Phytochemistry, 1973, Vol. 12, pp. 193 to 197. Pergamon Press. Printed in England.

β-PHENETHYLAMINE, TETRAHYDROISOQUINOLINE AND INDOLE ALKALOIDS OF *DESMODIUM TILIAEFOLIUM**

S. GHOSAL and R. S. SRIVASTAVA

Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India

(*Received* 23 *May* 1972. *Accepted* 15 *August* 1972)

Key Word Index—*Desmodium tiliaefolium*; Leguminosae; alkaloids; β-phenylamines; salsoline; abrine; hypaphorine; tetrahydro-isoquinolines.

Abstract—Ten alkaloids (I–X), five β-phenethylamines, two tetrahydroisoquinolines, and three indole-3-alkylamines, have been isolated from *Desmodium tiliaefolium*. Chemical transformations, spectral (UV, IR, NMR, MS) evidence, and in most cases comparison with reference materials established their identity as tyramine (I), hordenine (II), 3,4-dimethoxy-β-phenethylamine (III), *N,N*-dimethyl-3,4-dimethoxyphenethylamine (IV), *N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine (V), salsoline (VI), salsolidine (VII), tryptamine (VIII), abrine (IX), and hypahorine (X). Alkaloid (V) is a new naturally occurring compound, while no tetrahydroisoquinoline alkaloid has been encountered before in this genus. This is the first time that three different types of alkaloids have been reported in a single legume species. In addition to the above alkaloids, four quaternary β-phenethylamines and tetrahydroisoquinoline alkaloids have been detected, and choline and betaine have been isolated from the water-soluble alkaloid fraction of the roots.

INTRODUCTION

Desmodium tiliaefolium G. Don (Leguminosae), growing along the Himalayas from the upper Punjab to Tavoy, in temperate and tropical regions up to 9000 ft.,[1] is one of about three dozen species of this genus available in India.[1–3] Investigations of a number of *Desmodium* species, in the authors' laboratory, revealed[4–10] that they are rich in alkaloids. Considerable amounts of alkaloid were isolated from different parts of the above species, in preliminary work.[8,10] The present paper complements the previous reports regarding the isolation of alkaloids from the roots and structure elucidation of these alkaloids.

RESULTS AND DISCUSSION

Gradient-pH extraction,[11] fractionation into phenolic and non-phenolic bases over ion-exchange resin column, and separation into individual entities by column chromatography and preparative TLC[12] led to the isolation of five β-phenethylamines (I–V), two tetrahydroisoquinolines (VI, VII), and three indole-3-alkylamines (VIII–X), from the roots of

* Part V in the series "*Desmodium* Alkaloids". Part IV see S. GHOSAL, R. S. SRIVASTAVA, S. K. BHATTACHARYA and P. K. DEBNATH, *Planta Med.* **21** (1972) in press.

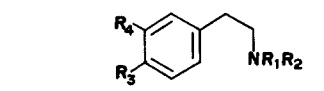
[1] R. N. CHOPRA, S. L. NAYAR and I. C. CHOPRA, *Glossary of Indian Medicinal Plants*, p. 94, C.S.I.R., New Delhi (1956).
[2] J. S. GAMBLE, *Flora of the Madras Presidency*, Vol. I, p. 243, Botanical Survey of India, Calcutta (1935).
[3] J. D. HOOKER, *Flora of British India*, Govt. Printer, London (1879).
[4] S. GHOSAL and B. MUKHERJEE, *J. Org. Chem.* **31**, 2284 (1966).
[5] P. K. BANERJEE and S. GHOSAL, *Austral. J. Chem.* **22**, 275 (1969).
[6] S. GHOSAL and P. K. BANERJEE, *Austral. J. Chem.* **22**, 2029 (1969).
[7] S. GHOSAL, P. K. BANERJEE and R. S. SRIVASTAVA, *Phytochem.* **10**, 3312 (1971).
[8] S. GHOSAL, U. K. MAZUMDER and R. MEHTA, *Phytochem.* **11**, 1863 (1972).
[9] S. GHOSAL, S. K. BANERJEE, S. K. BHATTACHARYA and A. K. SANYAL, *Planta Med.* **21**, 398 (1972).
[10] S. GHOSAL, *Planta Med.* **21**, 200 (1972).
[11] S. GHOSAL, P. K. BANERJEE and S. K. BANERJEE, *Phytochem.* **9**, 429 (1970).
[12] J. LUNDSTRÖM and S. AGURELL, *J. Chromatogr.* **30**, 271 (1967).

D. tiliaefolium. The alkaloids were identified by chemical transformations and comparison with authentic reference materials using TLC, UV, IR, NMR, and MS of the compounds and their derivatives. Alkaloid (V) has not been previously isolated from natural sources or prepared synthetically. No tetrahydroisoquinoline alkaloid has been encountered before in the genus *Desmodium*.

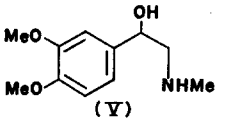
In addition to the above alkaloids, the ubiquitous choline, betaine, and four quaternary β-phenethylamine and tetrahydroisoquinoline bases were isolated from the water-soluble alkaloid fraction.

The stems of *D. tiliaefolium* contain essentially the same alkaloids, as in the roots, but in poorer yields. The amount of bases was least in the leaves, which contained mainly carboxylated tryptamines and only traces of the β-phenethylamines.

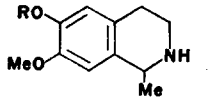
Phenolic β-phenethylamines and tetrahydroisoquinolines, containing a free NH group, develop cherry red or purple colours with Ehrlich reagent. The ready conversion of the two types of alkaloids into an indolic chromophore has precedence in *N*-norprotosinomenine and in some phenolic spiroamine (Erythrina) alkaloids.[13–15] A free-radical mediated transformation of salsoline (VI), into an indolic chromophore (Ehrlich-positive) has been accomplished. Dilute solutions of salsoline, in carbon tetrachloride, developed the characteristic yellow colouration of *ind-N*-vinyl polymers[16] in presence of sunlight. The resulting compound showed abundant fragment ions in its MS due to the entities (XI) and (XII) at *m/e* 164 and *m/e* 149, respectively. The reaction was sensitive to oxygen in rate and MW of the polymer.



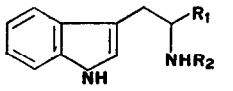
(I) $R_1 = R_2 = R_4 = H$; $R_3 = OH$
(II) $R_1 = R_2 = Me$; $R_3 = OH$; $R_4 = H$
(III) $R_1 = R_2 = H$; $R_3 = R_4 = OMe$
(IV) $R_1 = R_2 = Me$; $R_3 = R_4 = OMe$



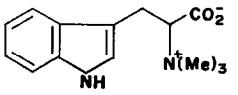
(V)



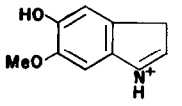
(VI) R = H
(VII) R = Me



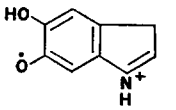
(VIII) $R_1 = R_2 = H$
(IX) $R_1 = CO_2H$; $R_2 = Me$



(X)



(XI)



(XII)

So far, all reported[4–10] alkaloids of about a dozen *Desmodium* species are either indole or β-phenethylamines or a combination of the two types. Co-occurrence of another combination of structural patterns, viz. β-phenethylamine and tetrahydroisoquinoline, is known in the Cactaceae, a family which liberally elaborates β-phenethylamines.[17] However, the tetrahydroisoquinoline alkaloids occurring in cactii species have different oxygenation and

[13] S. GHOSAL, S. K. MAJUMDAR and A. CHAKRABORTI, *Austral. J. Chem.* **24**, 2733 (1971).
[14] S. GHOSAL, R. S. SRIVASTAVA and A. CHAKRABORTI, *Phytochem.* **11**, 2101 (1972).
[15] S. GHOSAL, S. K. DUTTA and S. K. BHATTACHARYA, *J. Pharm. Sci.* **61**, 1274 (1972).
[16] M. BISWAS and S. GHOSAL, *Chem. & Ind.* 1717 (1966).
[17] J. J. WILLIAMAN and H. LI, *Lloydia* **33**, No. 3A (1970).

O-methylation patterns than those found in *D. tiliaefolium*. This is the first time that the simultaneous occurrence of three alkaloid types, viz. β-phenethylamine, tetrahydroisoquinoline, and indole-3-alkylamine, is reported in a single plant species.

Although β-phenethylamines are widely distributed in nature, simple tetrahydroisoquinoline alkaloids appear to be confined to a limited number of families, viz. Cactaceae, Fumariaceae, Chenopodiaceae, and Leguminosae. Of these, the tetrahydroisoquinolines found in the Cactaceae alone outnumber those reported in the other families combined. The occurrence of tetrahydroisoquinoline alkaloids in the Leguminosae has been previously reported only twice.[17] The accumulation of the varied alkaloid patterns in a number of *Desmodium* species could be interpreted in terms of 'divergence' phenomenon.[18] In this respect, *D. tiliaefolium* is comparable to *Calycotome spinosa* Link and *Genista purgens* Poir, two species which elaborate tetrahydroisoquinoline alkaloids calycotomine and salsolidine, respectively, while related species in the same genera synthesize papilionaceous alkaloids.[17,18] As pointed out earlier, one basic difference between the tetrahydroisoquinoline alkaloids occurring in cactus and those in leguminous plants, is the degree of oxygenation and the pattern of *O*-methylation. Thus, while the tetrahydroisoquinolines of the Cactaceae are derived from 3,4,5-trioxygenated β-phenethylamine precursors with a free hydroxyl group *ortho* to the point of ring closure,[19] those occurring in the Leguminosae are derived from 3,4-dioxygenated β-phenethylamines and, presumably, require a free hydroxyl group *para* to the point of ring closure. Laboratory analogies for the latter phenomenon have been provided.[20] *O*-Methylation, leading to the nonphenolic tetrahydroisoquinolines, salsolidine and calycotomine, would seem to take place at a later stage of their biogenesis. This hypothesis is now being tested with labelled precursors.

EXPERIMENTAL

M.ps were determined on a Toshniwal apparatus in open capillary and are uncorrected. UV were recorded in aldehyde-free EtOH. IR were determined in Nujol or KBr pellets and only the major bands are quoted. NMR were taken in $CDCl_3$ or $(CD_3)_2CO$. MS were determined by the Central Drug Research Institute, Lucknow. Separation by column chromatography was carried out over Brockmann neutral alumina (activity grade *ca.* III). TLC was carried out on silica gel G (Merck) using mainly $CHCl_3$–EtOH–$NHEt_2$ (17:1:2) (Solvent 1) and $CHCl_3$–*n*-BuOH–NH_4OH (20:20:1) (Solvent 2). Dragendorff (D), Ehrlich (E), and tetrazotized *ortho*-dianisidine (TDA), were used for staining purposes. Amberlite IRA-400 (OH^-) resin was used for separating the phenolic and non-phenolic alkaloids and De-Acidite FF (OH^-) was used for regeneration of bases from the reineckate salts.

Extraction of alkaloids with light petrol. Dried and ground roots of *D. tiliaefolium*[21] (2·3 kg) were continuously extracted with light petrol. (60–80°) for 16 hr. The petrol. extract was concentrated (*ca.* 200 ml) and poured into aqueous citric acid solution (12%, 200 ml). The suspension was stirred mechanically for 8 hr. The clarified acidic solution, upon basification (NH_4OH) and extraction with $CHCl_3$ (3 × 50 ml), afforded a thick brown basic gum (2·8 g). Preliminary purification was carried out by column chromatography (24 × 1·5 cm) with light petrol. (40–60°), benzene, $CHCl_3$, and $CHCl_3$–MeOH (19:1) as eluents. 40 ml-Fractions were collected. The middle $CHCl_3$ eluates gave a pale yellow syrup which showed two major spots on TLC, R_f 0·32 (D, positive; E, negative; TDA, yellow, Solvent 2), 0·71 (D, negative; E, purple changing to blue; TDA, negative). These were separated by preparative TLC using Solvent 2. Two zones were cut around R_f 0·3 and 0·7, the alkaloids being eluted with absolute methanol.

Tryptamine (VIII). The MeOH eluate of the upper zone gave a pale brown gum (17 mg), R_f 0·73 (co-TLC with authentic tryptamine); UV: λ_{max} 222 (log ε, 4·42), 272 (log ε, 3·93), 294 nm (log ε, 3·90); MS: *m/e* 160 (M^+, 12%), significant peaks at *m/e* 144 (8), 130 (100), 117 (14), 116 (32), 103 (9), 77 (5).

[18] R. HEGNAUER, in *Comparative Phytochemistry* (edited by T. SWAIN), p. 211, Academic Press, London (1966).
[19] S. AGURELL and J. LUNDSTROM, *Chem. Commun.* 1638 (1968).
[20] A. CHATTERJEE and N. ADITYACHAUDHURY, *J. Org. Chem.* **27**, 309 (1962).
[21] The plant material used in this investigation was obtained from Mr. R. SARKER, United Chemical & Allied Products, 10 Clive Row, Calcutta-1, where a voucher specimen has been preserved.

3,4-*Dimethoxy-β-phenethylamine* (III). The MeOH eluate of the lower zone gave a brown gum (14 mg); R_f 0·32 (co-TLC with a synthetic sample); UV: λ_{max} 205 (log ϵ, 4·55), 225–228 (log ϵ, 4·05), 282–285 nm (log ϵ, 3·59); MS: *m/e* 181 (M^+, 8%), significant peaks at *m/e* 166 (6), 152 (19), 151 (100), 137 (12), 136 (22), 30 (43).

Tyramine (I). The $CHCl_3$–MeOH (19:1) eluates afforded a brown gum from which tyramine crystallized out as straw coloured leaflets (48 mg) from alcohol, m.p. and m.m.p. 158–161°; R_f 0·28 (D, brown; E, faint purple; TDA, yellow; Solvent 1); UV: λ_{max} 208, 230, 275 nm; MS: *m/e* 137 (M^+, 24%), 108 (9), 107 (87), 94 (19), 93 (100), 41 (21), 30 (58).

Extraction of alkaloids with alcohol. The defatted plant material was continuously extracted with alcohol for 16 hr. The alcoholic extract was evaporated to yield a thick brown residue (105 g). It was poured into aq. HOAc (4%, 200 ml). The clarified acidic solution was extracted with $CHCl_3$ (4 × 50 ml) to remove the $CHCl_3$-soluble acetates (Fraction 1). The aq. solution was basified and the liberated bases were extracted with $CHCl_3$ (Fraction 2). The aq. alkaline mother liquor (pH ~ 9) was treated with a satd. aq. ammonium reineckate. The precipitated reineckate salt (Fraction 3) was filtered and the filtrate was brought to pH ~ 6. Ammonium reineckate solution was again added when a further crop of reineckate complex precipitated (Fraction 4). The aq. mother liquor was strongly acidified (pH < 1) and kept overnight at room temp. when reineckate salts of weakly basic water-soluble alkaloids precipitated (Fraction 5).

Chloroform-soluble acetates (*Fraction* 1). The residue (18 g) from the $CHCl_3$-soluble acetates was triturated with aq. HCl (2 N, 50 ml). The acidic solution was extracted with $CHCl_3$, to separate the soluble and insoluble alkaloid hydrochlorides. The $CHCl_3$-soluble hydrochloride fraction (228 mg) showed two major spots on TLC plates, one produced purple (phenolic) and the other brown colours (non-phenolic) with the TDA reagent. A concentrated methanolic solution of the bases was applied to a column of Amberlite IRA-400 (OH^-) (20 × 1 cm). The column was washed with aq. MeOH (30%, 200 ml) to yield the non-phenolic alkaloid. The phenolic alkaloid was obtained by elution of the column with MeOH–H_2O–HOAc (6:3:1).

Salsolidine (VII). The non-phenolic alkaloid was obtained as a thick brown liquid (72 mg); R_f 0·42 (D, orange; E, negative; TDA, brown; co-TLC with authentic salsolidine in Solvent 2); UV; λ_{max} 212 (log ϵ, 4·08), 232 (log ϵ, 3·99), and 285 nm (log ϵ, 3·84); MS: *m/e* 207 (M^+, < 2%), significant peaks at *m/e* 206 (14), 192 (100), 163 (7), 162 (3), 154 (6), 153 (17), 151 (5), 149 (8), 133 (4), 43 (21); $[\alpha]_D^{25}$ −0; NMR ($CDCl_3$): δ 6·30 (2H, aromatic protons), 3·92 (6H, OMe), 1·35 (3H, C_1–Me).

Salsoline (VI). The phenolic alkaloid crystallized from acetone containing a trace of MeOH as straw coloured micro needles (32 mg), m.p. 218–222°; R_f 0·48 (D, orange; E, cherry red on keeping; TDA, purple; Solvent 1); IR (KBr): ν_{max} 3450 (OH), 3318 (NH), 1627, 1532, 1030 cm^{-1}; UV: λ_{max} 210–212, 230 (sh), 285–288 nm; MS: *m/e* 193 (M^+, 3%), significant peaks at *m/e* 192 (12), 178 (100), 164 (5), 163 (17), 149 (11), 134 (7), 122 (6), 43 (25); NMR [$(CD_3)_2CO$]: δ 6·22 (*s*, 2H, aromatic protons), 3·88 (*s*, 3H, OMe), 4·16 (*q*, 1H, C_1–H), 1·38 (*d*, 3H, C_1–Me). Controlled (TLC) methylation of the alkaloid with ethereal diazomethane gave salsolidine (TLC, superimposable IR spectra). The aq. solution, containing the $CHCl_3$-insoluble hydrochlorides (from Fraction 1), upon basification and subsequent extraction with $CHCl_3$ afforded a brown amorphous material (117 mg). It was dissolved in $CHCl_3$ (5 ml) and the solution was passed over a column of alumina (24 × 1·5 cm). Elution was carried out with benzene, $CHCl_3$, and $CHCl_3$–MeOH (19:1). 40 ml-Fractions were collected.

N,N-*Dimethyl*-3,4-*dimethoxyphenethylamine* (IV). The first $CHCl_3$ eluates, upon evaporation, gave a brown liquid (41 mg); R_f 0·34 (major), 0·28 (minor) (D, orange; E, negative; TDA, yellow; Solvent 2). The major component was separated by preparative TLC; UV: λ_{max} 205 (log ϵ, 4·56), 230–232 (log ϵ, 4·15), and 284 nm (log ϵ, 3·95); MS: *m/e* 209 (M^+, < 3%), 194 (4), 179 (8), 152 (10), 151 (19), 149 (5), 136 (5), 121 (3), 58 (100); NMR ($CDCl_3$): δ 6·97 and 6·88 (3H, aromatic protons), 3·98 (3H, OCH_3), 3·92 (3H, OMe), 3·02 (4H, $-CH_2-$), 2·38 [$N(Me)_2$]. The base hydrochloride crystallized from $CHCl_3$ as microneedles, m.p. 192–194° (lit[22] 193–196°).

N-*Methyl*-3,4-*dimethoxy-β-hydroxyphenethylamine* (V). The later $CHCl_3$ eluates also showed two spots, R_f 0·28 (major) and 0·34 (minor). The major component was separated by preparative TLC. It was obtained as a brown gum (28 mg); UV: λ_{max} 208 (log ϵ, 4·45), 230 inflec. (log ϵ, 4·08), 285 nm (log ϵ, 3·95); $[\alpha]_D^{24}$ −38·4° (*c* 0·41, $CHCl_3$); MS: *m/e* 211 (M^+, 8%), significant peaks at *m/e* 196 (M-15, 5), 194 (M-17, 5), 193 (M-18, 3), 178 (14), 167 (12), 166 (17), 165 (42), 151 (100), 150 (62), 72 (7), 43 (58), 29 (21), the postulated structures of which are compatible with the expected fragmentation of the alkaloid.

Chloroform-soluble strong bases (*Fraction* 2). The alkaloids from this fraction showed four major Dragendorff-positive spots on TLC. The mixture of alkaloids was separated into phenolic and non-phenolic bases by passing its methanolic solution over Amberlite IRA-400 (OH^-) resin column. The non-phenolic alkaloid fraction gave a further crop of *N,N*-dimethyl-3,4-dimethoxyphenethylamine (9 mg) plus another unidentified β-phenethylamine (5 mg) by subsequent preparative TLC. The phenolic fraction afforded another crop of tyramine (traces) and hordenine.

[22] S. AGURELL, J. LUNDSTRÖM and A. MASOUD, *J. Pharm. Sci.* **58**, 1413 (1969).

Hordenine (II). It crystallized from light petrol.–benzene as light brown coloured needles, m.p. 115–117° (12 mg); R_f 0·50 (D, orange; E, faint purple upon keeping; Solvent 1); UV: λ_{max} 224 and 277 nm; Superimposable IR with authentic hordenine:[6] ν_{max} 3454, 2838, 1627 cm^{-1}; MS: *m/e* 165 (M^+, 14%), significant peaks at *m/e* 149 (5), 121 (18), 107 (16), 58 (100).

Water-soluble bases (*Fractions* 3, 4 *and* 5). The water-soluble bases were regenerated from their reineckate salts by passing the ethanolic solutions of the reineckate salts over De-Acidite FF (OH^-).[11]

Choline. The base from Fraction 3 was obtained as a hygroscopic solid (83 mg); R_f 0·20 (*n*-BuOH–AcOH–H_2O, 4:1:5); the TLC behavior and the pharmacological properties of the base were consistent with those of choline.[23] The base picrate crystallized from alcohol as orange needles, m.p. and m.m.p. 242°.

Quaternary β-phenethylamines and tetrahydroisoquinolines. The regenerated bases from Fraction 4 showed three major and one minor Dragendorff-positive spots on TLC, all of which had low R_fs, 0–0·16 (Solvents 1 and 2). The mixture did not give any molecular-ion peak in its MS as it fragmented before volatilization. TDA showed both yellow and purple spots with the mixture on TLC plates. UV: λ_{max} 205, 212, 230, 275 (sh), 280–285 nm, indicating the presence of phenolic β-phenethylamine and tetrahydroisoquinoline bases.

Hypaphorine (X). Fraction 5, upon processing in the usual way, afforded a colourless solid which crystallized from acetone-MeOH as needles (68 mg), m.p. 250–252°; UV: λ_{max} 224, 275, 294 nm; IR ν_{max} 1618 (CO_2^-, betaine); the alkaloid hydrochloride crystallized from alcohol as colourless needles, m p. and m.m.p. 232°.

Abrine (IX). The acetone–MeOH mother liquor showed 2 spots on TLC, R_f 0·52 (D, negative; E, purple changing to blue) and 0·18 (D, yellowish-orange; E, negative) (*n*-BuOH–AcOH–H_2O, 4:1:5). These were separated by preparative TLC. The upper spot (13 mg) showed UV: λ_{max} 222–224, 277, 292–294 nm; IR: ν_{max} 3481, 3395, 1710 cm^{-1}. Co-TLC and superimposable IR spectra indicated its identity with abrine.

Betaine. The lower spot (6 mg) was identified as betaine by co-chromatography with an authentic sample of betaine in three solvents and superimposable IR spectra.

Polymerization of salsoline. On keeping salsoline (*ca.* 0·2–0·5 M) in pure CCl_4 in the dark no colouration and no polymerization was detected even after 2 days. Similar solutions showed a yellow colouration within a few hr exposure to direct sunlight at 32° and furnished polymers (yield, *ca.* 2%/24 hr from a 0·25 M salsoline solution in CCl_4 in presence of air, m.p. > 360°; no molecular-ion peak, significant fragment-ions at *m/e* 164 and *m/e* 149; E, purple; Beilstein test for chlorine positive). In diffused sunlight the colouration was gradual and the yield of the polymer was low (< 0·5% 24 hr). In the absence of O_2, the rate of polymerization was very slow but the MW of the polymer was significantly increased.

Acknowledgments—The authors are grateful to Professor Gurbux Singh, Department of Chemistry, Banaras Hindu University, for kindly extending to them the facilities of using the UV, IR, and NMR instruments, and to Mr. Bepin Behari, for running the spectra. Grateful thanks are also accorded to Dr. Nitya Nand, Central Drug Research Institute, Lucknow, for the mass spectra, and to Dr. S. C. Pakrashi, Indian Institute for Experimental Medicine, Calcutta, for the optical rotation data. The junior author (RSS) thanks the C.S.I.R., New Delhi, for the award of a research fellowship.

[23] S. GHOSAL and S. K. DUTTA, *Phytochem.* **10**, 195 (1971).