Synthesis and Biological Evaluation of 14-Alkoxymorphinans, V[1]:

6-Deoxocyprodime, an Opioid Antagonist with Decreased μ Receptor Selectivity in Comparison to Cyprodime

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N-Cyclopropylmethyl-4,14-dimethoxymorphinan (**4**) and *N*-cyclopropylmethyl-4-hydroxy-14-methoxymorphinan (**5**) have been prepared from cyprodime (**1**) by *Wolff-Kishner* reduction. Pharmacological studies (mouse vas deferens and guinea pig ileum preparations) revealed that there was no significant decrease of **4** in antagonist activity but in μ selectivity when compared with **1**. The phenol **5** showed partial agonism at μ, κ and δ receptors.

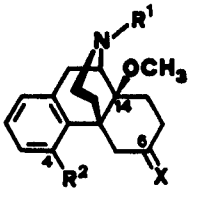
Synthese und Biologische Untersuchung von 14-Alkoxymorphinanen, 5. Mitt.[1]:

6-Deoxocyprodim, ein Opiatantagonist mit verminderter μ-Rezeptorselektivität im Vergleich zu Cyprodim

N-Cyclopropylmethyl-4,14-dimethoxymorphinan (**4**) und *N*-Cyclopropylmethyl-4-hydroxy-14-methoxymorphinan (**5**) wurden aus Cyprodim (**1**) durch eine *Wolff-Kishner*-Reduktion erhalten. Pharmakologische Studien (Mausvas-deferens- und Meerschweinchen-ileum-Präparation) zeigten keinen signifikanten Abfall der antagonsitischen Aktivität von **4** im Vergleich zu Cyprodim, aber einen Abfall der μ-Selektivität. Das Phenol **5** erwies sich als partieller Agonist an μ, κ und δ Rezeptoren.

Cyprodime (**1**) is a pure opioid antagonist with high selectivity for μ receptors [2,3]. Since it has the highest μ selectivity of nonpeptide, competitive μ opioid antagonists reported, this ligand is being used as pharmacological tool in opioid research [4,5]. It has already been tritium-labeled [6]. In an attempt to enhance its μ receptor affinity and/or μ selectivity a series of cyprodime-related compounds has been prepared and tested pharmacologically[4]. Besides other findings, this study revealed that increasing the chain length in position 4 resulted in a compound **2** with higher affinity for μ opioid receptors, but in very little changes in either selectivity or agonist activity at any of the opioid receptors, while decreasing the size of the substituent in position 4 (compound **3**) resulted in an appreciable increase in agonist activity at κ, μ, and δ receptors.

With the present study we wanted to examine the influence of the 6-keto function on μ receptor affinity, μ selectivity, and agonist and antagonist potency. Thus we prepared 6-deoxocyprodime (**4**) and *N*-cyclopropylmethyl-4-hydroxy-14-methoxymorphinan (**5**).



1 R^1 = CPM, R^2 = OCH_3, X = O

2 R^1 = CPM, R^2 = $O(CH_2)_3CH_3$ X = O

3 R^1 = CPM, R^2 = OH, X = O

4 R^1 = CPM, R^3 = OCH_3, X = O

5 R^1 = CPM, R^2 = OH, X = H_2

6 R^1 = CH_3, R^2 = OCH_3, X = O

7 R^1 = $CO_2CHClCH_3$, R^2 = OCH_3, X = O

8 R^1 = $CO_2CH_2CCl_3$, R^2 = OCH_3, X = O

9 R^1 = H, R^2 = OCH_3, X = O

CPM = cyclopropylmethyl

Chemistry

The starting material, cyprodime (**1**), was prepared by a slightly different route as described earlier[2,4]. Thus, *N*-demethylation of 4,14-dimethoxy-*N*-methylmorphinan-6-one (**6**)[7] was carried out using 1-chloroethyl chloroformate instead of 2,2,2-trichloroethyl chloroformate, since the corresponding carbamate **7** can be cleaved under milder conditions (reflux in MeOH)[8] than the carbamate **8** (reflux with Zn/NH_4Cl in MeOH). Alkylation of the *N*-normorphinan **9** with cyclopropylmethyl chloride yielded **1** as described.[2] *Wolff-Kishner* reduction of **1** afforded 6-deoxocyprodime (**4**) as the main product. During the course of the reaction, ether cleavage in position 4 has partly taken place under strong alkaline condition to give the phenol **5** as a side

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product. Compounds **4** and **5** were separated by column chromatography. Both compounds (**4** and **5**) did not show IR-carbonyl absorption. In contrast, the corresponding 6-oxo analogues **1** and **3** exhibit IR-carbonyl absorptions at 1705 and 1710 cm^{-1}, respectively.

Pharmacology

The cyprodime analogues were evaluated for opioid agonist and antagonist activity in the mouse vas deferens preparation (MVD)[2]. Antagonist potencies at the three opioid receptor sub-types were determined against normorphine (μ-6-fold increase in κ antagonist potency and a 4-fold increase in δ antagonist potency. In the MVD compound **5** exhibited some μ-selective antagonism, while with the parent 6-keto analogue **3** antagonist Ke values could not be determined because of its relatively high agonism at μ, κ, and δ receptors. Although both compounds possess δ agonist activity in the MVD, the inhibition of twitch produced by **3** was too great to allow determination of antagonist Ke values[4], whereas with **5** substantial shifts of the agonist dose response curves can be obtained with concentrations that produced negligible effects (< 15%) on the twitch height (Table).

Table: Opioid Antagonist Activities of **4**, **5**, and Cyprodime (**1**) in the MVD.

	Ke[a] (nM)			selectivity ratio	
Compound	NM[b](μ)	EKC[c](κ)	DADLE[d](δ)	κ/μ	δ/μ
4	72	245	1530	3.4	21
5	162	398	2900	2.5	18
cyprodime (1)	55.4	1551	6108	28	110

[a] Ke = [antagonist]/DR-1, where DR is dose ratio (i.e. ratio of equiactive concentrations of the test agonist in the presence and absence of the antagonist). The present Ke values were obtained using concentrations of the antagonist which produced a dose ratio of between 3 and 10 against the relevant agonist. [b] NM = normorphine. [c] EKC = ethylketocyclazocine. [d] DADLE = D-Ala^2, D-Leu^5 enkephalin.

selective agonist), ethylketocyclazocine (κ-selective agonist) and D-Ala^2, D-Leu^5 enkephalin (a mixed μ/δ agonist which is very δ selective in the MVD due to the high δ receptor reserve in this preparation). The compounds were also tested for agonist activity in the guinea pig isolated ileum preparation (GPI)[2], a preparation particularly sensitive to κ agonists.

Agonist effects were designated as being due to predominantly μ, κ, or δ receptor interactions on the basis of antagonism of the effect by the κ-selective antagonist norbinaltorphimine (3 nM)[9], the μ-selective antagonist cyprodime (1000 nM)[2,4], or the δ-selective antagonist naltrindole (10 nM)[10]. The compounds were tested up to a concentration of 50 μM. In the GPI, compounds which produced no inhibition of twitch height or produced an inhibition which was not antagonized by a combination of cyprodime (100 nM) plus norbinaltorphimine (3 nM) were considered devoid of any opioid agonist (μ, κ) activity. Compounds producing dose response effects only shifted by one of the antagonists were designated μ or κ agonists accordingly. Compounds producing a dose response effect which was shifted by cyprodime and further shifted by norbinaltorphimine (or vice versa) were designated as possessing both μ and κ agonist activity. In the MVD, compounds which produced no inhibition of twitch height or produced an inhibition which was not antagonized by naltrindole (10 nM) were considered devoid of any δ agonist activity.

In the GPI and MVD compound **4** was a pure antagonist, while **5** showed partial agonism at μ and κ receptors in the GPI and δ receptors in the MVD.

The μ affinity of **4** was a little bit lower than that of cyprodime, whereas the μ selectivity was much lower due to a

Conclusion

Removing the 6-keto function in cyprodime to from compound **4** produces only a small decrease in μ antagonist potency, but was accompanied by an increase in κ and δ antagonist potency, resulting in a much less μ selective compound. There was no measurable change in agonist activity, both compounds behaving as pure antagonists under the test conditions used.

A comparison of compound **5** with its present 6-keto analogue **3** shows that the removal of the 6-keto function has resulted in a decrease in agonist activity.

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Experimental Part

Chemistry. Melting points: Kofler melting point microscope, uncorrected. - Optical rotations: Perkin Elmer 141 polarimeter (concentration in g/100 ml, solvent). - IR spectra (in cm^{-1}): Beckman Accu Lab 2. - ^{1}H-NMR spectra: Bruker AM 300 (δ ppm, tetramethylsilane as int. reference). - Mass spectra (MS): Finnigan Mat 44S. - Elemental analyses: Dr. *J. Zak*, Institute of Physical Chemistry, University of Vienna. - Column chormatography: alumina basic (70-230 mesh, ASTM), Merck.

(-)-4,14-Dimethoxymorphinan-6-one Hydrochloride (**9**·HCl)

A mixture of 5.6 g (17.7 mmol) of **6**[7], 14.4 g (143.8 mmol) of $KHCO_3$, 15.7 ml (144.1 mmol) of 1-chloroethyl chloroformate and 50 ml of ETOH-free $ClCH_2CH_2Cl$ was stirred at 60-65°C (bath temp.) for 16 h. Then the mixture was filtered and the filtrate evaporated to give 6.73 g of **7** as a red

glassy solid (pure by TLC, not further characterized) which was refluxed in 30 ml of MeOH for 30 min. After cooling, the colorless precipitation was collected and washed with MeOH and Et_2O to give 4.86 g (81%) of **9** · HCl: mp. > 300°C (dec.). From a small portion of this material the free base was liberated and recrystallized from MeOH: mp 155-156°C (mp lit.[2]: 155-157°C). - This material was identical with an authentic sample by mixed melting point, TLC, and IR-spectrum.

(-)-N-Cyclopropylmethyl-4,14-dimethoxymorphinan (**4**) *and (-)-N-Cyclopropylmethyl-4-hydroxy-14-methoxymorphinan* (**5**)

A mixture of 480 mg (1.1 mmol) of **1**·HBr (prepared from **9**·HCl as described[2]), 2.4 ml (49.4 mmol) of hydrazine hydrate (64%) and 4.8 ml of triethylene glycol was stirred at 120-130°C (bath temp.) for 1.5 h. After cooling, 650 mg (11.6 mmol) of KOH pellets were added, and this mixture was stirred at 205°C (bath temp.) for 2 h. After cooling, the solution was acidified with 2N HCl, washed with Et_2O (discarded), rendered alkaline with conc. NH_4OH and extracted with CH_2Cl_2 (2x5 ml). The org. layer was washed with H_2O (3x10 ml) and saturated NaCl-solution (5 ml), dried (Na_2SO_4) and evaporated to give 310 mg of a yellowish crystalline residue which contained two products (TLC). Column chromatography (length of the column 30 cm, diameter 2.5 cm; alumina basic grade II; CH_2Cl_2) gave 162 mg (43%) of **4** and 41 mg (11%) of **5**. Recrystallization of **4** from MeOH: mp. 106-107°C. - $[\alpha]_D^{20}$ = - 65.3° (0.53, CH_2Cl_2). - ^{1}H-NMR ($CDCl_3$): δ (ppm) = 7.07 (t, 1 arom. H, J = 8 Hz), 6.69 (dxd, 2 arom. H, J = 8; 8 Hz), 3.75 (s, 3H, C-4-CH_3O), 3.26 (s, 3 H, C-14-CH_3O). - MS (Cl): m/z 342 $(M+1)^+$. - $C_{22}H_{31}NO_2$ (341.5) Calcd. C 77.4 H 9.15 N 4.1 found C 77.2 H 9.09 N 4.4.

Recrystallization of **5** from MeOH: mp. 181-184°C.- $[\alpha]_D^{20}$ = -85.6° (c 0.96, CH_2Cl_2). - IR (KBr): 3200 (OH). - ^{1}H-NMR ($CDCl_3$): δ (ppm) = 6.95 (t, 1 arom. H, J = 8 Hz), 6.68 (d, 1 arom. H, J = 8 Hz), 6.46 (d, 1 arom. H, J = 8 Hz), 3.27 (s, 3 H, CH_3O). - MS (Cl): m/z = 328 $(M+1)^+$. - $C_{21}H_{29}NO_2$ (327.5) Calcd. C 77.0 H 8.93 N 4.3 found C 77.0 H 9.12 N 4.2.

Pharmacology

Materials and Methods

Mouse Vas Deferens Preparation (MVD)

Vasa deferentia from adult male mice (strain MFI/OLA) heavier than 30 g were set up in a 50 ml organ bath containing oxygenated (95% O_2 and 5% CO_2) magnesium-free *Krebs* solution (mM: NaCl, 118; KCl, 4.75; $CaCl_2$, 2.54; $NaHCO_3$, 25; KH_2PO_4, 0.93; glucose, 11) thermostatically controlled at 30°C. The preparations were field stimulated between platinum electrodes at 0.1 Hz with 3.0 ms rectilinear pulses at a voltage of 30-50 V (measured across the electrodes with an oscilloscope) delivered from a computer-controlled stimulator made in the equipment development department at Reckitt & Colman. Dose-response curves were constructed by the cumulative addition of agonist to the organ bath. Contractions of the tissue were recorded with a Statham Goldcell Isometric Transducer connected to a Smiths Servoscribe flat bed potentiometric recorder. Essentially the method reported by *Henderson, Hughes,* and *Kosterlitz*[11] has been followed to perform this test.

Guinea Pig Isolated Ileum Preparation (GPI)

Male Duncan Hartley guinea pigs of weight > 300 g were killed by a blow on the head, and the ileum was dissected out. The last 15 cm from the ileocecal junction was discarded and the rest was placed in warm (36 °C) *Krebs* solution gassed with 95% O_2/5% CO_2. After the lumen was washed with warm *Krebs* solution, a piece of ileum approximately 4 cm long was removed and set up in oxygenated *Krebs* solution at 30 °C in a 50 ml organ bath between two platinum electrodes. One electrode was situated intraluminally and the other extraluminally. The tissue was stimulated with 1 ms square wave pulses at a rate of 0.1 Hz and just maximal voltage with a BBC microcomputer controlled stimulator developed in the equipment development laboratory at Reckitt & Colman. Agonist dose-response curves were constructed by using a cumulative dosing method - in all other respects the method described by *Kosterlitz* and *Watt*[12] has been essentially followed.

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