EXPERIMENTAL

Plant material of *G. indica* Chois was obtained from Sirsi, Karnatak, South India. All UV spectra were determined in MeOH, IR spectra as Nujol mulls. NMR spectra were measured on a Varian HA 100 instrument and MS with A.E.I. MS 12 and MS 9 spectrometers. TLC and PLC were carried out with Si gel (Merck Kieselgel G).

Extraction of G. indica. Heartwood was reduced to shavings (5 kg) and continuously extracted with hot $CHCl_3$ to give, after removal of solvent, 80 g crude extract. On standing the extract separated into 2 layers. The upper pale yellow oil was decanted off to leave a semi-solid black tar. A 16 g portion of the tar was chromatographed on Si gel and the column eluted with increasingly polar mixtures of PhMe–EtOAc and finally with MeOH. The early combined fractions gave more of the aliphatic oils previously decanted from the crude extract. Further elution gave a yellow oil R_f 0.7 (EtOAc–PhMe, 15:85) which was purified by PLC to give 1,7-dihydroxyxanthone (4 mg) mp 225–230° (lit [10], 239°) identical with an authentic sample. (Found: M^+, 228. Calc. for $C_{13}H_8O_4$ M, 228). Further elution gave dimethyl terephthalate (52 mg) as colourless needles mp 130–132° lit [13] 142°. UV λ_{max} nm ($\varepsilon \times 10^{-3}$) 275 (24.0), 289 (26.9), 321 (17.8). IR ν_{max} 1727, 1288, 1121, 1114, 1023, 961, 820, 740 and 734 cm^{-1}. NMR τ (CCl_4) 2.00 (*s*, 4H) 6.13 (*s*, 6H). (Found: M (mass spectrum), 194.0589. Calc. for $C_{10}H_{10}O_4$ M, 194.0579).

Concentration of the remaining fractions from the chromatography gave a mixture of biflavanoids (6.3 g). PLC of a portion of this mixture (300 mg) gave volkensiflavone (52 mg), and morelloflavone (190 mg) as amorphous yellow powders identical with authentic specimen. Methylation of the biflavanoids with dimethyl sulphate gave volkensiflavone hexamethyl ether M^+, 624 and morelloflavone heptamethyl ether M^+, 654.

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BIOSYNTHESIS OF GRAMINE IN *PHALARIS ARUNDINACEA*

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Key Word Index—*Phalaris arundinacea*; Gramineae; gramine; tryptophan; alkaloid biosynthesis.

Abstract—The administration of L-tryptophan-[3-^{14}C] to *Phalaris arundinacea* L. (Vantage strain) for 9 days resulted in the formation of radioactive gramine (8.2% absolute incorporation). A systematic degradation of the alkaloid indicated that essentially all its activity was located on the methylene group, indicating that its biosynthesis is the same as that occurring in *Hordeum* species and *Lupinus hartwegii*.

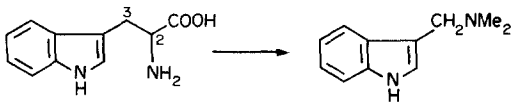
INTRODUCTION

Gramine (2) is found in reed canarygrass (*Phalaris arundinacea*, Gramineae) [1, 2]; several tryptamines and β-carbolines have also been isolated from this species [3, 4]. It has been established that different reed canary grass cultivars differ with respect to alkaloid concentration and the distribution patterns of specific alkaloids [4, 5]. The 'Vantage' cultivar contains only gramine, and this strain has been shown to be genetically recessive with respect to the tryptamine alkaloids [6].

It has been previously established that tryptophan (1) is a precursor of gramine in barley (*Hordeum* species) [7–9] and the lupin (Leguminosae): *Lupinus harwegii*

* Contribution No. 144 from this Laboratory.

[10]. It was thus of interest to determine whether gramine is produced in reed canarygrass by the same biosynthetic mechanism.



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RESULTS AND DISCUSSION

Accordingly L-tryptophan-[3-^{14}C] was fed by the wick method to *Phalaris arundinacea* (Vantage) for 9 days. The commercial L-tryptophan-[3-^{14}C] contained a significant amount of labelled tryptamine which was removed prior to feeding (see Experimental). Extraction of the aerial parts of the plant afforded gramine as the sole alkaloid. The absolute incorporation of activity into gramine was impressive (8.2%), much higher than that previously observed in *Lupinus hartwegii* (0.016–0.12% [10]) or *Hordeum distichum* (0.27% [8], 0.52% [11]). The radioactive gramine was degraded as previously described [10]. Gramine was converted to its methiodide which was subjected to solvolysis with sodium hydroxide affording 3-hydroxymethylindole and trimethylamine, collected as tetramethylammonium iodide. On boiling the 3-hydroxymethylindole with water, formaldehyde (derived from the methylene group of gramine) and 3,3′-diindolylmethane were obtained. The activities of these degradation products are recorded in the Table, and indicate that essentially all the activity of the gramine was located on its methylene group. This result is consistent with the formation of gramine from tryptophan by cleavage of the side chain between C-2 and C-3. The recent detection of 3-methylaminomethylindole (norgramine) in *Phalaris arundinacea* [12] supports the biosynthetic sequence: tryptophan → 3-aminomethylindole → 3-methylaminomethylindole → gramine.

Table 1. Activities of the L-tryptophan-[3-^{14}C] fed, and the isolated gramine and its degradation products

	Specific activity (dpm/mmol × 10^{-7})
L-Tryptophan-[3-^{14}C] [6.5 mg, 2.07 × 10^8 dpm]	650
Gramine [229 mg, 1.70 × 10^7 dpm]	1.29
Gramine methiodide	1.26*
3-Hydroxymethylindole	1.28
Tetramethylammonium iodide	inactive
Formaldehyde dimedone	1.26
3,3′-Diindolylmethane	1.32

All radioactive samples were crystallized to constant activity.
* The reported specific activities of the degradation products of gramine are average values obtained after carrying out the degradation in duplicate.

EXPERIMENTAL

General methods. A Nuclear Chicago Mark II liquid scintillation counter was used for assay of the radioactive compounds using dioxane–EtOH with the usual scintillators [13].

Feeding of the L-tryptophan-[3-^{14}C] to P. arundinacea *and isolation of the gramine.* L-Tryptophan-[3-^{14}C] (0.4 mg, nominal activity 0.1 mCi, New England Nuclear Corp. Boston, MA) was diluted to 6.5 mg with inactive L-tryptophan, and dissolved in H_2O (40 ml) containing a drop of conc. NH_3. This soln was extracted continuously with Et_2O for 18 hr. The aq soln containing tryptophan (2.07 × 10^8 dpm) was fed by the wick method to numerous *P. arundinacea* (Vantage) plants growing in soil in a greenhouse (14 hr daylight cycle, 21–23°). Cotton wicks were passed through several stems (10 or more) before being led back to the beaker which contained the tracer soln. The grass was 20 cm tall at the time of feeding, and this represented regrowth of plants which had been clipped 5 cm above ground level 3 weeks prior to feeding. The Et_2O extract of the tryptophan had an activity of 4.3 × 10^6 dpm, and was divided into two halves. Inactive tryptamine was added to one half, and gramine to the other. The reisolated tryptamine after repeated crystallization from $CHCl_3$/hexane had an activity amounting to 0.76% of the original tryptophan. The reisolated gramine had negligible activity (<0.01% of the original tryptophan). After 9 days grass was cut at soil level (fr. wt 195 g). The beakers which had contained the tryptophan soln contained 0.01% of the original activity fed. Leaves were macerated in a Waring blendor with MeOH (1.5 l) containing 1% NH_3. The filtered soln was evaporated to leave a residue which was stirred with 0.1 N HCl (250 ml) overnight. This soln was centrifuged, filtered, and its pH adjusted to 8–9 by the addition of Na_2CO_3. The soln was extracted with $CHCl_3$ (3 × 75 ml) which was then extracted with 10% KOH to remove any phenolic alkaloids. None was detected. $CHCl_3$ layer was washed with sat. NaCl and dried (Na_2SO_4). A pale brown crystalline residue (290 mg) was obtained on evaporation of the $CHCl_3$. TLC of a small fraction of this residue on Si gel GF-254 (Merck), developing with $CHCl_3$–MeOH–conc. NH_3 (10:1:1) indicated the presence of only gramine (R_f 0.4) detected as a dark spot in UV light. Crystallization of the residue from 50% aq Me_2CO afforded gramine (229 mg) as silvery plates, mp 131–132°, identical with an authentic specimen.

Degradation of the gramine. This was carried out on diluted material as previously described [10] with the following additional directions to isolate trimethylamine. Gramine methiodide was decomposed in aqueous NaOH in the presence of Et_2O which dissolved the 3-hydroxymethylindole. The aq soln was then distilled into a dry-ice-cooled receiver which contained MeI dissolved in Et_2O. After standing overnight distillate was evaporated to afford tetramethylammonium iodide.

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