletal muscles. The results indicated that the curative properties ascribed to the plant extracts are owing, at least in part, to the basic constituents isolated.

Zusammenfassung

Aus verschiedenen Organen von *Mucuna pruriens* – mit Ausnahme der Trichome der Hülsen – wurden vier Indol-3-alkylamine, nämlich N,N-Dimethyl-

tryptamin, dessen N_b-Oxid, Bufotenin und 5-Methoxy-N,N-dimethyltryptamin, ferner zwei uncharakterisierte 5-Oxy-indol-3-alkylamine und ein β-Carbolin isoliert. Die Trichome der Hülsen enthielten ausschließlich Serotonin. Darüber hinaus wurde in allen Teilen der Pflanze Cholin gefunden. Ein eingehendes pharmakologisches Screening des Totalextraktes und der einzelnen Alkaloide wurde durchgeführt.

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