the remaining residues (0.69 mmol) by standard solid-phase procedure,[51-53] resulted in Boc-Asp(OBzl)-Arg(NO_2)-Val-Tyr-(Bzl)-DL-Hfv-His(Tos)-Pro-Phe-resin, a hydrolysate of which showed Asp (0.25), Arg (0.30), Val (0.36), Tyr (0.35), Hfv (0.44), His (0.83), Pro (1.04), Phe (1.00). Treatment of this peptide-resin (600 mg) with liquid HF (10 mL) in the presence of anisole (1 g, 10 mmol) at 0 °C for 20 min gave 160 mg of crude peptide, which was separated into two fractions of K values of 0.27 and 0.47 by countercurrent distribution in 1-butanol–acetic acid–water (4:1:5) for 200 passages. Removal of the solvent followed by lyophilization resulted in 10.6 mg of peptide of K = 0.27 and 17.8 mg of peptide of K = 0.47. Further purification of the respective peptides by gel chromatography on Sephadex G25-120 (2 × 100 cm) in 1% AcOH gave 5 mg of the L diastereomer (**7**, K = 0.27) and 13 mg of the D diastereomer (**8**, K = 0.47). Amino acid analysis of a hydrolysate gave Asp (0.99), Arg (1.00), Val (0.96), Tyr (1.00), Hfv (0.98), His (1.09), Pro (1.20), and Phe (1.00) for compound **7** [R_f (iii) 0.26, R_f (iv) 0.49] and Asp (1.05), Arg (0.97), Val (1.00), Tyr (1.03), Hfv (1.07), His (0.98), Pro (1.10), and Phe (1.02) for compound **8** [R_f (iii) 0.27, R_f (iv) 0.57]. In the rat uterus assay, compound **7** was 133% as active as angiotensin II, while compound **8** had less than 1% activity.

Ac-Asn-Arg-Val-Tyr-Val-His-Pro (**9**) was prepared by standard solid-phase procedure, followed by sequential deprotection of the Ac-Asn-Arg(Tos)-Val-Tyr(Bzl)-Val-His(Tos)-Pro-resin with HBr/TFA and liquid HF. Purification of the peptide by countercurrent distribution in 8:1:2:9 of 1-butanol–pyridine–acetic acid–water for 1200 passages, followed by gel filtation of the appropriate fraction (K = 0.38) on Sephadex G25-120 (2 × 100 cm) in 10% AcOH, gave the peptide **9** in 36% yield, R_f (v) 0.17. Amino acid analysis of a hydrolysate gave Asp (1.10), Arg (0.99), Val (1.03), Tyr (0.92), Val (1.03), His (1.01), and Pro (0.92).

Ac-Asn-Arg-Val-Tyr-Val-His-Pro-DL-Hfv-OEt (**10**) was prepared from the heptapeptide **9** (100 mg, 0.12 mmol) and DL-Hfv-OEt (**2**; 63 mg, 0.25 mmol) by DCC (51 mg, 0.25 mmol) coupling in the presence of HOBt (19 mg, 0.12 mmol) in 5 mL of redistilled dimethylformamide (DMF) at 60 °C for 20 h. The mixture was evaporated to dryness in vacuo, and the residue was washed with chloroform, ethyl acetate, and ether and chromatographed on a vacuum-packed dry column (1 × 12 cm) of silica gel (CC-7, neutral, 100–200 μm, from Taylor Chemical Co., St. Louis, MO), which was prewashed with the eluting solvent and dried in vacuo at room temperature overnight. The dry column was eluted with the upper phase of the solvent mixture of 1-butanol–pyridine–acetic acid–water (8:1:2:9) at 12 mL/h, and 2-min fractions were collected and analyzed by TLC. The appropriate fractions (8 to 22 mL) were combined, evaporated to dryness, and precipitated from elution solvent–dioxane–ether (1:1:40) to give 55 mg. Further purification by ion-exchange chromatography on CM-Sephadex C-25-120 (2 × 100 cm) eluted with 0.5 M ammonium acetate in 10% acetic acid gave 45 mg (39%) of peptide **10**, R_f (v) 0.32. Amino acid analysis of a hydrolysate gave Asp (1.21), Arg (1.03), Val (0.97), Tyr (0.98), Val (0.97), His (0.90), Pro (0.97), and Hfv (0.93).

Ac-Asn-Arg-Val-Tyr-Val-His-Pro-DL-Hfv-OBzl (**11**) was prepared from **9** (100 mg, 0.12 mmol) and DL-Hfv-OBzl·HCl (**5**; 88 mg, 0.25 mmol) by DCC/HOBt coupling in DMF at room temperature for 3 h and at 60 °C for 3 h. The mixture was evaporated to dryness in vacuo, and the residue was washed with chloroform, ethyl acetate, and ether and chromatographed on a vacuum-packed dry column (1 × 20 cm) of silica gel as described for **10**. Isolation of the peptide, followed by precipitation from dimethylformamide–ether, gave 75 mg (61%) of **11**, R_f (v) 0.25. Amino acid analysis of a hydrolysate gave Asp (1.13), Arg (1.08), Val (0.93), Tyr (1.01), Val (0.93), His (0.92), Pro (1.10), and Hfv (0.95).

Ac-Asn-Arg-Val-Tyr-Val-His-Pro-DL-Hfv (**12**) was prepared by catalytic hydrogenation of peptide **11** (69 mg) in DMF (5 mL) with 10% Pd/C under 1 atm of H_2 for 2 h. The mixture was evaporated to dryness in vacuo, and the residue including the Pd catalyst was chromatographed on a vacuum-packed dry column (1 × 20 cm) of silica gel as described for **10**. Isolation of the peptide, followed by precipitation from dimethylformamide–ether, gave 54 mg (85%) of **12**, R_f (v) 0.10. Amino acid analysis of a hydrolysate gave Asp (1.14), Arg (1.02), Val (1.00), Tyr (0.97), Val (1.00), His (0.80), Pro (1.02), and Hfv (0.92).

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Phencyclidine Metabolism: Resolution, Structure, and Biological Activity of the Isomers of the Hydroxy Metabolite, 4-Phenyl-4-(1-piperidinyl)cyclohexanol

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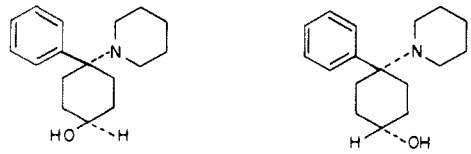
One of the major biotransformation pathways in the metabolism of phencyclidine is hydroxylation at C-4 of the cyclohexane ring to give 4-phenyl-4-(1-piperidinyl)cyclohexanol (**1**). Since the latter compound can exist as cis and trans isomers and the synthetic mixture has been reported to be biologically active, it was of interest to separate the isomers, test them for biological activity, and determine their ratio as metabolic products of phencyclidine. The synthetic mixture of **1** was separated by TLC and the individual isomers were characterized by ^{13}C and ^1H NMR and MS analyses. Preliminary testing of the isomers in the mouse rotarod assay indicates that the trans isomer (**1b**) is only slightly more active then the cis isomer (**1a**). Both isomers produced seizure activity and lethality at doses required to produce maximal ataxia.

Essential to the therapeutic management of drug overdosage is an understanding of the factors involved in the distribution and elimination of the active drug species. Metabolism may be an important consideration, particularly if the biotransformation products are active. The metabolites of 1-(1-phenylcyclohexyl)piperidine (phency-



1a (cis) 1b (trans)

Figure 1. Isomers of 4-phenyl-4-(1-piperidinyl)cyclohexanol. The cis and trans isomers are defined by the relative position of the phenyl and hydroxyl groups in relation to the plane of the cyclohexane ring.

clidine, PCP) generally have been assumed to be pharmacologically inactive;[1-5] however, two monohydroxy metabolites recently were reported to be active in the mouse rotarod assay[6] and the chronic spinal dog preparation.[7] These metabolites, 1-(1-phenylcyclohexyl)-4-piperidinol[8a] and 4-phenyl-4-(1-piperidinyl)cyclohexanol (**1**),[8b] were identified in the urine of man, rat, dog, and mouse following phencyclidine administration.[6,9,10] None of the analytical methods employed, however, were able to distinguish *r*-4-phenyl-4-(1-piperidinyl)-*c*-cyclohexanol (**1a**, cis isomer) and *r*-4-phenyl-4-(1-piperidinyl)-*t*-cyclohexanol (**1b**, trans isomer) (see Figure 1). In this paper we describe analytical methods for the separation and characterization of **1a** and **1b**, present their biological activity in the mouse rotarod assay, and discuss the stereoselectivity of hydroxylation of phencyclidine by the dog.

Results and Discussion

The synthetic scheme utilized for the preparation of **1** resulted in a mixture of cis and trans isomers (Figure 1). Separation of the isomers was successfully achieved on TLC. By careful control of chromatographic conditions (i.e., routine preparation of fresh solvent, plate activation, tank equilibration), a full 2.5-cm separation could be obtained between **1b** (R_f 0.67) and **1a** (R_f 0.51). Preparative TLC (1000-μm plates) provided sufficient quantities of the isomers for conversion to their hydrochloride salts and subsequent testing.

Structural assignments of the cis and trans isomers were made by ^{1}H and ^{13}C NMR spectroscopy. Previous carbon-13 NMR studies[11,12] have established that the preferred solution conformation of phencyclidine hydrochloride and analogues is that in which the protonated amine group occupies the equatorial position. Consequently, when in solution as the hydrochloride salt, the cis isomer (**1a**) will have an equatorial hydroxyl orientation and the trans isomer (**1b**) will have an axial hydroxyl orientation. The isomers were identified by comparing the observed chemical-shift values for the cyclohexane carbons of each isomer with values calculated from the chemical shifts of the corresponding carbons of phencyclidine hydrochloride[11,12] and the cyclohexane hydroxyl substituent parameters.[13] The comparison, summarized in Table I, is sufficiently close to identify unequivocally the cis isomer as the more polar and the trans isomer as the less polar isomer. The half-height width ($W_{1/2}$) of the -C*H*(OH) resonance was consistent with this assignment. Isomer **1a** showed δ 3.89 and $W_{1/2}$ = 18 Hz characteristic of an axial proton, whereas **1b** showed δ 3.86 and $W_{1/2}$ = 8 Hz typical of equatorial protons.

Table I. Carbon-13 Chemical-Shift Data for *cis*- and *trans*-4-Phenyl-4-(1-piperidinyl)cyclohexanol Hydrochloride

cyclohexane carbons[a]	cis (1a)		trans (1b)	
	calcd	obsd	calcd	obsd
4 (s)	68.73	69.69	69.64	70.47
3, 5 (t)	27.49	28.39	22.87	24.00
2, 6 (t)	30.96	31.47	28.39	29.52
1 (d)	68.72	67.84	63.52	61.60

[a] The letters in parentheses refer to the SFORD multiplicities: s = singlet; d = doublet; t = triplet.

Methane chemical-ionization mass spectrometry was also used for characterization of **1a** and **1b** (see Table II). Phencyclidine and the hydroxy metabolites (both free and derivatized) displayed a "triplet" of ions centered at the molecular ion (M^+) and a weak or nonexistent $(M + 29)^+$ ion. The most abundant ion for all species was that associated with the piperidine ring (*m/e* 86 for phencyclidine, **1a**, and **1b**; *m/e* 84 for the piperidinol metabolite). 1-(1-Phenylcyclohexyl)-4-piperidinol displayed a prominent ion at *m/e* 159 (loss of hydroxypiperidine), whereas both **1a** and **1b** could be distinguished from it by a prominent ion at *m/e* 157 (loss of piperidine and water). The effect of derivatization with heptafluorobutyric anhydride (HFBA) on the metabolites was to increase the molecular weight (*m/e* 455 vs. *m/e* 259) and the relative abundance of the ion at *m/e* 242 (loss of a heptafluorobutyryl or hydroxyl group).

The methane CI mass spectra of **1a** and **1b** were quite similar; the only differences were the relative abundances of certain ions. The ratio of *m/e* 242 to the M^+ ion for **1a** (both for the free and derivatized forms) was consistently greater than that of **1b** and could be used as a basis for isomer identification following separation.

GC analysis of untreated urine extracts from two dogs following a single subcutaneous dose of phencyclidine hydrochloride (0.5 mg/kg) indicated the presence of PCP and small amounts of unresolved **1** and 1-(1-phenylcyclohexyl)-4-piperidinol. Following acid hydrolysis, the relative amounts of **1** increased approximately 20-fold for both animals, representing about 10% of the administered dose, whereas the amount of the piperidinol metabolite doubled. The amount of phencyclidine in urine remained essentially the same following acid hydrolysis, and there were no interferences from control urine. Uncorrected retention times (min, 3% SE, 2 mm × 1.8 m, 190 °C, HFBA deriv-

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Table II. Methane Chemical-Ionization Mass Spectra of Phencyclidine and Metabolites

compd[a]	M_r	chemical-ionization mass spectra[b] (M + 29)$^+$	(M + 1)$^+$	M$^+$	(M − 1)$^+$	prominent fragment ions[c]
PCP	243	272 (1)	244 (22)	243 (47)	242 (30)	159 (32), 86 (100), 84 (40)
PCHP	259	288 (0)	260 (10)	259 (23)	258 (13)	242 (18), 160 (10), 159 (62), 102 (38), 100 (25), 91 (14), 84 (100), 83 (13), 82 (23), 80 (34)
PCHP·HFB	455	484 (0)	456 (1)	455 (3)	454 (2)	242 (28), 159 (29), 84 (100)
1a	259	288 (1)	260 (8)	259 (16)	258 (13)	242 (48), 157 (28), 86 (100), 84 (57)
1a·HFB	455	484 (0)	456 (3)	455 (4)	454 (1)	243 (11), 242 (59), 86 (100), 84 (18)
1b	259	288 (0)	260 (7)	259 (13)	258 (11)	242 (20), 157 (30), 86 (100), 84 (48)
1b·HFB	455	484 (0)	456 (13)	455 (16)	454 (9)	243 (28), 242 (80), 157 (20), 87 (12), 86 (100), 84 (30)

[a] Abbreviations used are as follows: PCP, phencyclidine; PCHP, 1-(1-phenylcyclohexyl)-4-piperidinol; HFB, heptafluorobutyrate. [b] *m/e* (percent relative abundance). [c] Only ions ≥10% relative abundance are reported.

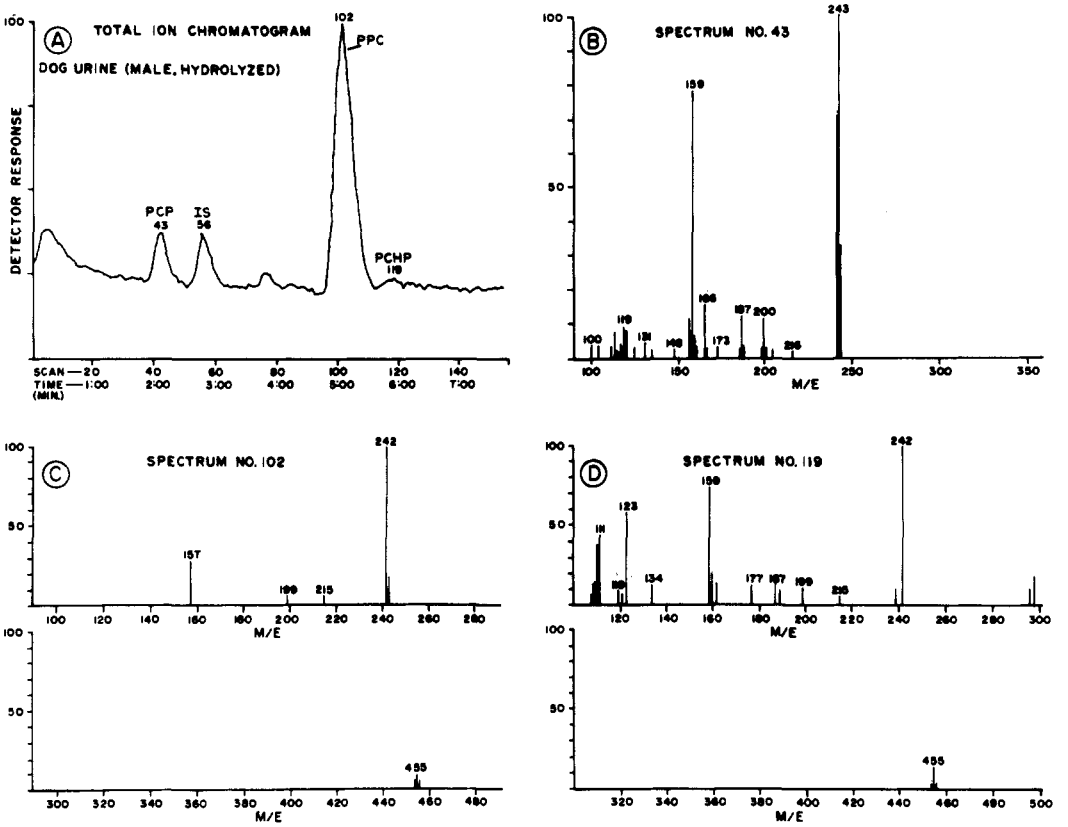

Figure 2. GC–chemical-ionization (methane) mass spectral analysis of dog urinary extract following phencyclidine administration. The range was scanned from 100 to 600 amu. A: integrated total ion current chromatogram of urine extract derivatized with heptafluorobutyric anhydride. PCP = phencyclidine, IS = internal standard, PPC = unseparated mixture of *cis*- and *trans*-4-phenyl-4-(1-piperidinyl)cyclohexanol, and PCHP = 1-(1-phenylcyclohexyl)-4-piperidinol. B: spectrum number 43 identified as PCP. C: spectrum number 102 identified as *cis*- and *trans*-PPC. D: spectrum number 119 identified as PCHP.

ative) for phencyclidine, **1a**, **1b**, and 1-(1-phenylcyclohexyl)-4-piperidinol were 4.1, 7.7, 7.9, and 8.7, respectively.

Analysis of the acid-hydrolyzed extract by GC/MS revealed a similar metabolite pattern and confirmed the structural assignments of phencyclidine, 1 (**1a** and **1b** are unresolved), and 1-(1-phenylcyclohexyl)-4-piperidinol (see Figure 2). Analyses of untreated extracts also were consistent with the GC results.

All analyses were performed in triplicate for both male and female dogs. The urinary excretion profile for the female dog was quite similar to that of the male, with only minor differences seen in the relative amounts of 1 excreted.

The trans/cis ratio of 1 in the 0–24 h hydrolyzed urine sample of the male dog was determined by visual comparison of extracts applied to TLC plates with standards and by elution from the TLC plate followed by GC analysis. Visual estimates of **1b**/**1a** indicated a range of 1.5–4 to 1 ratio. Elution from the TLC plate and measurement by GC gave a mean ± SE of 1.64 ± 0.07 (n = 3).

Biological Activity. Either three or four intraperitoneally administered doses of the cis and trans isomers of 1 or of the isomeric mixture of 1 were tested in the mouse rotarod assay. Convulsions and deaths were also recorded. The overall results (Table III) demonstrated that the trans isomer was slightly more potent in producing ataxia, convulsions, and death. A six-point bioassay comparing the ataxic effects of the trans (262, 328, and 410 μmol/kg) and cis (328, 410, and 512 μmol/kg) isomers demonstrated that the cis form was 0.87 times as potent as the trans, whose potency was set equal to 1.00. This difference in potency was not significant, since the 95% confidence limits were 0.54–1.10. Similar bioassays individually compared the isomeric mixture (262, 328, and 410 μmol/kg) to the individual isomers. Compared to the mixture 1, the cis isomer had a relative potency of 0.88 (0.69–1.02), and the trans isomer had a potency of 1.01 (0.75–1.39).

Table III. Ataxia and Toxicity Produced by the Intraperitoneal Administration of Phencyclidine and Hydroxy Metabolites in the Mouse[a]

drug	dose	ataxia (rotarod method)	convulsions	deaths
1a	328	0/5 (0)	0/5	0/5
	410	4/5 (76)	0/5	0/5
	512	1/1 (108)	3/5	1/5
1b	262	1/5 (16)	0/5	0/5
	328	3/5 (41)	0/5	0/5
	410	2/3 (64)	2/5	0/5
	512	0/0	3/5	5/5
1	262	0/5 (0)	0/5	0/5
	328	2/5 (36)	0/5	0/5
	410	3/4 (90)	1/5	0/5
PCP	17.6	4/5 (84)	0/5	0/5

[a] Doses are reported as micromoles per kilogram, and five mice were tested at each dose. Fractions represent the quantal responses for ataxia (falling off the rotarod), convulsions, and death. Parametric data for ataxia are presented within parentheses as the mean number of seconds out of a 120-s trial at peak effect that the mice were off the rotarod. Only those mice that did not convulse or die within the testing period are included in the ataxia measurements.

For comparison, the ED_{50} of phencyclidine (17.6 μmol/kg) was administered to five mice. In this group four of five mice fell, yielding an average off the rod of 84 s. These results provide a potency estimate of 0.05 for the mixture of isomers relative to phencyclidine. In general, the peak effect for all of the drugs occurred at 12 min after injection.

Other behaviors appeared to be empirically dose related for all three treatments. At low to moderate dose levels (262 and 328 μmol/kg), hyperactivity on the rotarod and hyperactivity and repetitive circling in the housing jars were seen in approximately half the mice. At the 410 μmol/kg level, two or three of every five mice tested became markedly tremulous. The Straub tail phenomenon was observed, with one exception, in all the mice that subsequently convulsed. Convulsions were either tonic or clonic or a combination of both. Two mice receiving 512 μmol/kg of the trans isomer died without convulsing. Not shown in Table III are the deaths of two mice caused by accidentally injecting 1024 μmol/kg of the cis isomer. Both died within 12 min after receiving the drug.

Conclusions

Although 1 has been identified as a urinary metabolite of phencyclidine in humans, dogs, rats, and mice,[6,9,10,14] no attempts have been reported to differentiate the isomeric composition of the metabolite. Recent reports of convulsant activity[15] and PCP-like activity[6,7] stimulated renewed interest in the pharmacology of the hydroxy metabolites of phencyclidine.

The present work demonstrates that the cis and trans isomers of 1 can be separated by TLC and provides a means of obtaining the individual isomers for chemical and pharmacological testing. Structural assignment of isomers was made by analysis of the ^{13}C and ^{1}H NMR data.

Gas chromatographic and mass spectral analysis of urinary extracts from two dogs which had received phencyclidine (0.5 mg/kg) indicated that a significant amount of the administered dose was excreted as the hydroxy metabolite, 1 (approximately 10%). Both TLC and GC analysis of the 0–24 h hydrolyzed urine extract from the male dog indicated that the trans isomer was present in greater amounts than the cis isomer. Although this represents data from a single animal, it suggests a degree of stereoselectivity in the biotransformation pathway involved in the hydroxylation of phencyclidine. Stereoselectivity has also been observed for hydroxylation of other centrally acting compounds.[16]

Preliminary testing of the cis (**1a**) and trans (**1b**) isomers of 1 for gross motor incoordination by the mouse rotarod assay indicates that **1b** is only slightly more active than **1a**. However, a clear statistical difference in potency between the isomers was not demonstrated with the available data. Both isomers were approximately one-twentieth as active as phencyclidine in this assay. Unlike phencyclidine, considerable seizure activity and lethality was found for both **1a** and **1b** at doses required to produce maximal ataxia. This suggests a lower margin of safety for the hydroxymetabolites which could become important in the case of phencyclidine overdose.

Experimental Section

Materials. Phencyclidine hydrochloride was obtained from Philip Roxane, Inc., St. Josephs, MO, through the courtesy of the National Institute on Drug Abuse. 1-(1-Phenylcyclohexyl)-morpholine (PCM, internal standard) was prepared as previously described.[12] 1-(1-Phenylcyclohexyl)-4-piperidinol was prepared using literature procedures.[9] 4-Phenyl-4-(1-piperidinyl)cyclohexanol (1) was synthesized from 4-(benzoyloxy)cyclohexanone[18] using literature procedures.[9] The structure and purity of these compounds were confirmed by TLC, MS, and ^{1}H and ^{13}C NMR. All other chemicals were of reagent grade quality.

Instrumentation. Analysis by gas chromatography–mass spectrometry was performed on a Finnigan Model 4021 Automated GC/MS/DS system operating in the methane chemical-ionization mode. The GC consisted of a glass column (2 mm × 1.83 m) packed with 3% SE-30 on Gas Chrom Q (100–120 mesh). The temperatures of the injector, column, interface oven, and ion source were maintained at 165, 190, 250, and 250 °C, respectively. The electron energy was set at 70 eV and the multiplier voltage at 1.35 kV.

For GC analysis, a Perkin-Elmer Sigma 2 gas chromatograph was employed which was equipped with a nitrogen-sensitive detector. The column was similar to that used for GC/MS analysis. The following conditions were used for analysis: bead voltage, 450 mV; carrier flow (helium), 20 mL/min; injector, 160 °C; detector, 250 °C; oven, 175 °C.

The ^{13}C NMR spectra were determined at 25.03 MHz on a modified JEOL JNM-PS-100 FT NMR interfaced with a Nicolet 1085 Fourier transform computer system. Samples (30 mg/0.30 mL) were spun in Me_2SO-d_6 solution in 5-mm o.d. tubes (Wilmad). The spectra were recorded at ambient temperature by using the deuterium resonance of the solvent as the internal lock signal. The values were in parts per million downfield from Me_4Si: $\delta(Me_4Si) = \delta(Me_2SO\text{-}d_6) + 39.56$. All proton lines were decoupled by a broad-band (~2500 Hz) irradiation from an incoherent 99.539-MHz source. Other spectral parameters included 8K output data points, 5000-Hz sweep width, 15-μs pulse width, and 2.0-s repetition time. High-resolution ^{1}H NMR spectra were obtained in the Fourier transform mode using a Bruker WM-250 spectrometer with Me_4Si as internal standard in $CDCl_3$.

Chromatographic Separation of the Cis (1a) and Trans (1b) Isomers. Preparative silica gel GF plates (Analtech Inc.) were activated for at least 2 h at 120 °C prior to application of the mixture. The plates were developed using the EtOAc/40% aqueous dimethylamine (99:1) solvent system, and the bands were

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marked under UV light. Identical silica gel bands from eight plates were scraped, combined, and soaked overnight in $CHCl_3$/MeOH (9:1). Afterwards, each suspension was filtered and the silica gel was washed copiously with $CHCl_3$. Evaporation of the combined filtrate and washings in each case provided isomerically pure material. Chromatography of 600 mg of the cis/trans mixture provided 133.5 mg of the less polar (top band) and 132.9 mg of the more polar isomer (bottom band).

The material isolated from the top band was dissolved in Et_2O, and the solution was filtered through a cotton plug to remove residual silica gel. The solution was then treated with HCl gas, the Et_2O was evaporated, and the residue was recrystallized from MeOH/Et_2O to obtain the pure hydrochloride salt as a white solid, mp 201–202 °C. A ^{13}C NMR analysis of this salt showed that it was *r*-4-phenyl-4-(1-piperidinyl)-*t*-cyclohexanol (**1b**) hydrochloride. There was no detectable amount of the cis isomer by either TLC or ^{13}C NMR. Anal. ($C_{17}H_{26}ClNO \cdot H_2O$) C, H, N.

Likewise, the material isolated from the bottom band was converted to a hydrochloride salt, mp 200–201 °C. A subsequent ^{13}C NMR analysis of this salt indicated that it was the cis (**1a**) hydrochloride. There was no detectable amount of the trans isomer in the sample. Anal. ($C_{17}H_{26}ClNO \cdot H_2O$) C, H, N.

Metabolic Studies in the Dog. The dogs were housed individually in cages equipped with stainless-steel urine collection pans. Feces remained separated from urine during collection periods and were discarded. Predrug control urine was collected the day before drug administration. One male and one female beagle dog were administered a single subcutaneous dose of PCP·HCl (0.5 mg/kg). Urine was collected in 24-h aliquots for 3 days. The urine samples were collected, filtered, and frozen until time of analysis.

An aliquot (10 mL) of the urine sample was mixed with internal standard (10 μg of PCM) and extracted either untreated or following acid hydrolysis. The samples to be hydrolyzed were treated with 10% concentrated hydrochloric acid and autoclaved at 115 °C with 15 psi pressure for 30 min. Control samples with and without added standards were included in all processes to ensure that artifacts were not produced.

Prior to extraction the pH of all samples was adjusted to 10, and 3 mL of 3 N potassium carbonate was added to give a final pH of 10.5. Sodium chloride (0.5 g) and hexane (12 mL) were added, and the contents were shaken and centrifuged. An aliquot of the organic phase was transferred to a clean tube containing 5 mL of 2 N hydrochloric acid. The tubes were shaken and centrifuged, and the organic phase was discarded. The pH was adjusted to a final value of 10.5 and the contents were extracted with hexane as before. An aliquot of the organic phase was transferred to a clean, conical tube and evaporated to dryness under nitrogen at 40 °C. The residue was analyzed by means of TLC, GC, and GC/MS.

For GC and GC/MS analysis the residue was treated with heptafluorobutyric anhydride (HFBA) (20 μL) in benzene (100 μL) and heated at 90 °C for 1 h. Excess HFBA was decomposed with ammonium hydroxide (1 mL, of 5% v/v). An aliquot of the organic phase (1–3 μL) was removed for analysis.

Procedures for the Estimation of the Cis/Trans Ratio of 1. The trans/cis ratio of 1 in hydrolyzed dog urine (male) was estimated using both TLC or GC. Elution of urinary extracts on the TLC system already described resulted in the complete separation of **1a**, 1-(1-phenylcyclohexyl)-4-piperidinol, **1b**, and phencyclidine with R_f values of 0.51, 0.58, 0.67, and 0.90, respectively. Following development and drying, the plate (standard 20 cm) was sprayed with potassium iodoplatinate solution. The metabolites appeared as dark brown spots. Visual comparison of the amounts of **1a** and **1b** in the extracts to standards applied to the same plate (range of 0.5–60 μg of cis isomer and 0–75 μg of trans isomer) provided a semiquantitative estimate of the trans/cis ratio.

A more quantitative procedure was developed for the estimation of the trans/cis ratio using GC. Unsprayed metabolite bands were removed from the plate and extracted with ether overnight in a Sohxlet extractor. Internal standard (PCM) was added to the extract, and the mixture was evaporated to dryness under nitrogen at 30 °C. The residue was dissolved in methanol and analyzed by GC. Peak height ratio comparisons to standards provided a quantitative estimate of the trans/cis ratio of 1 excreted by the dog.

In Vivo Testing of 1a and 1b in the Mouse. Ataxia was measured in mice using the rotarod method of Dunham and Miya[19] and the modification of Kalir et al.[20] Prior to testing, adult male SIM(SW)$_F$ BR mice weighing between 30 and 45 g were selected for their ability to remain on the rod rotating at 5 rpm for two 5-min periods. On test days, mice were given 12 120-s trials on the rotarod using the following sequence: two control trials, initiated at 12 and 6 min before drug administration, an ip injection, and trials beginning at 6-min intervals for the next hour. Data are presented as the fraction of mice falling off the rod and as the average time out of 120 s that the mice were off the rotarod based upon the peak drug effect for each mouse. In addition, gross behavioral effects, convulsions, and deaths were also recorded. The following drugs were employed: **1a**·HCl; **1b**·HCl; 1 (synthetic mixture of cis and trans isomers); PCP·HCl; vehicle. The hydrochloride salts were dissolved in a saline–double distilled water (1:1) vehicle. The free base forms of 1 and **1b** were dissolved in pH 4 phosphate–citrate buffer. The injection volume was 0.02 mL/g of body weight.

An initial testing dose of 328 μmol/kg for each treatment was selected as the estimated ED_{50} value for producing ataxia, similar to that initially determined for 1. Other doses were increments or decrements of 1.25. Five mice were tested at each dose. Parametric ataxia data were analyzed using bioassay statistics.

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