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OXINDOLES FROM *PHALARIS COERULESCENS*

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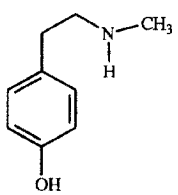
Key Word Index—*Phalaris coerulescens*; Gramineae; leaves; oxindoles; coerulescine; horsfiline; demethoxyhorsfiline.

Abstract—A phytochemical examination of various accessions of *Phalaris coerulescens* for the presence of toxic phenylethylamines, indoloamines and tetrahydro-β-carbolines revealed the presence of oxindoles previously not reported to occur in *Phalaris species*. One of the oxindoles, the structure of which was elucidated by spectroscopic methods, has not hitherto been reported to occur naturally and has been given the trivial name coerulescine. © 1998 Published by Elsevier Science Ltd. All rights reserved

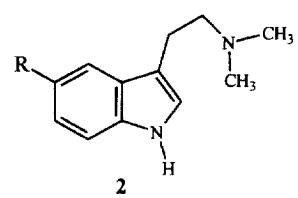
INTRODUCTION

Perennial *Phalaris* species, such as reed canary grass (*P. arundinacea*) and Toowoomba canary grass (*P. aquatica*), are useful pasture grasses especially in areas of poor soil and low rainfall, or poorly drained soils subject to occasional inundation [1]. However, neurotoxic and cardiotoxic effects have been observed in animals eating *Phalaris* and attributed to alkaloids such as *N*-methyltyramine (**1**), *N*,*N*-dimethyltryptamines (**2**) and 1,2,3,4-tetrahydro-β-carbolines (**3**) [2, 3]. Consequently, much effort has been directed at developing low alkaloid-content *Phalaris* cultivars for use as pasture [4]. However, poisoning cases have still been reported with animals grazing on these low-alkaloid cultivars. This suggests the presence of other unidentified toxins or the involvement of environmental or animal factors precipitating an acute toxic episode in chronically intoxicated but subclinical animals [5].

Phalaris coerulescens, a lesser known species of *Phalaris*, is a winter growing perennial with rapid germination, vigorous seedling growth and high production in its seedling year. Trials in Australia in the late 1950s and the 1960s produced conflicting reports on persistence and productivity [6]. The introduced strains were not commercialized but have since become volunteer species in higher rainfall areas, particularly in the state of Victoria, where it has also been circumstantially associated with equine fatalities.

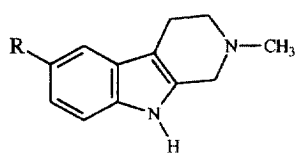


1



2

a R = H
b R = OCH_3
c R = OH bufotenine



3

a R = H
b R = OCH_3

A recent collection of grasses on the Iberian Peninsula has resulted in the reintroduction of *P. coerulescens* to Australia, in an attempt to provide a more persistent and productive grass, other than the traditional perennial veldt grass (*Ehrharta calycina*), for low water-holding, acid soils. Pastures on these soils, in low to moderate rainfall areas (475–550 mm per year), are generally poor and readily become infested with the problem annual grass, silver grass (*Vulpia* species). Trials of *P. coerulescens* accessions were established in the south east of South Australia. Under trial conditions, these accessions persisted and per-

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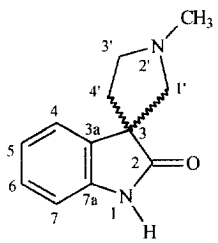
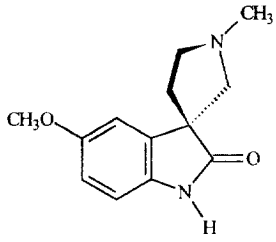
formed better than the *Phalaris* control *P. aquatica* cv. Sirosa and were the most productive of the grass accessions evaluated. As a consequence of the known potential toxicity of *Phalaris* and of a suspected association of *P. coerulescens* with horse fatalities on a farming property in Victoria in the late 1980s (unpublished data), it was considered necessary to investigate the accessions for alkaloid content before further agronomic development was pursued. In the process of evaluating the alkaloid content of the various *P. coerulescens* accessions by TLC, HPLC and GC-mass spectrometry methods a new alkaloid was identified.



4 (-)-coerulescine



5 (R)-(-)-horsfiline

RESULTS AND DISCUSSION

Leaf samples of several *P. coerulescens* accessions were extracted with 0.1 N HCl and the filtered extracts subjected to cation-exchange chromatography. Analysis of the extracts by GC-mass spectrometry indicated two main (alkaloids A and B) and two minor (alkaloids C and D) components. The retention times and mass spectra of alkaloids B and D were identical to those obtained from authentic sample of **3a** and **3b** and to that reported [7] for **3b**.

Extracts containing the unidentified alkaloid A were pooled and subjected to radial chromatography using silica gel as the adsorbent and chloroform-ammoniated methanol (4:1) as mobile phase. Under TLC conditions alkaloid A was reactive with Dragendorff's reagent (Rf 0.6 on silica gel) and, unlike the tryptamine, tyramine and tetrahydro-β-carboline alkaloids, was negative with acidified anisaldehyde reagent. A M_r of 202 was confirmed by chemical ionization mass spectrometry and a high resolution mass measurement of the $[M]^+$ indicated a molecular formula $C_{12}H_{14}N_2O$ for alkaloid A. The fragmentation pattern of the $[M]^+$ was similar to that expected for a tetrahydro-β-carboline and high resolution mass measurements on some fragment ions confirmed the association of the oxygen atom with the indole portion of the molecule. The alkaloid was readily acetylated (acetic anhydride-pyridine) or methylated (MeI-K_2CO_3-acetone) to yield the mono acetyl ($[M]^+$ *m/z* 244) or mono methyl ($[M]^+$ *m/z* 216) derivative, respectively.

Extensive NMR spectroscopic investigation (^1H, ^{13}C, ^1H-^1H COSY, ^1H-^{13}C DEPT, ^1H-^{13}C HMQC and ^1H-^{13}C HMBC) indicated that the unknown alkaloid was the oxindole **4**, subsequently given the trivial name coerulescine. The oxindole structure was differentiated from the isomeric pseudo-oxindole by the chemical shift of the spiro carbon (C-3, δ 53.5) and an observed three-bond correlation between the spiro carbon and H4 (δ 7.41) on the aromatic ring. Multiple bond correlations between the carbonyl C-2 of the indole entity and four of the methylene hydrogens (H1′a, H1′b, H4′a and H4′b) on the spiro pyrrolidine ring, in conjunction with the appropriate one bond ^1H-^{13}C correlations, confirmed the assignment of chemical shifts in this ring. The molecule is optically active but the stereochemistry of the spiro linkage remains undefined.

The related oxindole **5**, in conjunction with the tetrahydro-β-carboline **3b** and 5MeODMT (**2b**), has previously been isolated from the south-east Asian medicinal plant, *Horsfieldia superba* (Myristicaceae) and named (−)-horsfiline [8]. The absolute configuration of (−)-horsfiline has been shown, by synthesis from chiral tetrahydro-β-carboline precursors, to be (R)-(-)-horsfiline as shown in structure **5** [9]. The mass spectral data for the minor alkaloid C in the extracts of *P. coerulescens* were identical to those reported for horsfiline (**5**) [8]. Coerulescine (**4**) has not previously been reported from natural sources but has been synthesised in pilot studies towards the synthesis of horsfiline (**5**) [10] and vincadifformine [11].

To ensure that the coerulescine was not an artefact resulting from oxidative rearrangement of the related tetrahydro-β-carboline **3a**, the entire extraction and analytical procedure was repeated using a solution of **3a** in 0.1 N HCl. No oxindole was generated from **3a** under a variety of extraction and processing conditions involving time of exposure to the acid and/or cation-exchange resin.

EXPERIMENTAL

GC-MS data were recorded using a Finnigan GCQ instrument fitted with a 30 m × 0.25 mm J&W DU-5MS low bleed capillary column (0.25 μm film thickness). Standard EI (70 eV) HR measurements were recorded using a JEOL DX303 magnetic sector MS with the DA5000 data system using *ca* 1 μg of sample inserted *via* the direct inlet probe and using PFK as the relative standard for peak calibration. ^1H, ^{13}C, ^1H-^1H COSY, ^1H-^{13}C DEPT, ^1H-^{13}C HMQC and ^1H-^{13}C HMBC NMR expts were conducted using a Bruker spectrometer operating at 500 MHz for protons and 126 MHz for carbons.

Plant material

Seeds were collected on the Iberian Peninsula and germinated and multiplied at the Kybybolite Research Station in South Australia. In Portugal, the collections were made with the cooperation of the Estacao Nacional de Melhoramento de Plantas (National

Plant Breeding Station) at Elvas, whilst in Spain the collections were made with the cooperation of the Servicio de Investigacion Agraria de Extramadura near Badajoz. The identity of the plant as *P. coerulescens* was confirmed by Dr Rex Oram of the CSIRO Division of Plant Industry, Australian Capital Territory, Australia.

Extraction

Leaf samples of various accessions (20 × 20 g) were immersed in 0.1 N HCl and left, with occasional shaking, for 3 days. Filtration yielded reddish-pink aq. solns which were applied to Bakerbond SPE aromatic sulfonic acid cation-exchange columns preconditioned by washing with MeOH and H_2O. Elution of the columns with EtOH-NH_3) prepared by diln of 0.880 NH_3 with EtOH, 1:2), followed by immediate evapn of solvent under red. pres. and reconstitution of the residues in MeOH, yielded samples for GC-MS analysis.

Isolation and identification of coerulescine (**4**)

All *P. coerulescens* samples were positive by GC-MS for the unknown alkaloid A and were consequently pooled, concd and subjected to radial chromatography using CHa_3—ammoniated MeOH (prepd by saturating a vol. of methanol with NH_3 and then diluting with MeOH, 1:10) as the eluent (4:1). Frs containing the unknown alkaloid were pooled and evap to dryness to afford a pale yellow gum (5 mg, 0.001%). $[\alpha]_D^{20}$ −0.77° (0.022, MeOH). ^{13}C NMR ($CDCl_3$): δ 182.8, (*s*, C-2 carbonyl), 139.9 (*s*, C-7a), 136.1 (*s*, C-3a), 127.7 (*d*, C-6), 124.9 (*d*, C4), 123.3 (*d*, C5) 109.4 (*d*, C-7), 66.4 (*t*, C-1′), 56.8 (*t*, C-3′), 53.5 (*s*, C-3), 41.9 (*q*, *N*-CH_3), 37.9 (*t*, C-4′). ^1H NMR ($CDCl_3$): δ 8.24 (1H, *bs*, N-H), 7.41 (1H, *d*, $J_{4,5}$ = 7.5 Hz, H-4), 7.22 (1H, *bt*, $J_{6,5}$ = 7.5 Hz, $J_{6,7}$ = 7.5 Hz, H-6), 7.07 (1H, *bt*, $J_{5,4}$ = 7.5 Hz, $J_{5,6}$ = 7.5 Hz, H-5), 6.9 (1H, *d*, $J_{7,6}$ = 8 Hz, H-7), 3.01 (1H, *m*, H-3′a), 2.91 (1H, *d*, $J_{1'a,1'b}$ = 9.4 Hz, H-1′a), 2.86 (1H, *d*, $J_{1'b,1'a}$ = 9.4 Hz, H-1′b), 2.82 (1H, *m*, H-3′b), 2.47 (3H, *s*, *N*-CH_3), 2.43 (1H, *m*, H-4′a), 2.12 (1H, *m*, H-4′b). EIMS, *m/z* (rel. int.) 202 ([M]$^+$, 100), 185 (20), 159 ([M-CH_2NCH_3]$^+$12), 145 ([M-$(CH_2)_2NCH_3$]$^+$90), 130 (32), 117 (18). CIMS (CH_4) *m/z* 203 ([M + H]$^+$, 100%). HRCIMS, $C_{12}H_{14}N_2O$. H calcd 203.1184, found 203.1177. HREIMS, $C_{12}H_{14}N_2O$ calcd 202.1106, found 202.1104; $C_{10}H_9NO$ [M-CH_2NCH_3]$^+$ calcd 159.0684, found 159.0681; C_9H_7NO ([M-$(CH_2)_2NCH_3$)]$^+$ calcd 145.0528, found 145.0537.

REFERENCES

1. Cheeke, P. R., *Natural Toxicants in Feeds, Forages and Poisonous Plants* 2nd Ed. p. 275. Interstate Publishers Inc., Danville.
2. Anderton, N., Cockrum, P. A., Walker, D. W. and Edgar, J. A., in *Plant-associated Toxins: Agricultural, Phytochemical and Ecological Aspects* eds S. M. Colegate, and P. R. Dorling. CAB International, Wallingford, 1994, p. 269.
3. Bourke, C. A. in *Plant-associated Toxins: Agricultural, Phytochemical and Ecological Aspects* eds S. M. Colegate and P. R. Dorling. CAB International, Wallingford, 1994, p. 523.
4. Oram, R. N. (1970) *Proceedings of the XI International Grassland Congress*, p. 785.
5. Kennedy, D. J., Cregan, P. D., Glastonbury, J. R. W., Golland, D. T. and Day, D. G., *Australian Vet. J.*, 1986, **63**, 88.
6. Rhodes, I., *Fld. Stn. Rec. Div. Pl. Ind. CSIRO* 6 (no. 2), 1967, p. 69.
7. Gander, J. E., Marum, P., Marten, G. C. and Hovin, A. W., *Phytochemistry*, 1979, **15**, 737.
8. Jossang, A., Jossang, P., Hadi, H. A., Sévenet, T. and Bodo, B., *J. Org. Chem.*, 1991, **56**, 6527.
9. Pellegrini, C., Strässler, C., Weber, M. and Borschberg, H.-J., *Tetrahedron: Assymmetry*, 1994, **5**, 1979.
10. Bascop, S.-I., Sapi, J., Laronze, J. Y. and Lévy, J., *Heterocycles*, 1994, **38**, 725.
11. Kuehne, M. E., Roland, D. M. and Hafter, R., *J. Org. Chem.*, 1978, **43**, 3705.