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SYNTHESIS OF TRITIUM LABELLED (-)-U50,488, A SELECTIVE KAPPA OPIOID AGONIST

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SUMMARY

The preparation of ^{3}H labelled (-)-trans-3,4-dichloro-N-methyl-N-[2-(1-pyrrolidinyl)cyclohexyl]benzeneacetamide (U50,488) in four steps from N-methylcyclohexylaziridine is described. The synthesis of the pharmacologically active (-) isomer of U50,488 was accomplished through the resolution of the intermediate 2-[1-(3-pyrrolinyl)]-N-methylcyclohexylamine using (+)-mandelic acid .

Key words: U50,488, kappa opioid receptor agonist, (+)-trans-2-[1-(3-pyrrolinyl)]-N-methylcyclohexylamine, tritium.

INTRODUCTION

The heterogeneity of opiate receptors within the mammalian central nervous system has been demonstrated by many converging lines of pharmacological and biochemical investigation. The distinction of opiate receptors into three classes, referred to as mu, delta and kappa, has largely been attributable to the discovery of selective high affinity ligands for each of the sites (1). The pharmacological profiles of agonists within each class are often distinct. The kappa agonists are unique among the three classes in that they have the potential to deliver an analgesic effect to the central nervous system without the concomitant problems of constipation and respiratory depression (2). The phenylacetamide U50,488 (**4**) has been shown to be among the most selective agonists yet known for the kappa opiate receptor (2,3). Recently the enantiomers of U50,488 have been

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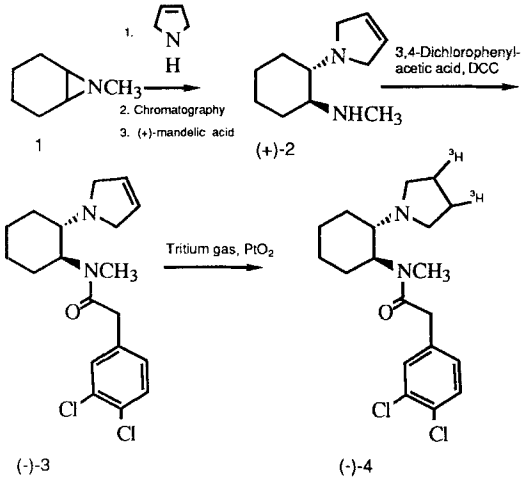
prepared and their absolute configuration determined by single crystal X-ray crystallography (4). Binding experiments using [^{3}H]bremazocine to label a kappa opioid receptor in guinea pig brain indicate that the kappa agonist action of U50,488 is isolated in the (-) enantiomer, having configuration 1S,2S. Optically pure tritium labelled (-)-U50,488 would be of great use to investigators interested in studying the kappa opiate receptor.

Scheme 1

Synthesis of Tritium Labelled U50,488



Results and Discussion

The synthetic pathway for preparing ^{3}H labelled 4 is shown in Scheme 1. The synthesis parallels that used by de Costa et al (4) to prepare unlabelled (-)-U50,488. Use of the unsaturated 3-pyrroline in place of pyrrolidine provides the opportunity for the introduction of the tritium label. The N-methylcyclohexyl-aziridine (1) used as a starting material was prepared as previously described (5). Compound 1 was condensed with commercially available 3-pyrroline (Aldrich Chemical Company, containing 25% pyrollidine) to provide racemic trans-2-[1-(3-pyrrolinyl)]-N-methylcyclohexylamine (2) which could be

chromatographically separated from its saturated impurity. Resolution of **2** could be accomplished by selective crystallization of the mandelic acid salts. The absolute configuration of (+)-**2** was confirmed by hydrogenation over platinum oxide to the known (1S,2S)-(+)-trans-2-(1-pyrrolidinyl)-N-methylcyclohexylamine (4). Reaction of (+)-**2** with 3,4-dichlorophenylacetyl chloride gave the unsaturated U50,488 derivative **3**. Catalytic hydrogenation of **3** using platinum oxide as the hydrogenation catalyst led to the desired **4**. Considerable experimentation was required to determine proper hydrogenation conditions which would minimize reduction of the aromatic chlorine atoms. The short hydrogenation time is of crucial importance as longer exposure resulted in considerable loss of the chlorine atoms. The monochloro and deschloro derivatives were easily separable on TLC from **4**.

EXPERIMENTAL

Materials and Methods

Radioactivity determinations were carried out with a Packard Model 2200CA liquid scintillation counter using Hydrofluor scintillation solvent. Thin layer chromatography (TLC) plates were analyzed with a Berthold Model LB 2760 TLC scanner. All synthetic and analytical operations were initially performed with unlabelled compounds, and the structures of the unlabelled intermediates and products were confirmed spectroscopically.

trans-2-[1-(3-Pyrrolinyl)]-N-methylcyclohexylamine (**2**).

3-Pyrroline (5.0 g, 72.5 mmol) was mixed with N-methylcyclohexylaziridine (1.6 g, 14.5 mmol). To the resulting mixture was added a catalytic amount of trifluoromethanesulfonic acid (100 mg) and the reaction warmed to 60° C for 13 h. Unreacted 3-pyrroline was removed by distillation at atmospheric

pressure after which the diamine products (2.1 g, 81%) were purified by fractional distillation (bp 78° C, 0.9 mm). The unsaturated compound 2 was purified from the pyrollidine derivative by chromatography on silica gel eluting with a 0.5/4.5/95 ratio of NH_4OH/methanol/chloroform to give 1.42 g of the desired product, b.p. 81°C (1.2 mm).

(1S,2S)-(+)-trans-2-[1-(3-Pyrrolinyl)]-N-methylcyclohexylamine [(+)-2].

The base (2, 1.39 g, 8.37 mmol) and (+)-mandelic acid (1.27 g, 1.0 equiv) were mixed together in methanol (20 mL) and the solution reduced to 5 mL on a hot plate. Ethyl acetate (60 mL) was added and the solution further reduced to a total volume of 50 mL before the solution was cooled to room temperature. The resulting crystalline material was filtered and washed with cold ethyl acetate to give 0.81 g of material which was recrystallized from 30 mL of ethyl acetate to give 0.66 g of (+)-2 mandelate as white needles, m.p. 151-152°C, $[\ ]^D_{23}$ +104.7° (c 0.31, MeOH). Anal. Calcd for $C_{19}H_{28}N_2O_3$: C, 68.64; H, 8.50; N, 8.43. Found: C, 68.54; H, 8.53; N, 8.34. A sample of (+)-2 was hydrogenated over platinum oxide and the resulting saturated amine was found to be identical in all respects (IR, NMR, MS, $[\ ]^D_{23}$) to an authentic sample of (1S,2S)-(+)-trans-2-(1-pyrrolidinyl)-N-methylcyclohexylamine.

(-)-trans-3,4-Dichloro-N-methyl-N-[2-[1-(3-pyrrolinyl)]-cyclohexyl]benzeneacetamide (3). To a mixture of (+)-2 mandelate (0.300 g, 0.9 mmol) and triethylamine (3.0 mL) in 10 mL of chloroform was added in one portion a solution of 3,4-dichlorophenylacetyl chloride (0.242 g, 1.1 mmol) in 2 mL of chloroform. After 10 min, the reaction was poured into a separatory funnel containing 50 mL of diethyl ether and 20 mL of

saturated sodium bicarbonate solution. The lower aqueous layer was removed and the organic layer was extracted with 50 mL of 10 % aqueous citric acid solution. The citric acid layer was removed, treated with 15 mL of NH_4OH and extracted with methylene chloride (2 X 50 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated to give 0.26 g (91 %) of the product as a colorless oil. The hydrochloride salt was prepared in methanol and recrystallized from ethyl acetate, m.p. 248-249°C, $[\ ]^{D}_{23}$ -18.4° (c 0.28, MeOH). Anal. Calcd for $C_{19}H_{25}Cl_3N_2O$ $3/2H_2O$: C, 52.97; H, 6.55; N, 6.50. Found: C, 53.24; H, 6.36; N, 6.46.

[^{3}H]-(-)-U50,488 (4). An atmosphere of carrier free tritium gas (58 Ci/mM) was applied for 20 min to a solution of 10 mg of 3 hydrochloride in 3 mL of methanol containing 3 mg of platinum oxide as the hydrogenation catalyst. After removal of the tritium atmosphere, the reaction was filtered and concentrated under a stream of nitrogen. Purification by preparative thin layer chromatography gave **4** (40 mCi, 21 Ci/mmol, 3% radiochemical yield). TLC R_f = 0.47 (9% methanol/90% chloroform/1% NH_4OH), identical to the cold material. The [^{3}H]-(-)-U50,488 was shown to be >99% radiochemically pure using the Berthold TLC scanner.

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