Phytochemistry, 1978, Vol 17, pp 301–303. Pergamon Press Printed in England

TLC LUMINESCENCE OF GRAMINE AND RELATED INDOLE ALKALOIDS IN *PHALARIS ARUNDINACEA*

WALTER MAJAK*, RUTH E. MCDIARMID* 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* 30 *April* 1977)

Key Word Index—*Phalaris arundinacea*; Gramineae; reed canarygrass; luminescence of indole alkaloids; fluorometric scanning.

Abstract—Luminescence characteristics of gramine, dimethyltryptamine and related indoles on Si gel TLC are described, chromatographic conditions are devised which enhance the luminescence, and a fluorometric TLC scanning procedure is developed for the quantitative determination of gramine in *Phalaris arundinacea*. 5-Methoxy- and 5-hydroxyindoles are readily distinguished from the indoles lacking a polar substituent at position 5.

INTRODUCTION

Recent studies have shown that the forage potential of reed canarygrass (*Phalaris arundinacea*) can be improved by developing alkaloid-deficient varieties [1]. Improved palatability and animal weight gains coincided with low levels of 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeODMT), gramine [3-dimethylaminomethyl)-indole] and 2-methyl-6-methoxy-1,2,3,4-tetrahydro-β-carboline [1]. Martin *et al.* [1]. however did not detect symptoms of 'phalaris staggers' during their two-year study. Earlier workers had linked this peracute disease, characterized by neurological disorders in sheep, to the occurrence of dimethylated tryptamines such as *N*,*N*-dimethyltryptamine (DMT), 5-MeODMT and 5-hydroxy-DMT (bufotenine) in *P. aquatica* (*P. tuberosa*) [2].

As new reed canarygrass varieties are introduced and distributed, screening procedures are required to assess the effects of different enviromental factors on alkaloid levels. Previously, we reported a quantitative TLC scanning procedure based on the acid-induced fluorescence of 5-methoxy- and 5-hydroxytryptamines in UV light [3]. We now describe TLC fluorescent properties of gramine and related indoles lacking a polar substituent at position 5. A fluorometric TLC scanning procedure has been developed to efficiently quantify gramine.

RESULTS AND DISCUSSION

There have been a number of different approaches to the quantitative and qualitative determination of gramine and related indole alkaloids. Procedures based on PC followed by elution and colorimetric estimation with the xanthydrol reagent are subject to the limitations of PC resolution [4]. We have also encountered difficulties in obtaining quantitative recoveries in preparative scale separations [3] and direct colorimetric analysis of crude indole alkaloid extracts by the xanthydrol reagent suffer from the obvious lack of specificity when more than one indolealkylamine is present [5]. While a quantitative GLC approach for gramine analysis looks promising [6, 7] it would be desirable to have available an alternative sensitive quantitative TLC procedure for gramine and related indole derivatives.

The direct fluorometric analysis of gramine and related indole derivatives in solution was examined earlier by Burnett and Audus [8] but their method appears to have received little further attention. At the same time, several methods based on the generation of the highly fluorescent carbolines from tryptamines by reaction with formaldehyde, ninhydrin and *o*-phthalaldehyde were desribed [9]. The formation of highly coloured charge-transfer complexes of gramine and other indole derivatives with a variety of electron acceptors has been described by Hutzinger *et al.* [10] on both silica gel layers and paper chromatograms. While these authors established a linear relationship between the concentration of indole acetic acid and the colour intensity of the complexes formed, no such dependence was established for gramine and related tryptamines.

The highly fluorescent products of reaction of paraformaldehyde with tryptamine derivatives were examined on silica gel TLC by Toneby [11]. However, the reaction failed for both *N*,*N*-dialkyltryptamines and gramine.

We observed that when gramine was separated by TLC on Avicel-silica gel (1 : 1) in isoPrOH–EtOAc–conc NH_4OH (60 : 15 : 3), and the plate was immediately examined under short-wave UV light the spot corresponding to gramine gave a violet fluorescence. Attempts to quantify gramine fluorometrically, however, were not reproducible since the intensity was not persistent. The loss of fluorescence coincided with the evaporation of solvent from the plate and could be prevented by incorporating one or more high boiling point solvents into the developing solvent system. For example, inclusion of 6% 2-ethoxy-EtOH in the aforementioned solvent system increased the persistence of fluorescence of gramine (10 μg) on a silica gel plate (EM Lab no. 5763) from 10 to 15 min. The best system proved to be (PrOH–EtOAc–conc NH_4OH–2–ethoxy–EtOH (60 : 15 .

‡ Contribution no.. 267

3.5, 'PENE'), where fluorescence under UV persisted for 30 min following development of the plate. It was further observed that when the developed plate was dried in the cold room (30 min, 2°, 88% r.h.) rather than in the laboratory as is the normal practice, a further improvement in the persistence and a 5-fold increase in intensity of fluorescence was observed. It appeared that fluorescence of gramine and other indolealkylamines of interest is strongly influenced by sorptive forces on the silica gel surface and quenching is substantially altered by the presence of residual solvents and possibly water. This was supported by the fact that the simple expedient of spraying the developed and dried silica gel plate with water fully restored the intense violet fluorescence of gramine. Fluorescence of gramine on silica gel TLC was also induced by chromatographic development in 20% aqueous solutions of monoethanol-amine or triethanol-amine or *N* NaOH. Ammonia fumes only induced gramine fluorescence on silica gel plates when the plate was initially treated with a fine spray of water.

The following indoles (2 μg) yielded a violet fluorescent colour following the TLC on silica gel in PENE: tryptamine hydrochloride ($R_f = 0.33$; relative intensity of luminescence = 100%); 5-methyltryptamine hydrochloride (0.35; 87%); tryptophan (0.06; 83%); DMT (0.57; 88%); *N*-methyltryptamine (0.23; 110%); gramine (0.39; 55%); and 3-methylindole (0.98; 140%). These indolealkylamines which lack polar substituents at position 5 were readily distinguishable from 5-hydroxy- and 5-methoxytryptamines which gave pale yellow fluorescent colours on silica gel TLC (PENE). The latter group of compounds could also be identified with increasing sensitivity by the acid-induced fluorescence on TLC (bright yellow) described previously [3].

The photophysical processes involved in substances adsorbed on solid surfaces are presumably complex [12] and the apparent contradiction between different studies has done little to clarify the issue. In mixed solvents, for example, indoles become increasingly more fluorescent with decreasing proportions of water [13], while the general statment has been made [14, 15] that substances adsorbed on thin layers of cellulose and silica gel fluoresce more intensely in the presence of solvent than on dry plates. Lloyd [16], by contrast cites examples in which direct interaction between analyte and adsorbate effectively enhances fluorescence and such interactions on cellulose [17, 18] and on silica gel [19] enabled the determination of a wide range of organic substances. The photophysics of indole derivatives vary greatly and Herschberger and Lumry [20] have shown 5-methoxy- and 5-hydroxyindole to be atypical indoles in that they are non-exciplex forming, as they are less basic in their excited than ground states and would therefore be expected to behave differently on silica gel than indoles lacking oxo substituents in the 5 position.

We adapted the alkali-induced fluorescence reaction on silica gel for the quantitative determination of gramine in reed canarygrass extracts. The fractionation procedure described previously [3] recovered authentic gramine in 93% yield. The fluorometric scanning technique was sensitive to 0.1 micrograms gramine, and gave a linear relationship over 0.5–4.0 μg range. Table 1 illustrates the range of values obtained for gramine and 5-MeOMT from 22 samples of reed canarygrass representing 3 varieties. The range in values for Castor points to the possible fluctuations within a variety. The levels in Table 1 for gramine fall within the range of values reported by others using the xanthydrol procedure for gramine determination in tryptamine–carboline-free strains of *P. arundinacea* [21].

Table 1. Concentrations (mg/100 g dry wt) of gramine and 5-methoxy-*N*-methyltryptamine (5-MeOMT) in 3 varieties of *P. arundinacea*

	Variety					
	NRG 741*		NRG 721*		Castor†	
Indole	Sample 1	Sample 2	Sample 1	Sample 2	Range	Mean
Gramine	59	63	27	74	28–78	56
5-MeOMT	115	67	0	0	9–51	25

* Experimental varieties with high (741) and low (721) tryptamine respectively.

† Mean derived from samples collected at 18 different plots.

The fluorescence on TLC under UV of a number of synthetic tetrahydro-β-carbolines, indicated that 6-methoxy- and 6-hydroxytetrahydro-β-carbolines could be detected via the acid-induced fluorescence reaction [3] and that the tetrahydro-β-carboline derivatives lacking the polar substituents at position 6 (equivalent to position 5 on the indole nucleus of tryptamines) were readily distinguished by the violet luminescence reaction on silica gel TLC (PENE). Tetrahydro-β-carbolines have been reported as constituents of *P. arundinacea* [22, 23] but these compounds were not detected in our screening experiments employing a variety of TLC solvent systems, the xanthydrol spray reagent [24] and the fluorescence reactions described above.

EXPERIMENTAL

Samples of *P. arundinacea* L. were collected at Beaverlodge, Alberta, in September 1976 from the aftermath following seed harvest. Fresh-frozen 10g portions were extracted, fractionated and the final $CHCl_3$ extract was concd to dryness and redissolved in 0.5 ml 2-methoxy-EtOH as in ref. [3] Following the initial extraction, glass-distilled solvents were used throughout the fractionation procedure and chromatography steps.

Quantitative determination. Duplicate 2 μl aliquots were used for quantifying the concn of 5-MeOMT [3]. For gramine determination, duplicate 2 μl aliquots were applied to a pre-coated Si gel 60 plate (250 μm thick, EM Laboratories No. 5763) under a stream of N_2. Adjacent standards (1 to 3 μg) were applied, the plate was developed in PENE and dried for 30 min at 2°. The gramine spots (violet under short-wave UV light) were located and the plate was allowed to equilibrate for 10 min in the Camag TLC scanner at room temp. The Camag scanner was attached to a Turner fluorometer (Model 111) employing a 7-54 primary filter, a 47B secondary filter and a far UV lamp (Turner No. 110-851). Alternatively for the secondary filter system a 47B can be combined with a 24 permitting a wider selection of slit widths and range selector settings. A narrow slit width (10% max) was essential at the × 3 range selector setting. The gramine spots were scanned at right angles to the solvent flow, yielding sharp symmetrical peaks and consequently peak height was used to determine concentration. The

fluorescence of the gramine spots was fugitive, however, resulting in a slight decrease (1 to 2%) in fluorescence intensity between scans of a 20 × 20 cm plate, and therefore it was essential to include standards with each run.

Acknowledgements—We thank Dr S. G. Bonin for supply of experimental reed canarygrass varieties and Dr G. R. Elliot for a gift of tetrahydro-β-carbolines.

REFERENCES

1. Marten, G. C., Jordan, R. M. and Hovin, A. W. (1976) *Agron. J.* **68**, 909.
2. Gallagher, C. H., Koch, J. H., Moore, R. M. and Steel, J. D. (1964) *Nature* **204**, 542.
3. Majak, W. and Bose, R. J. (1977) *Phytochemistry* **16**, 749.
4. Simons, A. B. and Marten, G. C. (1971) *Agron. J.* **63**, 915.
5. Woods, D. L. and Clark, K. W. (1971) *Can. J. Plant Sci.* **51**, 323.
6. Williams, M., Barnes, R. F. and Cassady, J. M. (1971) *Crop Sci.* **11**, 213.
7. Audette, R. C. S., Bolan, J. Vijayanagar, H. M., Bilous, R. and Clark, K. (1969) *J. Chromatog.* **43**, 295.
8. Burnett, D. and Audus, L. J. (1964) *Phytochemstry* **3**, 395.
9. Guilbault, G. G. (1973) *Practical Fluorescence, Theory, Methods and Techniques.* p. 306. Marcel Dekker, New York.
10. Hutzinger, O., Heacock, R. A., MacNeil, J. D. and Frei, R. W. (1972) *J. Chromatog.* **68**, 173.
11. Toneby, M. I. (1974) *J. Chromatog.* **97**, 47.
12. Nicholls, C. H. and Leermakers, P. A. (1971) *Adv. in Photochem.* **8**, 315.
13. Froehlich, P. M. and Yeates, M. (1976) *Analyt. Chimica Acta* **87**, 185.
14. Sawicki, E. and Johnson, H. (1964) *Microchemical J.* **8**, 85.
15. MacNeil, J. D., Hauser, M., Frei, W. and Hutzinger, O. (1972) *Analyt. Biochem.* **45**, 100.
16. Lloyd, J. B. F. (1975) *The Analyst* **100**, 529.
17. Wellons, S. L., Paynter, R. A. and Winefordner, J. D. (1974) *Spectrochim. Acta* **80A**, 2133.
18. Paynter, R. A., Wellons, S. L. and Winefordner, J. D. (1974) *Analyt. Chem.* **46**, 736.
19. von Wandruszka, R. M. A. and Hurtubise, R. J. (1976) *Analyt. Chem.* **48**, 1784.
20. Herschberger, M. V. and Lumry, R. W. (1976) *Photochem. Photobiol.* **23**, 391.
21. Coulman, B. E., Woods, D. L. and Clark, K. W. (1976) *Can. J. Plant Sci.* **56**, 837.
22. Gander, J. E., Marum, P., Marten, G. C. and Hovin, A. (1976) *Phytochemistry* **15**, 737.
23. Vijayanagar, H. M., Audette, R. C. S. and Bolan, J. (1975) *Lloydia* **38**, 442.
24. Dickman, R. S. and Crockett, A. L. (1956) *J. Biol. Chem.* **220**, 957.