Phytochemistry, Vol 22, No 1, pp 47–48, 1983
Printed in Great Britain

0031-9422/83/010047-02$03 00/0

N-METHYLTRANSFERASE ACTIVITIES IN *PHALARIS AQUATICA*

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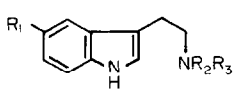
(*Received* 20 *May* 1982)

Key Word Index—*Phalaris aquatica*, Gramineae, alkaloid synthesis, *N*-methyltransferase activity, gramine, *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine, 3-aminomethylindole, 3-methylaminomethylindole

Abstract—The major alkaloids in 7-day-old seedlings of *Phalaris aquatica* are *N,N*-dimethyltryptamine, 5-methoxy-*N,N*-dimethyltryptamine and gramine Chromatographic evidence indicates that intermediates in the biosynthesis of the alkaloids are present These intermediates include tryptamine and *N*-methyltryptamine in *N,N*-dimethyltryptamine synthesis, 5-methoxytryptamine and 5-methoxy-*N*-methyltryptamine in 5-methoxy-*N,N*-dimethyltryptamine synthesis and 3-aminomethylindole and 3-methylaminomethylindole in gramine synthesis Two types of *N*-methyltransferase activities are present in crude extracts The first, consisting of two enzyme activities, is involved in the synthesis of *N,N*-dimethyl- and 5-methoxy-*N,N*-dimethyltryptamine Primary indolethylamine *N*-methyltransferase catalyses the transfer of methyl groups from *S*-adenosylmethionine to tryptamine and 5-methoxytryptamine, secondary indolethylamine *N*-methyltransferase catalyses the transfer of methyl groups from *S*-adenosylmethionine to *N*-methyl- and 5-methoxy-*N*-methyltryptamine The second, gramine-type *N*-methyltransferase, catalyses the methylation of 3-amino- and 3-methylaminomethylindole, this being a different enzyme from either primary or secondary indolethylamine *N*-methyltransferase

INTRODUCTION†

The three major indole alkaloids in *Phalaris aquatica* are DMT (**2**, R = H), 5MeODMT (**2**, R = OMe) [1] and gramine (**4**) [2]



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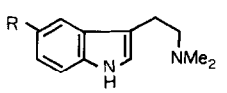

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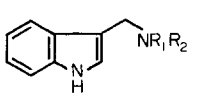

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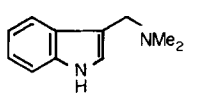

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The biosynthesis of each alkaloid requires two *N*-methylations catalysed by *N*-methyltransferases Those involved in the formation of DMT and 5MeODMT, PIM and SIM, have been partially purified but not separated [3] PIM catalyses the methylation of T (**1**, $R_1 = R_2 = R_3 = H$) and 5MeOT (**1**, $R_1 = OMe$, $R_2 = R_3 = H$) to MT (**1**, $R_1 = R_2 = H$, $R_3 = Me$) and 5MeOMT (**1**, $R_1 = OMe$, $R_2 = H$, $R_3 = Me$), respectively MT and 5MeOMT can then be converted to DMT and 5MeODMT by SIM All four methylations require *S*-adenosylmethionine (SAM) as the methyl donor The immediate precursors of gramine are thought to be AMI (**3**, $R_1 = R_2 = H$) and MAMI (**3**, $R_1 = H$, $R_2 = Me$) [4] These compounds can be *N*-methylated to gramine by enzymes present in a crude extract from barley, the reactions required SAM as the methyl donor [5]

In this paper we report the presence of AMI, MAMI, T, MT, 5MeOT and 5MeOMT in *P aquatica* seedlings and a method for the estimation of the *N*-methyltransferase activities in the gramine pathway The similarity of the *N*-methylation reactions involved in the synthesis of DMT, 5MeODMT and gramine raised the question whether PIM and SIM are also involved in gramine synthesis Preliminary data show that the *N*-methyl transfer reactions in the synthesis of gramine are catalysed by enzymes different from those required for the synthesis of DMT and 5MeODMT and that the synthesis of gramine is independent of the synthesis of DMT and 5MeODMT

RESULTS AND DISCUSSION

All of the six substrates involved in the *N*-methylation reactions, namely T, MT, 5MeOT, 5MeOMT, AMI and MAMI, as well as the alkaloids DMT, 5MeODMT and gramine, were present in the plant extract of *P aquatica* cv Australian Commercial and could be separated on the same 2D-TLC plate The compounds in the plant extract were identified by comparison with standards and estimated by their colour reactions with the van Urk–Salkowski reagent [6] This is the first time that

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†Abbreviations T, tryptamine, MT, *N*-methyltryptamine, 5MeOT, 5-methoxytryptamine, DMT, *N,N*-dimethyltryptamine, 5MeOMT, 5-methoxy-*N*-methyltryptamine, 5MeODMT, 5-methoxy-*N,N*-dimethyltryptamine, AMI, 3-aminomethylindole, MAMI, 3-methylaminomethylindole, PIM, primary indolethylamine *N*-methyltransferase, SIM, secondary indolethylamine *N*-methyltransferase, GTMT, gramine-type *N*-methyltransferase, SAM, *S*-adenosylmethionine

AMI and MAMI have been found in *P aquatica* The radioactive products obtained when the various intermediates were used as substrates with crude extracts of *P aquatica* cv Australian Commercial were identified by comparison with the standards after separation by TLC Thus T as substrate gave MT as the main (90%) radioactive product with some (10%) radioactive DMT, MT as substrate gave DMT as the only radioactive product Similarly in the gramine pathway AMI as substrate gave MAMI (95%) as the main product with some (5%) radioactive gramine, MAMI as substrate gave radioactive gramine as the only product Therefore, the radioactivity extracted into the scintillant was taken as a quantitative measure of the *N*-methyltransferase activities

The *N*-methyltransferase activities in the two cvs Australian Commercial and Sirocco, are compared in Table 1

Table 1 *N*-Methyltransferase activities in 7-day-old *P aquatica* seedlings

		Activity (units/100 seedlings)	
Substrate	Enzyme	cv Australian Commercial	cv Sirocco
T	PIM	242	128
MT	SIM	397	68
AMI	GTMT	782	689
MAMI	GTMT	988	915

PIM and SIM activities are much lower in cv Sirocco than in cv Australian Commercial whereas the gramine-type *N*-methyltransferase activities (GTMT) involved are similar GTMT activity has not previously been reported in *Phalaris* spp These data suggest that GTMT activity is distinct from PIM and SIM activities This idea is further supported by the distribution of the *N*-methyltransferase activities in different parts of the plant In the cv Australian Commercial PIM and SIM activities, as well as DMT and 5MeODMT, are found only in the leaves and not in the roots [3] However, 80% of the total GTMT activity involved in gramine synthesis is found in the leaves and 20% in the roots Gramine is similarly distributed between the leaves and the roots in the ratio 4 1 At present no evidence is available to show whether the two reactions catalysed by GTMT are due to separate enzymes

The distinction between GTMT activity and PIM and SIM activities is consistent with the idea that the pathways for DMT and 5MeODMT synthesis are independent of that of gramine synthesis DMT, 5MeODMT and gramine also occur in *P arundinacea* [7] Genetic evidence indicates that in *P arundinacea* the synthesis of gramine is independent of the synthesis of DMT and 5MeODMT [7] A comparison of the concentrations of DMT, 5MeODMT and gramine in 7-day-old seedlings of the two cvs of *P aquatica* shows that, although the concentrations of gramine in cv Australian Commercial and cv Sirocco are similar (200 and 175 nmol/100 seedlings, respectively), the concentrations of DMT and 5MeODMT are very different Thus the values for DMT are 280 and 24 nmol/100 seedlings in the Australian Commercial and Sirocco cvs, respectively, the corresponding values for 5MeODMT are 150 and 51 nmol/100 seedlings These results suggest that the synthesis of gramine is independent of the synthesis of DMT and 5MeODMT

EXPERIMENTAL

P aquatica seedlings were grown as described previously [3] AMI and MAMI were synthesized according to the method ref [4], other intermediates and alkaloids were purchased from Sigma

Alkaloid extraction and estimation Seedlings (10 g) were homogenized with HCl (0 01 M, 2 × 2 vol) and the homogenate was extracted with $CHCl_3$ (2 × 1 vol) The $CHCl_3$ soln was discarded and the aq soln was adjusted to pH 10 5 with NaOH (10 M) and extracted with EtOAc (3 × 1 vol) The organic phase was evaporated and the residue taken up in MeOH (500 μl) The alkaloids were separated on 2D-TLC (Merck Si Gel 20 × 20 cm plates, Cat No 5717) The plates were developed with (a) $CHCl_3$–MeOH–NH_4OH (80 15 1) and with (b) *n*-BuOH–EtOH–NH_4OH (20 2 1) The plates were sprayed with the van Urk–Salkowski reagent [6] and the alkaloids estimated by scanning with a RFT Transidyne 2955 scanning densitometer Scans of reference compounds run under the same conditions gave linear relationships between 0 2 and 2 0 nmol

Enzyme extracts 7-Day-old seedlings were ground with 0 05 M Tris–HCl buffer, pH 8 5 (1 2, w/v) and acid-washed sand in a mortar and pestle The homogenate was centrifuged at 5000 *g* for 5 min The supernatant fraction was chromatographed on a G-25 Sephadex (fine) column (1 × 8 cm/ml supernatant) to remove endogenous substrates The protein band containing the *crude enzyme fraction* was collected Extraction and gel filtration of the enzyme were at 0–4°

Enzyme assays 100 μl of assay mixture contained 1 mM substrate (T, MT, AMI or MAMI), 0 1 M Tris–HCl buffer, pH 8 5, 0 2 mM *S*-[methyl-^{14}C]adenosylmethionine (10 nCi, Amersham Australia) and 60 μl of the *crude enzyme fraction* The assay mixture was shaken in a scintillation vial at 25° for 20 min The reaction was stopped by the addition of 1 M H_3BO_3–Na_2CO_3, pH 10 (2 ml) and the radioactivity measured as described previously[3] A unit of activity is defined as 1 nmol product formed/hr

The radioactive products were extracted with toluene from the assay mixture after the addition of H_3BO_3–$NaCO_3$ The residue, after evaporating the toluene, was dissolved in MeOH (0 5 ml) and separated by TLC (Merck Si Gel F_{254} 5 × 20 cm plates, Cat No 5714) with $CHCl_3$–MeOH–NH_4OH (80 15 1) The spots were visualized under UV light and scanned with a Berthold LB2723 Thinlayer Scanner II

Acknowledgements—We thank Dr R Oram, CSIRO, Division of Plant Industry, Canberra for seeds of *P aquatica* cv Sirocco and Dale Lambert for synthesizing AMI and MAMI

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