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METHOXYLATED GRAMINE DERIVATIVES FROM *PHALARIS AQUATICA*

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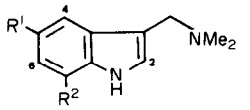
Key Word Index—*Phalaris aquatica*; Gramineae; roots; alkaloids; 5-methoxygramine; 7-methoxygramine; 5,7-dimethoxygramine.

Abstract—7-Methoxygramine and 5,7-dimethoxygramine were isolated from the roots of 12-day-old seedlings of *Phalaris aquatica*. The structures were elucidated by spectroscopic methods. 5-Methoxygramine also was detected by TLC. All three gramine derivatives are reported for the first time.

Phalaris aquatica L. is a major pasture grass of the temperate regions of the world and is known to contain several tryptamine derivatives [1] as well as gramine (**1**) [2]. We recently reported [3] a separation by TLC of alkaloids in young seedlings of *P. aquatica* cv Australian Commercial in which a number of unidentified indoles were noted in addition to the known tryptamine derivatives and gramine. Three of these unknown indoles have now been identified as new naturally occurring gramine derivatives. The first, a minor component, has been identified on the basis of chromatographic properties on TLC and colour reactions as 5-methoxygramine (**2**). The other two, the major unidentified alkaloids in the seedlings, have been isolated by preparative HPLC of the alkaloid fraction extracted from the root. These alkaloids are evenly distributed throughout the seedling, but it was convenient to isolate them from root extracts as these contain relatively less of the known tryptamines. The first of these two has been identified as 7-methoxygramine (**3**) by spectroscopic comparison with an authentic sample obtained by synthesis [4]. The ^{1}H NMR spectrum of the second compound showed three doublets centred at δ 6.30, 6.65 and 7.06. This is in good agreement with the published values for 5,7-dimethoxygramine (**4**) of δ 6.32, 6.70 and 6.98 for the aromatic protons at C-6, C-4 and C-2, respectively [5]. These three compounds were numbered 15 (5-methoxygramine), 17 (5,7-dimethoxygramine) and 18 (7-methoxygramine) in Fig. 1 in our previous publication [3].



1 $R^1 = R^2 = H$
2 $R^1 = OMe$, $R^2 = H$
3 $R^1 = H$, $R^2 = OMe$
4 $R^1 = R^2 = OMe$

EXPERIMENTAL

^{1}H NMR spectra were measured at 100 MHz in DMSO-d_6. HR-MS were recorded at 70 eV. Mps are uncor. HPLC runs were performed at ambient temp. at a flow rate of 3 ml/min using an RI detector.

Extraction. Roots of 12-day-old seedlings (500 g fr. wt) were grown [6] and extracted as previously described [3] to give an alkaloid fraction. The fraction was separated by prep. HPLC on a Waters μ Bondapak C_{18} column (7.8 mm i.d. × 30 cm) with 0.01 M diisopropylammonium phosphate in MeOH–H_2O (1:3), which was adjusted to pH 3 with H_3PO_4, as mobile phase. The mobile phase modified was subsequently removed from the purified alkaloids by rechromatography of the samples with MeOH–HOAc–H_2O (25:1:74) as eluant. The first compounds eluted were gramine and the known tryptamine derivatives [1] followed by 7-methoxygramine (R_f 9.7 min) (2 mg) and 5,7-dimethoxygramine (R_f 12.5 min) (2 mg).

7-Methoxygramine (**3**). Mp 107–109° (C_6H_6–petrol); slow (2–3 hr) development of a Bluebird Blue [7] colour on TLC at room temp. with diazotized sulphanilic acid (0.5% in 2 M HCl). ^{1}H NMR (CD_3SOCD_3): δ 10.94 (*s* (*br*), NH), 7.18 (*d* (B), J = 7.8 Hz, H-4), 7.07 (*d*, J = 2.4 Hz, H-2), 6.89 (*dd*, J = 7.8, 7.8 Hz, H-5), 6.62 (*dd*, J = 7.8, 0.8 Hz, H-6), 3.90 (*s*, OMe), 3.51 (*s*, CH_2), 2.13 (*s*, NMe_2). (Found: $[M]^{+\cdot}$, 204.1270. $C_{12}H_{16}N_2O$ requires $[M]^{+\cdot}$ 204.1262.) These data were identical with those obtained from an authentic sample obtained by synthesis [4]. The mp was not depressed on admixture with authentic **3**.

5,7-Dimethoxygramine (**4**). Mp 119–120° (C_6H_6–petrol) (lit. 121–122° [5]). Immediate development of a Claret Rose [7] colour on TLC at room temp. with diazotized sulphanilic acid (0.5% in 2 M HCl). ^{1}H NMR (CD_3SOCD_3): δ 10.68 (*s* (*br*), NH), 7.06 (*d*, J = 2.4 Hz, H-2), 6.65 (*d*, J = 2.0 Hz, H-4), 6.30 (*d*, J = 2.0 Hz, H-6), 3.87 and 3.74 (each *s*, 2 × OMe), 3.48 (*s*, CH_2), 2.08 (*s*, NMe_2). (Found: $[M]^{+\cdot}$ 234.1379. $C_{13}H_{18}N_2O_2$ requires: $[M]^{+\cdot}$ 234.1368.)

5-Methoxygramine (**2**). An aliquot of the alkaloid fraction was analysed by 2D-TLC (silica gel) [3]. By comparison of R_f values and colour reactions [3] with authentic 5-methoxygramine (Sigma) one of the previously unknown minor components was identified as 5-methoxygramine.

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