Comparative Pharmacology in the Rat of Ketamine and Its Two Principal Metabolites, Norketamine and (Z)-6-Hydroxynorketamine

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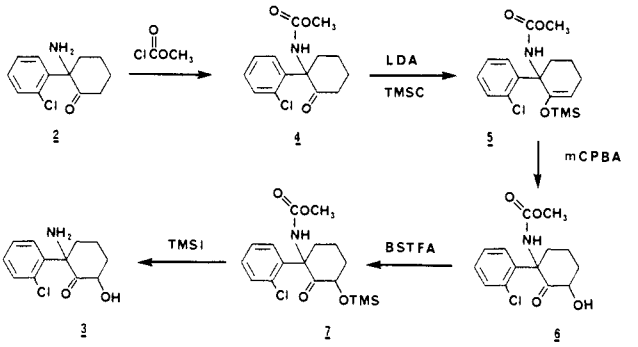
Received October 28, 1985

(Z)-6-Hydroxynorketamine (3), a secondary metabolite of the dissociative anesthetic agent ketamine (1), was synthesized, and its central nervous system (CNS) properties were compared to those of the parent drug and the primary metabolite, norketamine (2). Administration of compounds 1 and 2 to rats (40 mg/kg iv) produced general anesthesia and also led to marked increases in spontaneous locomotor activity during the postanesthetic recovery phase. These effects were of significantly longer duration with 1 than with 2. In contrast, the same dose of 3 produced neither general anesthesia nor CNS excitation, despite the fact that 3 entered brain tissue readily from the systemic circulation. It is concluded that the CNS effects of 1 are attenuated by metabolism to 2 and are abolished by subsequent hydroxylation to produce 3. Moreover, the results suggest that the desirable anesthetic properties of 1 and related arylcyclohexylamines may be inseparable from their ability to produce adverse postanesthetic emergence reactions.

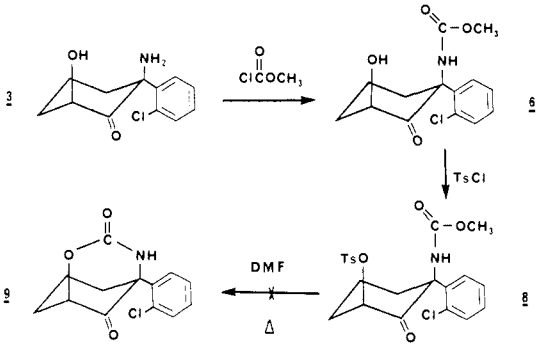
Ketamine, (±)-2-(o-chlorophenyl)-2-(methylamino)-cyclohexanone (1, Figure 1), is a short-acting parenteral anesthetic agent that has been in clinical use worldwide for some 15 years.[1] The drug produces profound analgesia at subanesthetic doses and lacks the cardiorespiratory depressive effects associated with most other general anesthetics. Despite these important clinical advantages, however, disturbing emergence reactions, including delirium and unpleasant dreams, frequently accompany ketamine therapy and have limited its clinical usefulness.[1] Although the origin of these adverse central nervous system (CNS) side-effects remains obscure, it has been speculated that metabolites of 1 may play an important role since circulating concentrations of the parent drug are very low during emergence from ketamine-induced anesthesia.[1]

Ketamine undergoes extensive metabolism by hepatic enzymes, yielding mainly the product of N-demethylation, norketamine (2).[2,3] This primary amine is metabolized further by hydroxylation of the alicyclic ring system at positions 4, 5, and 6 to produce a family of hydroxynorketamine isomers, the predominant member of which has been identified as the 6-hydroxy compound, 3.[4,5] Although 2 has been reported to be a weaker anesthetic and to produce less postanesthetic excitation than 1,[1,6,7] nothing is known about the pharmacological properties of hydroxylated metabolites of 1. It is of some interest to note, however, that a family of arylcyclohexylamine derivatives bearing a hydroxyl substituent on the cyclohexane ring system have proved to be extremely potent centrally acting analgesic agents.[8] In this paper, we describe the synthesis and stereochemical assignment of 3 and present the results of a comparative study in the rat of the anesthetic properties of compounds 1–3 and their effects on spontaneous locomotor activity (SLA).

Scheme I. Synthesis of 6-Hydroxynorketamine (3)



Scheme II. Attempted Conversion of 6-Hydroxynorketamine (3) to the Cyclic Carbamate 9



Results and Discussion

6-Hydroxynorketamine (3) was synthesized by a modification of the procedure reported recently by Woolf et al.[9] for the preparation of diastereoisomers of 6-hydroxyketamine. Thus, compound 2 was converted first to the methyl carbamate derivative 4, which was treated with lithium diisopropylamide (LDA) and trimethylsilyl chloride (Me_3SiCl) to yield the corresponding Me_3Si enol ether 5. Oxidation of 5 with m-chloroperoxybenzoic acid (mCPBA) gave the protected 6-hydroxynorketamine derivative 6, which was then converted to its Me_3Si ether 7 by reaction with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Removal of the methoxycarbonyl protecting group with trimethylsilyl iodide (Me_3SiI) and hydrolysis of the Me_3Si ether yielded the desired product, 3 (Scheme I).

(1) White, P. F.; Way, W. L.; Trevor, A. J. *Anesthesiology* **1982**, *56*, 119.
(2) Cohen, M. L.; Chan, S. L.; Way, W. L.; Trevor, A. J. *Anesthesiology* **1973**, *39*, 370.
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(4) Adams, J. D.; Baillie, T. A.; Trevor, A. J.; Castagnoli, N., Jr. *Biomed. Mass Spectrom.* **1981**, *8*, 527.
(5) Woolf, T. F. Ph.D. Dissertation, University of California at San Francisco, 1983.
(6) Cohen, M. L.; Trevor, A. J. *J. Pharmacol. Exp. Ther.* **1974**, *189*, 351.
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(8) Lednicer, D.; Von Voigtlander, P. F. *J. Med. Chem.* **1979**, *22*, 1157.
(9) Woolf, T. F.; Trevor, A. J.; Baillie, T. A.; Castagnoli, N., Jr. *J. Org. Chem.* **1984**, *49*, 3305.

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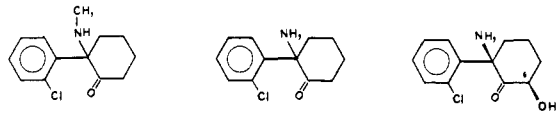

1 2 3

Figure 1. Structures of ketamine (1), norketamine (2), and (*Z*)-6-hydroxynorketamine (3).

Table I. CNS Activities of Compounds 1–3

compd[a]	duration of anesthesia, min	duration of increase in SLA, min
1	7.11 ± 0.96[b]	24.65 ± 4.50
2	3.24 ± 0.47	7.15 ± 0.59
3	0	0
control	0	0

[a] Test compounds were administered to male Sprague–Dawley rats (250–300 g) by iv bolus injection at a dose of 40 mg/kg. Control animals received vehicle only. See Experimental Section for details. [b] Results were expressed as mean values ± SEM (n = 4).

On the basis of the published synthesis of 6-hydroxyketamine,[9] it was anticipated that the above pathway would yield predominantly the *Z* isomer of **3**, in which the relative orientation of NH_2 and OH substituents was cis. Indeed, when **3** was converted to its tosylate derivative **8** and heated, the bicyclic carbamate **9** failed to form (Scheme II). This finding argues against a trans orientation for the NH_2 and OH functionalities in **3**, since ring closure to the bicyclic species occurs readily for the corresponding (*E*)-6-hydroxyketamine derivative but not for the *Z* isomer.[9] Woolf et al.[9] also noted that (*E*)-6-hydroxyketamine was much less stable in solution than its *Z* diastereoisomer and underwent a facile rearrangement/elimination reaction in which the methylamino substituent was lost. Compound **3** did not undergo such a reaction but appeared to be quite stable toward mild acid or base. (A recent, unsuccessful attempt by Lai et al.[10] to prepare **3** apparently did lead to this elimination product, although no information was presented on the stereochemical course of the synthesis.) Finally, compound **3**, as its pentafluoropropionyl (PFP) derivative, exhibited gas chromatographic and mass spectrometric properties identical with those of the *N*-desmethyl metabolite of (*Z*)-6-hydroxyketamine but different from those of the metabolite produced from (*E*)-6-hydroxyketamine.[5] On the basis of all of these considerations, **3** was assigned *Z* stereochemistry.

The results of the in vivo pharmacology studies performed with **1–3** are summarized in Table I. Of the three compounds, **1** produced the longest duration of anesthesia (measured by loss of righting reflex[11]) and also gave rise to the most pronounced increases in SLA. The duration of anesthesia induced by **2** was approximately half of that obtained with an equivalent dose of **1**, while increases in SLA during the recovery phase were of much shorter duration with **2** than with the parent drug. Compound **3**, however, was completely devoid of activity at the 40 mg/kg dose in both pharmacological tests.

In order to examine the relationship between the pharmacological activity of compounds **1–3** and their ability to enter the CNS, a series of experiments was performed in which these agents were given to rats by iv injection, and concentrations of each compound of interest were measured in matched samples of plasma and brain tissue obtained after 2, 5, and 10 min. The results of these preliminary pharmacokinetic experiments are depicted in Figure 2, which shows that all three compounds were taken up rapidly from the systemic circulation into the brain, from which they were cleared with an apparent $t_{1/2}$ of approximately 8–10 min. Hence, the diminished pharmacological activity of **2** relative to **1** and the lack of CNS activity of **3** cannot be ascribed to major differences in the blood–brain distribution properties of these compounds, but more likely reside in fundamental differences in their respective interactions with central receptor sites.

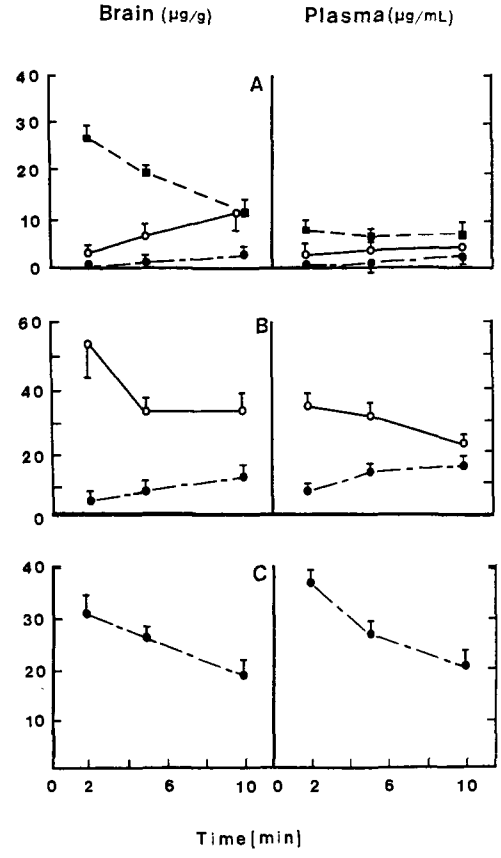

Figure 2. Concentrations in brain tissue and plasma of parent compounds and metabolites measured 2, 5, and 10 min after an iv dose (40 mg/kg) to rats. Compounds administered were (A) ketamine (1), (B) norketamine (2), and (C) (*Z*)-6-hydroxynorketamine (3). Results are depicted as mean values (n = 4), and error bars indicate the SEM. Symbols denote concentrations of 1 (■), 2 (○), and 3 (●).

When **1** was administered to rats, appreciable concentrations of **2** (and somewhat lower levels of **3**) were measured in plasma and brain samples as early as 2 min after injection, and concentrations of **2** and **3** increased steadily in these fluids up to the 10-min point (Figure 2A). Similarly, when **2** was given, **3** was detected in plasma and brain after 2 min and had more than doubled in concentration by 10 min (Figure 2B). These results demonstrate that metabolism of **1** (by N-demethylation) and of **2** (by 6-hydroxylation) occurs very rapidly in the rat in vivo. From the data presented in Table I and in Figure 2, it was estimated that animals anesthetized with **2** will have a mean brain concentration of this compound of 43 μg/g when they regain their righting reflex (at 3.2 min); that is, a figure of 43 μg/g may be considered to represent the "threshold" brain concentration for anesthesia induced by **2** in the rat. When animals were anesthetized with **1**, however, brain concentrations of the metabolite **2** averaged only 8.5 μg/g at the point of emergence from anesthesia

(10) Lai, I.; Hong, S. C.; Davisson, J. N. *J. Pharm. Sci.* **1985**, *74*, 486.
(11) Meliska, C. J.; Greenberg, A. J.; Trevor, A. J. *J. Pharmacol. Exp. Ther.* **1980**, *212*, 198.

Table II. Partition Coefficients of Compounds 1–3

compd	partition coefficient[a]		
	n-heptane	benzene	chloroform
1	6.36 ± 0.14	98.6 ± 10.3	299 ± 89
2	1.86 ± 0.09	52.6 ± 10.3	296 ± 119
3	(9.63 ± 0.70) × 10^{-3}	1.44 ± 0.10	19.4 ± 1.70

[a] Partition coefficients are expressed as the ratio of the equilibrium concentration of the test compound in the organic phase to that in an equal volume of pH 7.4 aqueous buffer. Results are expressed as mean values ± SEM for three determinations. See Experimental Section for details.

(7.1 min). Thus, it may be concluded that the desmethyl metabolite **2** contributes little to the anesthetic effects of **1** in the rat, while the secondary (hydroxylated) metabolite **3** makes no contribution to ketamine-induced anesthesia.

The data presented in Figure 2 also indicate that the ratio of drug concentration in brain tissue to the corresponding value in plasma was greater than unity at all time points for compounds **1** and **2** but slightly less than unity for metabolite **3** (whether given exogenously or formed in vivo). In order to determine whether these relatively subtle differences in brain/plasma concentration ratios reflected differences in the respective lipophilic properties of compounds **1–3**, measurements were made of partition coefficients in vitro using three different organic solvent/aqueous buffer systems. The results of these studies, which are summarized in Table II, show that of the three test compounds, **1** paritioned most favorably into *n*-heptane, benzene, and chloroform. Although partition coefficients for **2** were only slightly less than those for the parent drug, values for the hydroxylated metabolite **3** were substantially lower than for the other members of the group. Differences, therefore, in the brain/plasma concentration ratios in vivo for compounds **1–3** follow the order of their partition coefficients in vitro, although all three compounds evidently are sufficiently lipophilic to cross the blood–brain barrier readily.

Considered collectively, the findings of this investigation indicate that while **1** and its two major metabolites enter the CNS from blood plasma with almost equal ease, it is the parent compound that is responsible for the majority of both the anesthetic effects and the undesirable postanesthetic sequelae. Thus, production of anesthesia on the one hand and the occurrence of emergence reactions on the other may be inseparable characteristics of **1** and its arylcyclohexylamine congeners.

Experimental Section

Chemicals for synthetic procedures were of reagent grade, and all reactions were carried out under nitrogen. Compounds **1** and **2** were synthesized by published methods[12,13] and had melting points and spectroscopic properties (NMR, MS) in agreement with literature values. Melting points were recorded on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Proton NMR spectra (60 MHz) were recorded in $CDCl_3$ solution using a Varian EM-360 spectrometer; chemical shifts are reported in ppm downfield from Me_4Si. Gas chromatography was carried out on a Hewlett-Packard 5890 instrument, equipped with a fused silica capillary column (30 m × 0.32 mm i.d., DB-5; J and W Scientific, Ventura, CA). Helium (head pressure 20 psi) was employed as both carrier gas and detector make-up gas. Compounds eluting from the column were detected by means of a flame ionization detector and recorded with a Hewlett-Packard 3390A reporting integrator. Samples were injected with use of the splitless mode of operation (injection port temperature 250 °C) and were "cold-trapped" on the column at 50 °C. After a period of 30 s, the oven temperature was rasied rapidly to 140 °C and then programmed linearly at 16 °C/min to 250 °C. Low-resolution mass spectra were obtained on a Hewlett-Packard Model 5985 GC–MS instrument, equipped with a Model 5840A gas chromatograph and operated in the electron impact mode with an ionizing energy of 70 eV and ion source temperature of 200 °C. Quantitative determinations of **1** and its metabolites in biological samples were made by selected ion monitoring (SIM) GC–MS. Gas chromatographic conditions for these analyses were essentially identical to those described above for GC work. High-resolution ($m/\Delta m \sim 10\,000$) mass spectra were recorded by using a VG 70-70H instrument that was operated in the chemical-ionization mode with CH_4 as reagent gas (source pressure 0.5 torr). Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

Synthesis. 2-(*o*-Chlorophenyl)-2-[(methoxycarbonyl)amino]cyclohexanone (4). To a mixture of **2** (3.0 g, 14 mmol) in anhydrous benzene (100 mL) and Na_2CO_3 (4.5 g) was added a solution of methyl chloroformate (3.0 mL, 40 mmol) in anhydrous benzene (10 mL). After heating under reflux for 3 h, the reaction mixture was cooled to room temperature and washed in turn with H_2O, 10% Na_2CO_3, and H_2O again. The product was diluted with either, dried ($MgSO_4$), and concentrated under reduced pressure, when **4** precipitated as a white solid, yield 3.2 g (85%): mp 105–108 °C; NMR δ 6.7–7.4 (m, 4 H, Ar H), 3.5 (s, 3 H, OCH_3), 1.4–2.8 (m, 8 H, cyclohexanone CH_2 protons); MS, *m/z* 281 ($M^{+\cdot}$, 2%), 246 ($[M - Cl]^+$, 82%), 218 ($[M - Cl - CO]^+$, 100%).

2-(*o*-Chlorophenyl)-2-[(methoxycarbonyl)amino]-6-hydroxycyclohexanone (6). To a cooled (0 °C) mixture of diisopropylamine (4 mL, 28 mmol) and dry THF (30 mL) was added a solution of *n*-butyllithium in hexane (1.6 M, 17 mL, 28 mmol). The reaction mixture was stirred at 0 °C for 1 h, cooled to −78 °C, and treated dropwise with a solution of **4** (3.2 g, 11 mmol) in dry THF (20 mL). After stirring for a period of 2 h, Me_3SiCl (4 mL, 28 mmol) was added, and the reaction mixture was stirred for 20 min before warming to room temperature over 45 min. Hexane was then added, and the resulting solution was washed with 10% $NaHCO_3$ and H_2O and dried ($MgSO_4$), and the solvent was removed in vacuo to afford the crude product as a yellow oil. Purification by column chromatography on silica gel (75 g, 70–325 mesh, EtOAc as eluent) gave **5** as a clear yellow oil, yield 4.5 g (100%): NMR δ 7.0–7.6 (m, 4 H, Ar H), 3.6 (s, 3 H, OCH_3), 5.2 (m, 1 H, C_6H), 1.0–2.5 (m, 6 H, CH_2 groups); MS, *m/z* 338 ($[M - CH_3]^+$, 2%), 318 ($[M - Cl]^+$, 2%), 278 ($[M - H_2NCO_2CH_3]^{+\cdot}$, 35%); TLC (100% EtOAc) single spot, R_f 0.83.

To a mixture of **5** (4.46 g, 12.6 mmol) in hexane (60 mL) and Na_2CO_3 (2.5 g) was added mCPBA (4.5 g, 20.8 mmol). The reaction mixture was stirred at room temperature for 3 h, and the product was washed (10% Na_2SO_3, then H_2O), dried ($MgSO_4$), and evaporated to give a white solid. Column chromatography on silica gel (75 g, 70–325 mesh, 5% CH_3CN in CH_2Cl_2 as eluent) gave **6** as an oil (3.5 g, 78%): NMR δ 6.7–7.5 (m, 4 H, Ar H), 3.8–4.3 (m, 1 H, C_6H), 3.6 (s, 3 H, OCH_3), 3.2–3.5 (m, 1 H, exchangeable with D_2O, OH), 1.5–2.5 (m, 6 H, CH_2 groups); MS, *m/z* 297 ($M^{+\cdot}$, 3%), 279 ($[M - H_2O]^{+\cdot}$, 11%), 262 ($[M - Cl]^+$, 71%); TLC (5% CH_3CN in CH_2Cl_2) single spot, R_f 0.25. Anal. Calcd for $C_{14}H_{16}NO_4Cl$: C, 56.47; H, 5.42; N, 4.70. Found: C, 56.84; H, 5.40; N, 4.65.

2-(*o*-Chlorophenyl)-2-amino-6-hydroxycyclohexanone (6-Hydroxynorketamine) (3). To a solution of **6** (3.5 g, 12 mmol) in anhydrous CH_2Cl_2 (80 mL) was added BSTFA (4 mL, 15 mmol) and dry pyridine (0.2 mL). The mixture was heated under reflux for 1 h, and excess reagents were removed in vacuo to give **7** (4.5 g, 100%) as a white solid: NMR δ 6.7–7.5 (m, 4 H, Ar H), 4.0–4.4 (m, 1 H, C_6H), 3.5 (s, 3 H, OCH_3), 1.4–2.9 (m, 6 H, CH_2 groups), 0.2 (s, 9 H, Me_3Si); MS, *m/z* 354 ($[M - CH_3]^+$, 2%), 341 ($[M - CO]^{+\cdot}$, 2%), 306 ($[M - CO - Cl]^+$, 4%); TLC (5% CH_3CN in CH_2Cl_2) single spot, R_f 0.46.

A solution of **7** (4.5 g, 12 mmol) in dry CH_2Cl_2 (80 mL) was treated dropwise with Me_3SiI (3 mL, 17 mmol), and the resulting mixture was stirred at room temperature for 30 min. Methanol (80 mL) was then added, the mixture was washed (10% Na_2SO_3, then H_2O) and dried (Na_2SO_4), and the solvent was evaporated to give the crude product as a yellow oil (1.0 g). Column chro-

(12) Stevens, C. L. U.S. Patent 3 254 124; *Chem. Abstr.* **1966**, *65*, 6414.
(13) Parcell, R. F.; Sanchez, J. P. *J. Org. Chem.* **1981**, *46*, 5055.

matography on silica gel (10 g, 70–325 mesh, EtOAc as eluent) gave pure **3** as an oil, yield 0.8 g (28%) (overall yield from **2** 19%): NMR δ 7.1–7.8 (m, 4 H, Ar H), 4.0–4.4 (m, 1 H, C_6H), 1.0–2.4 (m, 6 H, CH_2 groups), 2.4–2.7 (br s, 3 H, exchangeable with D_2O, NH_2 and OH); CIMS, m/z 240.0771 (MH^+), $C_{12}H_{15}NO_2{}^{35}Cl$ requires 240.0791 (error = 8 ppm); TLC (EtOAc) single spot, R_f 0.17. Anal. Calcd for $C_{12}H_{14}NO_2Cl{\cdot}HCl$: C, 52.19; H, 5.47; N, 5.07. Found: C, 51.46; H, 5.33; N, 4.86.

Attempted Cyclization of 3 to Carbamate 9. A mixture of **3** (100 mg, 0.42 mmol), dry benzene (10 mL), and Na_2CO_3 (100 mg) was treated with methyl chloroformate (0.15 mL, 2.0 mmol), and the mixture was heated under reflux for 3 h. The reaction mixture was cooled to room temperature, washed in turn with H_2O, 10% $NaHCO_3$, and H_2O again, dried (Na_2SO_4), and concentrated to give an oily residue. Column chromatography on silica gel (2 g, 70–325 mesh, 5% CH_3CN in CH_2Cl_2 as eluent) gave **6** as an oil (80 mg, 65%).

The carbamate **6** (30 mg, 0.08 mmol) in dry toluene (2 mL) was then added to a mixture of (dimethylamino)pyridine (40 mg, 0.33 mmol), pyridine (1.5 mL), and dry toluene (2 mL) at 0 °C. Tosyl chloride (50 mg, 0.30 mmol) in dry toluene (1.5 mL) was then added dropwise, and the reaction mixture was allowed to warm up to room temperature and stirred overnight. The reaction mixture was then concentrated in vacuo and the crude product partitioned between CH_2Cl_2 and H_2O. The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated to give an oily residue. The tosylate **8** was isolated by column chromatography on silica gel (1.0 g, 70–325 mesh, 5% CH_3CN in CH_2Cl_2 as eluent), yield 15 mg (42%): NMR δ 1.2–1.8 (m, 6 H, CH_2 groups), 2.4 (s, 3 H, Ar CH_3), 3.5 (s, 3 H, OCH_3), 5.2–5.4 (m, 1 H, C_6H), 7.0–8.0 (m, 8 H, Ar H); MS, m/z 416 ($[M - Cl]^+$, 2%), 388 ($[M - Cl - CO]^+$, 7%), 268 ($[M - Ts - CO]^+$, 100%), 251 ($[M - TsOH - CO]^{+\cdot}$, 5%); TLC (5% CH_3CN in CH_2Cl_2) single spot, R_f 0.50.

The tosylate **8** (15 mg, 0.04 mmol) was dissolved in DMF (2 mL) and heated under reflux for 5 h. Analysis of the reaction mixture by TLC (5% CH_3CN in CH_2Cl_2), NMR, and MS showed only the presence of unreacted **8** and its hydrolysis product, **6**. No evidence was obtained for the formation of the cyclic carbamate **9**.

Pharmacology Studies. Pharmacology studies were carried out with male Sprague–Dawley rats (250–300 g), which were housed in individual cages mounted to the base of a stabilimeter platform (see below). For drug administration, animals were equipped with an indwelling catheter, inserted into the right jugular vein, and were allowed to recover for 48 h prior to use. Test compounds were given by bolus injection, at a dose level of 40 mg/kg, and were administered as 0.2 mL of a solution of either the free base dissolved in propylene glycol/ethanol/water (40/10/50 by volume) or the HCl salt dissolved in 0.9% saline. Control rats received the corresponding vehicle only. The duration of anesthesia was determined by the loss of the animal's righting reflex, and SLA was measured by the stabilimeter, which consisted of a sliding platform and pendulum–ring assembly (Model BRS-LVE, JPA-001). Excitation of the animal resulted in horizontal displacements of the cage, which, in turn, caused the pendulum to make contact with the ring assembly. Each time the pendulum contacted the ring, an electrical circuit was completed, and the resulting signal was registered on a recorder as a single event. Excitation was judged to have ceased when there were fewer than two events during a given 3-min period.

Blood–Brain Distribution Studies. Three groups of rats (four animals per group) were employed for these experiments. Each group received one of the test compounds (**1–3**), administered as an iv bolus as described above. After 2, 5, or 10 min, a blood sample was drawn from the indwelling catheter and the animal sacrificed immediately by decapitation. Concentrations of "total" (i.e., free plus protein-bound) drug and/or metabolites in plasma and brain tissue were then determined by stable isotope dilution GC–MS assay, as follows.

Samples of fresh brain tissue were homogenized in 0.9% saline solution (1:3, w/w), the homogenates were centrifuged at 100 000*g* for 30 min, and the clear supernatants were taken for analysis. Aliquots of plasma (0.1 mL) or brain supernatant (0.1-g tissue equivalent) were first treated with the internal standard, ketamine-d_2 hydrochloride (2.0 μg in 0.1 mL of H_2O). (This aromatic ring-labeled analogue of ketamine was prepared by acid-catalyzed exchange of ketamine in D_2O, followed by back-exchange of labile deuterium at C-6 under basic conditions.[4] The isotopic content of the product, which was determined by GC–MS analysis as described previously,[4] was found to be 1.0% D_0, 17.6% D_1, 60.2% D_2, 18.7% D_3, and 2.5% D_4.) Samples were then basified with NaOH solution (1.0 M, 0.2 mL) and extracted with diethyl ether (3.0 mL). The organic extracts were washed with H_2O (3.0 mL), dried (Na_2SO_4), filtered, and evaporated under a stream of N_2. To the residues were added EtOAc (150 μL) and pentafluoropropionic acid anhydride (PFPA; 20 μL), and formation of PFP derivatives was allowed to proceed at 60 °C for 30 min. Following evaporation of excess reagents under a gentle stream of dry N_2, samples were reconstituted in EtOAc (20 μL), from which duplicate aliquots (1–2 μL) were taken for analysis by GC–MS. Quantification of compounds **1–3** was based on SIM of the $[M - Cl - CO]^+$ ions in the mass spectra of their respective PFP derivatives,[4] viz. at m/z 320 (**1**), 306 (**2**), 468 (**3**), and 322 (internal standard). The ratios of peak areas (drug or metabolite:internal standard) in the resulting ion current chromatograms were used to determine concentrations by reference to calibration curves prepared by the addition of varying amounts of compounds **1–3**, together with a fixed quantity of internal standard, to drug-free samples of rat plasma and supernatant from brain homogenate. Calibration curves were linear ($r > 0.98$) within the concentration ranges of interest. Recovery of known amounts of **1–3** added to drug-free samples of plasma and supernatant from brain homogenates was found to be quantitative. Plasma and tissue concentrations are expressed as mean values ± SEM.

Determination of Partition Coefficients. Partition coefficients were measured at ambient temperature by mechanically shaking mixtures of compounds **1–3** (100 μg/mL in either *n*-heptane, benzene, or chloroform; 1.0 mL) with pH 7.4 aqueous sodium phosphate buffer (0.1 M, 1.0 mL). Ater a 30-min agitation, the organic and aqueous phases were separated by brief centrifugation, and each was treated with internal standard for subsequent GC analysis. Ketamine was used as internal standard for the assay of **2** and **3**, while **2** served as internal standard for the assay of **1**. Stock solutions (100 μg/mL) of these internal standards were prepared in each of the organic solvents used for partition experiments, and aliquots containing either 40 μg or 5 μg were added to the separated organic and aqueous layers, respectively. The organic phases were dried ($MgSO_4$) and evaporated under reduced pressure. The aqueous phases were basified by the addition of 1.0 M NaOH solution (0.1 mL) and extracted exhaustively with diethyl ether. The combined ether extracts were similarly dried and evaporated. The residues from both the organic and aqueous phases were converted, as described above, to PFP derivatives and analyzed quantitatively by GC. Concentrations of compounds **1–3** in each phase were calculated from the ratios of their GC peak areas to those of the internal standard. Partition coefficients were then expressed as the ratio of the concentration of each compound in the organic solvent to that in the corresponding aqueous buffer. Measurements of partition coefficient were performed (for each solvent) in triplicate and are expressed as mean values ± SEM.

Acknowledgment. We are grateful to William N. Howald for assistance with the mass spectrometry and to Drs. A. J. Trevor and N. Castagnoli, Jr. (University of California at San Francisco), for valuable discussions during the course of this work. These studies were supported by National Institutes of Health Research Grant NS 17956.

Registry No. (±)-**1**, 6740-88-1; (±)-**2**, 65329-10-4; (±)-**3**, 104010-58-4; (±)-**4**, 103904-69-4; (±)-**5**, 103933-03-5; (±)-**6**, 103904-70-7; (±)-**7**, 103904-71-8; (±)-**8**, 103904-72-9.