THE OCCURRENCE OF INDOLEALKYLAMINE ALKALOIDS IN *PHALARIS TUBEROSA* L. AND *P. ARUNDINACEA* L.*

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The perennial grass *Phalaris tuberosa* L. is a valuable component of improved pastures in many areas of south-eastern Australia. Under conditions not yet fully understood, it may produce disease in sheep, either a chronic form, "Phalaris staggers",[1,2,3] involving incoordination and disorder of the central nervous system; or an acute form,[1,4] in which there is sudden collapse followed by rapid death or apparent complete recovery. The nature of the toxin has remained unknown. Hypotheses that it might be fluoroacetate or a related fluoro-compound[5] or a cholinesterase inhibitor[6,7] have not received experimental support.

We wish to report the presence in *P. tuberosa* of tryptamine alkaloids, the known pharmacological properties of which are such that they may be responsible, at least in part, for the toxic effects of the grass. The two bases consistently present as major constituents have been identified as *NN*-dimethyltryptamine (I) and 5-methoxy-*NN*-dimethyltryptamine (II). Bufotenine (III) together with uncharacterized indole derivatives is also present.

Assays indicate that air-dried samples normally contain 0·05–0·08% total alkaloid, but that fresh grass may contain more than twice the amount that is present after drying. The total alkaloid from fresh grass contains a higher proportion of bufotenine and a lower proportion of the uncharacterized indole derivatives of high R_F value. Much of this latter material obtained from dried grass may thus be artefact. No large differences have been observed in alkaloid composition or content of samples from supposedly toxic and non-toxic pastures, but further investigation of this point is needed because many of the present assays were conducted on dried grass.

Wilkinson[8] has reported the occurrence of 5-methoxy-*N*-methyltryptamine (IV) and hordenine (V) in *Phalaris arundinacea* L. We have examined certain unpalatable strains of this species supplied by Drs. R. M. Moore and J. R. McWilliam (cf.[9]) and find the major base to be gramine (VI), present in amounts up to 0·3%.

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[1] McDonald, I. W., *Aust. Vet. J.*, 1942, **18**, 182.

[2] Lee, H. J., and Kuchel, R. E., *Aust. J. Agric. Res.*, 1953, **4**, 88.

[3] Lee, H. J., Kuchel, R. E., and Trowbridge, R. F., *Aust. J. Agric. Res.*, 1956, **7**, 333.

[4] Moore, R. M., Arnold, G. W., Hutchings, R. J., and Chapman, H. W., *Aust. J. Sci.*, 1961, **24**, 89.

[5] Jarrett, I. G., personal communication.

[6] Dick, A. T., and McKenzie, J. S., personal communication.

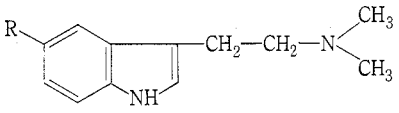
[7] Walker, D. J., *Nature*, 1959, **184**, 1411.

[8] Wilkinson, S., *J. Chem. Soc.*, 1958, 2079.

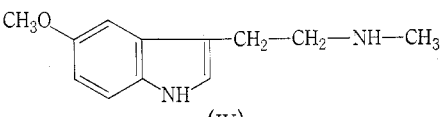
[9] Roe, E., and Mothershead, B. E., *Nature*, 1962, **193**, 255.

The bases (I), (II), and (III) are known to exert a strong action on the central nervous system and to interfere with some of the pharmacological effects of serotonin[10] (VII). Their possible role in the toxicity of *P. tuberosa* is under investigation elsewhere.

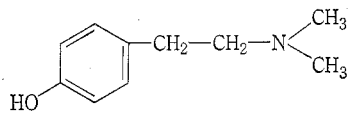
N-Methylated indolealkylamine and β-phenylethylamine derivatives, being simple metabolites of essential amino-acids, are probably fairly widely distributed in plant species and have been isolated so far from 35 species representing 23 genera and 7 families.[10,11] Among these, species of the Gramineae and Leguminosae are prominent. They have been found also in certain tissues of lower vertebrates, notably *Bufo* species, but not in mammals.



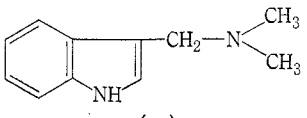
(I) R = H
(II) R = OCH_3
(III) R = OH



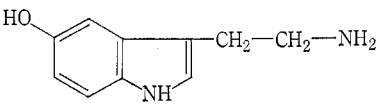
(IV)



(V)



(VI)



(VII)

Experimental

(*a*) *Alkaloid Assays*

Grass samples (approx. 100–200 g dry weight) were milled or macerated in a Waring blendor and extracted with methanol in a Soxhlet apparatus. Solvent was removed from the extract under reduced pressure and the residue extracted with dilute H_2SO_4. The aqueous solution was divided into two equal parts, one of which was made 2N with respect to H_2SO_4 and stirred with excess zinc dust for 4 hr. Both portions were then brought to pH 9 with ammonia and extracted with chloroform. The resulting crude base extracts were evaporated under reduced pressure and made up to standard volume (10 ml or 25 ml) in chloroform. Aliquots were titrated with 0·01N *p*-toluenesulphonic acid in chloroform, using dimethyl yellow as indicator. An approximate mean value of 200 for the equivalent weight was used to calculate alkaloid content as percentage dry weight With several representative samples, "reduced" and "unreduced" extracts differed by 10% or less, indicating that alkaloid *N*-oxides were either absent or present in very small amount. In subsequent assays, the reduction step was omitted.

Total Base Content of Phalaris tuberosa *Samples.*—The samples are air-dried unless described as "alcoholic", which means that the sample was placed in alcohol immediately after collecting: Glenroy, S.A., from field on which sheep were suffering "staggers", 0·06%; Dickson, A.C.T., from field on which "sudden death" had occurred in sheep, 0·06%; Tooradin, Vic., no evident

[10] Erspamer, V., *Progr. Drug Res.*, 1961, **3**, 151.
[11] Willaman, J. J., and Schubert, B. G., U.S. Dep. Agric. Tech. Bull. No. 1234 (1961).

toxicity, 0·07%; Ginninderra A, A.C.T., 0·05%; Ginninderra A, alcoholic, 0·12%; Ginninderra B, 0·07%; Ginninderra B, alcoholic, 0·08%; Ginninderra C, 0·05%; Ginninderra C, alcoholic, 0·12%; Ginninderra D, 0·02%; Ginninderra D, alcoholic, 0·07%; Deniliquin, N.S.W., 0·05%; Yass, N.S.W., 0·08%.

Total Base Content of P. arundinacea *Samples.*—Palatable strain supplied by Dr. Moore, 0·21%; unpalatable strain supplied by Dr. Moore, 0·50%; unpalatable strain, CPI 10446, supplied by Dr. McWilliam, 0·10%; Ginninderra, 0·05%; Ginninderra alcoholic, 0·18%; Deniliquin, 0·04%.

(*b*) *Paper Chromatography*

Paper chromatography of alkaloid fractions was carried out in butanol/acetic acid/water (80 : 3 : 17) with ascending solvent. Detecting reagents were iodine vapour or Dragendorff's reagent (red-brown with all bases), Ehrlich's reagent (blue-violet with indole derivatives), and α-nitroso-β-naphthol reagent (violet with 5-hydroxyindole derivatives, cf. Smith[12]).

R_F values and colour reactions (E, Ehrlich reagent; N, α-nitroso-β-naphthol) of the constituents of total base of *P. tuberosa* were as follows: 0·27 (bufotenine), E dark blue, N violet; 0·33, E dark blue, N violet; 0·38 (5-methoxy-*NN*-dimethyltryptamine), E dark blue, N weak brown; 0·48 (*NN*-dimethyltryptamine), E mauve changing to dark blue, N weak brown; 0·65, E red-brown, N nil; 0·74, E red-brown, N nil; 0·85 to 0·92, E red-brown, N nil. Dimethyltryptamine (R_F 0·48) and 5-methoxydimethyltryptamine (R_F 0·38) were the major components in all samples, either fresh or air-dried. Bufotenine (R_F 0·27) and the base of R_F 0·33, apparently also a 5-hydroxyindole derivative, were present in all fresh samples but were not always evident, and then only in considerably reduced proportion, in dried samples. The spots of R_F 0·65, 0·74, and 0·85–0·92, were somewhat variable in position and the last was relatively much stronger in air-dried samples.

Total base from palatable strains of *P. arundinacea* shows a similar pattern to that of *P. tuberosa*: a strong spot at R_F 0·48 (E mauve changing to dark blue) and weaker spots at 0·40 (E dark blue), 0·30 (E dark blue), and at 0·85 (E red-brown), 0·93 (green before treatment with reagents, E no colour). Thin-layer chromatography on alumina in chloroform/10% methanol shows that the R_F 0·48 material, although mostly dimethyltryptamine (R_F 0·66 in this system) also contains gramine (R_F 0·51). Total base from unpalatable strains of *P. arundinacea* was almost entirely of R_F 0·48, weakly staining with Ehrlich reagent and thus mostly gramine but probably with some dimethyltryptamine.

(*c*) *Isolation of Alkaloids from* P. tuberosa

Crude alkaloid (19 g) was obtained from partly dried grass from Glenroy, S.A., (260 lb dry weight equivalent) by the method described in the assay procedure but without the zinc reduction step. A 72-tube countercurrent distribution between chloroform and 0·2N HCl (150 ml phases) gave non-basic material (0·2 g) in tubes 10–30, base (0·17 g), R_F 0·90, in tubes 40–60, base (4·1 g), R_F 0·56, *0·48*, 0·40 in tubes 61–66, and base (14·5 g), R_F *0·50*, 0·41 in tubes 67–72. Part of the last fraction (4 g) was subjected to partition chromatography on a column of Pyrex glass powder (1880 g) moistened with K-phosphate buffer of pH 8·1 (320 ml). Elution with light petroleum (b.p. 60–80°; 7·6 l.) gave base of R_F 0·50 (0·65 g) but light petroleum/carbon tetrachloride (60 : 40, 2·1 l.) followed by solvents containing chloroform gave only a mixture of both bases (2·7 g).

When the base of R_F 0·50 was taken up in ether, a small amount of solid remained insoluble. Removed and washed with ether, this material had m.p. 212–214° (decomp.); it has not been identified, direct comparison showing that it is not bufotenine *N*-oxide which has a similar melting point. The ether-soluble material could not be induced to crystallize and was converted into a picrate which, after recrystallization from ethanol, formed orange crystals (Found: C, 52·0; H, 4·8; N, 16·8%. Calc. for $C_{18}H_{19}N_5O_7$: C, 51·8; H, 4·6; N, 16·8%), m.p. 167–167·5°, undepressed on admixture with authentic *NN*-dimethyltryptamine picrate, m.p. 168·5°.

[12] Smith, I., "Chromatographic and Electrophoretic Techniques." (2nd Ed.) p. 193. (Heinemann: London 1960.)

Mixed base, R_F 0·50, 0·41, was submitted to preparative paper chromatography on Whatman 3MM paper. After location with marker strips, the appropriate bands were separately eluted and the products converted into picrates. The picrate of the R_F 0·50 base formed yellow needles, m.p. 169°, undepressed on admixture with dimethyltryptamine picrate. The picrate of the R_F 0·41 base, recrystallized from ethanol, had m.p. 175·5–176°, undepressed on admixture with authentic 5-methoxy-*NN*-dimethyltryptamine picrate, m.p. 177–178°; infrared and nuclear magnetic resonance spectra of the picrate samples were also identical. The n.m.r. spectrum showed singlet peaks, δ 3·0 and 3·9, in 2 : 1 area ratio as expected for NMe_2 and OMe. Insufficient picrate was available for microanalysis.

Gas chromatography of crude base fractions was not successful in that some of the bases appeared to be adsorbed or decomposed. This may have been due, in some degree, to use of copper tubes. However, with a column of Celite/silicone E301 at 250°, dimethyltryptamine was eluted in 3·7 min. When a sample of total base of *P. tuberosa* was run on a preparative column, the appropriate peak collected and the product crystallized from light petroleum, dimethyltryptamine was obtained, m.p. 45°, undepressed on admixture with authentic base, m.p. 46·5° (Found: N, 14·9%. Calc. for $C_{12}H_{16}N_2$: N, 14·9%).

Mixed base (800 mg) derived from fresh grass and showing a well-defined spot at R_F 0·27 was chromatographed on cellulose in butanol/acetic acid solvent using an LKB Chromax column (280 g cellulose) and with collection of eluate in 5-ml fractions. Elution appeared to be faster than on Whatman No. 1 paper and separation of constituents was not complete. Fractions containing the base, R_F 0·27, were combined and rechromatographed. Appropriate fractions showed on paper chromatograms the spot R_F 0·27, but rapidly developed an additional spot R_F 0·84, pale blue fluorescence in u.v. light, yellow colour with Ehrlich's reagent. Attempted crystallization of the base, R_F 0·27, was unsuccessful and identification as bufotenine rests on the evidence of identical R_F values in butanol/acetic acid/water (80 : 3 : 17), R_F 0·27; *n*-propanol/1N ammonia (5 : 1), R_F 0·77; 3% aqueous ammonium chloride, R_F 0·51; and methylethylketone/t-butanol/diethylamine/water (10 : 10 : 1 : 5), R_F 0·95; and on identical colour reactions on paper chromatograms with Ehrlich's reagent and α-nitroso-β-naphthol reagent (cf. under (*b*) above), with u.v. light (weak purple fluorescence) and ninhydrin (no colour, whereas primary and secondary amines give a purple-black spot).

(*d*) *Isolation of Gramine from* Phalaris arundinacea (CPI 10446)

From dried grass (200 g), total base was isolated by the procedure described in (*a*). Both reduced and unreduced aqueous extracts gave the same amount of base, equivalent to 0·5% of starting material. Crude base showed only one spot, R_F 0·52, giving no immediate colour with Ehrlich reagent but slowly becoming yellow. Crystallization of total alkaloid from benzene gave gramine, m.p. 133–134°, unchanged on admixture with authentic material (Found: C, 75·4; H, 8·0; N, 15·7%. Calc. for $C_{11}H_{14}N_2$: C, 75·8; H, 8·1; N, 16·1%).

Acknowledgments

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