Table II—Within-Day and Between-Day Reproducibility of the Urine Assay [a]

Added, μg/mL	Within-Day Found, μg/mL	Within-Day CV, %	Between-Day Found, μg/mL	Between-Day CV, %
1.6	1.62 ± 0.03	1.8	1.62 ± 0.066	4.0
2.7	2.75 ± 0.04	1.5	2.78 ± 0.114	4.1
3.9	4.15 ± 0.14	3.4	4.18 ± 0.194	4.6

[a] Mean ± *SD*; $n = 4$.

same volume as the methanolic standards. Peak height ratio, however, would not be affected by volume difference. Since the extraction efficiencies of I and of the internal standard were independent of each other and the internal standard was completely recovered, recovery of I calculated from peak height ratio measurement would provide a more meaningful value. Using peak height ratio measurement, mean (±*SD*) recoveries of I (six samples) at 100, 500, and 1000 ng/mL were 99.0 ± 1.0, 98.5 ± 1.8, and 99.0 ± 1.5%, respectively.

Standard curves were linear and reproducible over the concentration range of 25–1000 ng/mL. Linear regression statistics for five standard curves prepared on different days over a period of 2 weeks produced a slope of 0.0044 (±0.0001), an intercept of −0.024 (±0.032), and a mean coefficient of determination (r^2) of 0.999.

Assay reproducibility was determined by repeated analysis of plasma samples containing I. Three different concentrations other than those used in the preparation of the standard curves were used. The mean concentrations, standard deviations, and coefficients of variation for within-day and between-day analysis are presented in Table I. These data indicate excellent assay reproducibility, which is further supported by the reproducible nature of the standard curves as shown above.

Plasma spiked with I (1 μg/mL) was stored in the refrigerator (0–4°C), and 1-mL aliquots were analyzed once a week for 6 weeks. Recoveries of I were similar over the 6-week period indicating good stability of I in plasma.

Recovery of I from urine was similar to that from plasma. Standard curves were linear and reproducible over the concentration range of 0.5 to 5 μg/mL with $r^2 > 0.999$. No interference was observed from the extractive constituents of blank urine. Both within-day and between-day assay reproducibility was excellent (Table II). The procedure can be used for concentrations down to 0.1 μg/mL with good reproducibility (coefficient of variation, 4.5%).

The lower sensitivity limit of the GC–MS method (16) was only ~100 ng/mL when 2 mL of plasma was used. Our GC method is simple, rapid, able to quantitate I down to 25 ng/mL with good precision (coefficient of variation, 6.0%), and requires only 1 mL of plasma. This is advantageous, since with greater assay sensitivity plasma concentrations can be monitored for a longer period of time and a smaller dose can be used. The versatile nature of the present method makes it particularly suitable for pharmacokinetic studies of I.

To further evaluate the usefulness of the assay procedure, a 14.5-kg dog was given a 10-mg/kg iv dose of I, and blood and urine samples were obtained. Figure 2 shows the chromatograms of ethereal extracts of dog plasma before and after the administration of I. After hydrolytic treatment of urine samples, the total recovery of I was 2.3% over a period of 96 h. Disposition studies in the dog are currently in progress.

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[^{14}C]Normacromerine Fate in the Rat

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Received August 19, 1982, from the *School of Pharmacy, Northeast Louisiana University, Monroe, LA 71209.* Accepted for publication March 17, 1983.

Abstract □ The biological fate of [^{14}C]normacromerine, a dimethoxylated phenethylamine derivative with putative hallucinogenic properties, was evaluated in male Sprague–Dawley rats at 100 mg/kg po. Urine was the primary elimination route accounting for 50% of administered carbon-14 after 24 h. Of this urine radioactivity, normacromerine comprised 30% at 8 h decreasing to nondetectable levels at 24 h. Carbon-14 in feces represented <10% of the administered dose at 24 h, and $^{14}CO_2$ expiration was not detected. Studies of normacromerine fate in comparison with previously studied phenethylamines may enhance evaluation of hallucinogenic potential of normacromerine.

Keyphrases □ Normacromerine—biological fate in the rat, hallucinogenic potential □ Biological fate—[^{14}C]normacromerine, rat, hallucinogenic potential □ Hallucinogens—normacromerine, biological fate in the rat

Normacromerine (*N*-methyl-3,4-dimethoxy-β-hydroxyphenethylamine), the major alkaloid in extracts of the Dona Ana cactus [*Coryphantha macromeris* (Engelm.) Br. and R.], has been implicated as the chemical responsible for hallucinogenic effects experienced by humans ingesting the cactus (1, 2). The potency of the Dona Ana cactus has been estimated to be one-fifth that of the peyote cactus [*Lophophora williamsii* (Lem.) Coult.] which contains mescaline, another natural hallucinogen (2, 3).

Laboratory studies of the behavioral effects of normacromerine in rats have provided both positive (4) and negative (5) results in support of the hallucinogenic properties of the drug. Absent from these studies has been a thorough evaluation of the mammalian disposition of normacromerine after ingestion. Studies of biological fate of the compound are unavailable in the literature.

Patterns of absorption, distribution, metabolism, and excretion of chemicals determine their extent and duration of action within biological systems (6). Such studies of norma-

0022-3549/84/0500-0692$01.00/0

Table I—Fate of Carbon-14 8, 16, and 24 h after Exposure to [^{14}C]-Normacromerine, 100 mg/kg po

Time, h	Percent of Administered Carbon-14[a]	
	Urine	Feces
8 (n = 6)	41.0 ± 2.6	0
16 (n = 4)	8.3 ± 1.85	8.0 ± 3
24 (n = 4)	1.4 ± 0.23	1.8 ± 0.32

[a] Total carbon-14 recovered (urine + feces + carcass + carbon dioxide) was 80 ± 3% of the administered dose. Each sample analysis was performed in duplicate. Values are the mean of the sample average ±SE; n is the number of animals evaluated.

cromerine may therefore provide a foundation for more enlightened evaluation of its hallucinogenic activity. Our initial objective in the evaluation of normacromerine was to characterize its previously unknown fate in a mammalian system, thereby establishing a framework for future metabolite studies.

EXPERIMENTAL

[^{14}C]Normacromerine—[^{14}C]Normacromerine (1 μCi/mmol, labeled in the β position) and nonlabeled normacromerine were synthesized *in vivo* from *Coryphantha macromeris* var. *runyonii*, as described by Keller (7, 8). Radiopurity (>99%) was evaluated using silica gel GF 254 TLC plates[1] developed in benzene–chloroform–methanol–58% ammonium hydroxide (8:6:5:1). All normacromerine analyses were performed using this chromatographic system.

Animal Exposures—Male Sprague-Dawley rats (300–400 g) were fasted 16–18 h prior to exposure; however, water was provided *ad libitum*. Each animal received 100 mg/kg of [^{14}C]normacromerine hydrochloride (in water) by oral gavage. This replicated dosage levels used in previous behavioral studies (5). After dosing, the animals were placed into metabolism chambers[2] for collection of expired CO_2, urine, and feces. Chamber air was drawn by vacuum (1 L/min) through 1 N KOH to collect the $^{14}CO_2$. One milliliter of KOH was sampled periodically throughout the initial 8-h postexposure. Each sample was placed into 15 ml of scintillation cocktail[3] for ^{14}C-radioassay using a liquid scintillation counter[4]. Urine and feces were collected and analyzed at 8, 16, and 24 h postexposure. At each time period collected urine was sampled into scintillation cocktail for total ^{14}C-radioassay and the remainder frozen until normacromerine analysis. Feces were collected, freeze-dried, ground to a fine powder, and combusted in a sample oxidizer[5] for total ^{14}C-radioassay. At 8 and 24 h, carcasses were also analyzed for total carbon-14. Following homogenization (1:1 with H_2O), samples of the carcass were combusted and radioassayed to provide a complete carbon-14 animal mass balance (carbon dioxide + urine + feces + carcass).

Urine Analysis—[^{14}C]Normacromerine was extracted (75% efficiency) from alkalinized urine with toluene. The toluene extract was then concentrated under nitrogen in a 40°C water bath[6]. [^{14}C]Normacromerine concentrates were spotted on TLC plates and developed by the standard solvent system. Co-chromatography with nonradioactive normacromerine standards allowed identification and isolation of [^{14}C]normacromerine. Silica gel spots containing [^{14}C]normacromerine were transferred (>90% recovery) into 15 mL of scintillation cocktail for subsequent radioassay.

RESULTS AND DISCUSSION

The 24-h carbon-14 elimination pattern is given in Table I. Urine was the major elimination route; feces accounted for minor excretion of the radiolabel. Elimination as $^{14}CO_2$ was not detected. Total carbon-14 recovery (urine + feces + carbon dioxide + carcass) was 80 ± 3% of the administered dose. This less than optimum recovery reflects an inability to accurately assay the total carbon-14 remaining in the animal at 8 and 24 h postexposure. Levels (<100 cpm/g of tissue) at two to three times the background radioactivity were measured in triplicate 500-mg tissue samples for each of 10 animals. The significant dilution of carbon-14 remaining in the animal (354 ± 72 dpm/g of tissue) was an unavoidable limitation due to the low normacromerine specific activity available from the *in vivo* synthesis.

[1] Fisher Scientific Co., Pittsburgh, Pa.
[2] Crown Scientific Glass Co., Orland Park, Ill.
[3] Packard Instruments Co., Downers Grove, Ill.
[4] Model LS-230 Liquid Scintillation System; Beckman, Fullerton, Calif.
[5] Model B306 Tri Carb Sample Oxidizer; Packard, Downers Grove, Ill.
[6] Organomation Associates, Inc., Northborough, Mass.

Table II—Percentage of Urinary Carbon-14 Analyzed as Normacromerine 8, 16, and 24 h after Exposure to [^{14}C]Normacromerine, 100 mg/kg po

Time, h	Percent Normacromerine[a]
8 (n = 4)	30.0 ± 3.8
16 (n = 4)	2.8 ± 1.5
24 (n = 4)	not detected

[a] Values are the mean of sample average ±SE; n is the number of animals evaluated. Each sample was analyzed in duplicate.

Since urine served as the primary elimination route, urine samples were further characterized to quantify [^{14}C]normacromerine. Table II indicates significant *in vivo* mammalian biotransformation of normacromerine. Thirty percent of urine radioactivity at 8 h was normacromerine, decreasing to 0% by 24 h. The remainder was accountable as water-soluble ^{14}C-labeled metabolites.

Our study in rats described normacromerine elimination primarily in urine, minimally in feces, and none as CO_2. Urine and feces analyses indicated normacromerine was significantly absorbed and metabolized to water-soluble metabolites.

Normacromerine is an *O*-methylated analogue of the endogenous neurotransmitter epinephrine and its singly *O*-methylated derivative, metanephrine. In fact, the biosynthesis of normacromerine in *Coryphantha macromeris* involves these catecholamines as precursors (7, 8). Consequently, the mammalian fate of normacromerine could be similar to the mammalian biotransformation of epinephrine and metanephrine. Previous studies of intravenously administered [^{3}H]epinephrine and [^{14}C]metanephrine in rats (9) demonstrated a similar biological fate as observed in these normacromerine studies. Urine was the significant elimination route (65–70% of administered dose after 48 h) and feces a secondary route (15 and 0.3% for epinephrine and metanephrine, respectively). Carbon dioxide was not analyzed. Since [^{14}C]-normacromerine was radiolabeled at the side-chain β position, loss of this carbon as $^{14}CO_2$ was not expected (10) and was not observed in our study. Cumulative carbon-14 recovery in urine and feces at 48 and 24 h postexposure was similar for metanephrine (65–68%) and normacromerine (61%), respectively. Free epinephrine and metanephrine accounted for <10% of urine radioactivity at 48 h. In comparison, no normacromerine was detected at 24 h postexposure. This may reflect enhanced oxidation of normacromerine by liver monoamine oxidases following the oral route of exposure (10). Metabolites of epinephrine and metanephrine in urine were products of monoamine oxidase reactions (3-methoxy-4-hydroxymandelic acid and 3-methoxy-4-hydroxyphenylglycol).

In vitro studies of methoxylated β-hydroxyphenethylamines, such as normacromerine, indicate significant interactions with mammalian monoamine oxidases (11–13). Therefore, the structural similarities between normacromerine, epinephrine, and metanephrine, as well as similar elimination patterns in the rat, suggest that normacromerine elimination products may be similar to monoamine oxidase products of its catecholamine analogues. The synthesis of these novel normacromerine metabolites and their *in vivo* identification, underway in our laboratory, are necessary for complete evaluation of normacromerine fate in the rat.

Understanding of normacromerine disposition will not only enhance evaluation of its hallucinogenic potential, but will provide further insight into comparative metabolism of xenobiotics by plant and animal systems.

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