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

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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 Rf'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 Rf 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

Further, the fragmentation pattern of the substance with a m/e, M⁺ of 216 was shown to be identical with authentic 2. Both the Rf in the paper chromatographic system used (Table 1) and the RR on the OV-1 GLC column
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 Rf'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 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 [23, 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 /3-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 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 of the unknown substance was identical with authentic 3 (Table 1). This /3-carboline has not been reported previously as a constituent of reed canarygrass.

The inheritance of these tryptamine and /3-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-NH4OH (25:1, v/v) and the alkaloids partially purified as described previously [1]. Alkaloids in the residue obtained were dissolved with 0.25 ml CHCl3. PC of the alkaloids on Whatman 3MM paper was carried out on 25 ml aliquots of the CHCl3 extract containing >250 mg of alkaloids [1,9] with (n-BuOH-HOAc-H2O, 80:3:1). 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 LKB9000 spectrometer containing 1.2m x 2mm 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 xanthydrol salts were measured in CHCl3.

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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REFERENCES