Table I—Effect of Id (50 μg/mL) on the Incorporation of [^{3}H]Thymidine and [^{3}H]Uracil into the DNA and RNA of *E. coli* GK-19.

Time, min	Counts per Minute of [^{3}H]-Thymidine[a]		Counts per Minute of [^{3}H]-Uracil[a]	
	Control	Treated	Control	Treated
5	100	100	100	100
10	102	100	294	193
20	106	99	650	224
30	108	97	650	237
40	112	96	650	255

[a] Figures in the table are relative to the counts per minute of labeled precursor in control experiments at the end of 5 min.

In addition, treatment of *E. coli* GK-19 with potassium cyanide, sodium azide, and 2,4-dinitrophenol, which are known inhibitors of respiration and energy production, was undertaken; Id was added simultaneously or at varying times after the addition of the inhibitors. The results are portrayed in Fig. 12A-C. With pretreatment times of 30 and 120 min, the antibacterial effect of Id is markedly reduced, which further indicates that cellular respiration or energy, in part at least, is likely required for the lytic effect of Id on *E. coli* to be manifested.

A similar study with chloramphenicol, which is an inhibitor of protein synthesis, showed that protein synthesis is required for the lysis of cells (Fig. 12D). It is concluded that in order for Id to exert its maximum lytic effect, normal respiration, energy production, and protein synthesis are required. Because of the cellular physiological functions that were affected by Id, it was suspected that macromolecular synthesis, in general, could also be affected. The conviction that protein synthesis was a site of action of this Mannich base was strengthened by its inhibition of the biosynthesis of the enzyme β-galactosidase. In *E. coli* GK-19, it is possible to induce the synthesis of this enzyme within 2-3 min with isopropylthiogalactoside (17), but addition of Id to a culture of this microorganism abolished the ability of the cells to synthesize this enzyme.

The effect of Id on the synthesis of DNA and RNA was measured using radioactively labeled thymidine and uracil, respectively, and the results are shown in Table I. The data indicate that while the synthesis of DNA is virtually unaffected, the inhibition of RNA synthesis commences after 5 min and is reduced by 66% after 20 min.

In conclusion, this study has unearthed some of the factors which affect the antibacterial activity of 1-(2,4-dichlorophenyl)-4-dimethylaminomethyl-1-nonen-3-one hydrochloride (Id) against *E. coli* GK-19. In addition, the bioactivity of this compound is due, at least in part, to its causation of lysis, and adverse effects on respiration, as well as protein and RNA biosynthesis, take place.

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Determination of Amphetamine, Norephedrine, and Their Phenolic Metabolites in Rat Brain by Gas Chromatography

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Received March 29, 1982, from the *Neurochemical Research Unit, Faculty of Pharmacy and Pharmaceutical Sciences* and *Department of Psychiatry, University of Alberta, Edmonton, Alberta T6G 2N8, Canada.* Accepted for publication April 25, 1983.

Abstract □ A specific analytical procedure for the quantitation of amphetamine (I), norephedrine (III), and their amphoteric metabolites, *p*-hydroxyamphetamine (II) and *p*-hydroxynorephedrine (IV), in biological samples using electron-capture gas chromatography (GC-EC) is described. The procedure utilizes the ion-pairing reagent, bis(2-ethylhexyl)phosphoric acid, which frees the amines from most contaminants and permits the efficient extraction of the amphoteric compounds (as acetates) from the aqueous solution. Amines I and III and acetylated amines II and IV were perfluoroacetylated prior to GC-EC analysis. Metabolism of I, II, and III in the rat brain was studied. Results indicate that both *in vivo* and *in vitro* amines I and III are *p*-hydroxylated to II and IV, respectively, and II is β-hydroxylated to give IV. Norephedrine (III) was not detected as a rat brain metabolite of amphetamine (I).

Keyphrases □ Amphetamine—rat brain metabolism, norephedrine, phenolic metabolites, gas chromatography □ Norephedrine—rat brain metabolites, amphetamine, phenolic metabolites, gas chromatography □ Metabolites, phenolic—amphetamine and norephedrine, rat brain metabolism, gas chromatography

When amphetamine (I) is administered systemically to rats, appreciable amounts of I and trace quantities of *p*-hydroxyamphetamine (II) and *p*-hydroxynorephedrine (IV) are detected in the brain tissue. In attempts to associate these metabolites with some of the pharmacological actions of I, their presence in various brain regions and other tissues have been

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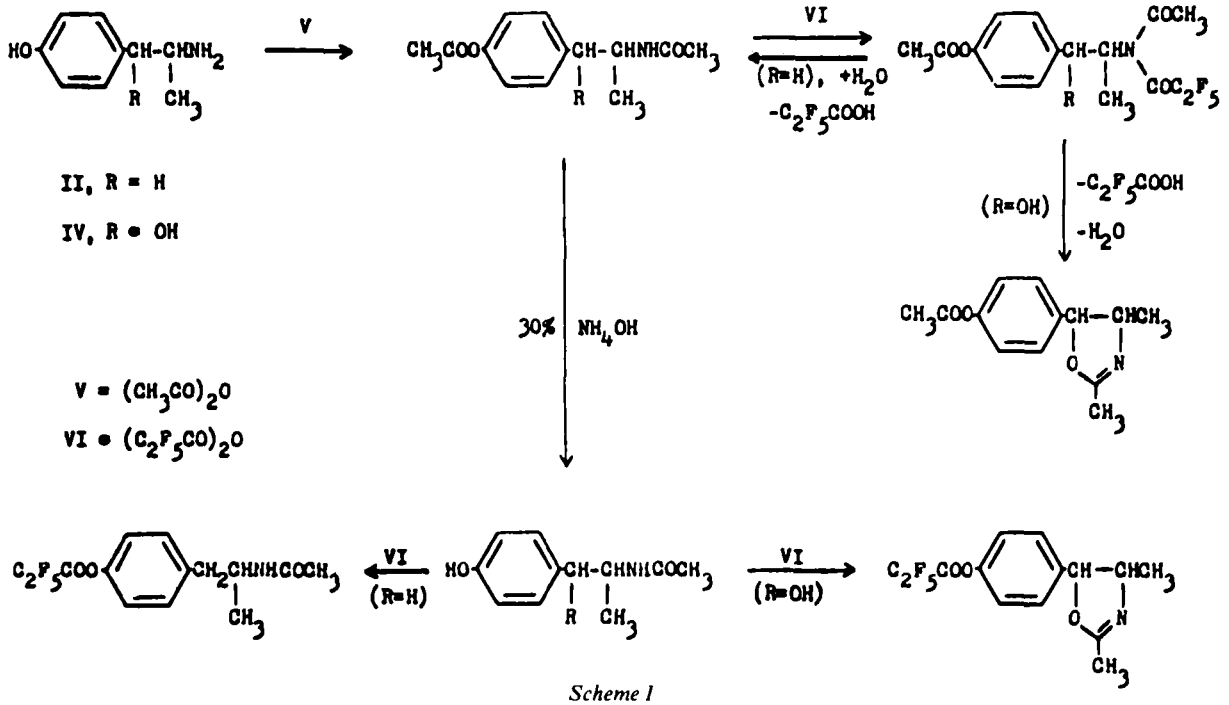


Scheme I

determined (1–11). It has been suggested (2, 5, 12, 13) that amphetamine (I) is first *p*-hydroxylated in the liver, and trace amounts of the resulting *p*-hydroxyamphetamine (II) penetrate the blood–brain barrier and accumulate in brain tissue where β-hydroxylation of II to *p*-hydroxynorephedrine (IV) occurs (14, 15). However, there is alternative evidence from *in vitro* studies to suggest that *p*-hydroxylation of I can also occur in the brain (9).

In a continuation of our studies on trace amines in the brain, we wished to determine whether analytical techniques previously developed (16–18) could be applied to the measurement of amphetamine (I), *p*-hydroxyamphetamine (II), norephedrine (III), and *p*-hydroxynorephedrine (IV) and provide information on the extent to which *p*- and β-hydroxylation occur in the brain tissue.

Current analytical procedures rely on the extraction of the amines into an organic solvent followed by derivatization with a reagent sensitive to electron-capture detection (EC) (2, 11, 19) or utilize technically more complex procedures involving tritiated compounds (1, 6, 8, 9, 11, 13, 14, 20) or mass fragmentography (3, 7). The simple extraction procedure is adequate for the analysis of many amines but usually demonstrates a poor gas chromatographic (GC) detection linearity over wide concentration ranges, as well as a poor sensitivity for phenolic amines, which are normally inefficiently extracted from aqueous solution.

We now report a rapid analytical method that permits the identification and efficient quantitation of amphoteric compounds (II and IV) in tissues and that can be employed to improve the GC sensitivity limits of simple amines (I and III). By utilizing this analytical procedure the levels of I–IV in rat brain tissues were determined after intraperitoneal administration of each amine. The results of an *in vitro* study on the metabolism of I–III with rat brain homogenate is also reported.

EXPERIMENTAL

Animal Studies—Male Sprague-Dawley rats (150 ± 20 g) were used. For the *in vivo* studies, rats were injected intraperitoneally with equimolar quantities (74 μmol/kg) of amphetamine hydrochloride[1], *p*-hydroxyamphetamine hydrobromide[1], norephedrine hydrochloride[2], and *p*-hydroxynorephedrine hydrobromide[2], (*i.e.*, 10.0, 11.19, 11.19, and 12.37 mg/kg, respectively, calculated as the free bases). Physiological saline was used as the vehicle, and injection volumes of ~0.3 mL were employed. Control rats received the same volume of vehicle without drug. All animals were sacrificed exactly 60 min after injection. The complete brain was rapidly removed, homogenized in three volumes of cold 0.4 M perchloric acid, and centrifuged at 10,000×*g* for 15 min. Supernatant equal to 1.0 g of whole brain tissue was used in the analyses.

For the *in vitro* studies, the whole brains from untreated rats were removed, homogenized in three volumes of ice-cold 1.15% KCl solution, and centrifuged at 10,000×*g* for 20 min. Supernatant corresponding to 0.5 g of the original tissue was incubated at 37°C with shaking in open 15-mL Erlenmeyer flasks containing an NADPH-generating system (20 μmol of glucose 6-phosphate[3], 20 μmol of magnesium chloride, and 4.4 μmol of NADP[4]) in Tris-HCl buffer (pH 7.4) and 1.0 μmol of the appropriate substrate (total volume 3.8 mL). Incubations were terminated after 60 min by the addition of 0.2 mL of 2.0 M perchloric acid. *In vitro* experiments were performed in triplicate.

Extraction and Derivatization—The samples [*in vivo* brain supernatant (4 mL) or *in vitro* incubation mixture (4 mL)], to which 1.0 μg of 2-(*p*-chlorophenyl)ethylamine[5] was added as internal standard, were adjusted to pH 7.8 by the addition of solid potassium bicarbonate and centrifuged (2000×*g*) to remove any precipitate formed. The supernatant was then extracted with 5.0 mL of a 2.5% solution of bis(2-ethylhexyl)phosphoric acid[6] in chloroform. After separation of the two phases, the aqueous layer was discarded and the chloroform layer was back-extracted with 3.0 mL of 0.5 M HCl.

Method a—For the analysis of I and III, the acid layer was adjusted to a pH value of >10.5 and extracted with 4.0 mL of ethyl acetate. The organic layer was evaporated to dryness under a stream of nitrogen, and the residue was pentafluoroacylated and treated further as described below.

Method b—For the analysis of II and IV, the acid layer was made slightly basic by addition of solid sodium bicarbonate, 300 μL of acetic anhydride (V) was added to the basified solution followed by small additional quantities of sodium bicarbonate with shaking until all effervescence ceased, and the so-

[1] Smith Kline and French Laboratories, Philadelphia, Pa.; lot no. 365-A for amphetamine sulfate, converted to hydrochloride, lot no. 988-64E for *p*-hydroxyamphetamine hydrobromide.

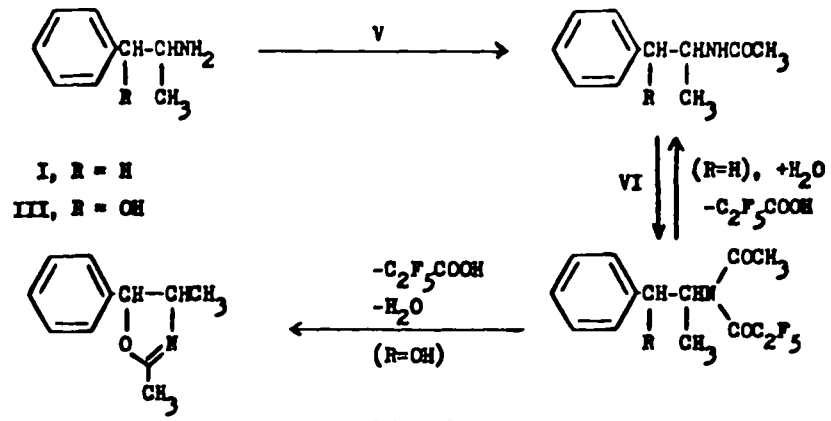
[2] Aldrich Chemical Co., Milwaukee, Wis.; lot no. 1414 TD for norephedrine hydrochloride, lot no. 122627PC for β-hydroxynorephedrine hydrobromide.

[3] Sigma grade, disodium salt; Sigma Chemical Co., St. Louis, Mo.

[4] Oxidized form, Sigma grade, monosodium salt; Sigma Chemical Co., St. Louis, Mo.

[5] Purchased as the free base (Sigma Chemical Co., St. Louis, Mo.) and converted to its hydrochloride in the usual way.

[6] Sigma Chemical Co., St. Louis, Mo.



Scheme II

lution was extracted with 4.0 mL of ethyl acetate. To the separated organic layer, 300 μL of a 30% NH_4OH solution was added and the mixture was vortexed for 40 min. After neutralization of the NH_4OH solution, the layers were separated by centrifugation. The ethyl acetate layer was then evaporated to dryness in a stream of nitrogen.

The residues obtained from each of the methods were separately dissolved in a mixture of 35 μL of ethyl acetate and 75 μL of pentafluoropropionic anhydride (VI)[7], and the ensuing reaction was allowed to proceed at 85°C for 1 h. Each mixture was reduced in volume under a stream of nitrogen and partitioned between toluene (200 μL) and 0.1 M sodium phosphate buffer (200 μL; pH 7.4). Each organic layer was retained for GC analysis.

Instrumentation—Analyses were performed with a gas chromatograph[8] equipped with a 15-mCi ^{63}Ni-source linear EC detector. A fused silica capillary column (Carbowax 20M, 12 m) was used with temperature programming, 80–220°C at 20°C/min, and injection port and detector temperatures of 250°C. The carrier gas was helium at 7 psi; argon–methane (95:5) at a flow rate of 36 mL/min was used as the make-up gas at the detector. The mass spectrometer[9] separator and ionization source temperatures were 180°C with an ionization energy of 70 eV. The gas chromatographic column and operating conditions were as described above. All spectra were consistent with the structures assigned; the mass spectra are described elsewhere[10].

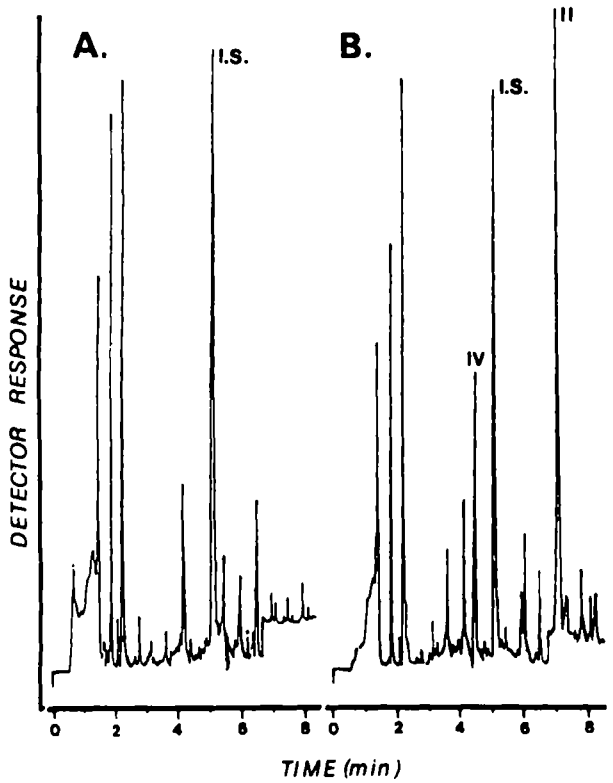


Figure 1—*Typical chromatograms of rat brain extracts which have been derivatized by method b. Key: (A) from rats injected with physiological saline; (B) from rats injected with norephedrine; (II) derivatized norephedrine; (IV) derivatized p-hydroxynorephedrine; (I.S.) derivatized internal standard.*

RESULTS AND DISCUSSION

Amphetamine (I), norephedrine (III), and their metabolites, *p*-hydroxyamphetamine (II) and *p*-hydroxynorephedrine (IV), were present in brain samples in trace concentrations. An analytical technique was required which would not only permit the adequate extraction of I–IV from aqueous solution, but also eliminate the interference created by the simultaneous extraction of endogenous contaminants. Such interference was found to be a particularly troublesome problem when utilizing GC–EC detection methods following the perfluoroacylation of sample extracts.

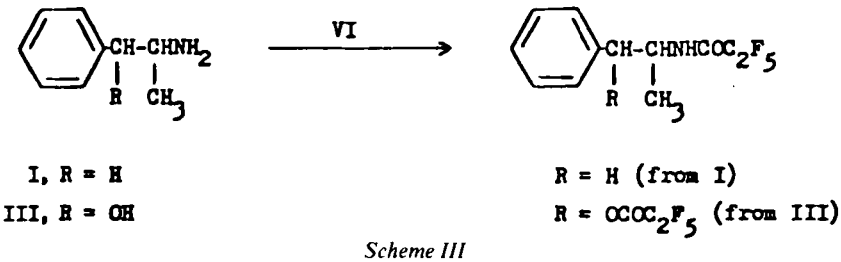
The described method proved to be a suitable analytical procedure. The four amines were efficiently extracted from brain supernatant or *in vitro* incubation mixtures with chloroform containing the ion-pairing reagent, bis(2-ethylhexyl)phosphoric acid (21) and were recovered from the organic solution by extracting the latter with dilute hydrochloric acid. The amine-containing aqueous solution was now free of most contaminants. The reextraction of the amphoteric metabolites II and IV into an organic solvent was made possible by their prior acetylation in the basified aqueous medium with acetic anhydride (V) to lipophillic *N,O*-diacetates (Scheme I). This acetylation procedure also converted amphetamine (I) and norephedrine (III) to their *N*-acetates (Scheme II). The β-hydroxyl group of either III or IV did not undergo acetylation.

Attempts were made to pentafluoroacylate the extracted acetates to increase their sensitivities to GC–EC. However, the *N*-pentafluoropropionyl derivatives of all four *N*-acetylated derivatives proved to be unstable. The derivatives of I and III rapidly lost the pentafluoroacetate moiety by hydrolysis, and the derivatives of III and IV cyclized to GC–EC-insensitive oxazoline products (Schemes I and II). The problem of insufficient sensitivity was overcome for II and IV by the preferential hydrolysis of the *O*-acetate moiety of their *N,O*-diacetates using ammonium hydroxide. This treatment freed the phenolic hydroxyl group for reaction with VI and resulted in the formation of the final derivatives identified in Scheme I. These *O*-pentafluoroacetyl derivatives were stable for at least 2 weeks if refrigerated. Typical GC traces are shown in Fig. 1.

Since neither amphetamine (I) nor norephedrine (III) contains a phenolic group, the analytical procedure just described for the analysis of II and IV was not applicable to the quantification of I and III; a separate analytical procedure was necessary for the latter two amines. They were directly removed from the aforementioned dilute hydrochloric acid solution containing I–IV by basifying the solution and extracting it with ethyl acetate. The extracts were treated with VI and the resulting *N*-pentafluoropropionyl derivatives (Scheme III) proved to be both stable to hydrolysis and GC–EC sensitive. This procedure permitted effective recovery and detection of both I and III without interference from the amphoteric metabolites.

Overall recoveries for the compounds (based on 100-ng amounts), using the methods described above, were as follows: I (87.7 ± 0.6%), II (65.0 ± 2.7%), III (80.3 ± 1.2%), IV (52.7 ± 2.5%), and internal standard (80.1 ± 1.7%); values represent mean ± *SD* ($n = 3$). The structures of all final de-

[7] Pierce Chemical Co., Rockford, Ill.
[8] Hewlett-Packard model 5830A.
[9] Hewlett-Packard model 5710 coupled to a Hewlett-Packard quadrupole mass spectrometer (model 5981A) and a model 5934A data system.
[10] Coutts, Baker, Pasutto, Liu, LeGatt, and Prelusky, Biomed. Mass Spectrum, in press.



Scheme III

rivatized products were confirmed by mass spectrometric analysis. Calibration plots of the derivatized form of all four amines were constructed using varying quantities of each amine and 2-(*p*-chlorophenyl)ethylamine as internal standard. All plots were linear over a 10–10,000-ng range. The minimal detection level of each amine was 10 ng/g of tissue sample.

The levels of substrates and metabolites found in whole brain tissue after intraperitoneal injection of rats with equimolar quantities of each amine are given in Table I. Levels of I, II, and IV after administration of amphetamine (I) are comparable to values obtained in other studies (2, 4, 5, 19, 20). Rat whole brain levels of III and IV after intraperitoneal injection of norephedrine (III), of *p*-hydroxynorephedrine (IV) after its intraperitoneal injection, and of II and IV after intraperitoneal injection of *p*-hydroxyamphetamine (II) have apparently not been determined previously.

Rat whole brain levels of *p*-hydroxyamphetamine (II) (114.1 ng/g), resulting from the administration of amphetamine (I) were significantly higher ($p < 0.025$; Student's t test) than those obtained (74.9 ng/g) when II was injected directly. Thus, even if all the intraperitoneally injected I had been metabolized to II in the liver (and this had not occurred—see Table I), this would not account for the levels of II observed. One possible explanation of these is that *p*-hydroxylation of I to II occurs in the rat brain to an appreciable extent. Similar results were observed when norephedrine (III) was the substrate. Although 80.9 ng/g of *p*-hydroxynorephedrine (IV) was measured in brain tissue after injection of IV, this does not fully account for the significantly higher ($p < 0.01$) brain levels (131.7 ng/g) of IV detected after injection of III.

Attempts to show that norephedrine (III) was an *in vivo* rat brain metabolite of amphetamine (I) were unsuccessful. If any III was so formed, it was in an amount which was below the detection limit of the assay (10 ng/g). This failure to detect III in rat tissues after intraperitoneal injection of I agrees with previous reports (6, 9, 15). In contrast, Lewander (2) detected [^{14}C]III in rat brain after injection of [^{14}C]I, although the levels measured (<10 ng/g of tissue) were below the detection limits of most other analytical procedures. Kuhn *et al.* (9) found appreciable amounts of III in brain following the intracisternal injection of amphetamine (I).

In vitro studies using rat brain 10,000×*g* homogenate supernatant confirmed that both *p*- and β-hydroxylating enzymes are present in brain tissue (Table II). Metabolism of amphetamine (I) (1.0 μmol) produced trace amounts of II (0.744 nmol) and IV (0.498 nmol), but no norephedrine (III). The incubation of III (1.0 μmol) similarly produced IV (0.789 nmol); conversion of II (1.0 μmol) to IV (1.117 nmol) was also demonstrated. These metabolic conversions were not observed *in vitro* in the absence of brain homogenate supernatant.

CONCLUSIONS

A sensitive analytical procedure has been developed that permits extraction and analysis of amphetamine, norephedrine, and their *p*-hydroxylated metabolites from rat brain. The phenolic metabolites are derivatized by a combination of aqueous acetylation, specific basic hydrolysis of phenolic acetyl groups, and perfluoroacylation under anhydrous conditions. The procedure permits the analysis of *p*-hydroxyamphetamine and *p*-hydroxynorephedrine without interference from amphetamine or norephedrine; this is particularly important in the study reported here, since in the *in vitro* investigation concentrations of the parent compounds were many times higher than those of the metabolites. The results obtained with this procedure suggest that (*a*) both amphetamine (I) and norephedrine (III) are *p*-hydroxylated to some extent in rat brain, both *in vivo* and *in vitro*, (*b*) *p*-hydroxyamphetamine (II) undergoes intracerebral conversion to *p*-hydroxynorephedrine (IV), and (*c*) norephedrine (III) could not be detected as either an *in vivo* or an *in vitro* brain metabolite. Thus, the results of this preliminary study suggest that a β-hydroxylation of *p*-hydroxyamphetamine, but not of amphetamine, occurs in rat brain. Factors such as differential metabolism and elimination rates in brain and liver must be considered, and future comprehensive time studies in these two organs may yield additional information on the formation of amphetamine metabolites.

Table I—Rat Whole Brain Levels of Amphetamine (I), Norephedrine (III), *p*-Hydroxyamphetamine (II), and *p*-Hydroxynorephedrine (IV)

Substrate[a]	Metabolite, nmol/g of Brain Tissue[b] I	II	III	IV
I	67.05 ± 4.50 (3)	0.756 ± 0.093 (15)	ND[c] (3)	0.498 ± 0.076 (12)
II	—	0.496 ± 0.074 (13)	—	0.318 ± 0.097 (13)
III	—	—	41.17 ± 4.05 (3)	0.789 ± 0.097 (11)
IV	—	—	—	0.484 ± 0.056 (9)

[a] 74.0 μmol/kg (free base) ip. [b] Mean ± *SD*; number of experiments in parentheses. [c] ND—not detected.

Table II—*In Vitro* Rat Brain Metabolism of Amphetamine (I), *p*-Hydroxyamphetamine (II), and Norephedrine (III)

Substrate[a]	Metabolite, nmol/g of Brain Incubation[b] I	II	III	IV
I	*[c]	0.744 ± 0.121 (10)	ND[d] (5)	0.211 ± 0.050 (10)
II	—	*[c]	—	1.117 ± 0.121 (6)
III	—	—	*[c]	0.533 ± 0.091 (6)

[a] Substrate (1.0 μmol) added as free base. [b] Mean ± *SD*; number of experiments in parentheses. [c] Recovery of substrate not determined. [d] ND—not detected.

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Hypolipidemic Activity of 4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone in Rodents

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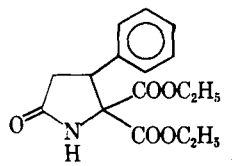
Abstract □ A series of pyrrolidinones have been reported to possess hypolipidemic activity in mice. The most active agent, 4-phenyl-5-5-dicarbethoxy-2-pyrrolidinone, effectively lowered both serum cholesterol and triglyceride levels at 20–30 mg/kg/d. The agent suppressed liver mitochondrial citrate exchange, phosphatidate phosphohydrolase, and *sn*-glycerol-3-phosphate acyl transferase activities. Lipid content of the liver, small intestine, and serum lipoprotein fractions was reduced by drug treatment, but lipid levels increased in the bile and fecal samples, suggesting the drug accelerated lipid excretion. The mode of action of the pyrrolidinone appears similar to that of the cyclic imides.

Keyphrases □ 4-Phenyl -5,5- dicarbethoxy -2- pyrrolidinone—hypolipidemic activity, rodents □ Hypolipidemic agents—potential, 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone, rodent screen

A series of substituted 2-pyrrolidinones have previously been examined for hypolipidemic activity in mice and have been observed to be active between 20–30 mg/kg/d. Similarly, types of moieties such as succinimide (1) also have hypolipidemic activity in the dosage range. One particular compound, 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone (2), demonstrated potent activity and lowered serum cholesterol and triglycerides >40% at 30 mg/kg/d after 16 d. The current study involves the mode of action of 4-phenyl-5,5-dicarbethoxy-2-pyrrolidinone in lowering lipid levels of the body.



4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone

EXPERIMENTAL

Antihyperlipidemic Screens in Normal Rodents—4-Phenyl-5,5-dicarbethoxy-2-pyrrolidinone was suspended in 1% aqueous carboxymethylcellulose and administered to male CF_1 mice (~25 g) intraperitoneally for 16 d or male Holtzman rats (~350 g) orally by an intubation needle for 14 d. On days 9 and 14 or 16, blood was obtained by tail vein bleeding, and the serum was separated by centrifugation for 3 min. The serum cholesterol levels were determined by a modification of the Liebermann–Burchard reaction (3). Serum was also collected on day 14 or 16, and the triglyceride content was determined using a commercial kit[1].

Testing in Atherogenic Mice—Male CF_1 mice (~25 g) were placed on a commercial diet[2] which contained butterfat (400 g), cellulose[3] (60 g), cholesterol (53 g), choline dihydrogen citrate (4 g), salt mixture oil[4] (40 g), sodium cholate (20 g), sucrose (223 g), vitamin-free casein (200 g), and total vitamin supplement for 10 d. After the cholesterol and triglyceride levels were assayed and observed to be elevated, the mice were administered the test drug at 20 mg/kg/d ip for an additional 12-d period. Serum cholesterol and triglyceride levels were measured after 12 d of drug administration.

Animal Weights and Food Intake—Periodic animal weights were obtained during the experiments and expressed as a percentage of the animals' weights on day 0. After dosing for 14 d with the test drug, selected organs were excised, trimmed of fat, and weighed.

Toxicity Studies—The acute toxicity (LD_{50} value) (4) was determined in male CF_1 mice by administering the test drug intraperitoneally from 100 mg to 2 g/kg as a single dose. The number of deaths were recorded over a 7-d period for each group.

Enzymatic Studies—*In vitro* enzymatic studies were determined using 10% homogenates of male CF_1 mouse liver with 50–200 μmol of the test drug. *In vivo* enzymatic studies were determined using 10% homogenates of liver obtained from male CF_1 mice after administering the agents for 16 d at a dose ranging from 10 to 60 mg/kg/d ip. The liver homogenates for both *in vitro* and *in vivo* studies were prepared in 0.25 M sucrose plus 0.001 M EDTA.

Acetyl CoA synthetase (5) and ATP-dependent citrate lyase (6) activities were determined spectrophotometrically at 540 nm as the hydroxamate of acetyl CoA formed after 30 min at 37°C. Mitochondrial citrate exchange was determined by the procedure of Robinson *et al.* (7, 8) using sodium [^{14}C]-bicarbonate (41 mCi/mmol) incorporated into mitochondrial [^{14}C]citrate after isolating rat mitochondria (9000×*g* for 10 min) from the homogenates. The exchanges of the [^{14}C]citrate were determined after incubating the mitochondrial fraction, which was loaded with labeled citrate and test drug for 10 min. The radioactivity was then measured in the mitochondrial and supernatant fractions in scintillation fluid[5] and expressed as a percentage. Cholesterol side-chain oxidation was determined by the method of Kritchevsky and Tepper (9) using [26-^{14}C]cholesterol (50 mCi/mmol) and mitochondria isolated from rat liver homogenates. After 18 h of incubation at 37°C with the test drug, the generated $^{14}CO_2$ was trapped in the center well in {2-[2-(*p* - 1,1,3,3-tetramethylbutylcresoxy)ethoxy]ethyl}dimethylbenzylammonium hydroxide[6] and counted[5]. 3-Hydroxy-3-methylglutaryl CoA reductase (HMG CoA reductase) activity was measured using [1-^{14}C]acetate (56 mCi/mmol) and a postmitochondrial supernatant (9000×*g* for 20 min) incubated for 60 min at 37°C (10). The digitonide derivative of cholesterol was isolated and counted (11). Acetyl CoA carboxylase activity was measured by the method of Greenspan and Lowenstein (12). Initially, the enzyme had to be polymerized for 30 min at 37°C and then the assay mixture containing sodium [^{14}C]bicarbonate (41.0 mCi/mmol) was added and incubated for 30 min at 37°C with the test drug. Fatty acid synthetase activity was determined by the method of Brady *et al.* (13) using [2-^{14}C]malonyl CoA (37.5 mCi/mmol), which was incorporated into newly synthesized fatty acids that were extracted

[1] Hycel Triglyceride Test Kit; Fisher.

[2] Basal atherogenic test diet; U.S. Biochemical Corp.
[3] Celufil.
[4] Wesson.
[5] Fisher Scintiverse in a Packard scintillation counter.
[6] Hyamine Hydroxide; New England Nuclear, Boston, Mass.

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