Synthesis and Pharmacological Evaluation of an 8β-Bis(2-chloroethyl)amino Opiate as a Nonequilibrium Opioid Receptor Probe

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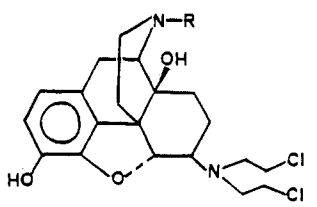
8β-[Bis(2-chloroethyl)amino]-6,7-didehydro-3-hydroxy-17-methyl-4,5α-epoxymorphinan (3) was synthesized from codeine, and its configuration at C-8 was determined by NMR. When evaluated in the guinea pig ileum and mouse vas deferens preparations, 3 was found to be a feeble, reversible agonist in both tissues without any irreversible agonist or antagonist activity. The fact that the 8β-bis-(2-hydroxyethyl)amino analogue was devoid of opioid activity suggests that steric hindrance to ligand–receptor association by a bulky 8-substituent may be responsible for this inactivity.

β-Chlornaltrexamine (1, β-CNA) and β-chloroxymorphamine (2, β-COA) are selective affinity labels for opioid receptors.[1,2] Both of these ligands bind covalently to opioid receptors in vitro and in vivo and are finding wide use as pharmacological tools.[3-14] The covalent binding capacity of β-CNA and β-COA is conferred by the nitrogen mustard group at the 6β-position of the opiate. Presumably, a neighboring nucleophile on the receptor becomes alkylated by the highly reactive aziridinium intermediate that is generated.

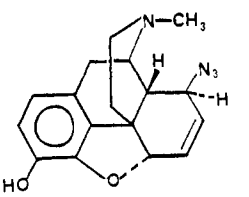


1 R=$CH_2CH(CH_2)_2$

2 R=CH_3

In an effort to explore the accessibility of other nucleophiles that might be present on opioid receptors, we decided to determine whether or not an electrophilic moiety attached to the C-8 position would give rise to irreversible activity. This report describes the synthesis and biological evaluation of such a ligand, 3.

Chemistry. The target compound 3 was synthesized from codeine (4) as outlined in Scheme I. Treatment of codeine with thionyl chloride in anhydrous chloroform afforded 6-chlorocodide (5).[15] Reaction of 5 with diethanolamine gave the S_N2' product 6 in which the diethanolamino group is in the 8β-position. The stereochemical assignment was based on the generally recognized S_N2' mode of nucleophilic attack[16] in 5 and on the magnitude of $J_{8,14}$ (10.4 Hz). This large J value obtained from the decoupled spectrum is in harmony with a diaxial arrangement between the C-8 and C-14 protons, and, therefore, the diethanolamino group of 6 possesses the β configuration. A comparable coupling constant has been reported[15] for the 8β-chloro compound ($J_{8,14}$ = 9.9 Hz). The 8β-azido compound 8 prepared by a published procedure,[17] also possessed a similar value ($J_{8,14}$ = 10.6 Hz). An important factor that contributes to the formation of the 8β isomer is the hindered α-face of 5. Demethylation of 6 with boron tribromide afforded compound 7, which then was converted to the target compound 3 with thionyl chloride. The product was unstable at pH 7.5, with approximately 10% decomposition occurring over a period of 30 min.



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Results and Discussion

Since the first step leading to covalent bonding of opioid receptors is the formation of a reversible ligand–receptor complex, we conducted a preliminary study with the easily accessible azide 8 in order to determine if a C-8 substituent would interfere with agonist activity. When tested on the guinea pig longitudinal muscle preparation[18] (GPI), 8 was found to be approximately one-fifth as potent (IC_{50} = 335 nM; n = 2) as morphine. Moreover, our observation that the agonist concentration–response curve was shifted to tenfold higher concentration with a decline in the maximum (50%) when the preparation was depleted of functional μ receptors with β-funaltrexamine[13] (β-FNA) suggested that 8 was mediating its agonist effect mainly

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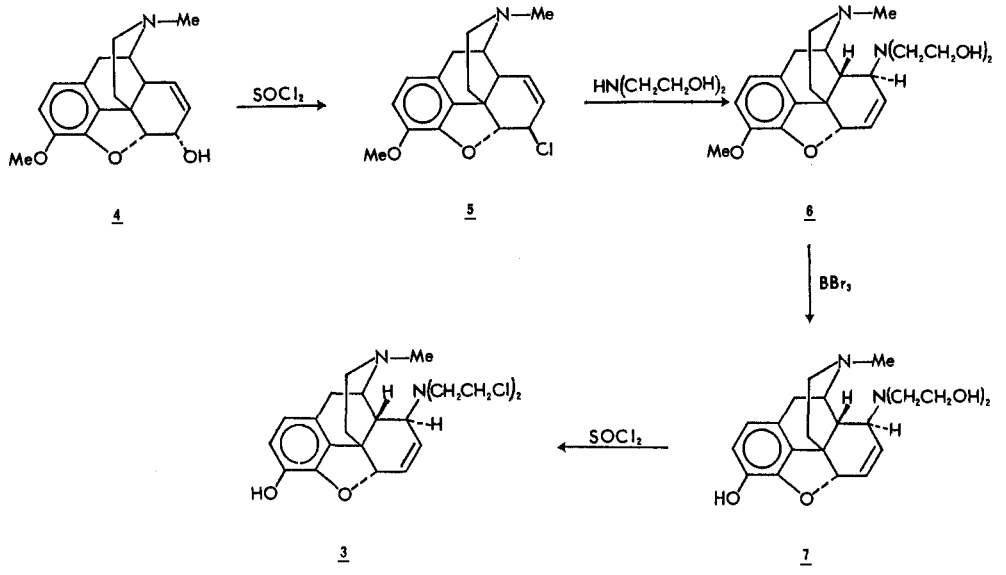
Scheme I



through μ receptors. On the mouse vas deferens (MVD),[19] azide **8** possessed 19 times greater potency (IC_{50} = 46 ± 18 nM; n = 4) than morphine and 200 times less activity than [D-Ala^2,D-Leu^5]enkephalin (DADLE).

Since these results suggested that substitution at C-8 would not seriously impair the reversible binding to opioid receptors, we then prepared the 8-substituted nitrogen mustard analogue **3**. When tested on the GPI, **3** behaved as a partial agonist (15% maximal response at 1 μM; n = 2) and showed no irreversible agonist or antagonist activity after incubation (200 nM for 30 min). Similarly, **3** exhibited partial agonist activity (25% maximal response at 200 nM; n = 2) in the MVD. At higher concentrations (10 μM), **3** potentiated the electrically stimulated contraction of this preparation. No significant irreversible agonist or antagonist activity was observed.

In view of the feeble agonist potency of **3**, the absence of irreversible activity might be a reflection of low affinity for opioid receptors. Indeed, it was found that its precursor alcohol **7** was devoid of reversible agonist or antagonist activity at a concentration 1 μM or greater, lending credence to the idea that substitution of a bulky substituent at C-8 is detrimental to the formation of a reversible complex. Thus, while smaller groups (e.g., azido) are tolerated at C-8, the present study reveals that this position cannot accommodate the variety of substituents that have been reported[20-24] to be attached to the C-6 position of the opiates.

Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ, and were within ±0.4% of the theoretical values. IR spectra were determined on a Perkin-Elmer Model 281 infrared spectrophotometer. NMR data (δ) were obtained at ambient temperature on a Nicolet 270 MHz nuclear magnetic resonance spectrometer with Me_4Si as internal standard. Mass spectra were obtained on an AEI MS-30 instrument.

6β-Chloro-7,8-didehydro-4,5α-epoxy-3-methoxy-17-methylmorphinan (5). To a mixture of codeine (1.5 g, 5 mmol) and DMF (2.01 g, 30 mmol) in anhydrous chloroform (10 mL) was added a solution of thionyl chloride (1.78 g, 15 mmol) in anhydrous chloroform (5 mL). The resulting mixture was heated at 80 °C for 4 h. After cooling, the mixture was poured into ice–water (100 mL), basified with NH_4OH, and extracted with $CHCl_3$ several times. The combined extracts were washed with water and dried ($MgSO_4$). The chloroform was evaporated, and the residue was crystallized from ethanol to afford 1.04 g (63%) of **5**, mp 150–152 °C (reported[25] mp 151–153 °C).

6,7-Didehydro-8β-[(2-hydroxyethyl)amino]-4,5α-epoxy-3-methoxy-17-methylmorphinan (6). Compound **5** (1.0 g, 3.1 mmol) was suspended in diethanolamine (1.32 g, 12.4 mmol) and heated in an oil bath at 110–115 °C for 6 h. After the mixture was cooled, water (5 mL) was added with vigorous stirring to afford 1.1 g (88%) of crude **6** as a solid, which was recrystallized from EtOAc–MeOH: mp 142–144 °C; EIMS, m/e 386 (M^+); NMR (base in $CHCl_3$) δ 4.95 (d, 1 H, $J_{5,6}$ = 2.8 Hz, H-5), 5.87–5.81 (m, 2 H, H-6 and H-7), 2.63 (m, 1 H, H-8), 2.33 (m, 1 H, H-14, $J_{8,14}$ = 10.4 Hz), 4.47 (2 H, OH). Anal. ($C_{22}H_{30}N_2O_4$) C, H, N.

6,7-Didehydro-8β-[(2-hydroxyethyl)amino]-4,5α-epoxy-3-hydroxy-17-methylmorphinan Dihydrochloride (7). To a solution of **6** (130 mg, 0.33 mmol) in anhydrous chloroform (2 mL) was added, with vigorous stirring, a solution of boron tribromide (0.45 mL, 1.9 mmol) in dry chloroform (8 mL). The temperature was maintained at −70 °C. The mixture then was warmed to −30 °C for 1 h and stirred at room temperature for an additional hour. The excess boron tribromide was destroyed with dilute NH_4OH (3 mL of NH_4OH in 4 g of ice–water). The water layer was extracted with $CHCl_3$ and methanol (4:1) several times, and the combined organic layers were dried ($MgSO_4$). The base was treated in EtOAc with ethanolic HCl to give the salt, which was recrystallized (EtOAc–MeOH) to afford 53 mg (47%) of **7**·2HCl. Free base of **7**: mp 194–196 °C; EIMS, m/e 372 (3, M^+), 329 (3, $M^+ - CH_2CH_2OH$), 268 [88, $M^+ - N(CH_2CH_2OH)_2$]; NMR (base in pyridine-d_5) δ 5.03 (d, 1 H, H-5), 5.87–5.85 (m, 2 H, H-6 and H-7), 5.85–5.67 (s, 2 H, OH), 5.00–4.73 (s, 1 H, phenolic OH), 2.61 (m, 1 H, H-8), 2.32 (m, 1 H, H-14). Anal. ($C_{21}H_{28}O_4N_2$·2HCl) C, H, N.

8β-[Bis(2-chloroethyl)amino]-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan Dihydrochloride (3·2HCl). A solution of thionyl chloride (200 mg, 1.85 mmol) in anhydrous

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acetonitrile (10 mL) was added with vigorous stirring to a solution of 7 (150 mg, 0.4 mmol) in dry acetonitrile (60 mL), and the mixture was maintained at room temperature for 6 h. After evaporation of solvent and excess thionyl chloride in vacuo, the residue was basified with NH_4OH and extracted with several portions of $CHCl_3$. The combined organic phases were dried ($MgSO_4$) and evaporated to give a syrup, which was subjected to dry column chromatography on silica gel with EtOAc–NH_4OH (100:1). Treatment of the base in EtOAc with ethanolic HCl gave the dihydrochloride salt, which was recrystallized from EtOAc to afford 91 mg (54%) of **3**·2HCl: mp 203–204 °C; EIMS, *m/e* 408, 410, 412 (15, 10, and 3 M^+), 345 (6, $M^+ - CH_2CH_2Cl$), 268 [20, $M^+ - N(CH_2CH_2Cl)_2$]; NMR (HCl salt in pyridine-d_5) δ 5.68–5.64 (m, 2 H, H-6 and H-7), 4.87 (d, 1 H, H-5), 3.02 (m, 1 H, H-8), 2.63 (m, 1 H, H-14). Anal. ($C_{21}H_{26}O_2N_2Cl_2$·2HCl·H_2O) C, H, N, Cl.

8β-Azido-6,7-didehydro-4,5α-epoxy-3-hydroxy-17-methylmorphinan (8). Compound 8 was synthesized by a method similar to that of Bognar et al.[17] Treatment of morphine with *p*-tosyl chloride afforded 7,8-didehydro-3,6-ditosyl-4,5α-epoxy-17-methylmorphinan, which was reacted with sodium azide to give 8β-azido-6,7-didehydro-4,5α-epoxy-17-methyl-3-tosylmorphinan. Detosylation with KOH–EtOH afforded compound 8. The free base was recrystallized from EtOAc: mp 185–187 °C; EIMS, *m/e* 310 (M^+); IR 2100 (azide band) cm^{-1}; NMR (base in $CDCl_3$) δ 5.87–5.87 (m, 2 H, H-6 and H-7), 2.58 (m, 1 H, H-8), 2.45 (m, 1 H, H-14). Anal. ($C_{17}H_{18}N_4O_2$) C, H, N.

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Registry No. **3**, 90246-16-5; **3**·2HCl, 90246-15-4; **4**, 76-57-3; **5**, 467-08-3; **6**, 90246-17-6; **7**·2HCl, 90246-18-7; **8**, 55781-29-8; **8** (tosylate), 90246-19-8; 7,8-didehydro-3,6-ditosyl-4,5α-epoxy-17-methylmorphinan, 90246-20-1; morphine, 57-27-2; diethanolamine, 111-42-2.

The Preferred Solution Conformation of Warfarin at the Active Site of Cytochrome P-450 Based on the CD Spectra in Octanol/Water Model System

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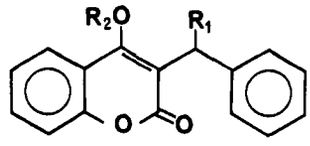
An octanol/water model system and circular dichroism (CD) spectroscopy have been used to study the solution conformation of warfarin in aqueous and lipid environments. Upon partitioning of (*S*)-warfarin from buffer pH 7.4 into octanol, the position of the absorption band due to the α,β-unsaturated carbonyl chromophore shifts from 210 nm in the aqueous phase to 220 nm in the octanol phase. The shift is coupled to an increase in the molecular ellipticity of the band, suggesting the formation of a dissymmetric chromophore. Comparison of CD spectra of conformationally fixed analogues of warfarin to that of warfarin in solution suggests that the compound shifts from the open side chain keto form in the aqueous phase at pH 7.4 to the cyclic hemiketal form after partitioning into the lipid octanol phase. On the basis of these results, the hemiketal form is proposed as the preferred solution conformation of warfarin in the lipid environment of the active site of cytochrome P-450 and the relationship between solution conformation and stereoselectivity of warfarin metabolism by β-naphthoflavone inducible cytochrome P-450 is discussed.

Warfarin (**1**) and phenprocoumon (**2**), two structurally related coumarin anticoagulants, are hydroxylated in the



1a, $R_1 = -CH_2COCH_3$; $R_2 = -H$

1b, $R_1R_2 = -CH_2C(OH)(CH_3)-$

2, $R_1 = -C_2H_5$; $R_2 = -H$

3, $R_1 = -CH_2COCH_3$; $R_2 = -CH_3$

4, $R_1R_2 = -CH_2C(OCH_3)(CH_3)-$

4′-, 6-, 7-, and 8-positions by rat liver microsomal preparations. Previous studies have shown the rate and regio- and stereoselectivity of aromatic hydroxylation are a function of induction state.[1] The highest rates of metabolism are observed with microsomes obtained from either 3-methylcholanthrene or β-naphthoflavone (BNF) pretreated rats. Induction with either compound leads to a high degree of regioselectivity for the 6- and 8-positions coupled to pronounced stereoselectivity but for opposite enantiomers of the two drugs. Thus, although a given absolute configuration for both compounds is spatially related about their asymmetric center, i.e., (*R*)-warfarin corresponds to (*R*)-phenprocoumon, the same enzymic preparation while selective for (*R*)-(+)-warfarin is also selective for (*S*)-(−)-phenprocoumon.

One possible explanation for the opposite stereoselectivity is provided by previous studies on the solution conformation of warfarin. Both nuclear magnetic resonance (NMR) and circular dichroism (CD) spectra of warfarin, phenprocoumon, and some conformationally restricted warfarin analogues indicate warfarin exists in solution in a tautomeric equilibrium of an open side chain keto form, **1a**, and a diastereomeric pair of ring closed hemiketals, **1b**.[2-4] In organic solvents warfarin exists principally as its hemiketal. It was the CD spectra of warfarin and phenprocoumon in organic solvent that suggested a structural relationship between opposite enantiomers of phenprocoumon and warfarin hemiketal. The CD spectra of like enantiomers of the two compounds are

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