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Synthesis and absolute configuration of optically pure enantiomers of a κ-opioid receptor selective agonist

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The enantiomers of U50,488 (**1**, in fig. 1), ligands highly selective for κ-opioid receptors, have been prepared by a refined procedure and their optical purity demonstrated. The absolute configuration of (+)-*trans*-2-pyrrolidinyl-*N*-methylcyclohexylamine, a chemically versatile intermediate for synthesis of analogs of κ-opioid receptor ligands with defined chirality, has been determined to be 1*S*,2*S* by X-ray crystallographic analysis. This intermediate has been used to synthesize the optically pure U50,488 enantiomers with known absolute configuration.

X-ray analysis; U50,488 synthesis; Absolute configuration; (+)-*trans*-2-Pyrrolidinyl-*N*-methylcyclohexylamine; κ-Opioid receptor agonist

1. INTRODUCTION

Many converging lines of pharmacological and biochemical investigation have provided convincing evidence for the existence of saturable, stereoselective and high-affinity μ-, δ- and κ-opioid receptor subpopulations in the mammalian CNS [1]. Such studies have raised many questions concerning the structure and function of these subtypes in the overall modulation of the CNS. A variety of structurally diverse opioid ligands have been required for demonstration of these subtypes and continue to be essential in defining receptor subtype function. In many cases, enantiomeric pairs of these ligands played a central role in detecting receptor mediated effects because of the high in vivo and in vitro selectivity for one member of the optical pair. Such a recent striking example was the utilization of the narcotic antagonist (−)-naloxone and the pharmacologically inert (+)-isomer as tools for demonstration of opioid receptor occupancy by [^{18}F]cyclofoxy in the living baboon brain by positron emission tomography (PET) scanning [2].

Our previous studies of the μ- and δ-opioid receptors [3,4] have now been extended to include the κ receptors. For our initial work, we selected U50,488 which is among the most selective ligands for the κ-opioid receptor subtype. Since it is well known that drug enantiomers can show distinctly different and in some cases opposite effects [5], it

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Abbreviation: U50,488, (±)-*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl-1]benzene-acetamide

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is imperative to utilize enantiomers of known optical purity for receptor studies. In addition, knowledge of the absolute configuration of a molecule, and bond angles and bond lengths around chiral centers determined from X-ray crystallographic analysis, are essential for molecular modeling and other studies aimed at detection of commonalities between structurally diverse classes of drugs which act at a given receptor subtype. We have now developed a refined synthesis of the enantiomers of U50,488 previously described in the patent literature [6,7], and have demonstrated their optical purity and determined their absolute configuration by single crystal X-ray analysis.

2. MATERIALS AND METHODS

2.1. *Chemical synthesis*

Melting points were determined on a Thomas Hoover capillary apparatus and are uncorrected. HPLC (high-performance liquid chromatography) was performed with a Gilson model 303 with a solvent mixer (model 811), and a Data Master (model 620). Optical rotations were measured with a Perkin Elmer 241 MC polarimeter, and GC (gas chromatography) on a Hewlett-Packard 5880A instrument, with an SE-30 capillary column. A Nicolet model R3M automatic X-ray diffractometer in $\theta 2\theta$ collection mode was used for X-ray crystallography [8]. All new compounds gave combustion analyses for carbon, hydrogen and nitrogen within $\pm 0.4\%$ of the calculated value and were performed by Atlantic Microlabs, Atlanta, GA. Each compound gave mass, NMR (nuclear magnetic resonance), and IR (infrared) spectra consistent with their assigned structure. TLC system A refers to Analtech silica gel (GF) plates, eluting with a mixture of chloroform/methanol/conc. NH_4OH (80:18:2) and system B refers to the same system with a ratio of 90:9:1.

2.2. *(±)-*trans*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(±)-2]*

Trifluoromethanesulfonic acid (4.9 ml, 0.1 equiv.) was added dropwise (with caution) to a stirred mixture of *N*-methylcyclohexaneaziridine [6,9,10] (62.0 g, 0.56 mol), and pyrrolidine (280 ml, 6 equiv.) at $-10°C$, and the solution was refluxed overnight under argon. Removal of excess pyrrolidine gave a two phase oily residue. The upper phase with product **2** was removed, and the lower phase was dissolved in water (100 ml), 20% KOH solution was added, and the mixture was ether extracted (4 × 100 ml). The ethereal solution was dried (KOH pellets), and the ether removed by distillation to give additional **2** which, combined with the upper phase, was fractionally distilled (b.p. 76–80°C/1.2 mm, in [10] b.p. 118–119°C/13 mm) to yield 94.8 g (93%) of **2** as a pure (by GC and TLC system A), clear colorless liquid. Treatment of **2** with HCl gas in isopropanol afforded a crystalline dihydrochloride hemihydrate salt: m.p. 242–243°C.

2.3. *(1S,2S)-(+)-*trans*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(+)-2]*

Initial resolution of (±)-**2** was carried out on a small scale by recrystallization (6×, isopropanol/ether) of the 2:1 salts of (±)-**2** and (+)- or (−)-camphor-10-sulfonic acid to give 99% diastereomerically pure salts: m.p. 188–190°C. Conversion of each of the salts to their free base (with NH_4OH), was followed by formation of their mandelate salt, on a small scale. These salts were used to seed the large scale preparation. In a large scale preparation, ethyl acetate (900 ml) was added to a solution of (±)-**2** (80.22 g, 440 mmol) and (*S*)-(+)-mandelic acid (66.95 g, 440 mmol) in methanol (300 ml). Crystallization occurred after distillation of the methanol from the solution, while maintaining a constant volume of 900 ml (by addition of ethyl acetate), and addition of a seed crystal. The crystals from the cooled mixture were washed twice with cold (0°C) ethyl acetate; recrystallization (3×, ethyl acetate) afforded 66.3 g (90%) of (+)-**2**. (+)-Mandelate: m.p. 168–169°C; $[\alpha]_D^{23}$ +106.4° (*c* 0.33, MeOH). The optical purity of (+)-**2** base (b.p. 72°C/0.7 mm; $[\alpha]_D^{23}$ +97.6° (*c* 0.29, MeOH)) was determined to be >99.5% (vide infra). (+)-**2**·HCl·½H_2O: m.p. 245–246°C, $[\alpha]_D^{23}$ +27.5° (*c* 0.276, MeOH); in [6] m.p. 247.5–248.5°C, $[\alpha]_D^{22}$ +30° (*c* 0.97, $CHCl_3$).

2.4. *(1R,2R)-(−)-*trans*-2-Pyrrolidinyl-*N*-methylcyclohexylamine [(−)-2]*

Solvent was removed from the combined mother liquors of the (+)-**2**. (+)-Mandelate salt (vide supra), water (300 ml) was added to the crystalline

residue, and the mixture rendered strongly alkaline with 30% aqueous NaOH (300 ml). Extraction with ether, drying (Na_2SO_4), and fractional distillation gave 40.1 g (91% recovery) of the mixed bases. The mixed bases were dissolved in MeOH (150 ml), *R*-(−)-mandelic acid (33.5 g, 1 equiv.) added, followed by ethyl acetate (900 ml). Distillation, crystallization (induced by a seed crystal), and recrystallization as above, gave 61.8 g (84%) of mandelate salt: m.p. 168–169°C; $[\alpha]_D^{23}$ −105.9° (*c* 0.35, MeOH). The base was generated as above for the (+)-isomer: b.p. 72°C/0.7 mm; $[\alpha]_D^{23}$ −97.4° (*c* 0.71, MeOH) (>99.5% optically pure, as determined below). (−)-**2**·HCl·½H_2O: m.p. 246–246.5°C, $[\alpha]_D^{23}$ −28.2° (*c* 0.28, MeOH); in [6] m.p. 240–242°C; $[\alpha]_D^{23}$ −28° (*c* 0.8, $CHCl_3$).

2.5. *Optically pure (R)-α-methylbenzylisocyanate*

A solution of (−)-tartaric acid (150.09 g, 1 mol) in DMF (1 l) was treated with 121.18 g (1 mol) of commercial (+)-α-methylbenzylamine that had a 3.8% optical impurity. After crystallization of the salt was complete (cooling to 20°C), the salt was filtered and washed thoroughly with DMF (3 × 500 ml), then thoroughly with ether (3 × 500 ml). The dried (in vacuo, 50°C) salt (220.1 g) was dissolved in DMF (660 ml, 120°C), and crystallized when cooled. It was filtered, pressed well, washed with ether (2 × 500 ml), and dried (in vacuo, 50°C) to give 180.25 g of product. Twice crystallized salt (175.0 g, 0.65 mol) was converted to the free base by addition of a cold solution of NaOH (102 g, in 600 ml of water). The base was washed with ether (200 ml, 100 ml), dried with $MgSO_4$ (3 g), filtered and distilled (b.p. 83–85°C/23 mm) to give (+)-α-methylbenzylamine (70.91 g). Redistillation gave the chemically and optically (by HPLC analysis of the ureas, below) pure free base (69.9 g): $[\alpha]_D^{24}$ +39.3° (neat, d = 0.9528). For determination of the enantiomeric purity of (+)-**2** and (−)-**2**, the isocyanate was prepared. A rapidly stirred solution of (+)-α-methylbenzylamine (6.0 g), rendered acidic with HCl gas, was treated dropwise at reflux with 25 ml of a 20% solution of phosgene in toluene. When the solution became homogeneous, it was distilled to yield the isocyanate (5.29 g, 73%): b.p. 55°C/0.35 mm; $[\alpha]_D^{23}$ +9.83°; (neat, d = 1.0428) (in [11] $[\alpha]_D^{19}$ +9.6°; (neat, d = 1.0428)).

2.6. *Determination of the optical purity of (+)-2 and (−)-2*

(*R*)-(+)-Methylbenzylisocyanate (1.2 equiv.) was added (under nitrogen, via syringe) to a stirred solution of (+)-**2** or (−)-**2** (10 mg, 0.055 mmol) in pentene stabilized chloroform (1 ml). The reaction was complete (by TLC, system A) after 10 min at room temperature. The respective diastereomeric ureas **3** and **4** (fig.1) were analyzed by HPLC on a 20 cm Waters μ-Porasil analytical column with 0.4:3.6:96 ammonia/methanol/chloroform as eluant (UV detection at 254 nm). The HPLC analysis showed >99.5% diastereoisomeric purity of **3** and **4**, indicating similar optical purity of (+)-**2** and (−)-**2**.

2.7. *(1S,2S)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]-benzeneacetamide [(−)-1]*

A solution of 3,4-dichlorophenylacetylchloride (2.94 g, 1.2 equiv.) in pentene stabilized chloroform (10 ml) was added to a rapidly stirred mixture of (+)-**2** (2.00 g, 11.0 mmol) in pentene stabilized chloroform (40 ml) and $NaHCO_3$ (saturated solution, 25 ml). After 15 min, TLC system B indicated that the reaction was complete. The organic phase was washed with water (50 ml) and dried (Na_2SO_4) to give a yellow oil. Citric acid was added (2 equiv., based on the theoretical quantity of product), followed by water (200 ml) and ether (200 ml), and the mixture shaken until homogeneous. The aqueous layer was extracted with ether (2 × 100 ml), and basified (conc. NH_4OH). Extraction with chloroform (3 × 100 ml) and drying through a plug of Na_2SO_4 afforded 3.75 g of (−)-**1** as an oil. Addition of l-tartaric acid (1.48 g, 1 equiv.) gave the tartrate salt. Crystallization from H_2O/isopropanol (1:19) gave the l-tartrate·monohydrate of (−)-**1** (4.99 g): m.p. 153–154°C; $[\alpha]_D^{23}$ −35.2° (*c* 0.28, MeOH). The HCl salt of (−)-**1** crystallized from 2 M HCl as a monohydrate: m.p. 91–94°C, $[\alpha]_D^{23}$ −32.9° (*c* 0.34, MeOH).

2.8. *(1R,2R)-trans-3,4-Dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl-1]-benzeneacetamide [(+)-1]*

The base (−)-**2** (2.00 g, 11.0 mmol), was converted to the d-tartrate·monohydrate salt of (+)-**1** (4.56 g), using the procedure described for (−)-**1**:

m.p. 155–156°C; $[\alpha]_D^{23}$ +36.9° (*c* 0.22; MeOH). The HCl salt of (+)-**1** crystallized from 2 M HCl as a monohydrate: m.p. 91–94°C, $[\alpha]_D^{23}$ +31.8° (*c* 0.31, MeOH).

2.9. *X-ray analysis*

The (*R*)-(−)-mandelate (1:1) salt of (+)-**2** crystallized in the triclinic space group P1 with two $C_{12}H_{23}N_2^+ \cdot C_8H_7O_3^-$ 'formula units' in the asymmetric unit. Cell dimensions are: a = 8.955(3), b = 9.782(2), c = 11.968(3) Å, α = 85.56(2), β = 71.30(2) and γ = 69.82(2)°. Vol. = 931.3(5) Å^3, d_{calc} = 1.193 g/cm^3, λ(Mo $K\alpha$) = 0.71069 Å, μ = 0.46 mm^{-1} and T = 225 K. The final R values are R = 0.049, and wR = 0.051 for the 2922 independent observed reflections. The goodness of fit parameter was 1.498 and the difference map was featureless.

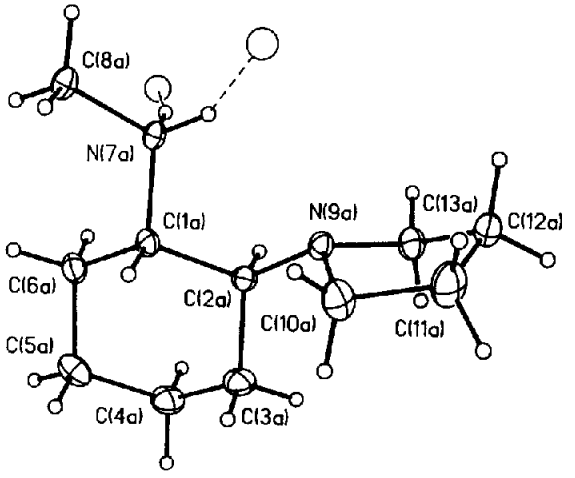

Fig.2. X-ray structure of (1*S*,2*S*)-(+)-*trans*-2-pyrrolidinyl-*N*-methylcyclohexylamine cation drawn from experimentally determined coordinates. The mandelate anion and the other crystallographically unique formulae units are omitted. The dashed lines are hydrogen bonds to mandelate anions.

3. RESULTS AND DISCUSSION

We have developed an efficient route to the optically pure enantiomers of U50,488 which make them available in quantity for various pharmacological studies. The route proceeds through a facile optical resolution of (±)-**2** to afford the enantiomeric intermediates (+)-**2** and (−)-**2** (fig.1). The absolute configurations of the U50,488 enantiomers, which follow from the absolute configuration of the enantiomer (+)-**2** (1*S*,2*S*, based on the known configurations of (*R*)-(−)-mandelic acid), will undoubtedly be of considerable value in establishing structure-activity relationships with

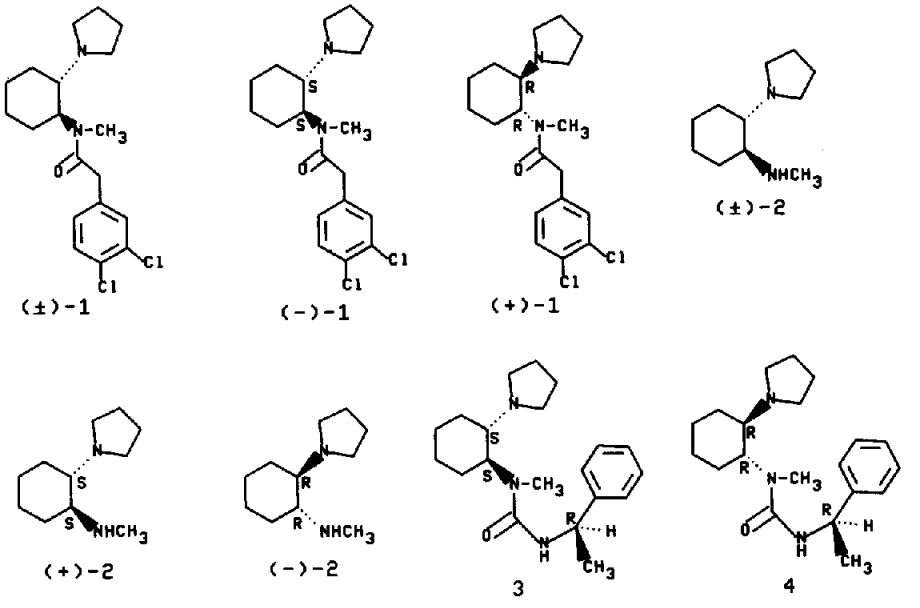

Fig.1. Structures of compounds.

other classes of ligands which interact with κ-opioid receptors. Fig.2 shows the result [8] of the X-ray analysis for the cation (+)-**2**. There are two intermolecular hydrogen bonds between the cation and mandelate anions with the amine nitrogens acting as donors to the mandelate oxygens. Intermolecular hydrogen bonding also occurs between the mandelate anions. The determination of the absolute configuration of the chemically versatile intermediate (+)-**2** (and, of course, (−)-**2**) allows the future synthesis of a host of different analogs of U50,488 which will have known absolute configuration at two centers of asymmetry. This will permit qualitative and quantitative research on the relationship of the structure of analogs of U50,488 to their experimentally determined activity on opioid receptor subtypes.

NOTE ADDED IN PROOF

Initial binding experiments using [^{3}H]bremazocine to label a κ-opioid receptor of guinea pig brain demostrated that (−)-**1** and (+)-**1** interacted with dissociation constants (K_d) of 124 and 90 300 nM, respectively (Rothman, R.B. et al., in preparation).

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