THE OCCURRENCE OF TETRAHYDRO-β-CARBOLINE ALKALOIDS IN *PHALARIS TUBEROSA* (GRAMINEAE)

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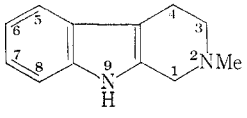
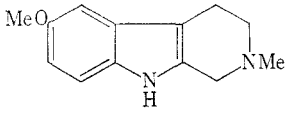
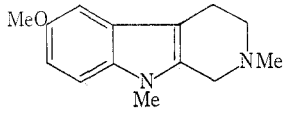
Grasses of the genus *Phalaris* seem to show wide variations in the nature and quantity of the indole derivatives present in selected strains grown as pasture in different places. We have now found that five strains of *Phalaris tuberosa* L. grown in one locality in South Australia consistently contain, as minor constituents at all stages of growth, 2-methyl-1,2,3,4-tetrahydro-β-carboline (1) and 6-methoxy-2-methyl-1,2,3,4-tetrahydro-β-carboline (2).



(1)



(2)



(3)

The major base of one locally grown strain (Sirocco) of *P. tuberosa* was 5-methoxy-*N*,*N*-dimethyltryptamine; of the others, it was *N*,*N*-dimethyltryptamine, usually accompanied by smaller amounts of the 5-methoxy derivative. Bufotenine, gramine, and hordenine were often detected as well. Culvenor, Dal Bon, and Smith[1] had examined several strains of *P. tuberosa* from Australian sources and consistently found *N*,*N*-dimethyltryptamine and the 5-methoxy derivative as major constituents, with smaller quantities of bufotenine and some unidentified indole derivatives also present. They also examined[1] Australian-grown *Phalaris arundinacea*, and found that, whereas strains palatable to sheep seemed to show a pattern of constituent bases similar to that shown by *P. tuberosa*, some unpalatable strains contained notably high concentrations of gramine. Wilkinson[2] had reported the presence of 5-methoxy-*N*-methyltryptamine and hordenine in *P. arundinacea* grown in England, and recently Audette, Vijayanagar, Bolan, and Clark[3] found that a Canadian strain of this species contains, in addition to a high proportion of hordenine and some gramine, 6-methoxy-2,9-dimethyl-1,2,3,4-tetrahydro-β-carboline (3), a previously unknown alkaloid closely related to the bases (1) and (2) now found in *P. tuberosa*.

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[1] Culvenor, C. C. J., Dal Bon, R., and Smith, L. W., *Aust. J. Chem.*, 1964, **17**, 1301.

[2] Wilkinson, S., *J. chem. Soc.*, 1958, 2079.

[3] Audette, R. C. S., Vijayanagar, H. M., Bolan, J., and Clark, K. W., *Can. J. Chem.*, 1970, **48**, 150.

The alkaloids (1) and (2) are already known as constituents (usually minor) of plants other than members of the Gramineae. For example, (2) is found[4,5] in the South American hallucinogenic plants *Virola theiodora*, *V. rufula*, and *Anadenanthera peregrina*, and (1) occurs in *Gymnacranthera paniculata*,[6] a tree of the New Guinea rain forest, as well as in *V. theiodora*,[5] *Arthrophytum leptocladum*,[7] and *Banisteriopsis rusbyana*.[8]

Paper electrophoresis was a convenient method for detecting the tetrahydro-β-carbolines (1) and (2) in extracts of *P. tuberosa*. In electrolytes at about pH 10 they behave as anions of low mobility, whereas gramine and the tryptamine derivatives are all cationic in the same electrolytes. In larger quantities, the bases (1) and (2) could be separated from each other and the accompanying indolealkylamines by chromatography on anion-exchange resin followed by thick-layer chromatography. The purified compounds were identified by comparison with authentic specimens.

Different groups of workers have tended to use different procedures for the separation and assay of the alkaloids mentioned above. Part of the apparent variability of different strains of *Phalaris* pastures may in fact be caused by preferential loss or destruction of some constituents through the use of a particular method of isolation or assay. As the presence of indole derivatives in *P. tuberosa* has been suggested as a possible cause of its occasional toxicity to ruminants,[1,9] it seems desirable to have any assays confirmed by an independent procedure. Paper electrophoresis by the enclosed-strip method under pressure seems to be one of the mildest procedures for the detection and roughly quantitative determination of a number of the common indolealkylamines and related compounds.

Experimental

Extraction and Fractionation of Alkaloids

The five strains of *P. tuberosa* growing at the Division's Field Station at O'Halloran Hill, South Australia (Seedmaster, Sirocco, GB81 and others designated "High Alkaloid" and "Low Alkaloid") were each sampled several times during their growth cycles. Depending upon the strain and its stage of growth, the total isolated alkaloidal content of the grass varied between 0·06 and 0·001% of its dry weight, being highest during periods of active growth after rain in autumn and early winter. As expected, the "Low Alkaloid" strain gave the least total yield of alkaloids but, on average, the "High Alkaloid" strain yielded, not more, but slightly less total alkaloids than Sirocco or Seedmaster.

Although alkaloids (1) and (2) were present in all strains at all times, each constituting about 5% of the total weight of alkaloids present in any extract, Seedmaster appeared to contain somewhat more of them than other strains. This was determined by electrophoresis on papers impregnated with 0·1N sodium carbonate as electrolyte and maintained at 2°, using the enclosed-strip apparatus described previously.[10] Seedmaster was therefore used for the following experiments.

[4] Agurell, S., Holmstedt, B., Lindgren, J. E., and Schultes, R. E., *Biochem. Pharmac.*, 1968, **17**, 2487.

[5] Agurell, S., Holmstedt, B., Lindgren, J. E., and Schultes, R. E., *Acta chem. scand.*, 1969, **23**, 903.

[6] Johns, S. R., Lamberton, J. A., and Occolowitz, J. L., *Aust. J. Chem.*, 1967, **20**, 1737.

[7] Platonova, T. F., Kuzovkov, A. D., and Massagetov, P. S., *Zh. obshch. Khim.*, 1958, **28**, 3128.

[8] Agurell, S., Holmstedt, B., and Lindgren, J. E., *Am. J. Pharm.*, 1968, **140**, 148.

[9] Gallagher, C. H., Koch, J. H., Moore, R. M., and Steel, J. D., *Nature*, 1964, **204**, 542.

[10] Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1959, **12**, 65.

The freshly cut grass (12 kg, corresponding to a dry-weight of 2·4 kg) was macerated in cold 0·1N HCl (40 l.) and the filtered extract neutralized with 17N NaOH and brought to about pH 10 by addition of sodium carbonate. The solution was saturated with sodium chloride and exhaustively extracted with chloroform. After drying over anhydrous magnesium sulphate, the chloroform extract was taken to dryness to yield a tar-like residue (9·8 g). This was thoroughly dispersed in 0·1N HCl (35 ml). The mixture was filtered and the clear, pale-brown filtrate was made alkaline with concentrated ammonia and extracted with chloroform to yield a crude mixture of bases (2·4 g).

Comparative tests conducted after the above extraction was completed indicated that alkaloids (1) and (2) are extractable from grass samples somewhat more conveniently with ethanol under conditions similar to those used by Audette *et al.*[3] for the extraction of *P. arundinacea*. It was also found that the alkaloids survive air-drying and storage of grass samples for at least 2 months prior to extraction.

Fractionation of the crude mixture of bases (0·6 g) was effected on a column (107 by 1·5 cm) of the cation-exchange resin, Bio-Rex 70 (Bio-Rad Laboratories, California), equilibrated in the H^+ form with formic acid–ammonium formate buffer (pH 3; 0·2M with respect to formate) and eluted with the same buffer until 220 tubes (15 ml) were collected. A further 50 tubes were collected after the formate buffer was adjusted to pH 2·6. Fractionation was followed spectrofluorometrically (using an Aminco–Bowman spectrofluorometer; range of λ excitation 285–310 nm, range of λ emission 338–360 nm). The contents of tubes were combined as appropriate, each fraction made alkaline with sodium hydroxide and saturated with sodium chloride, and the free bases extracted with chloroform. The extracts were taken to dryness and the fractions identified by thin-layer chromatography and paper electrophoresis. Fraction 1 (tubes 100–108, 20 mg) contained gramine; fraction 2 (tubes 110–180, 212 mg) contained *N*,*N*-dimethyltryptamine; fraction 3 (tubes 200–220, 32 mg) contained alkaloid (1) and 5-methoxy-*N*,*N*-dimethyltryptamine; fraction 4 (tubes 240–270, 15 mg) contained alkaloid (2) and an unidentified base. A little more of (2) was recovered on clearing the column with 0·1N HCl.

Alkaloids (1) and (2) were isolated from fractions 3 and 4, respectively, by chromatography on thick layers (2 mm) of silica gel using methanol–ammonia (50 : 1) as solvent. The bands (R_F 0·6) were each eluted from the silica gel with methanol–ammonia (10 : 1) and the eluate concentrated and extracted with chloroform. The extracts, taken to dryness, each yielded a syrupy residue, which was chromatographically and electrophoretically homogeneous.

Alkaloid (1) (10 mg), on crystallization from heptane–ethyl acetate (10 : 1) or benzene, gave colourless needles, m.p. 216–218° undepressed on admixture with a sample of 2-methyl-1,2,3,4-tetrahydro-β-carboline provided by Dr J. A. Lamberton, Division of Applied Chemistry, CSIRO, Melbourne. The ultraviolet and mass spectra of this compound described by Johns *et al.*[6] and reproduced by Agurell *et al.*[5] are identical with those of our specimen. This was also shown to be electrophoretically indistinguishable from the authentic compound in each of several electrolytes in the pH range 4·6–10·2. The alkaloid was located on papers exposed to the light of a Hanovia "Chromatolite" ultraviolet lamp as a blue fluorescent spot. It was also detected as a pink spot by heating papers (110°) after spraying them with a reagent prepared by dissolving xanthydrol (0·1 g) and syrupy phosphoric acid (5 ml) in absolute ethanol (95 ml). The analogous indolealkylamine, *N*,*N*-dimethyltryptamine, also gives a pink reaction with this reagent. Alkaloid (1) was also readily detectable on papers sprayed with a reagent containing chromium trioxide, potassium permanganate, and sulphuric acid, which is proving to be a detecting agent of wide application.[10–13]

Thin-layer chromatography of the alkaloid on silica gel in freshly prepared acetone–ammonia (75 : 1) yielded a spot, R_F 0·57.

Alkaloid (2) (9 mg) crystallized from heptane–ethyl acetate (10 : 1) to give fine needles, m.p. 209–211° undepressed on admixture with a sample of 6-methoxy-2-methyl-1,2,3,4-tetrahydro-β-carboline prepared from 5-methoxy-*N*,*N*-dimethyltryptamine according to published procedures.[14] Our specimen was electrophoretically indistinguishable from the synthetic compound

[11] Frahn, J. L., and Mills, J. A., *Aust. J. Chem.*, 1964, **17**, 256.

[12] Frahn, J. L., *J. Chromat.*, 1968, **37**, 279.

[13] Frahn, J. L., *Aust. J. Chem.*, 1969, **22**, 1655.

[14] Ghosal, S., and Mukherjee, B., *J. org. Chem.*, 1966, **31**, 2284.

and was detected on papers using the methods outlined above for alkaloid (1). Xanthydrol gives a blue reaction with (2) as it also does with the analogous indolealkylamine, 5-methoxy-*N*,*N*-dimethyltryptamine. Unlike the tryptamines, however, neither (1) nor (2) gives a strong colour reaction with Ehrlich's reagent (cf.[15]).

Thin-layer chromatography of (2) on silica gel in acetone–ammonia (75 : 1) yielded a spot, R_F 0·54.

Its ultraviolet spectrum showed λ_{max} 228 and 278 nm with shoulders at 289 and 307 nm in methanol solution (cf.[14]).

It was shown by mass spectrometry that (2) had a mol. wt. of 216 (M^+), that is, 2 mass units less than 5-methoxy-*N*,*N*-dimethyltryptamine, with prominent peaks in the spectrum at *m/e* 173 and *m/e* 158. These and other peaks are identical in position and relative intensities with those recorded by Agurell *et al.*[5] for this alkaloid.

Acknowledgments

We wish to thank Dr J. A. Lamberton for the gift of the sample of 2-methyl-1,2,3,4-tetrahydro-β-carboline and for access to ultraviolet and mass spectral data for this compound, and Dr J. A. Mills for helpful criticism of the manuscript. Thanks are also due to Mr A. C. K. Triffett for determining the mass spectra of our samples.

[15] Leonard, N. J., and Elderfield, R. C., *J. org. Chem.*, **1942**, **7**, 556.