UV light. The spots were detected by exposing the plate to iodine vapor.

RESULTS AND DISCUSSION

R_f values obtained with the five solvent systems are given in Table I. It is very interesting to note that the R_f values of the gallic acid ester in the two solvent groups (*N* and *A*) are reversed.

In a nonaqueous solvent system (Group *N*), the R_f values of the gallic acid ester increase with an increase in length of the carbon chain. In this case, the separation is mainly based on the adsorption or partition between the kieselguhr and the ester. In aqueous solvent systems (Group *A*), the R_f values decrease with an increase in length of the carbon chain. The separation mechanism may depend on the reversible formation of hydrogen bonds between the carbonyl-oxygen atom of polyamide and the hydrogen atom of phenolic group in the ester.

The layer is firmly bonded, does not crack, and can be stored easily. Both sides of the glass are independent of each other and chromatography can be performed simultaneously on each side.

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 9, 1969 from the *Department of Pharmacy, Taipei Medical College, Taipei, Taiwan (Republic of China)*.

Accepted for publication August 6, 1969.

Fate of 2-Phenethylamino-l-phenylethanol,2-^{14}C in Rats

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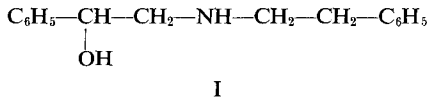
Abstract □ The fate of 2-phenethylamino-1-phenylethanol,2-^{14}C was determined following i.p. administration to rats. Little if any radioactivity was detected in tissue samples 2 days after dosage. The radioactivity was eliminated primarily in the urine. Recovery amounted to 91% in the urine and 6.8% in the feces after 3 days. The radioactivity in the urine was due to the administered compound. LD_{50} determination and *in vivo* MAO inhibition are also reported.

Keyphrases □ 2-Phenethylamino-1-phenylethanol—toxicity, *in vivo* inhibition and metabolic fate □ LD_{50} determination—2-phenethylamino-1-phenylethanol

2-Phenethylamino-1-phenylethanol (I) has been reported (1) to be a good inhibitor of monoamine oxidase (MAO) *in vitro*. The purpose of this study was to determine the toxicity, *in vivo* MAO inhibition, and metabolic fate of I.



I

EXPERIMENTAL

Methods and Materials—The LD_{50} of I was determined by the method of Horn (2). *In vivo* MAO inhibition was studied using the reserpine reversal technique (3). A liquid scintillation counter[1] was used to measure radioactivity of all samples. The background count rate of the instrument was stable at 25 to 30 c.p.m. throughout the study. Two counting formulations were employed: XDC scintillator for counting water-soluble samples consisted of 1 part xylene, 3 parts 2-ethoxyethanol, 1.0% 2,5-diphenyloxazole, 0.05% 1,4-bis-(4-methyl-5-phenyloxazolyl) benzene, and 8.0% naphthalene; TC scintillator for counting feces and tissue samples consisted of 1 part toluene, 1 part 2-ethoxyethanol, and 0.6% 2,5-diphenyloxazole. All counted samples were fortified with ^{14}C-toluene internal standard (15λ of 0.77 μc./ml.) and recounted to determine counting efficiency. All samples were counted in low-potassium counting vials.[2]

Thin-layer chromatograms (250 μ) spread with Silica Gel G[3] were activated at 110° for 0.5 hr. prior to use. Solvent systems used were ethanol–ammonium hydroxide (4:1) ethanol–acetic acid (4:1) and chloroform–methanol–water (75:22:3).

Labeled compounds were identified from autoradiograms using medical X-ray film.[4] Exposure times were based on exposure to the film of 10^8 disintegrations so that concentrations of 1% could be detected.

Reagent grade chemicals and ^{14}C-styrene[5] were used as received.

2-Phenethylamino-1-phenylethanol,2-^{14}C—Perbenzoic acid (30 ml. of a 15% solution in benzene) was placed in a 100-ml. three-necked flask equipped with stirrer and condenser. The flask was cooled to 0° and a solution of 1 mmole (104 mg.; 1 mc.) of 8-^{14}C styrene and 8.6 mmole (895.9 mg.) of styrene in 1.0 ml. of ether was added. After 24 hr. at 0° the solution was extracted with 3 × 30 ml. of 10% NaOH. The organic phase was washed with 3 × 30 ml. of water and then dried. Removal of the benzene *in vacuo* gave 0.85 ml. of 8-^{14}C-styrene oxide.

Table I—LD_{50} Determination of I·HCl in Mice[a]

Group[b]	Dose, i.p., mg./kg.	Deaths
1	46.4	5
2	31.6	2
3	21.5	0
4	10.0	0

[a] Male albino mice, 18–22 g. (Harland Industries). [b] Each group contained five mice.

[1] Beckman LS-100, Beckman Instruments Inc., Fullerton, Calif.

[2] Packard Instrument Co., Inc., Downers Grove, Ill.

[3] Brinkman Instruments Inc., Westbury, N. Y.

[4] No Screen, Eastman Kodak Co., Rochester N. Y.

[5] International Chemical and Nuclear Corp., City of Industry, Calif.

Table II—LD_{50} Determination of I·HCl in Rats[a]

Group[b]	Dose, i.p., mg./kg.	Deaths
1	500	3
2	300	3
3	200	3
4	100	3
5	50	2
6	30	0

[a] Male Sprague-Dawley rats, 165–185 g. (Harland Industries, Cumberland, Ind.).
[b] Each group contained three rats.

A 10-ml. flask containing β-phenethylamine (1.98 g., 16.33 mmoles) was fitted with a condenser and 1-ml. syringe *via* a Claisen head. 8-^{14}C-styrene oxide was added dropwise over 30 min. to refluxing β-phenethylamine. The mixture was heated under reflux for 2.5 hr., then allowed to cool to room temperature. Any droplets adhering to the condenser were washed into the flask with 7 ml. of hexane. The reaction mixture was cooled overnight and the resulting white solid was collected on a sintered-glass funnel. The solid was washed in order with hexane, water, and hexane. The solid (650 mg., 28.9% based on styrene) was air dried, m.p. 91° [lit. (4) 89.5–90°]. This material was sublimed (80°/1 mm., m.p. 91.5°) and was shown by autoradiography to be free of any other radioactive contaminants. The specific activity was 0.45 μc./mg.

The hydrochloride was prepared and air dried. Recrystallization from acetone–water gave a white solid, m.p. 210–211° [lit. (4) 205–206°]. Chemical and radiochemical purity were established by chromatography and autoradiography (3 days).

In Vivo Studies—The LD_{50} (i.p.) of I·HCl was found to be 35 mg./kg. in mice (Table I) and 27–30 mg./kg. in rats (Table II). The animals were dosed with the requisite amount of an aqueous solution (17.0 mg./ml.) of I·HCl. The animals were observed for 24 hr. following injection but all deaths occurred within 1 hr. of injection. Toxic doses led to convulsions and rapid death.

In vivo MAO inhibition as measured by the reserpine reversal (3) method in mice was not observed at a dose equal to 60% of the LD_{50}. One group of five mice[6] was dosed with 100 mg./kg. i.p. of pargyline hydrochloride. After 3 hr. the mice were given 20 mg./kg., i.p. of amitriptyline.[7] All mice became alert and active and salivated excessively. A control group (three mice) given only amitriptyline became sedated. Two groups of five mice each were then given 21 mg./kg. of I·HCl from an aqueous solution (17.0 mg./ml.). All mice exhibited slight depression. Amitriptyline (20 mg./kg. per dose) was then injected, i.p. at intervals of 1, 2, 6, 12, and 24 hr. All mice exhibited depression (no stimulation or salivation was observed) as did the control mice. Prolonged dosage (21 mg./kg. every 6 hr. for 72 hr.) followed by amitriptyline at the 73rd hr. gave similar results.

Six rats (165–185 g.) were dosed intraperitoneally with 0.24 ml. (2.25 μc.) of a stock solution of 2-phenethylamino-1-phenylethanol-2-^{14}C hydrochloride. The stock solution contained 0.125 g. of the hydrochloride in 6 ml. of water. The rats were placed in individual metabolism cages. Each rat received one dose every 8 hr. for the first 24 hr. (four doses per rat total) and urine was collected every 6–8 hr. The accumulated feces were combined at the termination of the experiment. All animals were allowed food and water *ad libitum*. One rat died 1 hr. after the second dose, thus a total of 49.5 μc. was administered in this study.

The accumulated feces of all six rats was oven dried at 110° for 48 hr. and pulverized. A 100-mg. sample was treated with 0.3 ml. of 72% perchloric acid and 0.2 ml. of 30% hydrogen peroxide and digested at 80° for 4 hr. (5). TC scintillator (15 ml.) was added and the samples were counted. A total of 3.4 μc. (6.9% recovery) was found in the feces.

The recovery of administered activity in the accumulated urine at each time interval is shown in Table III. A total of 46.39 μc. was recovered from the urine and feces at the end of 72 hr., representing 93.5% of the total radioactivity administered.

Tissue Distribution—Rats from the study were sacrified at the end of 72 hr. and the brain, kidney, liver, heart, and spleen, were removed and rinsed with saline to remove the adhering blood. Tissue samples (100–200 mg.) were digested in 0.3 ml. of 72% perchloric acid and 0.2 ml. of 30% hydrogen peroxide at 80° for 1 hr. Samples were counted in TC scintillator. None of the tissue samples registered statistically significant activity within several hours' counting time. The total activity was estimated to be 0.5–1.0% of the administered radioactivity.

Table III—Recovery of Radioactivity in Urine (2) of Rats Administered 2-Phenethylamino-1-phenylethanol-2^{14}C

No. of Doses	Hours after First Dose	Accumulative Administered μc.[a]	Recovered, μc.
1	8	13.50	1.80
2	17	27.00	3.26
3	24	38.25	4.24
4	32	49.50	4.04
—	48	—	21.80
—	60	—	6.20
—	72	—	1.65

[a] All urine samples were counted in XDC scintillator.

Urine Analysis—Urine samples collected were chromatographed on thin-layer Silica Gel G. The plates were autoradiographed for 48 hr. Development of the film showed one spot at an R_f equal to that of I (0.48 in ethanol:ammonium hydroxide). A mixture of I and the urine sample still chromatographed to one spot in all solvent systems used. Concentration *in vacuo* at room temperature of the urine to 0.25 the original volume followed by similar analysis uncovered no metabolities.

DISCUSSION

The toxicity of I is so great that one cannot measure the MAO inhibition *in vivo*. It is perhaps surprising that I is so toxic since it is an analog of 1-phenyl-2-aminoethanol which has an LD_{50} of 1100 mg./kg. in mice (SC) (6). The toxicity of I was characterized by severe convulsions.

It was hoped that a study of the metabolic fate of I would indicate an approach to the design of analogs which were less toxic. Since no metabolism was detected this goal was not met. Secondary amines are deaminated *in vivo*, but it is not unusual that a substituent such as phenethyl would eliminate deamination as a metabolic pathway (7).

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ACKNOWLEDGMENTS AND ADDRESSES

Received June 6, 1969 from the *Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, Lafayette, IN 47907*

Accepted for publication August 4, 1969.

Abstracted from the Ph.D. Thesis of William G. Andrus, Jr., Purdue University.

This investigation was conducted under National Institute of General Medical Sciencies Fellowship No. 1-F1-GM-34,131-01. Additional support was provided by the American Foundation for Pharmaceutical Education.

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