131. Tritium Labelling of Cyprodime (= (−)-17-(Cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one), a μ Receptor-Selective Opioid Antagonist

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Dedicated to Prof. Dr. *B. Witkop* on the occasion of his 75th birthday

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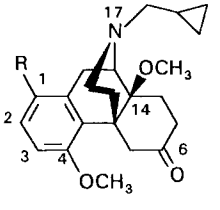
The preparation of [1-^{3}H]cyprodime (**2**), which has a specific activity of 31.6 Ci/mmol was carried out by catalytic tritiodehalogenation of 1-bromocyprodime (**3**). Bromination of cyprodime was performed by using chloroperoxidase, KBr, and H_2O_2.

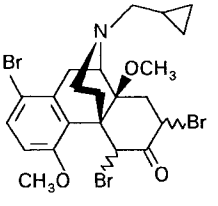
Introduction. – The existence of three major types of opioid receptors, μ, κ, and δ, is generally recognized. For studying these receptor types, selective agonists and antagonists are required. Recently, a highly selective μ opioid antagonist, cyprodime (**1**), has been discovered [1] [2]. Cyprodime (= (−)-17-(cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one) is a pure opioid antagonist with high selectivity for μ receptors. Since cyprodime shows the highest selectivity of nonpeptide, competitive μ opioid antagonists reported, this ligand is of interest as a pharmacological tool in opioid research [3], and particularly for the study of μ opioid receptors. Here, we describe the radiolabelling of cyprodime with tritium to give [1-^{3}H]cyprodime (**2**).



1 R = H (cyprodime)

2 R = ^{3}H

3 R = Br



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Chemistry. – Attempts to monobrominate cyprodime in the aromatic ring by conventional procedures (*e.g.* bromination with Br_2 in AcOH) did not result in the desired compound (1-bromocyprodime, **3**) but in a mixture of products (TLC). Thus, we prepared tribrominated cyprodime with the proposed structure of **4** by reaction of cyprodime with 3 equiv. of Br_2 in AcOH. Since tritiation of **4** did not produce a single product, we sought for an alternative method for the monobromination of **1**. We found that enzymatic monobromination using chloroperoxidase, KBr, and H_2O_2 gives a single product under mild conditions. According to the mass spectrum, the resulting compound had one Br-atom. ^{1}H-NMR revealed that the aromatic ring has been brominated. The assignment of the Br-atom at C(1) was unambiguously confirmed by NOE difference spectroscopy similarly to the assignment in 1-bromocodeine [4]. Irradiation of the 4-*Me*O *singlet* of **3** gave 2.6% enhancement of the *dublet* of H–C(3), and irradiation of the H–C(3) *dublet* 3.1% enhancement of the 4-*Me*O *singlet*. Analogously, the position of Br at C(1) in the aromatic ring of compound **4** was established. Dehalogenation of 1-bromocyprodime (**3**) was performed with tritium gas using $PdO/BaSO_4$ as catalyst and DMF as solvent. After purification by TLC, **2** had theoretical specific activity (31.6 Ci/mmol).

Biological studies performed with **2** are in progress and will be published elsewhere.

Experimental Part

General. Tritium gas was purchased from *Technabexport*, USSR, and contained at least 98% T_2. Chloroperoxidase (EC 1.11.1.10) was purchased from *Sigma Chemical Co.*, St. Louis, MO, USA. All materials used were anal. grade, but DMF and Et_3N were repurified by vacuum destillation and dried over molecular sieves prior to use. The amount of tritiated material was measured by UV detection on a *Shimadzu 160* spectrophotometer. Tritiated samples were counted in liquidfluor scintillant (from *BDH*, England) with a *Searl Delta 300* liquid scintillation counter. Anal. and radiochemical purity was checked with TLC on silica gel plates (Kieselgel *60 F254*, *Merck*) with a *Berthold Tracemaster*. M.p.: *Kofler* melting-point microscope, uncorrected. IR spectra (in cm^{-1}): *Beckman AccuLab 2* apparatus. ^{1}H-NMR: 400 MHz: *Bruker AM 400* spectrometer by the NMR Laboratory of the Szeged Regional Center for Scientific Instruments, Szeged, Hungary; 300 MHz: *Bruker AM 300* spectrometer; δ in ppm, J (apparent coupling constant) in Hz. MS: *VG Trio-2* mass spectrometer at the Analytical Laboratory of Alkaloida Chemical Factory, Tiszavasvari, Hungary.

1,5,7-Tribromo-17-(cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one Hydrobromide (**4**·HBr). 0.1M Br_2 soln. in AcOH (9.0 ml, 0.9 mmol) was added dropwise to a soln. of **1**·HBr (130 mg, 0.3 mmol) in 2 ml of AcOH at r.t. within 45 min under stirring. The resulting mixture was stirred at r.t. for 46 h. After filtration, the filtrate was evaporated to give 198 mg of a colorless crystalline solid which was recrystallized from MeOH to afford 136 mg (68%) of **4**·HBr. M.p. > 240° (dec.). IR (KBr): 3430 ($^+$NH), 1725 (CO). ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.58 (*s*, $^+$NH); 7.58 (*d*, J = 8.9, 1 arom. H); 6.96 (*d*, J = 8.9, 1 arom. H); 4.64 (*s*, H–C(5)); 3.83 (*s*, MeO–C(4)); 3.53 (*s*, MeO–C(14)). EI-MS: 589–595 (M^+). Anal. calc. for $C_{22}H_{26}Br_3NO_3$·HBr (673.11): C 39.25, H 4.04, N 2.08; found: C 39.41, H 4.01, N 2.31.

1-Bromo-17-(cyclopropylmethyl)-4,14-dimethoxymorphinan-6-one (**3**; *1-Bromocyprodime*). KBr (54.87 mg, 460 µmol) and 565 µl of 3% H_2O_2 soln. were added to a soln. of **1**·HBr (44.67 mg, 102 µmol) in 4 ml of AcOH/AcONa buffer (100 mM, pH 3.6). The reaction was started by addition of 229.5 units of chloroperoxidase enzyme and was completed after 10 min (TLC). Then, the pH of the mixture was adjusted to 8 by addition of conc. NH_4OH soln. After extractions with $CHCl_3$ (3 × 5 ml), the combined org. layers were dried and evaporated to yield 35.2 mg (79%) of **3** as colorless crystalline residue. Recrystallization from abs. EtOH gave 20.2 mg (45%) of **3** as anal. pure material. M.p. 158–162°. IR (KBr): 1715 (CO). ^{1}H-NMR (400 MHz, $CDCl_3$): 7.37 (*d*, J = 8.2, 1 arom. H); 6.64 (*d*, J = 8.2, 1 arom. H); 3.82 (*s*, MeO–C(4)); 3.42 (*s*, MeO–C(14)). CI-MS: 434, 436 ($[M + 1]^+$). Anal. calc. for $C_{22}H_{28}BrNO_3$ (434.37): C 60.83, H 6.50, N 3.23; found: C 60.99, H 6.71, N 3.14.

17-(Cyclopropylmethyl)-4,14-dimethoxy[1-^{3}H]morphinan-6-one (**2**; *[1-^{3}H]Cyprodime*). A mixture of **3** (2.38 mg, 5.45 µmol), 1 ml of DMF, 1 µl of Et_3N, and 20 mg of $PdO/BaSO_4$ catalyst (cont. 10% PdO, *Merck*) was treated

with tritium gas at r.t. in a vacuum manifold [5]. After 1 h of tritiation, the catalyst was filtered off and the filtrate evaporated under reduced pressure. Labile tritium was removed by repeated evaporation of the residue after dissolution in a mixture of $EtOH/H_2O$ 1:1. The crude product having 172 mCi of total activity was purified twice by TLC: The plates were previously washed [6] with spectroscopic-grade EtOH and activated at 110° for 1 h. Mobile phase first $CHCl_3$/MeOH/conc. NH_4OH soln. 90:10:1, subsequently CH_2Cl_2/MeOH 9:1. The amount of pure material was measured by UV spectroscopy at the wave length of 276.6 nm ($\varepsilon = 2200$). The total activity recovered was 32.9 mCi, and the specific activity of **3** was 31.6 Ci/mmol. The radiochemical purity of labelled cyprodime was > 95% as determined by TLC in two different systems (see above).

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