Stereochemical Studies of Demethylated Ketamine Enantiomers

SUK CHANG HONG and JOHN N. DAVISSON ˣ

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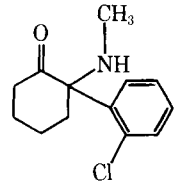
Abstract □ The enantiomorphs of norketamine, 2-(*o*-chlorophenyl)-2-aminocyclohexanone, were synthesized and screened for biological activity. Resolution was achieved by fractional crystallization of the tartrate salts. Stereochemical purity was determined using standard GC or GC–MS analysis. Preliminary pharmacological evaluations revealed that intraperitoneally injected dextrorotatory norketamine caused a greater duration of loss of righting reflex in mice than the levorotatory isomer.

Keyphrases □ Ketamine—stereochemical studies of demethylated ketamine enantiomers □ Enantiomers—stereochemical studies of demethylated ketamine enantiomers □ Stereochemistry—studies of demethylated ketamine enantiomers

Ketamine, 2-(*o*-chlorophenyl)-2-(methylamino)cyclohexanone (I), is rapidly and extensively metabolized (1, 2). The parent compound is demethylated to norketamine (II), which is believed to undergo further oxidation and conjugation reactions. Numerous investigations concerning the pharmacological properties and metabolism of ketamine have been conducted during the past several years (1–5). More recently, the pharmacological actions of the enantiomers of ketamine also have been evaluated (3, 6). Human studies have resulted in the suggestion that dextrorotatory ketamine hydrochloride may be more acceptable as an anesthetic agent when compared to the racemic or levorotatory drug (6). Additionally, levorotatory ketamine hydrochloride was shown to cause significantly higher incidences of unusual dreaming and posthypnotic phenomena than the dextrorotatory isomer. Norketamine resulting from the administration of levorotatory ketamine hydrochloride showed higher plasma levels than that derived from the dextrorotatory enantiomer, especially in the postanesthetic period. Animal experiments have revealed similar patterns of pharmacological activities for the ketamine enantiomers. Stereochemical differences in metabolism and the possibility of certain metabolites having prolonged biological half-lives have been offered as possible explanations for posthypnotic phenomena (3).

Pharmacological data have been reported for racemic norketamine, but no information is available for the purified enantiomers (5). As part of an investigation to determine stereochemical and pharmacological relationships between enantiomers of ketamine and those of metabolites, the synthesis, resolution, and preliminary pharmacological studies of enantiomerically purified norketamine have been completed and are described in this report.



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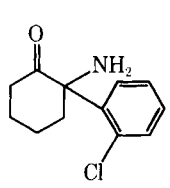

II

EXPERIMENTAL

Instrumentation—Melting points are reported as corrected values[1]. UV[2] and IR[3] spectra were recorded with double-beam spectrometers. NMR[4] spectra were recorded on a 60-MHz apparatus with tetramethylsilane as an internal reference. Optical rotation was measured with an automatic polarimeter[5]. GLC[6] conditions were as follows: glass column, 1.8 m × 2-mm i.d. packed with 3% SP2300 or 3% SP2401 on 100/120 mesh Supelcoport; carrier gas was nitrogen at 30 ml/min; the injector, column, and detector temperatures were 250, 210, and 275°, respectively. GC–MS[7] conditions were as follows: glass column, 1.8 m × 2-mm i.d. packed with 3% SP2300 or 3% SP2401 on 100/120 mesh Supelcoport; carrier gas was helium at 30 ml/min; injector, column, and source temperatures were 270, 230, and 225°, respectively. Ionization was at 75 eV.

Synthesis of Norketamine (II)—1-Bromocyclopentyl(o-chlorophenyl)ketone (III), 50 g (0.18 mole) (7, 8) was placed in a 500-ml round bottom flask containing 300 ml of 28% ammonium hydroxide solution which had been saturated with anhydrous ammonia. The mixture was agitated vigorously for 7 days at room temperature using a mechanical shaker. The dark brown bottom layer was separated, dissolved in 500 ml of cyclohexane–petroleum ether (5:1) solution, and placed in a refrigerator. After 2 days, 24 g (60% yield) of a white solid (mp 90.5–91.5°) was obtained. The IR spectrum revealed strong combined NH—OH stretching at 3225 cm^{-1} and C=N absorption at 1645 cm^{-1} consistent with the iminoalcohol intermediate (7). Without further purification, an isopropyl alcohol solution (200 ml) of 20 g (0.09 mole) of this solid was refluxed for 5 days. Crude norketamine was obtained by evaporation of the solvent under reduced pressure. The residue was dissolved in absolute ether and saturated with dry hydrogen chloride gas. The solid was filtered and dissolved in 50 ml of distilled water. The solution was neutralized with 10% sodium carbonate–ammonium hydroxide solution (1:1) and extracted with methylene chloride (3 × 200 ml). Evaporation of the solvent left a tan oily product (16 g) whose spectral data were identical to those of an analytical reference sample of racemic norketamine[8].

Resolution of Norketamine—A 20-g solution (0.09 mole) of racemic norketamine in 50 ml of methanol was mixed with a methanol solution (200 ml) of (+)-tartaric acid (15.1 g, 0.1 mole). The mixture was stirred overnight at room temperature and filtered. The solvent was evaporated at reduced pressure. The resulting crude solid was washed with 400 ml of 2-butanone and then dissolved in 5.4 liters of refluxing acetone. Fine needle-like crystals (7.5 g) were collected after storing the solution for 2 days at room temperature. The filtrate was saved for later isolation of the other enantiomer.

The bitartrate salt was recrystallized three additional times from acetone resulting in 4.8 g of fine needle-like crystals (mp 181–182°). The free base was obtained with 0.5 *N* NaOH solution and converted into the hydrochloride or bisuccinate salt using standard procedures. The hydrochloride salt was recrystallized from ethanol yielding a fine white solid, mp 175–177°; $[\alpha]_D^{25}$ −96.4° (*C* = 1.0, water). The bisuccinate salt was recrystallized from acetone yielding white platelets, mp 139–140°; $[\alpha]_D^{25}$ −66.2° (*C* = 1.0, water).

Anal.—Calc. for $C_{16}H_{20}ClNO_7$: C, 51.41; H, 5.39; N, 3.75; Cl, 9.48 O, 29.97. Found: C, 51.41; H, 5.39; N, 3.72; Cl, 9.43 O, 30.05. The combined filtrates remaining from the above procedures were evaporated to dryness. The remaining solid was dissolved in water, neutralized with 0.5 *N* NaOH, and extracted with methylene chloride. Following evaporation

[1] Thomas-Hoover Capillary Melting Point Apparatus.
[2] Perkin-Elmer Coleman model 124.
[3] Perkin-Elmer 710A Grating IR Spectrometer.
[4] Hitachi Perkin-Elmer NMR Spectrometer, model R-24A.
[5] Perkin-Elmer model 241.
[6] Hewlett Packard 402 High Efficiency Gas Chromatograph.
[7] DuPont Instruments Dimaspec 321 gas chromatograph-mass spectrometer interfaced with a DuPont Instruments 320 data system.
[8] Parke-Davis/Warner Lambert Co.

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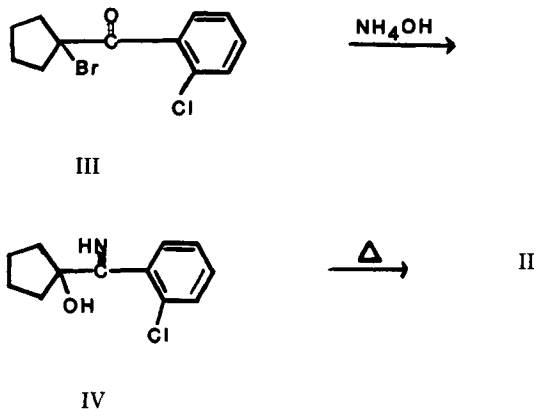


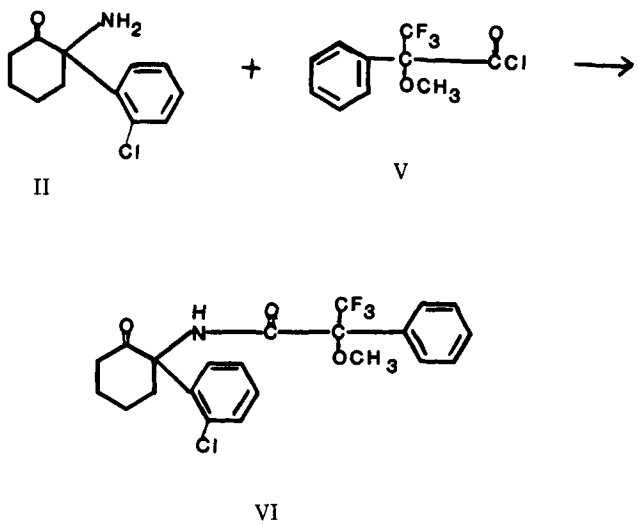
Scheme I—*Synthesis of norketamine.*

of the solvent, the residue was treated with (−)-tartaric acid in the same manner as described for (+)-tartaric acid. The resulting hydrochloride salt was recrystallized from ethanol yielding a fine white solid, mp 176–178°; $[\alpha]_D^{25}$ +95.0° (*C* = 1.0, *water*). The bisuccinate salt (mp 138–140°) was recrystallized from acetone; $[\alpha]_D^{25}$ +66.3° (*C* = 1.0, water).

Anal.—Calc. for $C_{16}H_{20}ClNO_7$: C, 51.41; H, 5.39; N, 3.75; Cl, 9.48 O, 29.97. Found: C, 51.43; H, 5.42; N, 3.73; Cl, 9.45 O, 30.05.

Determination of Stereochemical Purity—(+)-Norketamine bisuccinate, 2 mg (5.3 μmoles), was basified with 0.1 *N* NaOH and extracted with 3.0 ml of methylene chloride. The methylene chloride solution was separated and dried over anhydrous sodium sulfate. A 2-ml aliquot of this solution was mixed with 10 μl of a 0.5-*M* methylene chloride solution of (−)-α-methoxy-α-trifluoromethylphenylacetyl chloride (V) (9). The mixture was heated to 60° for 2 hr, cooled to room temperature and washed with 3 ml of 0.5 *N* HCl, 3 ml of 0.5 *N* NaOH, and 5 ml of distilled water. After drying over anhydrous sodium sulfate, the methylene chloride was evaporated under nitrogen, and the residue was dissolved in 25 μl of methanol. This solution (1–2 μl) was used for GLC and GC–MS analysis. (−)-Norketamine bisuccinate and racemic norketamine bisuccinate were derivatized in the same manner.

Animal Experiments—Male Swiss-Webster mice (25–32 g), provided with rodent laboratory chow[9] and water *ad libitum*, were housed in wire meshed cages (16 × 18 × 24 cm) suspended above indirect bedding. The temperature was maintained at 20–24° and the photoperiod was controlled to provide light from 6 am to 6 pm. Drugs were dissolved in distilled water and all injections were made intraperitoneally. Judgment of loss of righting reflex was made on an all-or-none basis after termination of drug injection. Mice that lost righting reflex were then placed on their backs in individual plastic cages and observed. Righting reflex was judged to be regained when an animal turned to a prone position



Scheme II—*Synthesis of the α-methoxy-α-trifluoromethylphenylacetamide analog of norketamine.*

[9] Purina.

Table 1—Effect of Norketamine Enantiomers upon the Duration of Loss of Righting Reflex in Mice

Compound	Dose[a], mg/kg	No. of Animals	Duration of Loss of Righting Reflex[b], min
(+)	100	8	6.8 ± 0.8
(−)	100	8	No Loss
(+)	200	14	46.1 ± 7.1[c]
(−)	200	14	25.2 ± 2.2[c]

[a] Animals received norketamine intraperitoneally and were observed for duration of loss of righting reflex. [b] Values represent the mean ± *SEM*. [c] $p < 0.01$ according to Student *t* test.

twice within a 10-sec period. All animal experiments were performed between 1 and 6 pm.

RESULTS AND DISCUSSION

The synthesis of norketamine was accomplished by utilizing an aminoketone synthesis introduced previously (7, 8) (Scheme I). The product was obtained directly from the thermal rearrangement of the intermediate Schiff base (IV) in a two-step sequence. This was accomplished in refluxing solvents ranging from methanol (bp 65°) to naphthalane[10] (bp 190°). Lower boiling alcohols produced greater yields with less decomposition than higher boiling solvents. In these studies refluxing IV in isopropyl alcohol resulted in an 80% yield of product after 5 days. Spectral data (IR, NMR) were consistent with the structure of norketamine, and the GLC retention time and mass spectrum were identical to those of an analytical reference sample.

Resolution of norketamine proved to be tedious. Formation of the diastereomeric bitartrate salts and subsequent recrystallization from acetone was found to be the most efficient method of resolution attempted. One major drawback was the large volumes of acetone needed to accomplish the purification.

Progress of the resolution was followed either by measuring optical activity or by GLC analysis. For the latter, diastereomers, formed by reacting the enantiomers with (−)-α-methoxy-α-trifluoromethylphen-

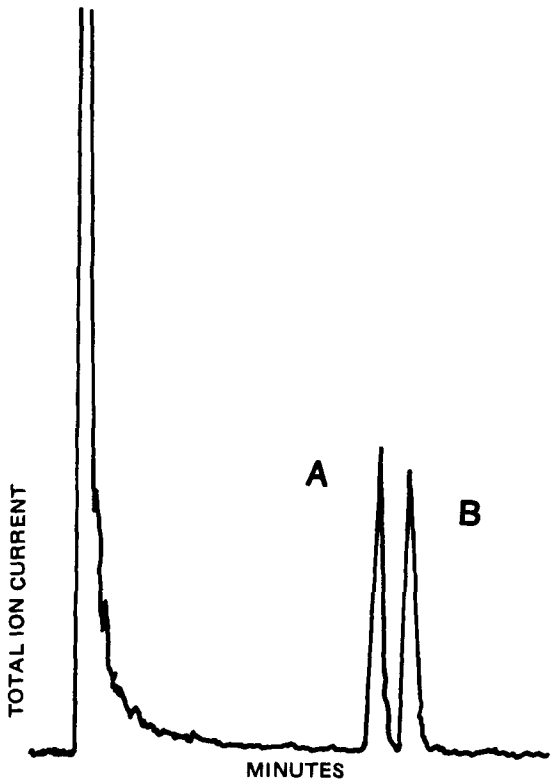


Figure 1—*Reconstructed gas chromatogram of the (−)-α-methoxy-α-trifluoromethylphenylacetamide analogs of (+)-norketamine (A) and (−)-norketamine (B).*

[10] Decalin.

ylacetyl chloride, were chromatographed over 3% SP2401 (9) (Scheme II). Using this system good separation of diastereomers was achieved (Fig. 1). Retention times of derivatized (+)-norketamine and (−)-norketamine were 13.5 and 14.9 min, respectively. These compounds failed to show a parent ion (m/z = 439) using electron impact mass spectroscopy, but when subjected to chemical ionization conditions, both displayed significant M + 1 ions. Fragmentation patterns also were consistent with the structures. When purified enantiomers were derivatized and subjected individually to GLC analysis, no contamination from the other stereoisomer could be detected, indicating that the resolution procedure had resulted in highly stereochemically pure norketamine.

The enantiomers were converted into either hydrochloride or bisuccinate salts. The former proved to be quite hygroscopic making the bisuccinate salts more convenient for subsequent studies. The signs of optical rotation were the same for both salts. The free bases were not highly purified to permit accurate measurements of their optical rotations, but using partially purified samples, it was determined that the signs of rotation for the free bases and the salts were the same. This observation is in contrast to reported optical rotations of ketamine (10). The free bases and hydrochloride salts for this compound show opposite signs of rotation.

Initial pharmacological evaluation revealed that intraperitoneally injected dextrorotatory norketamine bisuccinate caused a greater duration of loss of righting reflex in mice than the levorotatory isomer (Table I). At doses of 100 mg/kg (calculated on free base content) the levorotatory isomer failed to induce loss of righting reflex, whereas this same dose appeared to be near the ED_{50} value for the dextrorotatory form. At 200 mg/kg all animals lost righting reflex with the dextrorotatory compound, producing a significantly greater duration of loss (Table I). These preliminary studies also suggested that levorotatory norketamine bisuccinate may cause greater amounts of central excitation than the dextrorotatory form. This is based on the observation that animals receiving the levorotatory drug appeared to show greater amounts of spontaneous locomotor activity than those receiving the dextrorotatory isomer.

Similar actions as to loss of righting reflex and central excitation have been reported for the ketamine enantiomers (3). Also, it has been shown that racemic norketamine and ketamine are qualitatively similar with regard to central nervous system depressant effects and posthypnotic excitation (5). Because of the these findings, it is tempting to speculate that the levorotatory salts of ketamine and norketamine have identical stereochemical configurations; the same being true for the dextrorotatory forms. However, additional studies will be needed to establish the exact stereochemical relationships between these compounds as well as the pharmacological significance of the norketamine enantiomers.

REFERENCES

(1) T. Chang, W. A. Dill, and A. J. Glazko, *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, **24**, 268 (1965).

(2) M. L. Cohen and A. J. Trevor, *Anesthesiology*, **39**, 370 (1973).

(3) M. P. Marietta, W. L. Way, N. Castagnoli, Jr., and A. J. Trevor, *J. Pharmacol. Exp. Ther.*, **202**, 157 (1977).

(4) D. A. McCathy, G. Chen, D. H. Kaump, and C. Ensor, *J. New Drugs*, **5**, 21 (1965).

(5) M. L. Cohen and A. J. Trevor, *J. Pharmacol. Exp. Ther.*, **189**, 351 (1974).

(6) P. F. White, J. Ham, W. Way, and A. J. Trevor, *Anesthesiology*, **52**, 231 (1980).

(7) C. L. Stevens, A. Thuillier, K. G. Taylor, F. A. Daniher, J. P. Dickerson, H. T. Hanson, N. A. Nielsin, N. A. Tikotkar, and R. M. Weier, *J. Org. Chem.*, **31**, 2601 (1966).

(8) C. L. Stevens, A. Thuillier, and F. A. Daniher, *ibid.*, **30**, 2962 (1965).

(9) J. Gal, *J. Pharm. Sci.*, **66**, 169 (1977).

(10) P. Newman, "Optical Resolution Procedures for Chemical Compounds," Optical Resolution Information Center, Manhattan College, Riverdale, N.Y., 1978, pp. 252–253.

Preparations of Solid Particulates of Theophylline–Ethylenediamine Complex by a Spray-Drying Technique

H. TAKENAKA *, Y. KAWASHIMA *ˣ, S. Y. LIN *, and Y. ANDO ‡

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Abstract □ Aqueous solutions of ethylenediamine and theophylline were spray dried to obtain solid particulates of theophylline–ethylenediamine complex to improve solubility of theophylline. Packing and flow properties of the spray-dried products were much improved when compared with those of original theophylline particles, due to their spherical shapes which were confirmed by a scanning electron microscope. The solubility of theophylline in the resultant products was found to be three to five times higher than that of original theophylline. The solubilities of the products decreased with increasing drying temperature and rotation speed of the atomizer, which was interpreted in terms of the contents of ethylenediamine in the products. The products were confirmed to be a mixture of aminophylline, α-aminophylline, and theophylline by X-ray analysis and NMR spectroscopy. The logarithm of the relative intensity of the X-ray diffraction peak of α-aminophylline to that of theophylline decreased linearly with drying temperature and rotation speed of the atomizer. Thermal decomposition of the spray-dried products involved liberations of crystal water at 100° and ethylenediamine between 110 and 127°. Liberation of ethylenediamine occurred *via* three steps for aminophylline, but with different steps for the spray-dried products.

Keyphrases □ Complexation—theophylline–ethylenediamine, preparations, spray-drying technique, solid particulates □ Theophylline—complexation with ethylenediamine, preparations, solid particulates, spray-drying technique □ Ethylenediamine—complexation with theophylline, preparations, solid particulates, spray-drying technique

The spray-drying technique has been accepted as a favorable method for drying a variety of heat-sensitive materials, such as foods, pharmaceuticals, enzymes, *etc.* The preparation of particulate solids from liquid droplets by chemical reaction during drying is one of the recent uses of this technique. Microcapsules of barbituric acid and phenobarbital with a tensioactive precondensate of the hexamethylolmelamine type, which form macromolecules under the influence of heat, have been prepared previously (1, 2). An ammonium sulfate sphere has been produced by the reaction of a single drop of phosphoric acid with gaseous ammonia (3).

The objective of the present study was to prepare solid particulates of the theophylline–ethylenediamine complex (*e.g.*, aminophylline) by a spray-drying technique. The manufacturing method of aminophylline referred to in the

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