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THE OCCURRENCE OF 2-METHYL-1,2,3,4-TETRAHYDRO-β-CARBOLINE AND VARIATION IN ALKALOIDS IN *PHALARIS ARUNDINACEA**

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Key Word Index—*Phalaris arundinacea*; Gramineae; reed canarygrass; indole alkaloids; β-carboline; tryptamine; gramine; hordenine

Abstract—2-Methyl-1,2,3,4-tetrahydro-β-carboline was isolated from reed canarygrass (*Phalaris arundinacea* L.) and the occurrence of 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline confirmed. Clones of reed canarygrass that contained *N*,*N*-dimethyltryptamine or 2-methyl-1,2,3,4-tetrahydro-β-carboline did not contain their respective methoxy or hydroxy derivatives. Five of the 12 clones tested contained either or both of 5-methoxy-*N*,*N*-dimethyltryptamine and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline. The data suggest that clones that contain gramine are not likely to contain *N*,*N*-dimethyltryptamine and/or β-carbolines. Thus, an inverse biosynthetic relationship between gramine and the tryptamine and β-carboline alkaloids seems to exist. However, further work is needed to firmly establish any such relationship between these alkaloids.

INTRODUCTION

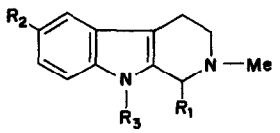
The total basic alkaloid concentration of genotypes of reed canarygrass (*Phalaris arundinacea* L.) is negatively correlated with their palatability to ruminant animals [1,2]. The tryptamine alkaloids present in some reed canarygrass lines are in fact potentially toxic to sheep and cattle [3]. At least 8 alkaloids are known to occur in the plant including one phenol (hordenine), 5 indoles (gramine and 4 derivatives of tryptamine), and 2 derivatives of β-carboline [4]. The carbolines include 2,9-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (**1**) and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (**2**). *Phalaris aquatica* L. (*P. tuberosa* L.) sometimes contains **2** and 2-methyl-1,2,3,4-tetrahydro-β-carboline (**3**) [7]. Minor quantities of 1,2-dimethyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline (**4**) also were reported to occur in *Virola* spp. and in *Anadenanthera* (*Piptadenia*) *peregrina* [8]. Alkaloids **3** or **4** have not been reported to occur in reed canarygrass.

This paper describes the isolation of **2** and **3** from selected genotypes of reed canarygrass. Their color reactions to xanthydrol and their R_f's on paper chromatograms [1] may have caused them to be previously identified incorrectly as two tryptamine analogues, 5-methoxy-*N*,*N*-dimethyl-tryptamine (5-MeO-DMT) and *N*,*N*-dimethyltryptamine (DMT), respectively.

RESULTS AND DISCUSSION

The alkaloid content of 12 clones of reed canarygrass was examined by GC–MS. The results showed that 5 clones contained one or both of 5-MeO–DMT and a substance with a *m/e*, M^+ of 216. Paper chromatographic analysis of the extracts revealed 5-MeO–DMT and a substance migrating at R_f 0.47 which was previously thought to be DMT. However, GC–MS analysis of these extracts revealed no detectable amount of DMT.

β - Carboline derivatives



	R_1	R_2	R_3
(1)	H	MeO	Me
(2)	H	MeO	H
(3)	H	H	H
(4)	Me	MeO	H

Further, the fragmentation pattern of the substance with a *m/e*, M^+ of 216 was shown to be identical with authentic **2**. Both the R_f in the paper chromatographic system used (Table 1) and the RR_f on the OV-1 GLC column

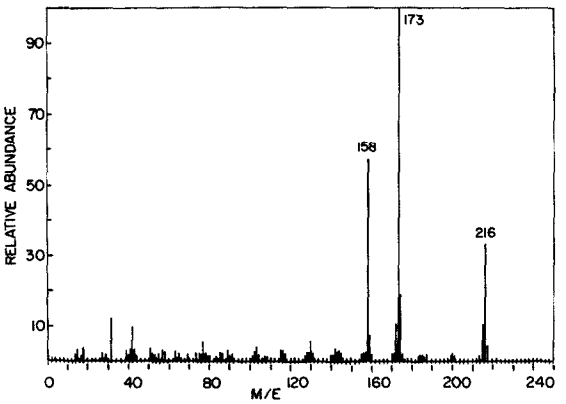


Fig. 1.

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Table 1. R_f, RR_t of tryptamine and β-carboline derivatives, and the color and λ_{max} and λ_{min} of their xanthydrol reaction products

Substance	R_f§	RR_t* (min)	Xanthydrol reaction products† color	λ_{max} (nm)	λ_{min} (nm)
5-MeO-DMT	0.42	5.4	Blue	590	425–500
DMT	0.48	3.3	Lavender	510	400
2	0.47	6.5	Blue-Violet	560	425–500
3	0.51	4.4	Lavender	505	427

* The RR_t of the substance was measured in a GC–MS containing an OV-1 column as described in the text.

† Developed paper chromatograms were sprayed with 0.1% xanthydrol reagent (0.1 g xanthydrol in EtOH:11.7 N HCl 19:1).

§ On paper in *n*-BuOH(HOAc–H_2O (80:3:17)

were the same as authentic **2** confirming earlier report of its occurrence in reed canarygrass [5,6]. Table 1 shows that DMT and **2** are not resolved by the paper chromatographic system, and thus clones reported [1,2] to contain DMT in mixture with 5-MeO–DMT may contain either DMT or **2**, or possibly both. Although these substances (DMT and **2**) have similar R_f's, the colors of their xanthydrol reaction products are quite different (Table 1) in contrast, to those of DMT and **3**. However, all 4 alkaloids (5-MeO–DMT, DMT, **2**, and **3**) are readily resolved by GLC on an OV-1 column as shown by their RR_t values.

The distribution of hordenine, gramine, DMT, MTHC, 5-MeO-DMT and 6-MeO-THC in 12 clones of 3–4-week-old reed canarygrass regrowth is shown in Table 2. The quantity, but not the type [2], of total basic alkaloids in the vegetation is influenced by age, and by environment [4]. Hordenine occurred in 7 of the 12 clones tested. The results also show that in those clones containing gramine, the tryptamine and β-carboline derivatives were absent, and *vice versa*. However, paper chromatographic analysis of extracts from numerous other clones [1,2] has revealed an occasional clone containing both gramine and tryptamine derivatives. These results need to be confirmed with GC–MS. Further, the occurrence of either 5-MeO–DMT or **2** in 5 of the 12 clones and the lack of occurrence of either DMT or MTHC, or their respective hydroxy derivatives, in these 5 clones

Table 2. Occurrence of amines and β-carboline alkaloids in selected reed canarygrass clones†

Clone	Hordenine	Gramine	DMT	**3**	5-MeO-DMT	**2**
m/e, M^+	165	174	188	186	218	216
R34	+++	++				
R99	+++	+++				
R22	+	+++				
R21		+++				
R504	++		+++			
R16			+++	+		
R38			+	+++		
R96	+				+++	+
R37	++				+	+++
R52	++					+++
R51					+++	
R5					+++	+

† 5 g fr. grass from the indicated clones were extracted as described in Experimental section and aliquots were subjected to GC–MS. The various substances were identified on the basis of RR_t and fragmentation patterns. The relative quantities of alkaloids are: +++, large; ++, intermediate; +, trace.

provides indirect evidence that hydroxylation, not methylation, constitutes the limiting reaction in those clones lacking the methoxy groups.

Clone R38 was of particular interest in that it contained no 5-MeO–DMT or **2**, but contained a relatively large quantity of a substance with *m/e*, M^+ of 186 as well as traces of DMT. The fragmentation pattern, PC, and RR_t of the unknown substance was identical with authentic **3** (Table 1). This β-carboline has not been reported previously as a constituent of reed canarygrass.

The inheritance of these tryptamine and β-carboline alkaloid derivatives in reed canarygrass is under investigation at this institution. Preliminary results show that among progenies in a cross between R37 and R38, 3 contained 5-MeO–DMT with a trace of **2** and **3**. At no other times have we observed plants to contain 5-MeO–DMT with **3**. Crosses between appropriate clones may show a somewhat different distribution of these alkaloids.

EXPERIMENTAL

Reagents. All reagents used were analytical reagent grade.

Methods. 5.0 g fr. grass from each clone were extracted with 50 ml MeOH–NH_4OH (25:1, v/v) and the alkaloids partially purified as described previously [1]. Alkaloids in the residue obtained were dissolved with 0.25 ml $CHCl_3$. PC of the alkaloids on Whatman 3MM paper was carried out on 25 μl aliquots of the $CHCl_3$ extract containing >250 μg of alkaloids [1,9] with (*n*-BuOH–HOAc–H_2O, (80:3:17). Alkaloids were visualized by spraying it with 0.1% (w/v) xanthydrol reagent [9]. Sample extracts were compared to adjacent reference compounds. GC–MS was conducted with an LKB 9000 spectrometer containing 1.2 m × 2 mm column of 80–100 mesh Supelco coated with 3% OV-1. The column was temperature programmed at 8°/min from 150° to 250°. Spectra of the xanthydryl salts were measured in $CHCl_3$.

Plant materials. Clones R5 and R16 were received from R. F. Barnes, U.S. Regional Pasture Research Laboratory, University Park, Pennsylvania, under the designations 405-9 and 369-3, respectively. The other clones trace to a highly diverse source population used in plant breeding and genetic studies at the University of Minnesota. Department of Agronomy and Plant Genetics.

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