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N,N-Dimethyltryptamine Production in *Phalaris aquatica* Seedlings

A MATHEMATICAL MODEL FOR ITS SYNTHESIS

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ABSTRACT

The activities of three enzymes and the concentration of intermediates involved in the synthesis of *N,N*-dimethyltryptamine (DMT) from endogenous tryptophan (TRP) have been measured *in vitro* in seedlings of *Phalaris aquatica* L. cv Australian Commercial over 16 days after planting. The activities of tryptophan decarboxylase and the two *N*-methyltransferases increased rapidly to maximal rates of substrate conversion at day 5 of 95, 1000, and 2200 micromoles per hour per milliliter, respectively. After these maximal rates, the activities decreased rapidly. The concentration of intermediates increased rapidly from zero in the seeds to maximal values of 25 and 53 micromolar at day 5 for tryptamine (T) and *N*-methyltryptamine (MT), respectively, 1000 micromolar at day 6 for TRP, and 650 micromolar at day 8 for DMT. The concentration of DMT and of all the intermediates in its synthesis declined rapidly after the maximal value had been reached. A mathematical model of the pathway from TRP to DMT using these enzymes correctly predicts the concentrations of T and MT, intermediates whose concentration is determined only by the pathway, and confirms that these three enzymes are responsible for the *in vivo* synthesis of DMT. Kinetic studies are reported for these enzymes. Tryptophan decarboxylase uses pyridoxal phosphate (PALP) as a coenzyme and has the following kinetic constants: K_m^{PALP} = 2.5 micromolar, K_m^{TRP} = 200 micromolar, K_i^{MT} = 5 millimolar, and K_i^{DMT} = 4 millimolar. The *N*-methyltransferases use *S*-adenosylmethionine (SAM) as substrate; *S*-adenosylhomocysteine (SAH) is assumed to be the product. The mechanism of secondary indolethylamine-*N*-methyltransferase, determined by initial velocity studies, is rapid equilibrium random with formation of both dead end complexes. Secondary indolethylamine-*N*-methyltransferase methylates both MT and 5-methoxy-*N*-methyltryptamine (5MeOMT). The kinetic constants for the methylation of MT are: K_{MT} = 40 ± 6, K_{SAM} = 55 ± 15, K_{DMT} = 60, K_{SAH} = 4.3 ± 0.4 micromolar with unity interaction factors. The kinetic constants for the conversion of 5MeOMT to 5-methoxy-*N,N*-dimethyltryptamine (5MeODMT) are K_{5MeOMT} = 40 ± 10, K_{SAM} = 90 ± 40, and K_{SAH} = 2.9 ± 0.3 micromolar with unity interaction factors, except for SAM-5MeODMT = 2.0 ± 0.9 and SAH-5MeOMT = 0.45 ± 0.25. The kinetic constants for primary indolethylamine *N*-methyltransferase are K_m^T = 20, K_m^{SAM} = 40, K_i^{DMT} = 450 micromolar with the substrates binding independently.

Phalaris spp., notably *Phalaris aquatica* and *Phalaris arundinacea*, are major pasture grasses in many parts of the world. Because there are undesirable features associated with these grasses, particularly unpalatability to sheep, which has been linked in *P. aquatica* to the gramine content (4), a considerable amount is known of the factors involved in alkaloid production. For example, genetic evidence in *P. arundinacea* indicates that gramine synthesis is independent of DMT[1] and 5MeODMT formation (11). This is further supported by the finding that the *N*-methyltransferase activities involved in gramine synthesis are different from those involved in the synthesis of DMT and 5MeODMT (13).

Feeding experiments with *P. aquatica* have established the biosynthesis and turnover of DMT and 5MeODMT (1), while the biosynthesis of gramine has been studied in the closely related reed canary grass, *P. arundinacea* (9). Enzymes from *P. aquatica* that catalyze the *in vitro* formation of DMT have been partially characterized, namely TDase (2) and the two SAM-dependent *N*-methyltransferases, PIMase and SIMase (10), which methylate T and MT, respectively. Because of the small number of enzymes involved, the synthesis of DMT from TRP offers the possibility of completely characterizing an alkaloid-synthesizing system and of testing the hypothesis that the enzymes are responsible for the *in vivo* synthesis of DMT.

This paper reports the activities of enzymes and concentrations of reactants of the DMT synthesizing pathway as a function of age of *P. aquatica* seedlings for the first 16 d of seedling growth. These data together with the kinetic constants of the enzymes have been used to model the DMT synthesizing system mathematically during this initial 16 d of seedling growth.

MATERIALS AND METHODS

Labeled Compounds and Chemicals. L-[^{14}C-Methylene]tryptophan ([^{14}C]TRP) and [^{14}C-methyl]SAM ([^{14}C]SAM) were purchased from Amersham Australia Pty Ltd. PALP and the substrates were obtained from Sigma Chemical Co., St Louis, MO. [^{14}C]SAM used was assayed for SAH content by chromatography on Whatman 3MM paper using butanol/acetic acid/water (12:3:5). The nucleosides were detected with Cd-ninhydrin (3), eluted with methanol, and quantitated by absorbance at 500 nm. The SAM used in the assay contained 0.024% SAH, which, at the highest concentration of SAM used (200 μM), gives an SAH

[1] Abbreviations: DMT, *N,N*-dimethyltryptamine; 5MeODMT, 5-methoxy-*N,N*-dimethyltryptamine; TDase, tryptophan decarboxylase; SAM, *S*-adenosylmethionine; PIMase, primary indolethylamine *N*-methyltransferase; SIMase, secondary indolethylamine *N*-methyltransferase. T, tryptamine; MT, *N*-methyltryptamine; TRP, tryptophan; PALP, pyridoxal phosphate; SAH, *S*-adenosylhomocysteine; wrt, with respect to.

concentration in the assay of 0.05 μM. This is small compared to K_{SAH} for the enzymes and the concentration of SAH used in the inhibitor binding studies.

Preparation of Enzyme Extracts. Seedlings of *Phalaris aquatica* L. cv Australian Commercial were grown as described previously (10). Whole seedlings (*i.e.* all plant material including the testa for pre-emergent seedlings) (5 g) were homogenized in the assay buffer (1:2, v/w) with sand. An additional 1% (v/v) of mercaptoethanol was added to the homogenate, which was then centrifuged at 5000*g* for 5 min. The supernatant (1 mL) was passed through a Sephadex G-25 (fine) column (1.5 × 6 cm) to separate the proteins from endogenous alkaloids and other low mol wt compounds. The excluded protein fraction (3 mL), referred to as the enzyme extract, was collected and used for enzyme assays. It behaved similarly with respect to linearity with time and enzyme concentration and gave the same kinetic constants as the original preparation. No differences in kinetic constants were seen for preparations from leaves or whole plants. The kinetics were investigated at constant pH and the effect of hydrogen ion concentration was not investigated.

Enzyme Assays. *TDase.* The assay mixture consisted of enzyme extract (300 μL), [^{14}C]TRP (1.5 mM; specific activity 0.07 μCi/mmol), PALP (25 μM), Na-phosphate (0.1 M; pH 7.6) in a total volume of 400 μL. This was incubated for 2 h at 25°C in a scintillation vial. The reaction was stopped by adding 1 M H_3BO_3-Na_2CO_3 (2 mL; pH 10.0), and T was extracted into toluene scintillant as previously described (10). Activity is measured as nmol T h^{-1} mL^{-1} of cell fluid. Cell fluid was calculated from the difference between fresh and dried (100°C for 1 h) weights of the seedlings.

PIMase and SIMase. The assay mixture consisted of enzyme extract (60 μL), T (for estimating PIMase activity) (1 mM), MT (for estimating SIMase activity) (1 mM), [^{14}C]SAM (0.4 mM; specific activity 0.5 mCi/mmol), and Tris buffer (0.25 M; pH 8.5) in a total volume of 100 μL. This was incubated for 30 min at 25°C in a scintillation vial. Activity is measured as nmol of ^{14}C-methyl from SAM incorporated per h into the tryptamine alkaloids per mL of cell fluid. The reactions were stopped by adding 1 M H_3BO_3-Na_2CO_3 (2 mL; pH 10.0), and MT (or DMT) was extracted into toluene scintillant as described previously (10).

Extraction and Estimation of Tryptamines and TRP. T, MT, DMT, and TRP were extracted from plant material as described previously (14). T, MT, and DMT were separated and estimated by two-dimensional TLC (14). This method did not separate TRP from 5-methyoxytryptophan. Accordingly, the extract was evaporated to dryness, dissolved in 1 mL of 0.2 M (wrt Na^+) citrate buffer, pH 2.2 (12), and estimated on a JLC 6AH amino acid analyser (JEOL Co. Ltd, Tokyo) using LCR-2 cation exchange resin on a column (45 cm × 8 mm) at 53°C at a flow rate of 0.84 mL/min. Three citrate buffers (12) were used sequentially for differing lengths of time, namely (wrt Na^+) 0.2 M buffer (pH 3.3) for 110 min, 0.2 M buffer (pH 4.46) for 50 min, and 0.35 M buffer (pH 5.40) for 130 min. Under these conditions, TRP separated from 5-methoxytryptophan and the other amino acids in the extract and was eluted at 69 mL after the buffer change from pH 4.46 to 5.40.

The TRP in the proteins in the seeds was estimated as follows: 100 mg of pulverized dry seeds were incubated for 18 h at 40°C with pronase solution (1.0 mL; 5.5 mg/mL in 0.2 M phosphate buffer, pH 7.4). TRP in aliquots (0.1 mL) of the incubation mixture was estimated by boiling the aliquot with 1.0 mL of van Urk-Salkowski reagent (14) for 10 min, cooling, and reading the absorbance at 600 nm. Appropriate controls were included to ensure that the pronase was not self-digesting.

Reproducibility of Results. The concentrations of intermediates and the activities of enzymes were measured three times. The trends were the same in each experiment, but there was a variation by up to half a day of the time at which the plumule emerged through the sand. As the maximal activities of the enzymes and the concentrations of intermediates were related to this event, it was felt it would be more appropriate to present the results from a single experiment rather than as SE means.

Total Nitrogen Determination. Forty mg of seedlings were digested with 3.0 mL of 18 M H_2SO_4, 1.5 g of K_2SO_4, and 0.1 g of HgO at 300°C; NH_3 produced was estimated by the phenol/hypochlorite method (7).

Estimation of SAM. Five g of seedlings were homogenized with TCA (10%; 15 mL) and were centrifuged at 2000*g*, and the supernatant was washed with diethyl ether (3 × 15 mL). The extract was made 15 mM wrt to HCl and then chromatographed as previously described (5).

RESULTS

Changes in Tryptophan and Alkaloid Concentrations. Cell fluid weight increased from 8 to 90% of the fresh weight in the first 16 d of seedling growth. Over the 16 d of the experiment, the nitrogen content of the seedlings remained constant at 105 ± 10 μmol/100 seedlings. No free indole compounds could be detected in the dry seeds, and the amount of TRP in the seed proteins was 5 μmol/100 seeds. The concentration of SAM at d 6, the day at which TRP concentration was maximal, was 30 μM. The earliest time that any of the 3 compounds involved in the synthesis of DMT, namely TRP, T, and MT, could be detected was 3 d after planting (Table I and Fig. 1). The concentration of TRP increased rapidly to 1000 μM in seedlings at d 6 (Table I). The plumule emerged through the sand between d 6 and 7. Thereafter, the concentration of TRP decreased steadily. Similarly, the concentrations of T and MT increased rapidly from d 3, the earliest stage at which they could be detected, until d 5, and then they decreased rapidly (Fig. 1). The maximum concentrations of T and MT were 25 and 53 μM, respectively. The maximum concentration of DMT was 650 μM at d 8, thereafter declining quickly.

Activities of TDase, PIMase, and SIMase. None of the enzymes, namely TDase, PIMase, and SIMase, could be detected in *P. aquatica* before the 3rd d after sowing (Table I); they increased rapidly until just before the seedlings emerged from the sand (d 6) and then declined until d 14. The maximum activity of TDase was 95 μmol $h^{-1}mL^{-1}$. The profiles of the changes in concentration of the two *N*-methyltransferases were similar and decreased at a slower rate than TDase.

The maximum activities for PIMase and SIMase were 1000

Table I. *Variation with Time of the Concentration of TRP and the Activities of TDase, PIMase, and SIMase in P. aquatica*

Day	TRP	TDase	PIMase	SIMase
	μM		μmol^{-1} mL^{-1}	
3	333	6.77	222	583
4	444	55.6	687	1630
5	733	94.7	1000	2190
6	1000	55.9	767	1500
7	767	26.7	606	1370
8	826	17.4	601	1210
9	728	12.0	440	982
10	630	6.67	381	851
11	533	2.78	330	737
12	436	1.16	286	639
13	340	0.72	217	490
14	267	0.45	164	376
15	217	0.27	157	343
16	167	0.0	150	315

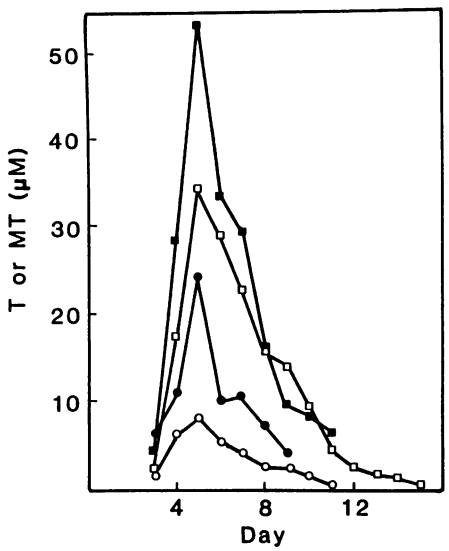


FIG. 1. Variation with time of T and MT in *P. aquatica* seedlings. Results presented for T (●) and MT (■) are from one experiment; values calculated from the numerical simulation are T (○) and MT (□).

and 2200 μmol $h^{-1}mL^{-1}$, respectively. The high levels of PIMase and SIMase relative to that of TDase could easily convert the T produced by TDase to DMT and resulted in a low concentration of the intermediates T and MT.

Kinetic Analysis. Inhibition type nomenclature and interaction factors were used according to Segel (15). Interaction factors are the ratio of K_{diss} from the ternary complex to K_{diss} from the binary enzyme complex.

Kinetic constants were determined using initial velocity experiments, and the data were analyzed by a least square fitting of intersecting straight lines to Lineweaver-Burk plots using the program Labtech Notebook (Laboratory Technologies Corp, Cambridge, MA) run on an IBM PC computer. Data for the Lineweaver-Burk plots were weighted inversely to the Lineweaver-Burk $y(=1/v)$ value to reflect equal weighting for each of the original data points. Results were not significantly different with an unweighted fit, indicating no bias in the data at high or low reaction velocity.

The mechanisms of TDase and PIMase were not determined, and kinetic constants used steady state nomenclature of K_m and K_i. The binding of TRP to TDase, which was tested at concentrations of 5 and 25 μM of PALP, had a small slope and intercept effect of about equal size giving $K_m^{PALP} \approx 2.5$ (μM)). MT and DMT are competitive inhibitors of TDase. K_i for MT and DMT for the E-PALP complex was high relative to the concentrations of inhibitor used (1 mM), giving $K_i^{MT} \approx 5$ mM and $K_i^{DMT} \approx 4$ mM. These K_i values were large compared to K_m^{TRP}, consistent with the inability of these product analogs to form an imine with PALP.

Kinetic constants derived from initial velocity experiments are listed for PIMase in Table II, for the methylation of MT by SIMase in Table III, and for the methylation of 5MeOMT by SIMase in Table IV.

The inhibition patterns for SIMase are for a rapid equilibrium random bi-bi mechanism with formation of both dead end complexes. The product inhibition plots intersect near the axes. The binding pairs, DMT-MT and SAH-SAM, were good fits to a competitive inhibition mechanism, and, on the assumption that these pairs of ligands bind mutually exclusively, the data were fitted to a Lineweaver-Burk plot constrained to intersect on the $1/v$ axis. The fits to the graphs for the DMT-SAM and SAH-MT pairs were not constrained for the position of intersection. The binding constant for any ligand should be independent of the other ligands that are used in the experiment. For example, K_{SAM} should be the same whether it was determined for experiments with MT or 5MeOMT as the varied ligand and with or without any inhibitors present. Kinetic constants vary by up to a factor of 2 (Table III) indicating that the partially purified enzyme preparations used in any particular experiment were not always identical. Thus, the magnitudes of the K_{diss} values cannot be regarded as being more accurate than a factor of 2. Inspection of interaction factors shows that with the normal number of methyl groups for the reaction at the active site of SIMase, *i.e.* SAM and MT or 5MeOMT, the ligands bind independently. An extra methyl group, *i.e.* SAM and DMT, decreases the binding of the second ligand by a factor of 3, or, with 5MeODMT, a factor of 2. One less methyl group, *i.e.* SAH and MT, increases the binding of the second ligand by a factor of 5, or with 5 MeOT, a factor of 2.

Mathematical Model for DMT Synthesis. To determine if the synthesis of T, MT, and DMT could be described in terms of the enzymes TDase, PIMase, and SIMase, a model for the synthesis of DMT from TRP was constructed from the data in Table I and the kinetic constants in Scheme I. These constants are derived from the kinetic data in Tables II to IV. Velocities for the three enzymes for the numerical simulation were calculated using the rate equation for the mechanism. The mechanism of SIMase is rapid equilibrium random bi-bi with both dead-end complexes formed. Because of the many similarities between PIMase and SIMase, the mechanism for PIMase was assumed to

Table II. *Kinetic Constants for TDase and PIMase*

Enzyme	Varied Ligand	Inhibitor	Inhibition Type[a]	Constant		Interaction Factor
					μM	
TDase	PALP, TRP[b]			K_m^{TRP}	200	1.0
				K_m^{PALP}	2.5	
	TRP[c]	MT	C	K_i^{MT}	5000	
	TRP[c]	DMT	C	K_i^{DMT}	4000	
PIMase	T, SAM[d]			K_m^{T}	17 ± 3	1.2 ± 0.4
				K_m^{SAM}	38 ± 7	
	T[e]	DMT	C	K_i^{DMT}	450	

[a] C, competitive. [b] Initial velocities were measured at five concentrations of TRP (100–1000 μM) in the presence of two concentrations of PALP (5 and 25 μM). [c] Initial velocities were measured at eight concentrations of TRP (40–500 μM) in the presence of two concentrations of MT or DMT (0 and 1 mM). [d] Initial velocities were measured at five concentrations of T (0.01–0.075 mM) in the presence of four concentrations of SAM (5–40 μM). [e] Initial velocities were measured at five concentrations of T (0.01–0.075 mM) and two concentrations of DMT (0 and 1 mM).

Table III. *Kinetic Constants for SIMase with MT and SAM as Substrates*

Varied Ligand	Inhibitor	Inhibition Type[a]	Constant		Interaction Factor
				μM	
SAM, MT[b]			K_{MT}	40 ± 6	1.15 ± 0.20
			K_{SAM}	55 ± 15	
MT[c]	DMT	C	K_{MT}	120 ± 20	
			K_{DMT}	105 ± 10	
SAM[d]	DMT	NC	K_{SAM}	130 ± 20	3.1 ± 0.9
			K_{DMT}	35 ± 10	
MT[e]	SAH	NC	K_{MT}	120 ± 40	0.18 ± 0.08
			K_{SAH}	25 ± 10	
SAM[f]	SAH	C	K_{SAM}	45 ± 5	
			K_{SAH}	4.3 ± 0.4	

[a] C, competitive; NC, noncompetitive. [b] Initial velocities were measured at five concentrations of MT (100–2000 μM) in the presence of four concentrations of SAM (20–200 μM). [c] Initial velocities were measured at four concentrations of MT (100–1000 μM) in the presence of five concentrations of DMT (0–1 mM). Concentration of SAM = 200 μM. [d] Initial velocities were measured at 3 concentrations of SAM (40–200 μM) in the presence of five concentrations of DMT (0–1 mM). Concentrations of MT = 1000 μM. [e] Initial velocities were measured at four concentrations of MT (100–1000 μM) in the presence of five concentrations of SAH (0–1 mM). Concentration of SAM = 200 μM. [f] Initial velocities were measured at four concentrations of SAM (20–200 μM) in the presence of six concentrations of SAH (0–20 μM). Concentration of MT = 1000 μM.

Table IV. *Kinetic Constants for SIMase with 5MeOMT and SAM as Substrates*

Varied Ligand	Inhibitor	Inhibition Type[a]	Constant		Interaction Factors
				μM	
SAM, 5MeOMT[b]			K_{5MeOMT}	40 ± 10	0.85 ± 0.15
			K_{SAM}	90 ± 40	
5MeOMT[c]	5MeODMT	C	K_{5MeOMT}	75 ± 10	
			$K_{5MeODMT}$	83 ± 6	
SAM[d]	5MeODMT	NC	K_{SAM}	66 ± 5	2.0 ± 0.8
			$K_{5MeODMT}$	40 ± 10	
5MeOMT[e]	SAH	NC	K_{5MeOMT}	60 ± 25	0.45 ± 0.25
			K_{SAH}	7 ± 4	
SAM[f]	SAH	C	K_{SAM}	25 ± 4	
			K_{SAH}	2.9 ± 0.3	

[a] C, competitive; NC, noncompetitive. [b] Initial velocities were measured at five concentrations of 5MeOMT (100–2000 μM) in the presence of four concentrations of SAM (20–200 μM). [c] Initial velocities were measured at four concentrations of 5MeOMT (100–1000 μM) in the presence of five concentrations of 5MeODMT (0–1 mM). Concentration of SAM = 200 μM. [d] Initial velocities were measured at four concentrations of SAM (20–200 μM) in the presence of five concentrations of 5MeODMT (0–1 mM). Concentration of 5MeOMT = 1000 μM. [e] Initial velocities were measured at four concentrations of 5MeOMT (100–1000 μM) in the presence of five concentrations of SAH (0–1 mM). Concentration of SAM = 200 μM. [f] Initial velocities were measured at five concentrations of SAM (20–200 μM) in the presence of six concentrations of SAH (0–20 μM). Concentration of 5MeOMT = 1000 μM.

be the same as the SIMase. For TDase, PALP was assumed to be saturating and the enzyme was treated as a uni-uni enzyme.

Inspection of the experimentally determined concentrations of intermediates T and MT (Fig. 1) shows that their pool size is small compared to the amount of DMT produced and that the rate of change of concentration of these intermediates is small compared to the flux of the pathway. For all practical purposes, all TRP consumed by the pathway appears as DMT, and the concentration of the indolethylamine intermediates is steady state. The worst case approximation occurs on day 4, when the pathway begins to function, when the ratio of the rate of change of concentration to flux is 1:20. The profile of the changes in the concentrations of DMT (Fig. 2, inset) was very similar to that of TRP (Table I) indicating that DMT was being further metabolized and was only present when being produced from TRP.

The behavior of this system was analyzed by numerical methods using the Euler method (6), which gives the exact calculated concentrations for all the intermediates (Figs. 1 and 2), and, by assuming the concentration of T and MT is small and steady state, deriving approximate analytic equations, which show how the parameters of the model affect its results.

The following information and assumptions were made about reactants that interact with outside pathways and hence are not controlled by the alkaloid pathway:

1. TRP: TRP was determined experimentally (Table I).
2. PALP: There are no data for the *in vivo* concentration of PALP; $K_{m\text{-}PALP}^{TDase}$ is low (~2 μM), and it was assumed that PALP was always saturating.

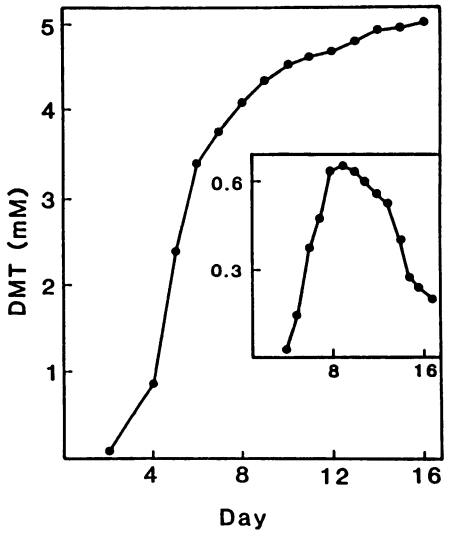


FIG. 2. Variation with time of DMT in *P. aquatica* seedlings. Values calculated from the numerical simulation; inset, results presented from one experiment.

3. Binding of MT and DMT to TDase: K_i^{TDase} for MT and DMT are large compared to the concentration of these ligands and their binding was ignored in the simulation.

4. SAH: No information was available about the concentration *in vivo* of SAH. Since it is a strong inhibitor of the methyltransferases and mechanisms for its removal in other systems are known (8), the concentration of SAH was assumed to be zero.

5. DMT: DMT is turned over in seedlings of *P. aquatica* (1), and the data presented here show that the alkaloid synthesizing pathway produces more DMT than is found *in vivo*. The model continually removed DMT so that the concentration of DMT in the model never exceeded that *in vivo*. Thus, the enzymes in the simulation are presented with experimentally determined concentrations of TRP and DMT.

6. SAM: The analytic expression for the concentration of the intermediate MT can be simplified for SIMase which is mostly bound to DMT giving

$$MT' = \frac{Flux}{V_3} \cdot \frac{DMT'}{SAM'} \quad (1)$$

in which

$$Flux = \text{activity of TDase} = V_1 TRP'/(1+TRP') \quad (2)$$

where, V_3 = activity of SIMase saturated with substrates and V_1 = activity of TDase saturated with substrates.

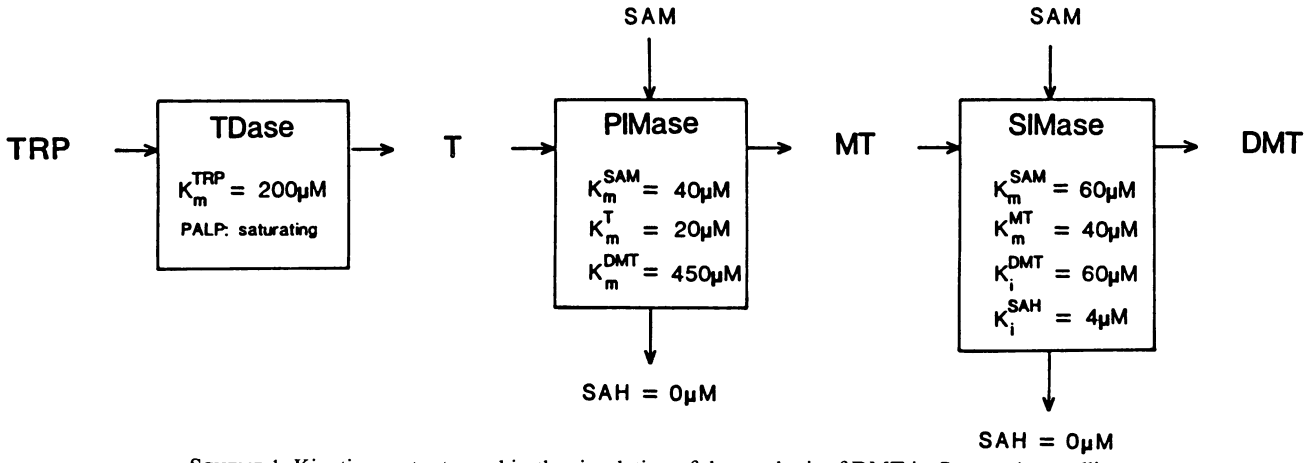
Prime superscripts represent the reduced concentration of ligands, *i.e.* the concentration of the ligand divided by the K_m, K_i, or K_{diss} for the enzyme.

The approximation used to derive Equation 1 is reasonable between days and 5 and 12, while at day 4 this approximation gives results low by a factor of 2 compared to the exact numerical method. For SAM and MT not saturating, Equations 1 and 2 show that SAM has no effect on the flux of the pathway, only on the concentration of intermediates. Thus, a decrease in the concentration of SAM leads to an initial decrease in methyltransferase velocity followed by a compensating increase in the concentration of MT. Since, except at d 6, the concentration of SAM is unknown, this property of SIMase was used to test two simple hypotheses about the time course of SAM concentration. SAM concentration when assumed to be constant led to calculated MT concentrations which, relative to concentrations at d 6 were too low, the disparity increasing the further the age from d 6. This indicated that the concentration of SAM used in the simulation is too high at days other than d 6. The concentration of SAM, when allowed to follow the time curve of TRP, gave calculated MT concentrations which stayed in step with their experimentally determined concentrations. This latter hypothesis is reasonable, in that both TRP and SAM are being produced by the same metabolic machinery during mobilization of the seed storage material, and it was used for the results presented here.

DISCUSSION

The concentrations of the intermediates T and MT are determined solely by the pathway and are used as the measure of worth of the model. From Equation 1 the errors in determining the concentration of MT are mainly due to three kinetic constants which have errors of a factor of 2 (errors in concentrations or activities are only about 10%). In the worst case, where all three kinetic constants contribute equally and independently, the final error is a factor of 3. Estimates of errors for the concentration of T are difficult to determine as, unlike SIMase, PIMase is not mostly bound to DMT. In this case the expected errors for MT are used as an estimate for the error of the concentration of T. The data of Figure 1 show that the ratio calculated/experimental for the concentrations of T and MT is less than a factor of 3, showing the pathway as modelled to be an acceptable fit to the data.

TDase activity is the rate limiting step in the pathway, resulting in bound concentrations of the intermediates T and MT. Rerunning the simulation by increasing only the activity of TDase gave



SCHEME 1. Kinetic constants used in the simulation of the synthesis of DMT in *P. aquatica* seedlings.

bound concentrations for the intermediates until TDase reached 0.1 to 0.5 times the activity of SIMase, when the concentrations of T and MT increased without limit. The highest TDase/SIMase ratio found experimentally is 0.05 on d 5 indicating that the pathway is always more than a factor of two inside the requirements for a bound solution for the intermediates. Within the time of the simulation, TDase activity varies over a factor of 350 and TDase/SIMase varies over a factor of 65. These numbers are large compared to the margin of 2 required for maintenance of TDase as the rate limiting step in the pathway, and it is reasonable to suppose that the TDase is rate limiting through selective pressure, rather than coincidence. TRP is close to saturating at the time when the amount of TDase is highest (around day 6) indicating that a change in the pathway to produce unbound concentrations of intermediates would only come from increasing the amount of TDase, rather than increasing the concentration of TRP.

The affinity of DMT for the enzymes is in the order SIMase>PIMase>TDase, decreasing by a factor of about 10 for each enzyme. The very weak binding of DMT to the PALP-TDase complex is expected from the chemistry of the TDase reaction, where an imine cannot be formed. The weak (relative to SIMase) binding of DMT to PIMase, when considered with the similar chemistry of the two enzymes, indicates that PIMase is designed to preclude binding of DMT. DMT only binds strongly to SIMase because it is the enzyme product. Larger amounts of SIMase, relative to the other two enzymes in the pathway, are thus required to overcome inhibition by DMT. The enzymes of the DMT-synthesizing pathway thus are not designed to use DMT as a feedback-regulating element for the pathway, and the ratio of enzyme activities of the enzymes (in reverse order of their affinities for DMT) reflects a requirement for low concentrations of the indolethylamine intermediates in the presence of DMT.

The two criteria for the acceptance of simulation, the ratio of the calculated to experimentally found concentration of the intermediates and that the concentration of intermediates is bound, both show that the model is an acceptable representation of the actual DMT synthesizing system. We conclude that TDase, PIMase, and SIMase are the enzymes responsible for the *in vivo* synthesis of DMT.

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LITERATURE CITED

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