DESMODIUM ALKALOIDS
PART II[1] CHEMICAL AND PHARMACOLOGICAL EVALUATION OF *D. GANGETICUM*

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Introduction

The members of the genus *Desmodium (Papilionaceae)* are mostly shrubs, widely distributed in tropical and sub-tropical habitats and particularly abundant in India. They are well known for their various medicinal uses in the Indian system of medicine (Chopra et al., 1956). Among about four dozen *Desmodium* species available in this country, chemical and pharmacological evaluation of *D. pulchellum* Benth. ex Baker (Ghosal et al., 1971) and only preliminary chemical investigation of the entitled species (Ghosal and Banerjee, 1969) have been reported so far. The medicinal uses of the plant extracts and the high alkaloid content of the two aforementioned species prompted us to examine in detail the alkaloid content and pharmacological properties of the available *Desmodium* species. Such investigation would also serve to locate the active principles of the plants with respect to the reported medicinal uses of their crude extracts. The initial chemical investigation with *D. gangeticum* DC has now been complemented by detail chemical and pharmacological evaluation of the species at different stages of its development. The results are reported in this paper.

Experimental

The general procedure for the separation and identification of the $CHCl_3$-soluble alkaloids involved gradient-pH extraction from aqueous AcOH solution; column chromatographic resolution over Brockman neutral alumina; paper chromatography (Whatman 3MM papers) and TLC (silica gel G, E. Merck) of the eluates from column chromatorgaphic runs in presence of markers (Ghosal and Mukherjee, 1966; Ghosal et al., 1971a); preparation of picrate, hydrochloride, and methiodide where possible; and determination of UV, IR, NMR and mass spectra of the single entities. The H_2O-soluble bases were isolated through their reineckate salts under alkaline and acidic conditions and decomposition of EtOH-solution of the complex salts over De-Acidite FF (Ghosal et al., 1970).

Assays with fresh green or air-dried and preserved plants indicated that the alkaloid content of the aerial portions of green plant materials was more than three times the amount present in dry and preserved samples. In case of roots, however, no such difference was discernible.

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[1] Part I in the Series: Chemical and Pharmacological Evalution of *D. pulchellum* Benth. ex Baker, – S. Ghosal, S. K. Banerjee, S. K. Bhattacharya, and A. K. Sanyal, Planta Med. (1972).

Isolation of Alkaloids from the Stem and Leaves of D. gangeticum

The green plant materials (2 kg, wet wt.) were macerated in a Waring blender with a mixture of $CHCl_3$ (6 l) and NH_4OH (15N, 100 ml). The mixture was kept at room temperature, with occasional shaking, for one week. The extract was filtered and the two layers were separated. The $CHCl_3$ layer was extracted with aqueous AcOH (4%, 100 ml), the organic layer containing the $CHCl_3$-soluble acetates (extractives at pH level 4; yield, 3.8 g) was processed in the usual way (Ghosal and Banerjee, 1969). The acidic layer was basified (NH_4OH) and the liberated bases were extracted with $CHCl_3$ (extractives at pH level 9; yield, 5.1 g). The total alkaloids from this fraction showed several Ehrlich and Dragendorff positive spots, not all of which could be isolated by column chromatography. The H_2O-soluble bases remaining in the aqueous mother liquor were isolated through ther reineckates (yield of total bases, 1.3 g). The alkaloids isolated and identified from the aerial portions are listed in Table 1.

Isolation of Alkaloids from the Roots of D. gangeticum

Dried and milled roots (1.6 kg) were continuously extracted (12 hr) with light petroleum (60–80°). The petroleum extract was concentrated under reduced pressure and the concentrate extracted with aqueous citric acid (12%, 200 ml). The crude mixture of bases (0.4 g), obtained from the acidic solution, was separated by column chromatography and fractional crystallization from petroleum (40–60°). The defatted plant material was continuously extracted with EtOH (16 hr). The EtOH-concentrate was poured into aqueous AcOH, the alkaloids were isolated from the clarified acidic solution at three pH levels (extractives at pH 4, yield, 0.9 g; pH 7, 0.95 g; pH 9, 0.55 g). The H_2O-soluble bases were isolated through the reineckate salts in the usual way (yield of total bases, 0.32 g). The alkaloids isolated and identified from the roots are recorded in Table 1.

Isolation of Alkaloids from the Seeds of D. gangeticum

Dried and finely grounds seeds (1 kg) were continuously extracted with petroleum (30 hr) and then with EtOH (16 hr). The petroleum extract did not give any test for alkaloids and was rejected. The alcoholic extract was processed in the usual way for $CHCl_3$-soluble alkaloids (extractives at pH levels 4 and 9, yield, 0.08 g and 0.068 g. respectively) The aqueous mother liquor did not give any appreciable test for alkaloids.

Pharmacology

Among the more important uses of the plant in the Indian system of medicine are the application of the root extracts in diarrhoea, dysentery, in chronic fever and in asthma and those of the aerial portions in eye diseases, biliousness, as a uterine stimulant and aphrodisiac. Pharmacological studies were conducted with the alkaloids, from different parts of the plant, to test the validiy of the aforementioned uses of the plant. The total alkaloids from the roots, stem-leaves, and seeds were used in the present studies, since the individual alkaloids were scarce for complete screening.

Smooth muscle. The total alkaloids from the stem-leaves/seeds in doses of 2–5 mcg/ml elicited spasm in rat uterus which were antagonised in higher doses of the drugs. In doses of 10–20 mcg/ml the drugs showed non-specific spasmolytic action against acetylcholine (0.01 mcg/ml), histamine (0.01 mcg/ml) and barium chloride (0.2 mg/ml) induced spasms in guinea-pig ileum. Similar spasmolytic effects were observed against acetylcholine (0.1 mcg/ml), serotonin (0.01 mcg/ml) and oxytocin (0.01 mcg/ml) in isolated uterus of albino rats. The initial antagonism, in the latter

case, was followed by potentiation of the spasmogenic action upon washing the tissue. In very low concentrations (10^{-7}–10^{-6}), the drugs directly facilitated the action of serotonin in estrus rat uterus. The spasmogenic activity of the alkaloids was blocked by pretreatment of the tissue (rat uterus) with LSD (1 mcg/ml) and the facilitation of serotonin responses was blocked by chlorpromazine (5 mcg/ml). The above effects are typical of drugs which cause hallucination (Costa, 1956; Ghosal, 1971; Ghosal et al., 1971b).

The root alkaloids ($CHCl_3$-solubles) (0.1 mg/kg, I.V.) produced spasm in dog's intestine *in situ* and in isolated rabbit intestine (2–5 mcg/ml). The spasms were blocked by pentolinium.

Skeletal muscle. The total alkaloids from the aerial portions (50–75 mcg/ml) specifically blocked the acetylcholine (5 mcg/ml) induced spasm in frog rectus abdominis muscle without altering the action of KCl (3 mg/ml) in this tissue. The spasmolytic ED_{50} against acetylcholine was calculated by plotting log dose percentage inhibition curves and was found to be 68.5 mcg/ml as against 1.05 mcg/ml of d-tubocurarine.

The root alkaloids (2 mcg/ml) elicited spasm in isolated frog rectus abdominis muscle which was blocked by d-tubocurarine. In higher concentrations (4 mcg/ml), the drug blocked its own spasmogenic action and also of the acetylcholine.

Behavioral study (Observational methods). The effect of the stem-leaves alkaloids (8–10 mg/kg) on oral and parenteral administrations to albino rats and mice were observed with reference to appearance of excitation, hyperactivity, tremors, paralysis of hind limbs, tapping with forelimbs, convulsion and respiratory arrest. The effects of the drug due to pretreatment of the animals with chlorpromazine (5 mg/kg), atropine (100 mg/kg), reserpine (5 mg/kg), and nialamide (10 mg/kg) were also examined. The overt effects, produced by the drug, with the exception of hindlimb paralysis, were almost completely antagonized by pretreatment of the animals with chlorpromazine and reserpine, but not by atropine. The effects were potentiated by nialamide.

The alkaloids of the seeds elicited similar behavioral effects, while the root alkaloids did not produce any significant behavioral change.

Anticholinesterase activity. The anticholinesterase activity of the total alkaloids from the aerial portions was investigated and compared with that of physostigmine using both *in vitro* and *in vivo* methods. *In vitro* studies were conducted by the biological allay technique of Hemsworth and West (1970). The results showed that while physostigmine was active at a concentrations of 2.0 mcg/ml against both true and pseudocholinesterases, the equieffective concentrations of the total alkaloids were 22.8 (sem-leaves) and 28.5 mcg/ml (seeds). *In vivo* studies were carried out by using the chromodacryorrhea test (Burger; 1949). Physostigmine and the drugs were injected I. P., in different doses, to groups of rats 30 minutes before the injection of smaller doses of acetylcholine (0.2 mg/kg, S. C.). The rats were examined for the red tears at 2 min. intervals for 14 min. The doses of physostigmine and the drugs required to produce a positive response in all the animals have been determined as mean of three experiments. The results showed that physostigmine was active at a dose of 0.1 mg/kg while the total alkaloids showed similar actions in doses of 1.78 mg/kg (stem-leaves) and 2.52 mg/kg (seeds).

The root alkaloids did not exhibit any appreciable anticholinesterase activity.

Blood pressure. The total alkaloids from the aerial portions (1 mg/kg, I. V.) produced a depressor response on pentobarbitone sodium (35 mg/kg, I. P.) anesthetised dog's carotid blood pressure. The fall was sharp and the recovery was slow and gradual. There was a slight stimulation of respiration. Subsequent doses of the drug caused tachyphylaxis. The depressor response was either absent or very much reduced when the drug was administered after petreatment of

the animal with one or two doses of d-tubocurarine (0.1 mg/kg, I. V.). Similar changes were observed when the drug was administered first followed by d-tubocurarine. These observations suggest that the mode of depressor response and tachyphylaxis produced by the drug (consisting of indolic bases) are histamine-release mediated (Ghosal et al., 1969).

The root alkaloids (0.1 mg/kg, I. V.) elicited a biphasic response (initial fall followed by a sharp rise) in dog's carotid blood pressure. The onset of action was immediate and the duration was short. The drug also caused a transitory bradycardia followed by a profound tachycardia. The rate and amplitude of respiration were also increased. Pentolinium (5 mg/kg, I. V.) blocked these effects of the drug.

Results and Discussion

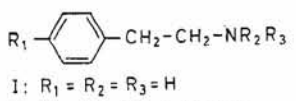
Several carboxylated and decarboxylated tryptamines together with a number of β-phenethylamines were isolated from the roots of *D. gangeticum* while the stem and leaves furnished indole-3-alkylamines and β-carbolines. The number and content of alkaloids in the seeds were considerably poor than in other parts of the plant. The alkaloids isolated and identified so far from the individual parts of *D. gangeticum* are recorded in Table I.

Table I

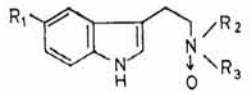
Alkaloids of *Desmodium gangeticum*

Part of plant	Petroleum extract	Extractives at pH levels 4	7	9	H_2O-Soluble Bases
Roots	I, IV	II, IV–VI	III, IX	III, VIII	VIII, XII
Stem-leaves	IV	IV, V, XI	–	IV–VII	X, quaternary indole bases
Seeds	–	β-Carboline, unidentified indole bases	–	IV, VI	–

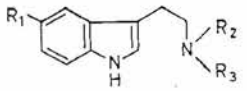
A new paper and thin layer chromatographic staining reagent was developed for detecting *p*-hydroxy-β-phenethylamines present in the roots of *D. gangeticum*. The reagent, α-nitroso-β-naphthol-nitrous acid, was very sensitive to the above alkaloids and only 5–7 mcg base was required to produce a reddish-purple colouration. The corresponding methoxy compound did not give any characteristic colour with this reagent. The reagent was previously used by Udenfriend et al. (1955) for detecting hydroxyindoles. It also served in the present investigation to distinguish between *p*-hydroxy-β-phenethylamines and 5-hydroxy/methoxy tryptamines, since the reddish-purple colour produced by the hydroxy-β-phenethylamines turned yellow within a few hours while the violet colour produced by the indolic bases remained stable for a long time.



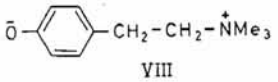
I: $R_1 = R_2 = R_3 = H$
II: $R_1 = OH$; $R_2 = H$; $R_3 = Me$
III: $R_1 = OH$; $R_2 = R_3 = Me$



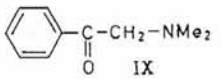
IV: $R_1 = H$; $R_2 = R_3 = Me$
V: $R_1 = OMe$; $R_2 = R_3 = Me$



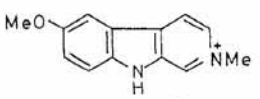
VI: $R_1 = H$; $R_2 = R_3 = Me$
VII: $R_1 = OMe$; $R_2 = R_3 = Me$



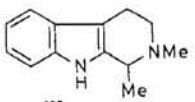
VIII



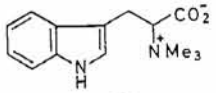
IX



X



XI



XII

Although tetrahydroharman and N_b-methyltetrahydroharman are not uncommon in the family *Leguminosae* (Johns et al., 1966), the quaternary β-carbolinium alkaloid of the type (X) appears to have been isolated only from the *Loganiaceae* (Bächli et al., 1957). This is for the first time that the quarternary β-carboline alkaloid (X) has been isolated from a natural source. The corresponding O, N-didemethyl tetrahydro analogue of (X) has previously been reported to occur in *Plectocomiopsis geminiflorus* Becc. (Palmae) (Kiang et al., 1967). Further, the isolation of candicine (VIII) from a leguminous plant as this is also a new observation.

The alkaloid versatility of *D. gangeticum* obviously points it to be a phylogenetically well developed species in the sub-family *Papilionaceae*. Although other kind of chemical information, e. g., elaboration of branched chain *n*-alkanes, oxygen heterocycles, and triterpenes by plant species are often employed in many instances for systematic studies, no significant information from the study of non-nitrogenous constituents of *D. gangeticum* was available. This fraction from the plant consisted of only *n*-alkanes (C_{27}-C_{29}), *n*-alkanols (C_{26}-C_{30}), a typical mixture of β-sitosterol, stigmasterol and campesterol, and traces of an unidentified phenolic compound. Similar observation was also made during the study of non-nitrogenous constituents of the sister species, *D. pulchellum*. The significance of the alkaloid information (variation in number, content and types) in the systematic classification of plants of this genus is thus established.

The alkaloid variation of the different parts of *D. pulchellum*, at different stages of its development, has already been reported (Ghosal et al., 1971). The alkaloid content of the individual parts of *D. gangeticum* in young seedlings was found to be much smaller than in the mature plants (in fruits). Moreover, the elaboration of β-carbolines by the plant was discernible only at a later stage of its development.

The plants, in the present investigation, were collected from the University area during June-September 1967 and were properly identified. A dried specimen of the mature plant from the plant population under investigation has been preserved.

The pharmacological properties exhibited by the alkaloids of *D. gangeticum* would seem to indicate that the medicinal properties ascribed to the plant extracts reside essentially in the alkaloidal constituents. It is also apparent from these studies the reason for using different portions of the species for different purposes. The anticholinesterase, smooth muscle stimulant and CNS stimulant effects, produced by the alkaloids from the aerial portions, are consistent with the reported uses of the vegetable drug in eye diseases, as a uterine stimulant and as an aphrodisiac, respectively. The presence of catecholamine liberators (β-phenethylamines) in the roots, on the other hand, is consistent with the uses of the root extracts in the treatment of asthma and in related respiratory troubles. Most of the other curative properties, e. g., diuretic and antidysenteric properties, ascribed to these parts of the plant would seem to be due to the major base, hordenine, which is known to increase the flow of urine and as a remedy for diarrhoea and dysentery (Barger and Dale, 1910).

Summary

Twelve alkaloids (I–XII) comprising of four broad structural types, viz., carboxylated and decarboxylated tryptamines, β-carbolines and β-phenethylamines, have been isolated from different parts of *Desmodium gangeticum* DC at different stages of its development. Two alkaloids, viz., (IX, X), were previously unreported in nature. The occurrence of candicine (VIII) in a leguminous plant is also a new observation.

The alkaloid versatility of *D. gangeticum* points it to be a phylogenetically well developed species in the sub-family *Papilionaceae*. The significance of alkaloid information in the systematic classification of plants belonging to the genus *Desmodium* has also been demonstrated.

Pharmacological screening was conducted with the total alkaloids from different parts of the plant to examine the validity of the reported uses of the plant extracts in the Indian system of medicine. Four major responses were discernible from the examination of alkaloids from the aerial portions. These are anticholinesterase, smooth muscle stimulant, CNS stimulant and depressor responses. The presence of catecholamine liberators (*tert*-β-phenethylamines) and candicine in the roots is responsible for a nicotinelike effect on dog's intestine in situ and carotid blood pressure. The results would seem to indicate that the medicinal properties of the plant essentially reside in the contained alkaloids.

References

Barger, G., and Dale, H. H.: J. Physiol. **41**, 19 (1910)
Bächli, E., Vamvacas, C., and Schmid, H.: Helv. chim. Acta **40**, 1167 (1957)
Burger, A. S. V.: Brit. J. Pharmacol. **4**, 185 (1949)
Chopra, R. N., Nayar, S. L., and Chopra, I. C.: Glossary of Indian Medicinal Plants, p. 94 (C.S.I.R., New Delhi: 1956)
Costa, E.: Psychiat. Res. Rep. **4**, 11 (1956)
Ghosal, S., and Mukherjee, B.: J. Org. Chem. **31**, 2284 (1966)
Ghosal, S., and Banerjee, P. K.: Aust. J. Chem. **22**, 2029 (1969)
Ghosal, S., Dutta, S. K., Sanyal, A. K., and Bhattacharya, S. K.: J. Med. Chem. **12**, 480 (1969)
Ghosal, S., Banerjee, P. K., and Banerjee, S. K.: Phytochem. **9**, 429 (1970)
Ghosal, S.: Planta Med. **21**, 200 (1972)
Ghosal, S., Banerjee, S. K., Bhattacharya, S. K., and Sanyal, A. K.: Planta Med. **21**, 398 (1972)
Ghosal, S., Singh, S., and Bhattacharya, S. K.: Planta Med. **19**, 279 (1971a)
Ghosal, S., Majumder, U. K., and Bhattacharya, S. K.: J. Pharm. Sci. **60**, 1209 (1971b)
Hemsworth, B. A., and West, G. B.: J. Pharm. Sci. **59**, 118 (1970)
Johns, S. R., Lamberton, J. A., and Sioumis, A. A.: Aust. J. Chem. **19**, 1539 (1966)
Kiang, A. K., Chan, K. C., and Taylor, W. I.: Lloydia **30**, 189 (1967)
Udenfriend, S., Weisbach, H., and Clark, C. T.: J. Biol. Chem. **215**, 337 (1955)

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