Phytochemistry, 1977, Vol. 16, pp 749–752. Pergamon Press. Printed in England.

FURTHER CHARACTERIZATION AND QUANTITATIVE DETERMINATION OF 5-METHOXY-*N*-METHYLTRYPTAMINE IN *PHALARIS ARUNDINACEA*

WALTER MAJAK*

and

ROBERT J. BOSE†

* Research Station, Agriculture Canada, Kamloops, British Columbia, Canada‡; † Vancouver Laboratory, Fisheries and Marine Service, 6640 N.W. Marine Drive, Vancouver, British Columbia, Canada

(*Received* 12 *November* 1976)

Key Word Index—*Phalaris arundinacea*; Gramineae; reed canarygrass; isolation and fluorometric determination; indole alkaloids.

Abstract—The 100 MHz PMR spectrum of 5-methoxy-*N*-methyltryptamine (5MMT) isolated from *Phalaris arundinacea* is elucidated, acid-induced fluorescence characteristics of 5-methoxy- and 5-hydroxytryptamine alkaloids on Avicel and silica gel TLC are described, and the concentration of 5MMT in *P. arundinacea* is determined by fluorometric TLC scanning.

INTRODUCTION

In British Columbia, large areas of native wet meadows, both Organic and Gleysolic [1], as well as cultivated pastures in areas of medium to high rainfall are being reseeded to *Phalaris arundinacea* L. (reed canarygrass) [2]. Under certain conditions, however, ruminants grazing reed canarygrass can be debilitated. Reduced intake and poor performance could be related to palatability which was found to be negatively associated with total alkaloid (mainly indolealkylamine) concentration of reed canarygrass [3, 4]. *N,N*-dimethyltryptamine (DMT) and 5-methoxy-*N,N*-dimethyltryptamine (5DMT) have been detected in both *P. arundinacea* [3, 5] and *P. aquatica* L. (*P. tuberosa* L.) [6], and in the case of the latter species these compounds have been linked to the occurrence of 'phalaris staggers', an acute disorder of the central nervous system in sheep [7, 8]. The tryptamines of *P. arundinacea* also have been implicated in pasture-mediated bovine pulmonary emphysema [9]. In this work reed canarygrass samples from representative wet meadow sites were screened to determine their indolealkylamine composition, the structure of the principal indolealkylamine was confirmed by PMR, and a fluorometric TLC scanning procedure was developed to quantify 5-methoxy- and 5-hydroxy-tryptamine alkaloids.

RESULTS AND DISCUSSION

We detected 5-methoxy-*N*-methyltryptamine (5MMT) in reed canarygrass from wet meadows of the interior of the province. Although this compound was originally isolated from reed canarygrass and synthesized by Wilkinson [10] in 1958, most recent reports [8, 9, 11–13], largely based on chromatographic evidence, identify other indole derivatives as major alkaloids of reed canarygrass. In view of the importance of these substances in relation to increased seedings of the crop throughout British Columbia, it would seem advisable to establish their identity in a more rigorous manner. Because of the lack of a ready source of authentic 5MMT, identification in this work was based on the PMR spectrum of the isolate and comparison with spectra of related commercially available substituted indoles.

As described in the Experimental section, 5MMT was purified by fractionation on neutral aluminium oxide followed by PLC on Avicel-Silica Gel 7 and the yield was 0.001% (fr. wt). Attempted isolations of 5MMT by PC or 100% cellulose PLC were unsuccessful since the compound was found to be strongly adsorbed on these layers.

The PMR spectrum of 5MMT in deuteromethanol showed sharp singlets for the *N*-methyl and *O*-methyl protons at δ2.71 and δ3.85 respectively, a multiplet corresponding to aminoethyl protons in the region of δ3.20 was partially obscured by the residual protons in deuteromethanol, and a singlet for the H_2 proton on indole occurred at δ7.13. The remaining protons appear as an AMX pattern with H_4 as a closely spaced doublet (J 2.7) at δ7.05 and H_7 as a doublet with a coupling constant of 8.5 Hz and centered at δ7.26. H_6 appears as a quartet in the spectrum of 5MMT centered at δ6.78 with the afore-mentioned coupling constants. Apart from the methylaminoethyl protons, the remainder of the spectrum of 5MMT bears a close resemblance

‡Contribution number: 263.

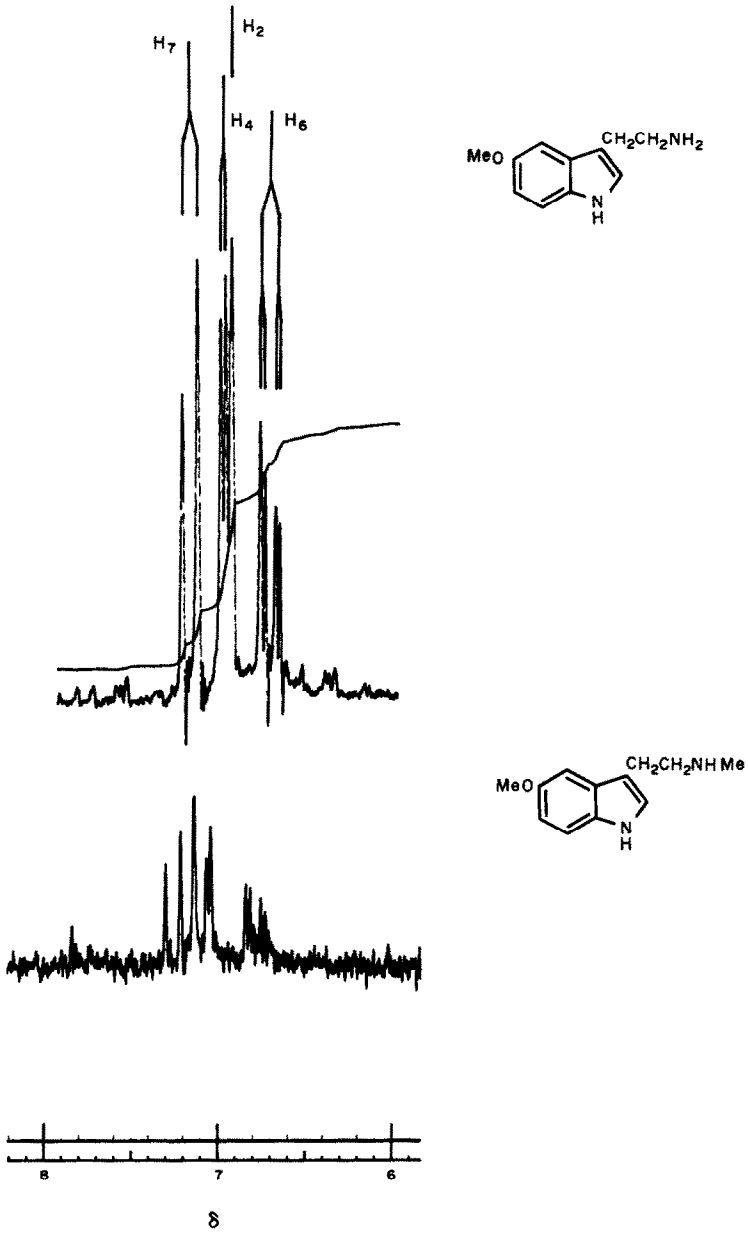

Fig. 1. The partial ^{1}H PMR spectra of 5-methoxytryptamine and 5-methoxy-*N*-methyltryptamine.

(Fig. 1), as expected, to the spectrum of 5-methoxytryptamine for which an authentic sample was readily available. The latter gave a 3H singlet for the 5-methoxy group at δ3.76, and a singlet for the proton on carbon 2 at δ6.94. H_4 appears as a 2.7 Hz doublet at δ6.98, and a doublet appears at δ7.17 with a coupling of 8.7 Hz for H_7, while H_6 gives a quartet centered at δ6.72 with both 2.7 and 8.7 Hz couplings. The AMX pattern for the aromatic protons of both 5-methoxytryptamine and 5MMT readily distinguish them from indole derivatives lacking the 5-substituents and from the carbolines, which are devoid of the indolyl H_2, previously reported in reed canarygrass [13, 14].

During the extraction procedure the compound was visualized on cellulose-TLC (Avicel or Avicel-silica gel 7) by acid-induced fluorescence and/or spraying with Ehrlich's reagent [12]. 5-Methoxy- and 5-hydroxytryptamines yield bright yellow fluorescent spots under UV light (254 nm) on exposure to HCl vapour or on chromatography in acidified solvents such as MeOH–conc HCl (9 : 1) or BuOH–2M HCl (see below). The acid-induced visible (near 550 nm) fluorescence of serotonin and related 5-substituted indoles has been shown to be due to the exciplex formed by protonation of the substituted indoles in their excited states, and not to excitation of protonated ground states [15]. However, less intense fluorescence (15%) was observed for 5MMT at the 0.05–0.5 μg level on silica gel plates as compared to cellulose plates exposed to HCl. MacNeil *et al.* [16] have shown that for a number of 5-substituted indoles adsorbed on silica gel and exposed to dilute acid spray, there is only the normal UV fluorescence (347–360 nm)

corresponding to uncomplexed compound and a weak concentration dependent excimer fluorescence (430–475 nm). Further, it is recognized from frequency changes in the OH stretch of the silanol groups on silica gel [17] that there is increasing interaction between adsorbent and adsorbate on the addition of polarizable functional groups, particularly ether and amino substituents, to organic molecules which profoundly affect the spectroscopic properties of the adsorbed substance. It has also been shown [18] that for nitrogen heterocycles and polycyclic aromatic hydrocarbons, the visible fluorescence arising from exciplex formation (protonation of excited states by silanol OH groups) on silica gel, diminishes with increasing basicity and consequent protonation of the ground states by the absorbent. The behaviour of 5MMT on silica gel is explicable in terms of ground state interaction between the silanol OH groups and the methoxyl and methylaminoethyl substituents. By contrast, there is probably little such interaction between 5MMT and cellulose, and protonation of the excited state is more probable and could account for the intense visible fluorescence observed on exposing 5MMT to HCl vapour on cellulose-TLC.

Although intensive studies have been carried out on the proximate analysis of reed canarygrass alkaloids, the determination of specific indolealkylamine levels has received little attention. Consequently, we adapted the acid-induced fluorescence reaction to the quantitative determination of 5MMT. The TLC scanning procedure (see Experimental) lends itself as well to the determination of serotonin (5-hydroxy-tryptamine), 5DMT and bufotenine (5-hydroxy-*N*,*N*-dimethyltryptamine) which was found in *P. aquatica* [6]. The method is sensitive to 5 ppm 5MMT (recorder output, 1 mv: fluorometer range selector, ×3) but it was sufficient to work in the 50–500 ppm 5MMT range (10 mv, ×3) for the reed canarygrass samples. Our determinations yielded the following levels (mg/100 g dry wt) of 5MMT based on random samplings of five reed canarygrass stands: 6.7; 1.8; 0.2; 5.2 and 1.4. Concentrations of 5MMT in *P. arundinacea* have not been previously reported but the above values are comparable to the levels of bufotenine and lower than the concentrations of DMT and 5DMT reported in *P. aquatica* [19, 20]. The latter 3 compounds, however, in addition to *N*-methyltryptamine [5, 12] were not detected in the British Columbia samples. Our screening procedure eliminated these compounds by two simple TLC checks: the TLC system employed in the quantitative determination resolved DMT (R_f 0.6) and *N*-methyltryptamine (R_f 0.6) from 5MMT (R_f 0.43), 5DMT (R_f 0.43) and bufotenine (R_f 0.31). 5MMT and 5DMT were resolved on Avicel-Silica Gel 7 TLC in isoPrOH–EtOAc–conc NH_4OH (60:15:3) [5], yielding R_fs of 0.51 and 0.83 respectively. On the other hand, the presence of gramine and hordenine was confirmed in all the samples by co-chromatography with authentic standards and by colour reactions with diazotised *p*-nitroaniline (System A [21]), the former compound yielding a mauve colour and the latter a magenta colour on Avicel TLC.

EXPERIMENTAL

Plant material. For large-scale isolations, *P. arundinacea* L. (commercial variety) was harvested in July 1976, at Strachan Lake, a wet meadow near Kamloops, B.C., which was reseeded to reed canarygrass in the spring of 1975. Other samples of reed canarygrass were obtained from established meadows in the Kamloops-Cariboo district during June to September 1976.

Large-scale isolation. Fresh-frozen plant material (4 kg) was chopped in a cold room, twice extracted in a Waring blender with EtOH and the filtrate was concentrated to 10 l. by evaporation in a fume hood for 2 days. The concentrate was filtered again, reduced in vol in a rotary evaporator to 2 l. adjusted to pH 9.5 with NaOH and extracted ×3 with 800 ml portions of $CHCl_3$. One quarter of the $CHCl_3$ extract was washed once with an equal vol. of H_2O and the $CHCl_3$ layer was concentrated and applied to a 14 × 3 cm Al_2O_3 (Woelm, neutral, activity grade 1) column equilibrated in $CHCl_3$. The column was eluted with the following order of solvents: $CHCl_3$ (100 ml); Me_2CO (100 ml); Me_2CO–EtOH (1:1, 100 ml); PrOH (200 ml); 5MMT was finally eluted with PrOH–EtOH (1:1, 300 ml). The fraction containing 5MMT was concentrated and applied to 4 Avicel (pre-washed)-Si gel 7 (Baker No. 3406) (1:1, 20 × 40 cm, 1 mm thick) plates which had been developed in MeOH and dried before sample application. The plates were chromatographed in BuOH–HCO_2H–H_2O (16:1:3), and the 5MMT band (detected near the edge by HCl-induced yellow fluorescence) was eluted with MeOH and yielded pale yellow feathery crystals on drying.

Quantitative determination. To ensure complete extraction, fresh-frozen plant material (10 g) was chopped, extracted for 10 sec in a Waring blender with 100 ml $CHCl_3$–MeOH–conc NH_4OH (26:33:1) [22], filtered through glass wool and the residue was re-extracted and washed with 50 ml portions of the same solvent to a final vol. of 300 ml. One third of the filtrate was acidified with 2N H_2SO_4, the $CHCl_3$ phase was discarded, and the aq. phase was washed ×3 with $CHCl_3$. The aq. phase was adjusted to pH 9.3 with conc NH_4OH, extracted ×3 with 50 ml portions of $CHCl_3$ and the $CHCl_3$ extract washed ×3 with H_2O. The washed $CHCl_3$ extract was concentrated to dryness, redissolved in 0.5 ml 2-methoxy-EtOH, and duplicate 2 µl aliquots were applied to a precoated cellulose TLC plate (100 µm thick, EM Laboratories No. 5757) under a stream of N_2. Adjacent standards (0.05 to 0.5 µg) were applied, the plate was developed in BuOH–2N HCl (1:1, upper phase) to a height of 7 cm and dried for 20 min in a fume hood in a uniform current of air away from direct light. The 5MMT spots (bright yellow when irradiated with short-wave UV light) were marked and immediately scanned at right angles to the solvent flow in a Camag TLC scanner attached to a Turner fluorometer (Model 111) employing a 7–54 primary filter, a 2A-15 secondary filter and a far-UV lamp (Turner No. 110-851). The 5MMT spots were photosensitive, however, resulting in a 3% decrease in fluorescence intensity in 6 min (the time required for scanning one 20 × 20 cm plate) and therefore it was essential to include standards with each run. A narrow slit width (20% of maximum) minimized baseline drift, and produced sharp, symmetrical peaks, and consequently peak ht was used to determine concentration. When an authentic sample of 5MMT (100 µg) was subjected to the above fractionation procedure, the compound was recovered in 86% yield.

Acknowledgements—The authors are indebted to Ruth McDiarmid for her excellent technical assistance and to Klaas Broersma for providing plant material.

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