ALKALOIDS OF DESMODIUM ADSCENDENS

By

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Summary

Five alkaloids: three β-phenethylamine, one tetrahydroisoquinoline and one indole-3-alkylamine have been isolated from the stem-leaves of Desmodium adscendens, a plant used in Ghana for the management of asthma. Spectral (Ultraviolet, infrared, mass spectral) evidence and comparison with reference materials established their identities as tyramine, N,N-dimethyltyramine, 3,4-dimethoxy-β-phenethylamine, salsoline and N,N-dimethyltryptamine. In addition several unidentified indole and other minor basic components were detected by thin layer chromatography.

Key Words: Desmodium adscendens, Papilionaceae, antiasthmatic, β-phenethylamines, salsoline, N,N-dimethyltryptamine.

Introduction

Desmodium adscendens (SW) DC. var. adscendens (Papilionaceae) is a forest shrub that grows up to 90m under the shades of cocoa trees in many parts of Ghana. Sixteen species of the genus Desmodium are identified in tropical Africa, nine of which have been reported in Ghana[1]. .Forty-eight species of the genus have been identified in India. Phytochemical work on some of the species in India by Ghosal and coworkers has revealed the occurence of several simple indole-3-alkylamines, β-phenethylamines, tetrahydro-isoquinolines and α-carbolines[2-9]. These alkaloids show a wide range of interesting biological properties including anti-asthmatic activity. The occurrence of these alkaloids has also been reported in some species outside India [10].

In Ghana, the stem-leaves of *D. adscendens* are used by the rural population for the management of asthma. The plant is also under investigation at the Centre for Scientific Research into Plant Medicine, where a herbal clinic is in operation[11].

Laboratory investigation into the scientific basis for the therapeutic use of the plant material revealed that extracts administered orally to guinea pigs reduced anaphylactic contractions and inhibited histamine-induced contration of isolated ileum[12]. The extract also reduced the amount of histamine present in, and the quantity of this and other spasmogens released from the lung tissues of sensitized guinea pigs. These effects were shown to be dose-dependent[13].

The only phytochemical work reported on *D. adscendens* is contained in a research project which gave preliminary report of the presence of some tryptamine derivatives[14]. However, the identi-

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ties of these derivatives could not be established due to lack of reference materials. Since alkaloids of some Desmodium species have been reported to have anti-asthmatic properties[7,8], the alkaloids of *D. adscendens* have been characterized as part of our study of the anti-asthmatic potential of this species.

Materials and Methods

Materials

Tyramine, hordenine (N,N-dimethyltyramine), 3,4-dimethoxy-β-phenethylamine, N,N-dimethyltryptamine and salsoline were purchased from Sigma (St. Louis, MO, U.S.A.). *Desmodium adscendens* was collected from under cocoa trees on the plantation belonging to the Cocoa Research Institute of Ghana (CRIG). The plant was identified by the resident taxonomist of the Ghana Herbarium where a voucher specimen has been deposited.

Methods

Fractionation and Isolation

Stem-leaves of *D. adscendens* harvested from CRIG were freed of sand and other debris and solar dried using a solar dryer. The dried material was pulverished and 1.6 kg of sample were initially de-fatted with hot hexane and then extracted with hot methanol. The methanol extract was evaporated and freeze dried, the residue weighed 148g. It was dissolved in 4% aqueous acetic acid solution and extracted at two pH levels (4 and 9) with chloroform and 1-butanol to obtain the chloroform-solubles (1.6g) and the 1-butanol-solubles (4.8g).

Purification

Preliminary purification of the alkaloids from the chloroform-soluble fraction was by silica gel column chromatography (35 x 3 cm). The column was eluted with chloroform and then chloroform-methanol (7:3). The alkaloids from the butanol-soluble fraction were purified using the same type of column with 95:5 and 85:15 chloroform-methanol as eluents. 20-ml fractions were collected. The eluates were further purified by preparative thin layer chromatography using isopropanol-ammonia-water (9:1:1) as solvent.

Identification

Thin Layer Chromatography was accomplished using 0.25 mm coated plates of silica gel $60F_{254}$, and the solvents isopropanol-ammonia-water (9:1:1) and chloroform-ethanol (9:1). Dragendorff, Ehrlich and ultraviolet light were used for visualisation. Melting points were uncorrected, and ultraviolet spectra were recorded in aldehyde-free ethanol. The identification of compounds was by comparison of spectra with authentic reference samples. In addition, crystalline compounds were identified by mixed melting point determination. Ultraviolet absorption spectral determinations were done using Shimadzu 190 DU spectrophotometer. Infrared spectra were obtained on JASCO Infrared spectrophotometer as thin films on small plates, potassium bromide pellets or chloroform solutions. Mass spectra were obtained using Hitachi mass spectrometer. Electron impact ionization mass spectra were recorded under the following conditions: ionization energy 70 ev, ionizing current 300 μA and accelerating voltage 3 KV.

Results

Chloroform-Soluble Bases

Salsoline and 3,4-dimethoxy-β-phenethylamine were obtained from the crude mixture of chloroform-soluble bases.

Salsoline

Salsoline was obtained from the chloroform eluate by preparative thin layer chromatography and it crystallized from acetone as colourless crystals (34mg). Melting point: 220-222 °C. The sample co-chromatographed with an authentic reference compound with the same Rf

value of 0.70 in the solvent system isopropanol-ammonia-water (9:1:1). The ultraviolet absorption spectrum showed peaks with maximum absorption at the wavelengths (nm) 212, 230, 232 and 286-288. The infrared spectrum (potassium bromide disc) showed hydroxyl group at 3460 cm^{-1} and amine (NH) group at 3318 cm^{-1}. The mass spectrum showed the molecular ion (M^+) peak at mass/charge (m/z) 193 (with 5% relative intensity). Other significant peaks associated with the sample molecule were obtained at m/z 178 (100%), 163 (12%) 149 (11%), 132 (15%), 122 (12%) and 43 (27%). The chromatographic and the spectral data of the sample were entirely consistent with an authentic salsoline compound.

3,4-dimethoxy-β-phenethylamine

The chloroform-methanol (7:3) eluate from the silica gel column gave a pale-yellow gum (27 mg). Thin layer chromatography on silica gel of the sample with an authentic reference compound of 3,4-dimethoxy-β-phenethylamine showed that they have the same Rf value of 0.84 in the solvent system, isopropanol-ammonia-water (9:1:1). The ultraviolet absorption spectrum showed peaks with maximum absorption at the wavelengths (nm) 204-205, 224-227 and 284. The mass spectrum indicated a strong molecular ion (M^+) at m/z 181 (with 12% relative intensity). Other significant peaks occurred at m/z 166 (8%), 151 (100%), 136 (32%) and 30 (47%). The sample was identified as 3,4-dimethoxy-β-phenethylamine by direct comparison of the spectral and chromatographic data with that of a synthetic reference compound.

Five minor bases (73 mg) were also isolated by preparative thin layer chromatography from the chlorofrom-soluble fraction. The identities of these could not be established.

Butanol-soluble Bases

Three alkaloids, hordenine, tyramine and N,N-dimethyltryptamine were isolated from the crude butanol extract.

Hordenine

Hordenine was purified from the chloroform and the chloroform-methanol (95:5) eluates by preparative thin layer chromatography (23 mg). The sample co-chromatographed with authentic hordenine compound with the same Rf value of 0.45 in the solvent system, isopropanol-ammonia-water (9:1:1). The sample gave a brown coloration with Dragendorff reagent and a purple coloration (developed slowly) with Ehrlich reagent. The ultraviolet absorption spectrum showed peaks with maximum absorption at the wavelengths (nm) 224, 227 and 284. The sample gave infrared (in chloroform) bands at 3560 cm^{-1} due to hydroxyl group, 2850 cm^{-1} due to amine (NCH_3) group and 1612 cm^{-1} due to substituted benzene. The mass spectrum showed a strong molecular ion (M^+) peak at m/z 165 (with 17% relative intensity). Other significant peaks due to fragmentation of the sample molecule occurred at m/z 136 (66%), 135 (100%), 123 (37%), 107 (57%) and 58 (92%). This fraction was identified as hordenine by direct comparison of the spectral and chromatographic data with that of an authentic hordenine compound.

Tyramine

Tyramine was obtained from a chloroform-methanol (95:5) eluate and purified by preparative thin layer chromatography. It crystalized from ethanol as colourless crystals (37 mg). Melting

point 157-160 °C. The sample co-chromatographed with an authentic reference compound with the same Rf value of 0.30 in the solvent system isopropanol-ammonia-water (9:1:1). It reacted with Ehrlich reagent to produce a purple colour, and with Dragendorff reagent to produce a brown colour. The ultraviolet absorption spectrum showed peaks with maximum absorption at the wavelengths (nm) 230, and 275. The mass spectrum showed a very strong molecular ion (M^+) peak at m/z 137 (with 43% relative intensity). The base peak was m/z 94, and m/z, 121 (85%) and 120 (35%) were the only other significant peaks obtained in the mass spectrum. Direct comparison of the spectral and chromatographic data of the sample with synthetic reference tyramine identified the fraction as tyramine.

N,N-dimethyltryptamine

N,N-dimethyltryptamine was obtained from a chloroform-methanol (85:15) eluate as a brown oil. It crystallized from petroleum ether as colourless crystals (62 mg). Melting point: 49-50 49-50 °C. The sample co-chromatographed with an authentic reference compound with the same Rf value of 0.34 in the solvent system isopropanol-ammonia-water (9:1:1). The ultraviolet absorption spectrum showed peaks with masimum absorption at wavelengths (nm) 223, 283 and 291. The mass spectrum showed a strong melecular ion (M^+) peak at m/z 188 (with 51% relative intensity).

Other peaks due to fragmentation of the sample occurred at m/z 143 (15%), 130 (40%), 115 (21%), 103 (27%) and 58 (100%). This fraction was identified as N,N-dimethyltryptamine by direct comparison of the spectral and chromatographic data with that of synthetic N,N-dimethltryptamine compound.

Discussion

Five alkaloids representing three broad structural types, viz: indole-3-alkylamine, β-phenethylamine and hydroisoquinoline were isolated from the stem-leaves of *D. adscendens*, a plant used by rural populations in Ghana for the management of asthma.

Previous pharmacological evaluation of *D. adscendens* has shown that the plant has anti-allergic properties. Extracts of the plant administered orally to guinea pigs reduced anaphylactic contractions and inhibited histamine-induced contractions of isolated ileum[12]. It also reduced the amount of histamine present in, and the quantity of this and other spasmogens released from the lung tissues of sensitized guinea pigs[13]. Other species of Desmodium are known for their medicinal properties, which are usually ascribed to the total alkaloidal content of the various parts of the plant[6-9]. *Desmodium triflorum* is used in the treatment of asthma and cough in India and it has been suggested that the anti-asthmatic property of this species is due to the total alkaloidal content of the plant. The plant material also offers protection against acetylcholine- and histamine-aerosol-induced bronchrospasms[7]. Several mediating substances are involved in allergic reactions and in asthmatics serotonin is reported to be a smooth muscle agonist. Indole compounds are known to act as serotonin antagonist[15]. The indole alkaloid, N,N-dimethyltryptamine (ca 25% of total alkaloid content), shown to be present in this plant could act as serotonin antagonist and therefore help in overcoming the asthmatic attacks.

The effects of some of the mediators released in allergic reactions eg. histamine are counteracted by catecholamines[15]. β-phenethylamines have been shown to liberate catecholamines[8,15]. In

the present study one third of the total alkaloidal fraction of the stem-leaves of *Desmodium adscendens* was made up of β-phenethylamines. The effectiveness of the plant extracts against asthmatic attacks could be due in part to the β-phenethylamine content.

Further work is needed to characterise the anti-asthmatic properties of the compounds isolated in this study.

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References

1. Irvine, F.R.: Woody Plants of Ghana. London, Oxford University Press, 1961, p. 375.
2. Ghosal, S. and Mukherjee B.: Alkaloids of *Desmodium pulchellum.* Chem Ind. 1964; 1800.
3. Ghosal, S. and Mukherjee B.: Occurrence of 5-Methoxy-N, N-dimethyl-tryptamine oxide and other Tryptamines in *Desmodium pulchellum.* Chem Ind. 1965; 793-794.
4. Ghosal, S. and Banerjee P.K.: Simple Indole Bases of *Desmodium gangeticum.* Aust. J. Chem 1969; 22: 275-277.
5. Ghosal, S., Banerjee P.K. and Banerjee S.K.: A general method for the isolation of naturally occurring water-soluble bases. Phytochem. 1970; 9: 429-432.
6. Ghosal, S., Srivastava, R.S., Banerjee, P.K. and Dutta, S.K.: Alkaloids of *Desmodium triflorum.* Phytochem 1971; 10: 3312-3314.
7. Ghosal, S., Srivastava, R.S. Bhatacharya, S.K. and Debnath, P.K.: Desmodium alkaloids. IV. Chemical and pharmacological evaluation of *Desmodium triflorum.* Planta Medica. 1973; 23: 321-329.
8. Ghosal, S.: Desmodium Alkaloids. II. Chemical and pharmacological evaluation of *Desmodium gangeticum.* Planta Medica. 1972; 21: 434-440.
9. Ghosal, S. and Srivastava, R.S.: β-phene thylamine, Tetrahydroisoquinoline and indole alkaloids of *Desmodium titiafolium.* Phytochem. 1973; 12: 193-196.
10. Urano, A., Ikeya, V., Fukushima, S., Noro, T., Morinaga, K. and Kwano, H.: Studies on the contituents of *Desmodium caudatum.* Chem. Pharm. Bull, 1978; 26: 2411-2414.
11. Ampofo, D.: Plants that heal. World Health 1977; 26-30.
12. Addy, M.E. and Awumey, E.M.K.: Effects of the extracts of *Desmodium adscendens* on anaphylaxis. J. Ethnopharmacol. 1984; 11: 283-292.
13. Addy, M.E., and Dzandu, W.K.: Dose-response effects of *Desmodium adscendens,* aqueous extracts on histamine response, content and anaphylactic reactions in the guinea pig. J. Ethnopharmacol. 1986; 18: 13-20.
14. Gbowonyo, W.S.K.: Effects of *Desmodium adscendens:* Inhibitory effects of anaphylactic contraction of isolated guinea pig ileum. MSc Thesis, University of Ghana, Legon, Ghana, 1980.
15. Goodman, L.S. and Gilman, A.: The Pharmacological Basis of Therapeutics, (5th Edn). New York, Macmillan Publishing Co. Ltd., 1972 613.