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Inhibition of Dimethyltryptamine Biosynthesis by N,N'-Bis-(3-methyl-2-thiazolidinylidene)Succinamide (I) and 2-Imino-3-Methylthiazolidine (II)

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SUMMARY

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N,N'-bis-(3-methyl-2-thiazolidinylidene)succinamide (*I*) and 2-imino-3-methylthiazolidine (*II*) are inhibitors of indoleamine-N-methyltransferase, the enzyme which biosynthesizes N,N-dimethyltryptamine. *I* was designed as a neutral compound which will form the active basic metabolite *II* in rabbits. *In Vitro*, *I* is not an effective inhibitor, while *II* is a potent inhibitor of the rabbit and human lung enzyme ($IC_{50} \leq 3$ μM). When administered to rabbits at 4-5 mg/kg i.v., both compounds produce a 60% reduction in the specific activity of the lung methyltransferase. A single oral dose of *I* produces 50% inhibition of enzyme activity when the animals are sacrificed 2 hr after dosage (8 mg/kg) or at 7 hr (68 mg/kg). Administration of *II* in the drinking water for 12 days at a daily dose equivalent to about 7 mg/kg results in a 36% inhibition in the activity of the lung enzyme. When enzyme preparations from drug-treated rabbits were dialyzed, the specific activity returned to control values, suggesting a reversible type inhibition. *I* was also evaluated orally for its effect on dimethyltryptamine biosynthesis in rabbits; at single doses of 100 mg/kg or at 25 mg/kg twice daily for four days, there was a 65% reduction in the conversion of [^{14}C]N-methyltryptamine (administered i.v.) to [^{14}C]dimethyltryptamine in lung. The level of carotid arterial DMT appearing over 1 minute after the intravenous injection of NMT (10 mg/kg) was also reduced 75%. Thus, inhibition of indoleamine-N-methyltransferase results in a block in the *in vivo* biosynthesis of dimethyltryptamine.

INTRODUCTION

The possible relationship of methylated indoles such as DMT[1] to psychiatric disorders has stimulated over two decades of research and has been the subject of several recent reviews on biochemical aspects of schizophrenia (1-10). DMT is a psychotogenic compound for which there is an established biosynthetic mechanism. Axelrod first reported on the occurrence of the DMT-forming enzyme INMT in rabbit lung (11). Many tissues are now known to possess an enzyme activity capable of biosynthesizing DMT; its distribution has been reviewed recently (5).

[1] The abbreviations used are DMT, N,N-dimethyltryptamine; INMT, indoleamine-N-methyltransferase; NMT, N-methyltryptamine.

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Several naturally occurring and synthetic inhibitors of INMT have also been described. Inhibitors have been found in rat brain (12), human lung (13), human blood (14), rabbit lung (15), bovine pineal extracts (16), and rabbit brain (17). Synthetic compounds reported to inhibit INMT activity include chlorpromazine and related structures (11, 17, 18), S-adenosylhomocysteine and analogues (19–21), 3,4-dihydroxyphenylacetic acid (22), 1-[2-(3,4,5,6-tetrahydropyridyl)]-1,3-pentadiene (23), 2-(2-aminoethyl)-5,6-dichlorobenzimidazole, N-(2-aminoethyl)pyrrolidine and 2-(2-aminoethyl)pyridine (24), 1,5-diazobicyclo-[4.3.0]-non-5-ene; DBN (25), aliphatic diamines (26), and tetrahydro-β- and γ-carbolines (27). Substrates for INMT such as NMT and 5-methoxy NMT, and its reaction products DMT and bufotenin (N,N-dimethylserotonin) have also been found to inhibit enzyme activity *in vitro* (28–30).

This paper describes two novel INMT inhibitors; the structures of N,N′-bis-(3-methyl-2-thiazolidinylidene)succinamide (*I*) and 2-imino-3-methylthiazolidine (*II*) as the hydrochloride hydrate are shown in Fig. 1. Compound *I* is converted to the active metabolite, compound *II*, upon administration to rabbits; the metabolic studies will be published elsewhere.[2] Thus *in vitro*, *I* is not an effective DMT biosynthesis inhibitor, while *II* is a potent inhibitor of the rabbit and human lung INMT ($IC_{50} \leq 3\ \mu M$). When administered orally to rabbits, both compounds reduce the specific activity of lung INMT markedly, and this is associated with a block in the conversion of NMT to DMT in lung and blood.

MATERIALS AND METHODS

INMT was prepared from rabbit and human lung by methods described previously (13, 28). The rabbit enzyme was used as the 40–60% ammonium sulfate fraction or it was purified further through the Sephadex G-150 step. Reaction mixtures (0.1 ml) contained 20 μmoles potassium phosphate buffer pH 7.9, 2 nmoles S-adenosylmethionine-methyl-[^{14}C] (160,000 cpm), 35 nmoles NMT, enzyme and test compound (dissolved in water or 5% DMSO). The samples were incubated 60–90 min at 37° and the reactions terminated by the addition of 0.2 ml of 0.125 M sodium tetraborate pH 10. The [^{14}C]DMT was extracted into 2 ml of water-saturated isoamyl alcohol or 97% toluene-3% isoamyl alcohol. After centrifugation the radioactivity in 0.5–1.0 ml of the upper phase was determined.

The human lung enzyme was prepared from post-mortem specimens by homogenizing the tissue in 2–5 vol of 0.15 M KCl containing 10^{-4} M each sodium phosphate pH 7.9, EDTA and dithiothreitol. After centrifugation at 55,000 × *g* for 30–60 min at 0°, the supernatant fluid was dialyzed against several hundred volumes of the EDTA-sodium phosphate-dithiothreitol solution with two changes over 18 hr at 4°. The dialyzed preparation was used as the enzyme source for most of the experiments. In some cases a more purified enzyme preparation (carried through the Sephadex G-150 step) was used (13). Reaction mixtures (0.1 ml) contained 15–30 μmoles potassium phosphate buffer pH 7.9, 4 nmoles S-adenosylmethionine methyl-[^{14}C], 0.2–0.4 μmoles NMT, enzyme and test compound. Samples were incubated 60–120 min at 37° and the reactions terminated as outlined above, following the addition of 25–100 μg carrier DMT. After centrifugation, an aliquot of the upper phase was evaporated to dryness *in vacuo* at room temperature. The radioactivity in the residue was determined at this stage, or the sample was taken up in a small volume of methanol and applied to silica gel GF plates for thin layer chromatography using methanol—1N ammonium hydroxide (5:1) or n-butanol-acetic acid-water (72:18:30) as the developing solvent. The DMT was recovered from the plates by elution with methanol and the radioactivity determined in a Packard liquid scintillation counter.

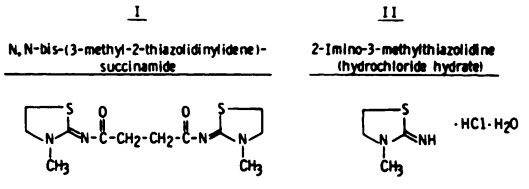

FIG. 1. *Structure and nomenclature of compounds I and II.*

[2] Zacchei *et al.*, manuscript in preparation.

The chromatographic step tended to reduce blank (no NMT or zero time incubations) values, but had no significant effect on the overall pattern of data obtained.

A crude DMT-forming enzyme from human liver was obtained by homogenizing the tissue in 3 vol of 0.01 M sodium phosphate buffer pH 7.9 and centrifuging at 78,000 × *g* for 60 min at 0°. The supernatant fluid was dialyzed against 300 vol of the buffer (with two changes) for 20 hr at 4°. Reaction mixtures (0.5 ml) contained 0.43 ml of enzyme, 2.5 μmoles of NMT and 8 nmoles of S-adenosylmethionine-methyl-[^{14}C]. Samples were incubated 90 min at 37° and reactions terminated with the addition of 25 μg carrier DMT, 0.6 ml of 0.125 M borate, and 6 ml of 97% toluene-3% isoamyl alcohol. The radioactivity in the DMT zone was determined following thin layer chromatography. This procedure was employed because of the relatively crude nature of the enzyme preparations from the human tissues.

Bovine adrenal phenethanolamine-N-methyltransferase was prepared and assayed by the method of Axelrod using normetanephrine as substrate (31). Protein was measured by the method of Oyama and Eagle (32).

For the experiments *in vivo,* rabbits were given compounds *I* and *II* orally with a stomach tube, intravenously (ear vein), or by maintaining animals on the compound in the drinking water. Lung INMT was prepared and assayed by the procedure described previously (25). Studies on the conversion of NMT (administered i.v.) to DMT in carotid arterial blood were carried out according to the method of Mandel *et al.* (33). Experiments designed to measure the conversion of [^{14}C]labeled NMT (i.v.) to [^{14}C]DMT in rabbit lung were performed by minor modification of methods also reported previously (25, 33). These publications also present the sources for the chemicals, animals and supplies used in the experiments reported herein. The synthesis of compounds *I* and *II* will be presented in separate publications.[3]

RESULTS

The inhibitory effect of *I* and *II* on the activity of rabbit and human lung INMT was studied in *in vitro* experiments initially. The data in Table 1 show that *I* was a relatively weak inhibitor of the rabbit enzyme (IC_{50} = 6 μg/ml; 20 μM), and it had no effect on the enzyme from human lung. These findings were expected since *I* was designed to possess activity *in vivo,* but not necessarily *in vitro. II* was a potent inhibitor of the enzyme from both sources; it produced 49% inhibition of the rabbit lung INMT and 66% inhibition of the human lung enzyme at 0.5 μg/ml (3 μM). The kinetics of inhibition of the rabbit lung INMT (40–60% ammonium sulfate fraction) was also studied. The results, illustrated in Fig. 2, show a noncompetitive type inhibition; the K_i value is of the order of 4 μM and the K_m value for N-methyltryptamine (NMT)

TABLE 1

Inhibition of lung INMT by I and II in Vitro

Compound	Enzyme source	% inhibition at[a]			
		25 μg/ml	2.5 μg/ml	0.5 μg/ml	0.25 μg/ml
I	rabbit	70	38	14	7
	human	+12	0	—	—
II	rabbit	88	69	49	39
	human	92	86	65	—

[a] Data are average values from 2–4 determinations.

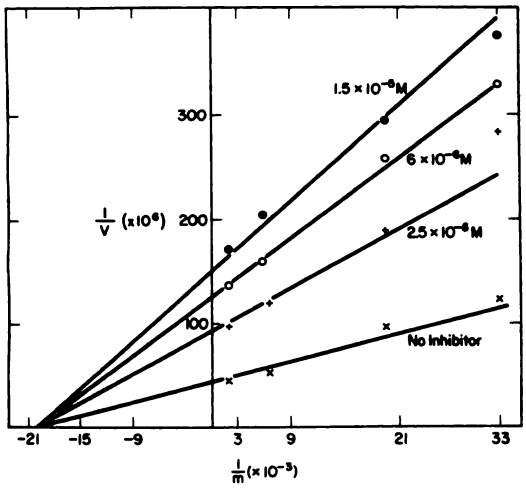

FIG. 2. *Kinetic analysis of INMT inhibition in vitro produced by II*

Units on abscissa refer to the concentration of NMT; data for ordinate based upon radioactivity (cpm values) in DMT fraction. Final concentrations [M] of compound *II* are shown on the lines.

[3] Rokach *et al.,* manuscripts in preparation.

is 50 μM. The kinetics of inhibition against S-adenosylmethionine was not determined.

The effect of *II* on the N-methylation of several other amines by S-adenosylmethionine-methyl-[^{14}C] and the rabbit lung INMT are presented in Table 2. At 25 μg/ml the inhibition of the methylation of catecholamines was 57–88%; these data are based upon radioactivity in organic solvent extracts of reaction mixtures. The conversion of 5-methoxy NMT to 5-methoxy DMT, a compound known to be even more potent a psychotogen than DMT (34), was inhibited 96%. The data here are based upon a thin layer chromatographic separation in the methanol-1N ammonium hydroxide (5:1) solvent system. *II* did not inhibit bovine adrenal phenethanolamine-N-methyltransferase or a DMT-forming enzyme activity in human liver extracts at levels about 100-fold higher than that required to block INMT (Table 3).

The *in vivo* effect of *I* and *II* on the specific activity of rabbit lung INMT was evaluated in several experiments. Male NZW rabbits (0.7–1.2 kg), fasted overnight, were given *I* as a solution (in dilute HCl at pH 3.5–3.9) or suspension (in dimethylacetamide-emulphor-water 4:16:80) or *II* (in water) with a stomach tube. Two to seven hours later, the animals were decapitated, exsanguinated, and the lungs homogenized in 3–10 vol of 0.15 M KCl at 0°. The homogenates were centrifuged at 55,000 × *g* for 30–60 min at 0° and the supernatant fluids assayed directly for INMT activity (see MATERIALS AND METHODS). Fig. 3 illustrates some representative enzyme activity curves for control animals and rabbits treated with compound *I*. The specific activity of the enzymes (cpm in [^{14}C]DMT fraction/mg protein/hr at 37°) was calculated from the slope, using the linear portion of the curves. Fig. 4a summarizes the data for the effect of compound *I* on INMT. When the rabbits were dosed with 8–25 mg/kg and sacrificed 2–3 hr later, inhibition of enzyme activity ranged from 51 to 85%. Sacrifice at 7 hr following a 25 mg/kg dose gave 36% inhibition in one experiment and 68 mg/kg resulted in over a 50% reduction of INMT specific activity in three experiments. Panel b in Fig. 4 shows the effect of *II* on the specific activity of rabbit lung INMT. When the animals were dosed at 4–7 mg/kg and killed 2 hr later, inhibition was over 60%. Sacrifice at 3 hr following 25 mg/kg gave 87% inhibition in one experiment. When the rabbits were decapitated 7 hr after dosage at 25–35 mg/kg, inhibition was variable (29–56%).

Both compounds were also evaluated for activity by intravenous administration. About 40 min after a single i.v. dose (ear vein) of 4–5 mg/kg, there was over a 60% reduction in the specific activity of INMT (Table 4). Thus, *I* and *II* are potent inhibitors of INMT *in vivo* when administered by injection or orally, while *I* is not strikingly active *in vitro* when compared to *II*.

An experiment was designed to determine whether administration of a relatively low dose of a DMT biosynthesis inhibitor for a relatively longer time period maintains a reduction in INMT activity. Rabbits were placed on *II* (selected because of its better water solubility) at 0.02% and 0.005% in the drinking water for 12 days, up until the time they were decapitated. There were five animals in each group, and weight changes as

TABLE 2

Effect of II on the methylation of other amines by rabbit lung INMT

Substrate	Product	% Inhibition by 25 μg/ml *II*
mM		
5-methoxy NMT (0.2)	5-methoxy DMT	96
normetanephrine (0.5)	metanephrine	88
dopamine (0.6)	epinine	57
norepinephrine (0.6)	epinephrine	57
3-0-methyldopamine (0.1)	3-0-methylepinine	70
4-0-methyldopamine (0.1)	4-0-methylepinine	65

TABLE 3

Effect of II on other N-methyltransferases in Vitro

Enzyme	μg/ml of *II*	% inhibition
human liver DMT-forming enzyme	26	0
bovine adrenal phenethanolamine-N-methyltransferase	25	7
	2.5	0

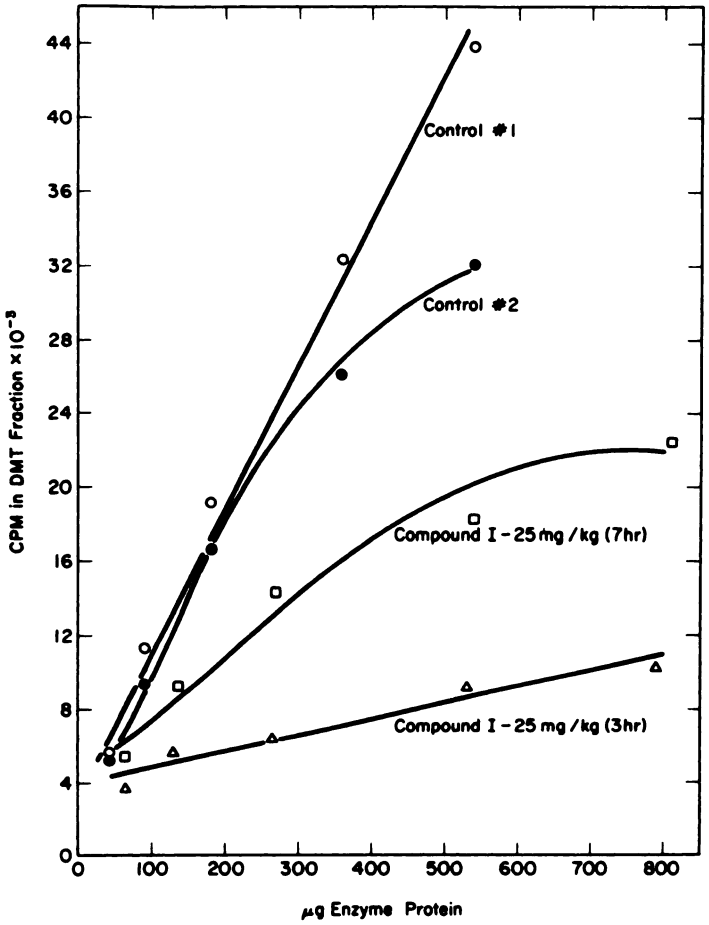

FIG. 3. *INMT activity curves from control animals and rabbits treated with compound I.*

well as daily consumption of medicated and nonmedicated drinking water were monitored. The data in Table 5 show that at the 0.02% level (equivalent to a daily dose of about 22–32 mg/kg), *II* inhibited INMT 76%; at 0.005% (equivalent to 5–7 mg/kg/day) inhibition was 36%. These results demonstrate that a reasonably low oral daily dose of a DMT biosynthesis inhibitor can produce a reduction in INMT after 12 days. To establish whether the inhibitory effect of *II* on INMT was reversible, an enzyme preparation from a treated rabbit was assayed before and after dialysis against 2000 vol of 1 mM potassium phosphate buffer pH 7.1. Fig. 5 shows that before dialysis INMT was markedly inhibited (80% reduction), but after dialysis activity was indistinguishable from that of the control enzyme preparation; a reversible type inhibition is suggested.

Experiments were devised to determine whether inhibition of INMT in rabbits treated with *I* was accompanied by a block in the conversion of precursor NMT to DMT. In the first approach, the conversion of intravenously administered NMT to DMT in carotid arterial blood was studied (33). Animals were pretreated with *I* orally at 25 mg/kg three times a day for two days, then twice on the third day. About 2 hr after the last of the eight doses, 10 mg/kg NMT was injected in the ear vein. The DMT formed during the first minute after NMT injection was measured by minor modification of our gas chromatographic-mass spectrometric method (35); all analyses were carried out with coded samples.

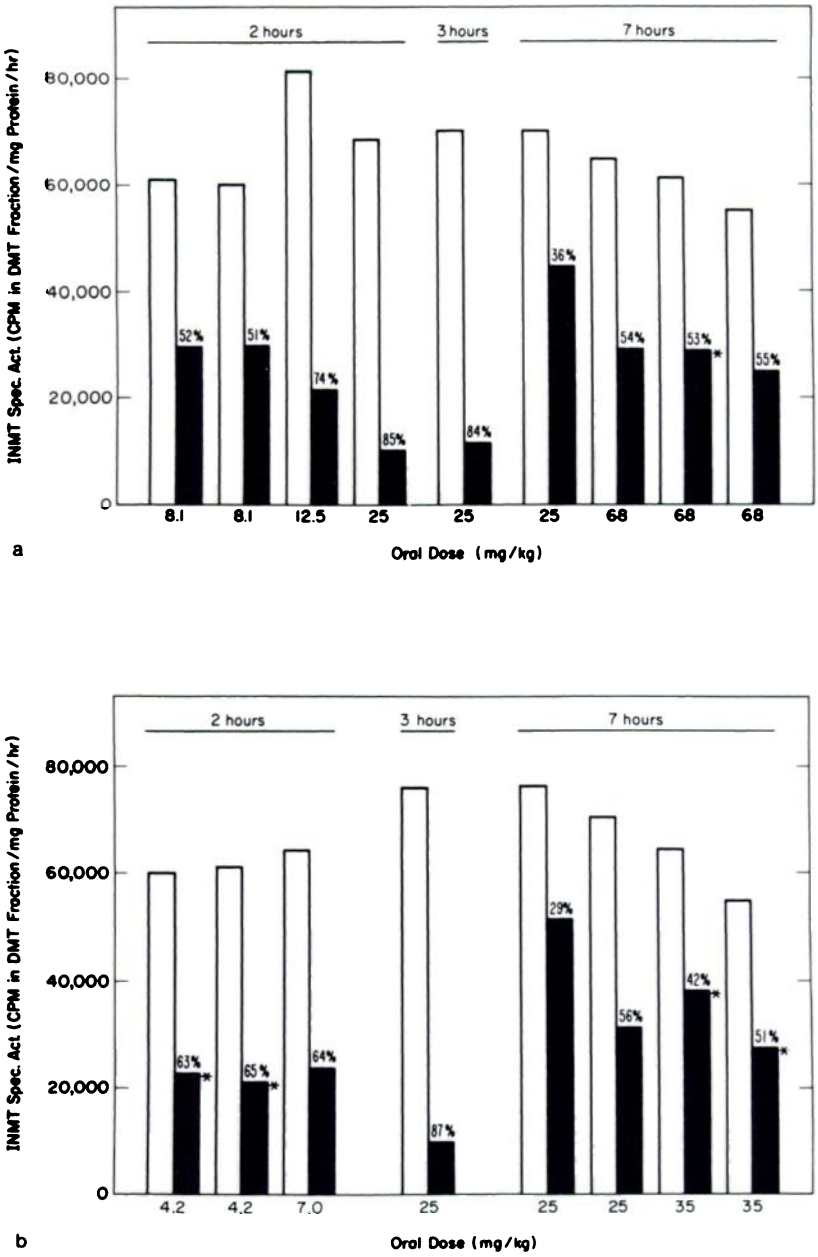

FIG. 4. *Summary of the effect of I (panel a) and II (panel b) on the specific activity of rabbit lung INMT*
The open bars are for the control animals, the solid bars for drug-treated animals. There were 2 rabbits per group in all experiments, except for the ones marked with the asterisk (1 rabbit). The sacrifice time is shown at the top of the figure and the percent inhibition values ($p < 0.01$) are given over the closed bar. Each set of bars represents a separate experiment.

The data in Table 6 show that there was a 75% reduction in DMT biosynthesis under conditions where INMT activity was suppressed markedly. Control rabbits produced 26–55 ng DMT/ml carotid blood, while the drug-treated animals produced only 1.4 ng DMT/ml. In similar experiments, *I* also produced inhibition of DMT

TABLE 4

Effect of I and II on rabbit lung INMT activity (i.v. dosage)

Compound	Dose	Time of sacrifice after i.v. dosage	Enzyme specific activity[a]		% Inhibition
			treated	control	
	mg/kg	*min*			
I	4	36	45,662	117,160	61 ($p < 0.01$)
II	5	40	37,043	103,280	64 ($p < 0.05$)

[a] The specific activity is defined as the cpm in the [^{14}C]DMT fraction/mg protein/hr at 37°. The values were obtained from the slopes of the titration curves (see text and Fig. 3); there were two rabbits/group. Error term pooled over the two experiments used for statistical analysis (*t*-test).

TABLE 5

Effect of II on INMT activity in a 12-day study[a]

Treatment group	mg/kg *II* consumed based upon		Enzyme specific activity	% Inhibition
	starting wt.	final wt.		
water control	—	—	65,290 ± 4,486	—
0.02% *II* in drinking water	32.24 ± 2.47	21.88 ± 0.92	15,671 ± 1,717	76 ($p < .001$)
0.005% *II* in drinking water	7.40 ± 0.44	5.08 ± 0.26	41,697 ± 3,707	36 ($p < .001$)

[a] All data are average values (±SEM) from five rabbits per group. The weight range of the 15 animals which were randomly distributed into the three groups was 835–1275 g at the start of the experiment and 1285–1685 g at the end of the study. There was no statistically significant difference in the mean weight gain among the three groups.

formation from NMT at single or multiple oral doses of 100 mg/kg in one-day experiments (data not shown).

The second approach taken to demonstrate that *I* blocked DMT biosynthesis was based upon the conversion of [^{14}C]labeled NMT (administered i.v.) to [^{14}C]-DMT isolated from rabbit lung (33). This organ is the principal site of INMT in the rabbit (11). Male NZW rabbits (0.4–0.8 kg) were given oral doses of *I* in one and three day experiments. One to two hours after the last oral dose the animals (pretreated with the monoamine oxidase inhibitor pheniprazine at 10 mg/kg i.p.) were given [^{14}C]NMT in the ear vein. Four minutes later they were decapitated, exsanguinated and the lungs frozen at −196° for the subsequent isolation of the [^{14}C]DMT. The tissues were analyzed in pairs (one control and one drug-treated), and the readout was based upon thin-layer chromatography. In some experiments the identification of the [^{14}C]DMT was confirmed by reverse isotope dilution assay (36). All tissue samples were coded prior to analysis. As shown in Table 7, treatment of rabbits with three doses of 100 mg/kg each of compound *I* over 6 hr produced an 89% inhibition in DMT formation; with a single 100 mg/kg dose, inhibition was 66%. When the compound was administered in several doses of 25 mg/kg over 3–4 days, there was a 70% reduction in [^{14}C]DMT formation. In experiments #3 and #4 the specific radioactivity of DMT (cpm/μg carrier DMT) was determined along with the cpm/gm lung. In experiment #3 (3 days), compound *I* produced a 73% reduction, while in experiment #4 (4 days), 64% inhibition resulted. *I* also produced a significant reduction (65%; $p < 0.05$) in [^{14}C]DMT accumulation in rabbit kidney in experiment #3 (data not shown). The effect of *II* on [^{14}C]DMT synthesis was determined in one experiment, and a 90% inhibition resulted after three doses of 125 mg/kg each over 6 hr. Control animals (3/group) gave 829 cpm [^{14}C]DMT/gm lung while treated rabbits produced an average of 61 cpm/gm lung. These data, taken with the results from the experiments employing non-radioactive NMT, demonstrate that a reduction in rabbit lung INMT activity is, as expected, associated with a block in the conversion of NMT to DMT in blood and lung.

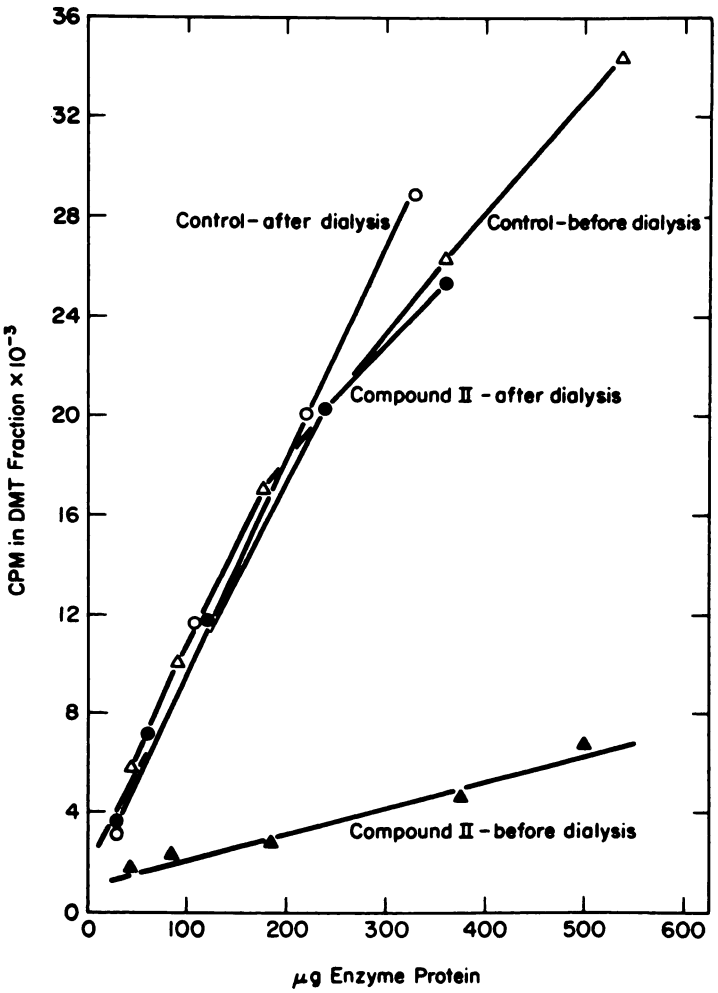

FIG. 5. *Effect of dialysis on INMT inhibition produced by compound II.*

DISCUSSION

The studies described in this paper demonstrate that *I* is a potent inhibitor of INMT *in vivo* and that the active metabolite *II* is also effective *in vitro*. Compound *II* is about 10 times more potent than other INMT inhibitors *in vitro* including DBN (25). Associated with inhibition of the enzyme, there is a block in the conversion of NMT to DMT in rabbit lung and blood. *II* also inhibits the N-methylation of several other amines *in vitro*, including that of 5-methoxy-NMT to 5-methoxy-DMT; however, it does not affect phenethanolamine-N-methyltransferase or a DMT-forming enzyme in liver extracts. Neither *I* nor *II* produced any overt behavioral or physiological effects in rabbits when given in three relatively large oral doses of 100–125 mg/kg each over a 6 hr period. Both compounds have also been evaluated in a variety of other assay systems in our laboratories, but no striking activity has been observed. As examples, they are inactive as antibacterial agents, as coccidiostats and fasciolicides, they have no cardiotonic or antihypertensive activity, and they do not inhibit enzymes such as the renin converting enzyme, glycolate oxidase and histidine decarboxylase *in vitro*. Neither compound is positive in bacterial mutagenicity tests. In acute toxicity studies in mice, *I* has an oral LD_{50}

TABLE 6

Effect of I (25 mg/kg × 8 over 3 days) on the conversion of NMT to DMT in blood

Animal	ng DMT/ml carotid arterial blood						
	Blank values[a]		(Avg.)	Experimental values		(Avg.)	Change (avg.)[b]
control	19.4	11.5	(15.5)	34.6	48.1	(41.4)	25.9
control	6.7	11.1	(8.9)	42.1	85.1	(63.6)	54.7
treated	18.1	17.3	(17.7)	17.2	20.9	(19.1)	1.4
treated	15.4	11.9	(13.7)	17.0	13.2	(15.1)	1.4

[a] Blank values were obtained by measuring DMT in freshly drawn blood samples to which an amount of NMT was added in proportion to that to be injected i.v. This was done to correct for the trace (0.0027%) of DMT present in the NMT. Experimental values are data obtained after injecting 10 mg/kg NMT. All analyses were carried out using coded sample numbers.

[b] Using ratios of geometric means, there was a 75% reduction ($p < 0.05$) in DMT formation due to *I*.

TABLE 7

Effect of I on the conversion of [^{14}C]NMT (i.v.) to [^{14}C]DMT in rabbit lung

Exp. no.	Oral dose of *I*	[^{14}C]NMT injected	cpm in [^{14}C]DMT[a]				% Inhibition of DMT formation[c]
			Per g lung control vs. treated		Per μg DMT[b] control vs. treated		
1	100 mg/kg × 3 over 6 hr	33 μc (0.6 mg/kg)	6131	563	—	—	89
2	100 mg/kg × 1	21 μc (1.6 mg/kg)	5888	1755	—	—	66
3	25 mg/kg twice daily for 3 days	40 μc (0.42 mg/kg)	11095	3583	861	301	73
4	25 mg/kg twice daily for 4 days	16 μc (0.17 mg/kg)	5164	1840	270	100	64

[a] Data are average values from 3 to 4 rabbits per group.

[b] Carrier DMT (100 μgm) was added at the outset of the isolation procedure. These data are the cpm in [^{14}C]DMT per μg of carrier DMT.

[c] Reductions calculated from the ratio of geometric means. $p < 0.05$ in experiments 1 and 4 whether data were analyzed as paired or unpaired samples. $p < 0.05$ in experiments 2 and 3 when data were analyzed as unpaired values (analysis of variance based upon log transformation).

of 1460 mg/kg, while the value for *II* is 432 mg/kg. The parenteral LD_{50} is also higher for *I* (1250 mg/kg i.p.) compared to *II* (118 mg/kg i.v.). Details of these studies will be published separately.

Because *I* and *II* block the biosynthesis of DMT, they would be of interest in the treatment of schizophrenia. The acute toxicity data and lower basicity for *I* make it the preferred compound. The evidence that DMT may be involved in some phases of the disease has been discussed in detail in several review articles (1–10). To summarize, DMT has well-established psychedelic effects in man, producing visual colors and distortions; there are reports that it is present in body fluids (although its occurrence at higher levels in schizophrenics is not established); precursors and an enzyme for DMT (and 5-methoxy DMT) synthesis are in human tissue; and behavioral tolerance may not develop in man. Thus DMT, one of several substances proposed as a candidate for an endogenous schizotoxin, meets many of the required criteria of Hollister (37).

It has also been pointed out that neuroleptic drugs should inhibit the synthesis, increase the metabolism or antagonize the behavioral effects of the compound proposed as an endogenous psychotogen. While some agents may interfere with DMT biosynthesis or its behavioral effects in animals (11, 38), it is not known whether antipsychotic drugs affect DMT metabolism in humans. The clinical utility of a DMT synthesis inhibitor or antagonist in schizophrenia is still hypothetical. In this

regard, one could test the DMT hypothesis of schizophrenia with a compound such as *I*. Should such an evaluation be undertaken, it would be desirable to try to correlate changes in the clinical status with DMT levels in body fluids. Whether a novel therapeutic agent will emerge from this approach remains to be established.

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