

0.1 M dithiothreitol, 17% glycerol]. Each sample was sonicated to reduce viscosity and heated to 90 °C for 5 min. Samples (7 μ L each) were analyzed by polyacrylamide gel electrophoresis, using 15% acrylamide gels. The gels were run overnight at 30 V, and fluorography was carried out with 2,5-diphenyloxazole-dimethyl sulfoxide (20% wt/wt). The dried gels were exposed with use of XS-5 X-ray films (Kodak). Densitometric profiles of the films were performed in an Optronics P 1700 microdensitometer.

Assay of Infections Units. HeLa cells were infected with HSV-1 at a multiplicity of 0.5 pfu/cell in the presence of several concentrations of compound 13. Forty-eight-hour postinfection cells were collected and lysed by sonication with a MSE sonicator. Serial dilutions were made in saline phosphate buffer containing

1% calf serum. Vero cell monolayers were infected with 0.5 mL of each virus dilution. After 1 h at 37 °C, the cells were overloaded with DMEM containing 0.7% agar and 1% calf serum and incubated at 37 °C. After 4 days the medium was removed and 0.5 mL of 5% trichloroacetic acid was added over the monolayer, and plaques were counted.

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Metabolism of Phencyclidine. The Role of the Carbinolamine Intermediate in the Formation of Lactam and Amino Acid Metabolites of Nitrogen Heterocycles

John K. Baker* and Thomas L. Little

Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, Mississippi 38677.
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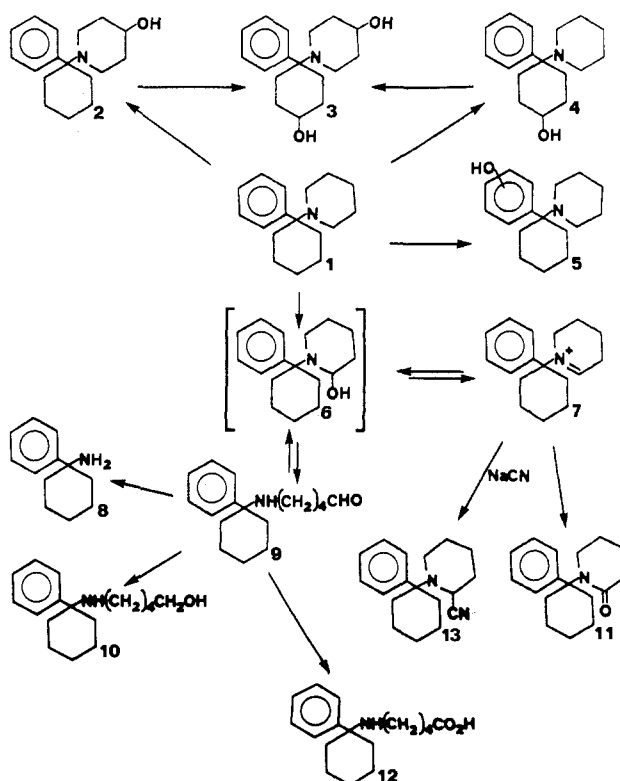
The transformation of phencyclidine in a mouse liver microsome preparation to several oxidative metabolites was studied. With use of GLC and HPLC methods with internal standards, phencyclidine and six metabolites were quantitated and the amino acid 12, resulting from the α -oxidation of the piperidine ring, was produced in 10-50 times greater amounts than the other metabolites. While most piperidines and pyrrolidines produce an amino acid and a corresponding lactam, it was found that phencyclidine was not converted to the lactam 11.

The metabolism of phencyclidine (1) has been of considerable interest because some of the delayed-onset effects or biphasic effects of the drug have been attributed to metabolites (of unknown structure) that may have a pharmacological profile very distinct from that of phencyclidine itself. In man, acute intoxication is characterized by hypotension, but the blood pressure may rise above normal 2 or 3 days later.¹ A schizophreniform condition appears in a small number of the subjects and the psychosis may persist for several weeks.²⁻⁵

Previous studies with several species including man have shown that phencyclidine is metabolized to small quantities of *cis*- and *trans*-4-phenyl-4-piperidinocyclohexanol (4)^{6,7} and 1-(1-phenylcyclohexyl)-4-hydroxypiperidine (2),⁶ and the structures of these metabolites were verified by comparisons to synthetic standards. GC-mass spectral data have been utilized to tentatively identify the dihydroxy metabolite 3 and the phenol 5.⁸

The major metabolic pathway for phencyclidine appears to be through oxidation of the α -carbon of the piperidine ring. The major metabolite of phencyclidine was first detected in dogs and found to be 5-[N-(1-phenylcyclohexyl)amino]pentanoic acid (12).⁹ This amino acid metabolite has also been detected in the urine of humans,¹⁰

as well. Other metabolites arising from the α -oxidation route that have been detected are the amine⁸ (8) and the amino alcohol¹¹ (10) metabolites.

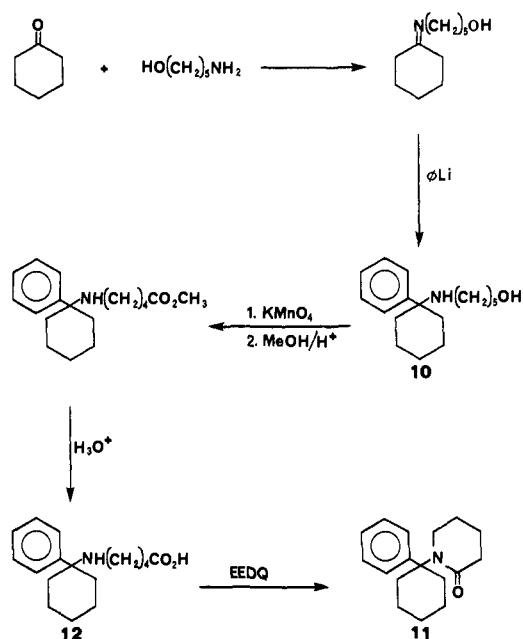


Most of the studies in the literature on the metabolism of piperidines and pyrrolidines have shown that this class of compounds undergo α -oxidation leading to the formation of a lactam and the corresponding amino acid. For

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Scheme I



example, the pyrrolidine ring of nicotine is metabolized to a mixture of cotinine and the corresponding amino acid¹² and the piperidine ring of diphenidol is also converted to a lactam and open-chain amino acid.¹³ Similar transformations have been reported for tremorine,¹⁴ phenmetrazine,¹⁵ and nitrimidazole.¹⁶

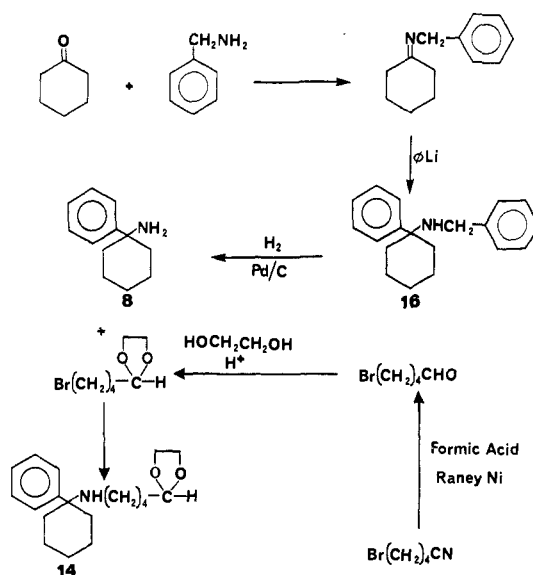
Since the amino acid 12 is the major known metabolite of phencyclidine, it could reasonably be expected that the lactam 11 would also be a metabolite of phencyclidine. The objectives of the present study were to prepare reference standards of 11 and 9 and to further investigate the role of the carbinolamine intermediate in the transformations of phencyclidine.

Results and Discussion

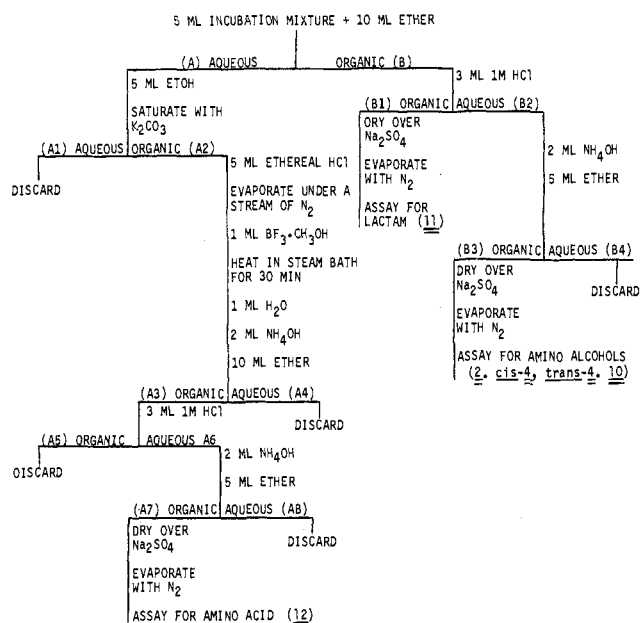
Chemistry. The amino acid metabolite 12 and the amino alcohol metabolite 10 were prepared by using a slightly modified version of a previously published procedure⁹ (Scheme I). Conversion of 12 to 11 did not go as easily as expected. Refluxing the methyl ester of 12 in toluene, heating 12 at 200 °C for 48 h in a stainless steel bomb, and the use of sodium hydride all failed to produce 11. Dicyclohexylcarbodiimide with 12 gave the desired product in 10% yield, but the highest yield (67%) was obtained with *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ).

Though it was recognized that the purported phencyclidine metabolite 9 would be extremely unstable, it was hoped that it might be generated from some stable precursor immediately before use in the metabolism studies. Numerous oxidative procedures using 10 as the precursor were attempted and found to be unsatisfactory. The procedure of Ito et al.¹⁷ for reducing amino esters to the

Scheme II



Scheme III



corresponding amino aldehydes using diisobutylaluminum hydride was attempted for the conversion of the methyl ester of 12 to 9 and found to be unsuccessful.

The stable acetal derivative of the amino aldehyde 14 was prepared as shown in Scheme II. The product was obtained with a 39% yield and the crystalline hydrochloride salt of 14 was found to be quite stable. However it was found that 14 in aqueous 0.5 N HCl at room temperature was completely hydrolyzed in 1 h as followed by HPLC analysis. The aldehyde 9 produced from the hydrolysis of the acetal was stable for at least 3 h under acidic conditions. However, when the solution was adjusted to the pH 7–8 range, the aldehyde 9 disappeared with a half-life of ~45 min and seven distinct decomposition products appeared. Using the same reversed-phase HPLC system that was also utilized for the metabolism studies, the aldehyde 9 was found to have a retention time of 7.9 min. Using the same HPLC system, 14 was observed at 9.0 min and 10 at 6.1 min. From measurements of the absorbance ratio at 254 and 280 nm using dual HPLC detectors, the chromophores for 9, 10, and 14 appeared to be nearly identical while the chromophores for many of

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Table I. Levels of in Vitro Metabolites of Phencyclidine^a

time, h	lactam 11	amino acid 12, μg	alcohol 10, μg	2, μg	<i>cis</i> -4, μg	<i>trans</i> -4, μg
0.0	ND ^b	ND ^c	ND ^d	ND ^d	ND ^d	ND ^d
1.0	ND	110	2.8	9.3	6.7	3.1
2.0	ND	123	2.4	9.2	7.7	3.6
3.0	ND	135	1.4	10.3	8.0	5.1
3.0 ^e	ND	ND	ND	ND	ND	ND

^a Five micromoles of substrate (1215 μg)/incubation vessel. ^b Not detected (less than 0.42 μg /vessel). ^c Not detected (less than 8 μg /vessel). ^d Not detected (less than 2 μg /vessel). ^e Heat-inactivated control.

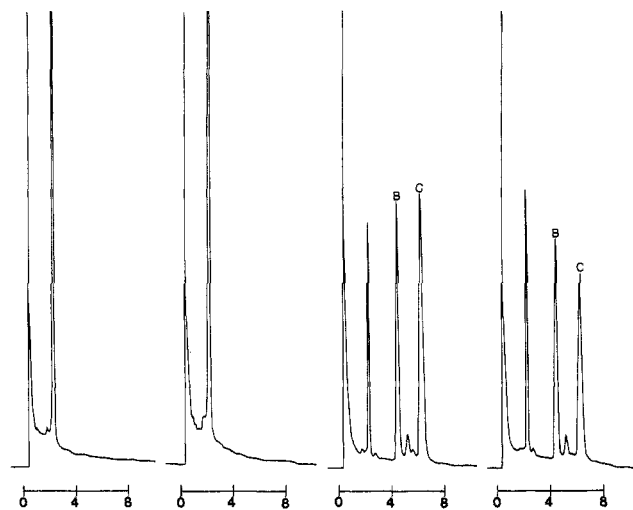


Figure 1. GLC analysis of the amino acid metabolite. First panel, heat-inactivated control; second panel, time-zero negative control; third panel, positive control (B, *N*-benzylphenylalanine; C, 144 μg of 12); fourth panel, 1-h test sample.

the seven decomposition products of 9 were markedly different.

Analysis of Phencyclidine Metabolites. When the incubations were complete, the two internal standards, ketamine and *N*-benzylphenylalanine, were added to each of the 5-mL incubation mixtures and then processed according to Scheme III. In the first extraction step at pH 7.4, the zwitterionic 12 and its internal standard remained in the aqueous phase. The aqueous phase was then mixed with an equal volume of ethanol and made biphasic by the addition of K_2CO_3 . Following the subsequent extraction and derivatization steps, 12 and its internal standard were chromatographed by using a nitrogen detector–GLC system (Figure 1). The assay was calibrated by adding five different quantities of 12 and the internal standard to the complete microsomal incubation mixture, immediately quenching the reaction by placing in an ice bath, then measuring the relative peak heights of derivatized 12 and *N*-benzylphenylalanine. The peak-height ratios were found to show very good correlation ($r = 0.99$) with the amount of 12 present. The heat-inactivated microsomal controls (Figure 1, first panel) showed that both peaks were free from any interferences. The assay for the lactam 11 was also calibrated by spiking a microsomal blank and then chromatographing using the nitrogen detector–GLC system (Figure 2). In this matrix, concentrations as small as 84 ng/mL of 11 could be detected.

HPLC analysis using ketamine as the internal standard was used to quantitate 1, 2, *cis*-4, *trans*-4, and 10. The method was calibrated by spiking the microsomal preparation mixtures with known quantities of the metabolites and correlating these with the peak-height ratios to the internal standard (ketamine). The time-zero controls and the heat-inactivated controls were found to be free of interfering peaks and calibration curves were found to give good correlation coefficients (*cis*-4, $r = 0.99$; 2, $r = 0.91$;

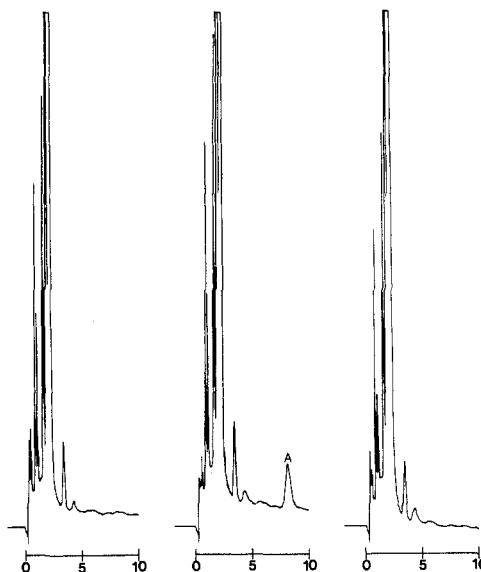


Figure 2. GLC analysis of the lactam metabolite. First panel, time-zero control; second panel, positive control ($A = 1.27 \mu\text{g}$ of 11); third panel, 3-h test sample.

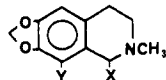
10, $r = 0.96$). Since a pure sample of *trans*-4 was not available, its detector response was assumed to be the same as that of *cis*-4.

Microsomal Metabolism Studies. It was found that the amino acid metabolite 12 was formed rapidly and was found in 10–50 times higher concentrations than any other metabolite (Table I). The yields of 2, *cis*-4, and *trans*-4 were similar to that reported in previous studies, except that in the present in vitro study using mice liver microsomes, there was a slight preference for the *cis* isomer. Previous in vivo studies on 4 have reported *cis*/*trans* ratios of 1:1.4 for man¹⁰ and 1:1.5–4.0 for dogs,⁷ while in vitro studies with mice¹⁸ have shown the *cis* isomer to predominate.

Only very small quantities of 10 were detected, and this compound has been previously reported¹ to be the result of an enzymatic reduction of the aldehyde 9. In both the present study and the previous study, 10 was formed in small quantities and it appeared to be further metabolized (presumably to 12). It appeared that the compound could very likely be an artifact resulting from the simple chemical decomposition of 6 or 9 to 10 during the sample workup. As discussed in the chemistry section of this report, when synthetic 9 was adjusted to pH 7–8, direct HPLC analysis showed that it was rapidly ($t_{1/2} = 45 \text{ min}$) converted to seven components, one of which had a retention time identical with that of the alcohol 10. Hydrastinine and cotarine are also carbinolamines, but these compounds are stable enough to isolate. Both of these compounds also undergo a disproportionation reaction to yield a mixture of the corresponding alcohol and carboxylic acid.^{19,20} Thus

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it appears that the small, transient amount of the alcohol 10 that was detected in the microsomal incubations of phencyclidine might have been an artifact resulting from a disproportionation reaction during the processing of the sample under alkaline conditions. In the previous report¹¹ where a pH 9.5 buffer was used in the sample extraction, 10 was obtained in a 15% yield; while in the present study where a pH 7.4 buffer was used, the yield of 10 dropped to only 0.2%.



hydrastinine, X = OH, Y = H
15, X = CN, Y = H
cotarine, X = OH, Y = OCH₃

While most of the pyrrolidines and piperidines that have been studied were transformed to both the lactam and corresponding amino acid, the present study showed that phencyclidine was transformed to the amino acid 12 in high yield, but none of the lactam 11 was detected (Table I). It was also found that when synthetic 11 was incubated in the same microsomal preparation, there was no transformation to the amino acid. However, following a 3-h incubation, 11 was converted in ~20% yield to an unknown compound having a GLC retention time of 0.81 relative to 11. Thus, it appeared that 12 resulted from the enzymatic oxidation of 9 and that the sequence 6 to 7 to 11 to 12 did not contribute to all.

When phencyclidine was incubated in a microsomal system in the presence of KCN, it has been shown that 13 is formed and some of the phencyclidine is covalently bonded to protein.²¹ These observations have been interpreted as indicating the existence of the iminium ion 7. Since the iminium ion is also believed to be the actual substrate leading to the formation of lactams,²² one could conclude that phencyclidine is transformed to 7, but the iminium ion is not enzymatically oxidized to 11. This would seem plausible because the phenyl and cyclohexyl rings in 7 would form a very bulky "wing-like" structure which would prevent the substrate from properly interacting with the enzyme surface.

Though the above explanation appeared to be the most likely, it also might have been possible that 11 was not formed because 7 did not exist. It has been shown that the carbinolamine hydrastinine, under solvent conditions that would preclude the formation of the iminium ion, HCN displaced the hydroxyl group by a S_N2 reaction to form 15.^{19,20} By analogy, it is at least remotely possible that 13 might have come directly from 6, and 11 was not formed because 7 was not available as a substrate for the "aldehyde oxidase".²²

In general, it would appear that pyrrolidines and piperidines can be converted to both amino acid metabolites and lactam metabolites, but the ratio of the two products is dependent on the steric and electronic properties of the substrate substituents. The mixed-function oxidase converts these substrates to a carbinolamine which can be further oxidized to the lactam by aldehyde oxidase or it can spontaneously open up to the open-chain amino aldehyde which is then oxidized to the amino acid by al-

dehyde dehydrogenase. For most simple pyrrolidines and piperidines with a fairly basic nitrogen, the carbinolamine is too unstable to isolate. However, in the case of piromidic acid²³ and medazepam,²⁴ which have an aromatic substituent on the nitrogen, the carbinolamine intermediate is often stable enough to isolate. Medozepam is converted to a mixture of the lactam and open-chain metabolites; while piromidic acid and phencyclidine, which have very large bulky groups on the nitrogen, are converted *only* to the open-chain amino acid.

Experimental Section

Chemistry. ¹H NMR spectra were obtained on a Varian Model EM390 (90 MHz) spectrometer and ¹³C NMR spectra on a JEOL JNM FX-60 (15.03 MHz) Fourier transform spectrometer. Infrared spectra were obtained on a Perkin-Elmer Model 281B spectrometer and electron-impact (70eV) mass spectra were obtained on a Finnigan 3200 MS/DS system. Melting points were on a Mel-Temp apparatus and were uncorrected.

1-(1-Phenylcyclohexyl)-2-piperidone (11). By using a previously published procedure,⁹ metabolite 10 was prepared in 47% yield from cyclohexanone (Scheme I). The product was then converted to the hydrochloride salt (mp 189–190 °C from EtOH/ether). Then 10 was oxidized with 15% aqueous KMnO₄, converted to the methyl ester to aid in purification, and then hydrolyzed to give (47% yield) metabolite 12 [mp 213–214 °C (lit.⁹ mp 214–215 °C)]. A solution of 1 g (3.2 mmol) of the HCl salt of 12, 1 equiv of triethylamine, and 50 mL of MeOH was stirred at room temperature for 30 min. The excess MeOH was removed in vacuo and the residue was dissolved in 1 L of THF. After the solution was stirred for 5 min, 841 mg (3.4 mmol) of *N*-(ethoxycarbonyl)-2-ethoxy-1,2-dihydroquinoline (EEDQ) was added and this mixture was stirred at room temperature for 72 h. The excess THF was removed in vacuo and the residue was placed under a high vacuum (0.15 mm) for 12 h to remove the quinoline byproduct. The product was purified by using flash chromatography (silica gel) with CHCl₃/EtOAc (95:5) as the mobile phase. The fractions containing the product were combined and concentrated in vacuo. Recrystallization from MeOH/H₂O gave 560 mg (67%) of white crystals: mp 81–82 °C; ¹H NMR (CDCl₃) δ 1.3–2.0 (m, 12), 2.42 (t, 2, CH₂C=O), 2.7–3.0 (m, 2), 3.2 (t, 2, CH₂N), 7.1–7.5 (m, 5, Ar H); IR (KBr) 1622 (C=O), 751, 700 cm⁻¹ (phenyl); ¹³C NMR (CDCl₃) δ 172.6 (C=O, s), 146.6 (1-phenyl, s), 128.3 (phenyl, d), 126.5 (phenyl, d), 126.2 (phenyl, d), 66.1 (1-cyclohexyl, s), 44.8 (t), 36.7 (t), 35.1 (t), 26.0 (t), 24.0 (t), 23.5 (t), 20.5 (t); mass spectrum (70 eV), *m/e* (relative intensity) 257 (M⁺, 0.52), 200 (11.8), 158 (84.9), 143 (31.5), 130 (39.1), 115 (20.8), 100 (100), 91 (74.2). Anal. (C₁₇H₂₃NO) C, H, N.

5-[*N*-(1-Phenylcyclohexyl)amino]valeraldehyde Ethylene Acetal (14, Scheme II). By use of the general procedure of Maddox et al.,²⁵ cyclohexanone and benzylamine were refluxed in anhydrous benzene with a Dean-Stark trap for 8 h, treated with freshly prepared phenyllithium for 1 h, and then chromatographed to give a 32% yield of 16 [16·HCl, mp 231–232 °C (lit.²⁵ mp 226–227 °C)]. Then 4 g of 16·HCl and 25 mL of anhydrous EtOH (4 drops of concentrated HCl) was hydrogenated at 50 °C (50 psi) with 1 g of 10% Pd-C for 24 h to give near-quantitative conversion to metabolite 8 [8·HCl, mp 250–251 °C (lit.²⁵ mp 247–248 °C)].

For the preparation of the bromo acetal intermediate, 5 g (30.9 mmol) of 5-bromovaleronitrile, 5 g of Raney nickel, and 75 mL of 75% formic acid was heated at 65 °C for 6 h and cooled, and then 50 mL of H₂O was added. The solution was then extracted with CHCl₃ and then chromatographed (silica gel, benzene) to give 3.3 g (65%) of the bromo aldehyde: ¹H NMR (CDCl₃) δ 1.65–2.05 (m, 4, CH₂CH₂), 2.5 (t, 2, CH₂CHO), 3.42 (t, 2, CH₂Br), 9.8 (s, 1, CHO). The aldehyde (18.2 mmol), ethylene glycol (36.4

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mmol), a few crystals of *p*-toluenesulfonic acid, and 75 mL of anhyd benzene were heated under reflux for 8 h with a Dean-Stark trap. The mixture was washed with 10% NaHCO₃, dried, and then concentrated in vacuo. Purification using flash chromatography (silica gel, benzene) gave 3.1 g (81%) of 5-bromovaleraldehyde ethylene acetal: ¹H NMR (CDCl₃) δ 1.45–2.1 (m, 6), 3.4 (t, 2, CH₂Br), 3.7–4.1 (m, 4, OCH₂CH₂O), 4.84 (t, 1, OCHO).

A solution of 900 mg (5.14 mmol) of 8, 2.15 g (10.3 mmol) of 5-bromovaleraldehyde ethylene acetal, 1.03 g (10.3 mmol) of anhydrous CaCO₃, and 50 mL of DMF was stirred for 36 h at room temperature. The DMF was then removed under high vacuum (0.15 mm) at room temperature, the residue diluted with 100 mL of diethyl ether, and filtered and then the filtrate evaporated in vacuo, leaving a pale yellow oil. The product was purified by using flash chromatography (silica gel; EtOAc:NH₄OH, 10 mL:1 drop) to give 600 mg (39%) of a clear oil. The product was dissolved in 25 mL of anhydrous diethyl ether and then placed in a desiccator that also contained 1 mL of concentrated HCl in a separate beaker. The 14-HCl crystal were collected and recrystallized (EtOH/ether) to give the final product as white crystals: mp 191–192.5 °C; IR (KBr) 1579 cm⁻¹ (phenyl), 769, 707 cm⁻¹ (phenyl); ¹H NMR (HCl salt, CDCl₃) δ 1.15–3.0 (m, 18), 3.75–4.0 (m, 4, OCH₂CH₂O) 4.75 (t, 1, RO₂CH), 7.35–7.9 (m, 5, Ar H), 9.3–9.8 (br s, 2, NH₂⁺); ¹³C NMR (free base, CDCl₃) δ 147.6 (phenyl, s), 128.1 (phenyl, d), 126.2 (phenyl, d), 126.0 (phenyl, d), 104.6 (CO₂, d), 64.8 (ethylene, t), 57.2 (1-cyclohexyl, s) 41.5 (t), 36.3 (t), 33.8 (t), 30.8 (t), 26.0 (t), 22.2 (t), 21.9 (t); mass spectrum (70 eV), *m/e* (relative intensity) 303 (M⁺, 2.3), 260 (74.5), 160 (70), 117 (47), 91 (100). Anal. (C₁₉H₃₀NO₂Cl) C, H, N.

***N*-Benzylphenylalanine.** By use of a procedure similar to that of Kanao,^{26,27} *L*-phenylalanine was converted to the methyl ester by refluxing with H₂SO₄ in anhydrous MeOH. A solution of 900 mg (5.03 mmol) of the methyl ester, 1.39 g (10.6 mmol) of anhydrous K₂CO₃, 1.29 g (7.55 mmol) of benzyl bromide, and 50 mL of acetone was stirred for 8 h at reflux. After cooling, the mixture was concentrated in vacuo and the residue dissolved in 150 mL of diethyl ether and then extracted with 100 mL of 10% HCl. The aqueous layer was then back-extracted into ether and then dried with Na₂SO₄. The ether extract was then treated with ethereal HCl to give 1.0 g (65%) of the HCl salt of *N*-benzylphenylalanine methyl ester: mp 151–152 °C. A solution of 500 mg of the HCl salt, 4 drops of HCl, and 25 mL of H₂O was refluxed for 24 h and concentrated in vacuo to give a quantitative conversion to *N*-benzylphenylalanine hydrochloride: mp 225–226 °C (free base mp 229–231 °C, lit.²⁶ mp 234 °C); IR (KBr) 1723 (C=O) 744, 694 cm⁻¹ (phenyl); ¹H NMR (HCl salt, Me₂SO-*d*₆) δ 3.05–3.67 (m, 2, Ar CH₂CH), 4.05–4.22 (m, 3, CHCO₂ and CH₂NH), 7.4–7.8 (m, 10, Ar H).

Analytical Procedures. A 5.0-mL sample of the microsome incubation mixture was mixed with two internal standards [500 nmol of *N*-benzylphenylalanine hydrochloride (146 μg) and 100 nmol of ketamine hydrochloride (27 μg)] and then processed according to Scheme III. For calibration of the method, varying quantities of 11, 12, 2,²⁸ *cis*-4,²⁸ and 10 were added to a blank microsome incubation mixture and then processed in the same manner as the test samples. The 5.0-mL microsome samples were extracted with 10 mL of diethyl ether, then transferred to a 20-mL screw-top centrifuge tube, and stored at -4 °C until further use (Fr-B). The aqueous layer (Fr-A) was mixed with 5 mL of EtOH, saturated with anhydrous K₂CO₃, and then centrifuged. The top EtOH layer (Fr-A2) was then transferred to a 20-mL centrifuge tube and 5 mL of ethereal HCl was slowly added. After filtering through a glass wool plug, the solvent was removed at slightly above room temperature with a stream of N₂, then 1.0 mL of

BF₃·MeOH was added, and the mixture was heated on a steam bath for 30 min. After the mixture cooled, 1 mL of H₂O was added, and 5 min later 2 mL of concentrated NH₄OH was added and then extracted with 10 mL of diethyl ether. The ether layer (Fr-A3) was transferred to a centrifuge tube containing 3 mL of 1.0 M HCl and then shaken for 5 min. The ether layer was discarded, and then the aqueous layer (Fr-A6) was mixed with 2 mL of concentrated NH₄OH and then extracted with 5 mL of diethyl ether. The ether layer (Fr-A7) was passed through a funnel containing 2 g of anhydrous Na₂SO₄ and then evaporated (low heat) under a stream of N₂. The residue was dissolved in 25 μL of ethyl acetate for GLC analysis as the methyl ester of 12.

Fraction B was mixed with 3 mL of 1.0 M HCl, shaken for 5 min, and centrifuged, and then the aqueous layer (Fr-B2) was removed and stored at -4 °C until later use. The ether layer (Fr-B1) was passed through 2 g of anhydrous Na₂SO₄ and then evaporated under a stream of N₂. The residue was dissolved in 25 μL of ethyl acetate for the GLC analysis of 11.

Fraction B₂ was shaken for 5 min with 2 mL of concentrated NH₄OH and 5 mL of diethyl ether and then centrifuged. The ether layer (Fr-B3) was passed through 2 g of anhydrous Na₂SO₄ and then evaporated (low heat) under a stream of N₂. The residue was dissolved in 25 μL of MeOH for the HPLC analysis of 2, *cis*-4, *trans*-4, and 10.

The GLC analysis of the residues were conducted with a Perkin-Elmer Model 900 chromatograph using a single 2 mm × 183 cm glass column whose output was equally split between a nitrogen selective detector and a flame ionization detector. The column was packed with 2% OV-17 on 100–120-mesh Gas Chrom Q and operated at 190 °C for the determination of 12 and at 195 °C for the determination of 11. Helium (30 mL/min) was used as the carrier gas and the nitrogen detector was operated at 6.8 ma.

HPLC analysis was conducted with a Waters Model 6000A pump (1.5 mL/min), a μ-Bondapak C-18 reversed-phase column (10 μM), and a Waters Model 440 dual detector system operating at 254 and 280 nm. The mobile phase was prepared with 1.4 L of MeOH, 2.6 L of H₂O, 6.6 g of K₂HPO₄, 8.4 g of KH₂PO₄, and 4 g of *N,N*-dimethyl-*n*-octylamine.

Microsome Preparations. The livers of 10 male ICR mice were homogenized in a Potter-Elvehjem tube with ice-cooled, pH 7.4, 0.05 Tris buffer containing 0.15 M KCl (3 mL of buffer/g of liver). The homogenates were centrifuged at 12500g, 4 °C, for 10 min. The supernatant was removed and stored at -70 °C for later use (within 15 h). Incubations were performed in 25-mL beakers containing a glass marble at 120 osc/min at 37 °C under a 100% O₂ atmosphere. Each beaker contained 3 mL of cofactor (0.64 mg/mL of NADP·4.5H₂O, 2.3 mg/mL of glucose 6-phosphate, 0.81 mg/mL of nicotinamide, 2.1 mg/mL of M₉SO₄·7H₂O in Tris buffer), 1 mL of 12500g supernatant, and 1 mL of substrate in Tris buffer.

In addition to the test samples, two types of controls were also used. In one control, the reaction was stopped immediately after the addition of the substrate. In the second type of control, the 12500g supernatant was heat inactivated before the substrate, and cofactors were added. The latter control was incubated and assayed along with the test samples.

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Registry No. 1, 77-10-1; 2, 60232-85-1; *trans*-4, 78165-07-8; *cis*-4, 78165-06-7; 8-HCl, 1934-71-0; 10, 77160-82-8; 10-HCl, 93185-16-1; 11, 93185-13-8; 12, 77160-83-9; 12-HCl, 85089-77-6; 14, 93185-14-9; 14-HCl, 93185-15-0; 16-HCl, 1934-58-3; cyclohexanone, 108-94-1; benzylamine, 100-46-9; phenyllithium, 591-51-5; 5-bromovaleronitrile, 5414-21-1; 5-bromovaleraldehyde, 1191-30-6; 5-bromovaleraldehyde ethylene acetal, 87227-41-6; *N*-benzylphenylalanine, 19461-04-2; *N*-benzylphenylalanine methyl ester, 2577-90-4; benzyl bromide, 100-39-0; *N*-benzylphenylalanine methyl ester hydrochloride, 7703-09-5; *N*-benzylphenylalanine hydrochloride, 23514-03-6.

(26) Kanao, S. *J. Pharm. Soc. Jpn.* 1946, 66, 4.

(27) Though presently discontinued, this material which is needed as the internal standard is available in limited quantities from Vega Biochemicals, Tucson, AZ.

(28) Reference samples of 2, *cis*-4, and a mixture of *cis*- and *trans*-4 were obtained from the National Institute on Drug Abuse through F. I. Carroll at the Research Triangle Institute.