30. Synthesis and Biological Evaluation of 14-Alkoxymorphinans

Part 9[1])

14-*O*-Ethyl-5-methylnaltrexone, an Opioid Antagonist with Unusual Selectivity

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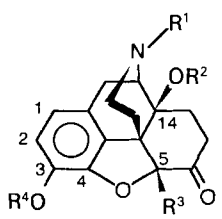
14-*O*-Ethyl-5-methylnaloxone (**7**) and 14-*O*-ethyl-5-methylnaltrexone (**8**) have been prepared starting from 14-*O*-ethyl-5-methyloxycodone (**9**) in several steps. Both, **7** and **8**, were found to be opioid antagonists *in vitro* and *in vivo*. Compound **7** exhibited some selectivity for μ opioid receptors, whereas compound **8** did not show selectivity for any of the receptor types. In the AcOH-writhing antagonism test, **8** was not able to antagonize morphine-induced antinociception, but antagonized fentanyl- and sufentanil-induced antinociception.

Introduction. – The introduction of a 14-*O*-alkyl group (MeO, EtO) into naloxone (**1**) and naltrexone (**2**), respectively, did not significantly alter the *in vitro* and *in vivo* potencies of these two opioid antagonists [2], while introduction of a 5-Me group reduced the antagonist properties and enhanced the agonist effects producing partial agonists [3]. In the *N*-methylmorphinan-6-one series of opioid agonists, a 5-Me group slightly decreased



1 $R^1 = CH_2=CH-CH_2$, $R^2 = R^3 = R^4 = H$ (naloxone)
2 R^1 = cyclopopylmethyl, $R^2 = R^3 = R^4 = H$ (naltrexone)
3 $R^1 = R^2 = R^3 = Me$, $R^4 = H$
4 $R^1 = Me$, $R^2 = R^3 = R^4 = H$
5 $R^1 = CH_2=CH-CH_2$, $R^2 = R^4 = H$, $R^3 = Me$
6 R^1 = cyclopropylmethyl, $R^2 = R^4 = H$, $R^3 = Me$
7 $R^1 = CH_2=CH-CH_2$, $R^2 = Et$, $R^3 = Me$, $R^4 = H$
8 R^1 = cyclopropylmethyl, $R^2 = Et$, $R^3 = Me$, $R^4 = H$
9 $R^1 = R^3 = R^4 = Me$, $R^2 = Et$
10 $R^1 = CH_3CHCl-COO$, $R^2 = Et$, $R^3 = R^4 = Me$
11 $R^1 = H$, $R^2 = Et$, $R^3 = R^4 = Me$
12 $R^1 = CH_2=CH-CH_2$, $R^2 = Et$, $R^3 = R^4 = Me$
13 R^1 = cyclopropylmenthyl, $R^2 = Et$, $R^3 = R^4 = Me$

[1]) Part 8: [1].

opioid-agonist properties [4], whereas a 14-*O*-alkyl group enhanced opioid-agonist effects significantly [5] [6]. In 14-methoxymetopon (**3**), where a 5-Me group exists in addition to a 14-*O*-Me group, the opioid-agonist effects were further increased, producing a compound that had *ca.* 1500 times higher antinociceptive potency in the AcOH writhing test in mice than oxymorphone (**4**) [1]. Thus, a 5-Me group together with a 14-*O*-alkyl group could enhance opioid agonist properties in *N*-methylmorphinan-6-ones dramatically.

To clarify structure-activity relationships relating to 5-Me and 14-alkoxy groups, it was of interest to determine the influence of 14-*O*-alkylation on the partial agonists 5-methylnaloxone (**5**) and 5-methylnaltrexone (**6**). Thus, we prepared 14-*O*-ethyl-5-methylnaloxone (**7**) and 14-*O*-ethyl-5-methylnaltrexone (**8**) and compared these compounds in the AcOH-writhing test with **5** and **6**, respectively.

Chemistry. – Starting material for the synthesis of compounds **7** and **8** was 14-*O*-ethyl-5-methyloxycodone (= 4,5α-epoxy-14-ethoxy-3-methoxy-5β,17-dimethylmorphinan-6-one; **9**) which is available from thebaine *via* 5-methylthebaine [7] [8] in four steps [1] [4]. *N*-Demethylation using 1-chloroethyl chloroformate [9] afforded carbamate **10** which was refluxed in MeOH to yield *N*-normorphinan **11**. Alkylation of **11** with either allyl bromide or cyclopropylmethyl chloride in DMF in the presence of K_2CO_3 gave **12** and **13**, respectively. Ether cleavage was carried out with 48% HBr solution to afford 14-*O*-ethyl-5-methylnaloxone (**7**) and 14-*O*-ethyl-5-methylnaltrexone (**8**).

Pharmacological Evaluation. – Compounds **7** and **8** were evaluated *in vitro* in opioid-receptor-binding studies (*Table 1*), in the isolated guinea-pig myenteric plexus-longitudinal muscle preparation (GPI), and in the mouse vas deferns preparation (MVD) (*Table 2*). [^{3}H]DAMGO (μ-selective agonist), [^{3}H]U-69593 (κ-selective agonist), and

Table 1. *Opioid-Receptor-Binding Studies*

Compound	[^{3}H]DAMGO (μ) *Ki* [nM]	[^{3}H]EKC (κ) *Ki* [nM]	[^{3}H]DPDPE (δ) *Ki* [nM]
7	1.9	15.2	26.6
8	1.1	1.4	4.5
Naloxone (**1**)[a])	1.8	17.2	27.0

[a]) Values taken from [10].

Table 2. *Tissue Preparations* (GPI and MVD)

Compound	*Ke*[a]) [nM]			Selectivity ratio	
	Normorphine (μ)[b])	EKC (κ)[b])	DPDPE (δ)[c])	κ/μ	δ/μ
7	1.36	5.14	44.2	3.8	33
8	2.83	5.04	69.6	1.8	25
Naloxone (**1**)	2.10	19.6	20.4	9.3	9.7
Cyprodime	31	1157	6108	37	219

[a]) *Ke* = [antagonist]/DR-1, where DR is the dose ratio (*i.e.* ratio of equiactive concentrations of the test agonist in the presence of the antagonist).
[b]) Determined in the GPI.
[c]) Determined in the MVD.

[^{3}H]DPDPE (D-Pen^2, D-Pen^5; δ-selective agonist) were used as ligands in the receptor-binding studies, while in the GPI and MVD normorphine, EKC (ethylketocyclazocine), and DPDPE were used as μ, κ, and δ agonists, respectively.

In opioid receptor binding, **7** and **8** showed approximately the same potency as naloxone in displacing [^{3}H]DAMGO (μ-selective) from its binding sites. Compound **7** exhibited some selectivity for μ receptors, showing 8-fold and 14-fold preference for μ receptors over κ and δ receptors, respectively. In contrast, compound **8** was not selective.

The *Ke* (antagonist dissociation constant) values of compounds **7** and **8** were determined in the GPI and MVD (*Table 2*). From these tissue studies, it is seen that the selectivity of **7** for μ receptors over κ receptors was lost, although the μ/δ selectivity was retained. Indeed, compounds **7** and **8** exhibited similar antagonist profiles and, in comparison with the μ selectivity of the opioid antagonist cyprodime [11] [12], both **7** and **8** showed much lower selectivity ratios.

In the GPI, both compounds, **7** and **8** , showed partial agonist properties at high concentrations reaching 50% inhibition of the electrically induced contractions at 2884 ± 121 nM and 4886 ± 219 nM, respectively. In the presence of 30 nM naloxone, no shift in the dose-effect curves was seen suggesting that the agonist response was mediated *via* κ receptors.

In the AcOH-writhing antagonism test[2]), **7** showed *ca.* 1/20 of the potency of naloxone (**1**), *ca.* 1/2 of the potency of 5-methylnaloxone (**5**), and *ca.* 1/6 of the potency of 5-methylnaltrexone (**6**) in antagonizing morphine-induced antinociception, while an AD_{50} against U-50,488-induced antinociception was not determinable. In contrast, with compound **8** no AD_{50} values against the μ-selective agonist morphine and the κ-selective agonist U-50,488 were obtained. On the other hand, the two short acting μ-selective agonists fentanyl and sufentanil were antagonized by low doses of **8** (*Table 3*).

Table 3. *AcOH-Writhing Antagonism Test in Mice*

Compound	Morphine (μ) (1.25 mg/kg; s.c.) AD_{50}[a])[b])	Fentanyl (μ) (0.04 mg/kg; s.c.) AD_{50}[a])[b])	Sufentanil (μ) (0.0025 mg/kg; s.c.) AD_{50}[a])[b])	U-50,488 (κ) (2.5 mg/kg; s.c.) AD_{50}[a])[b])
7	1.79	–	–	NE[c])
8	NE[c])	0.0018	0.0056	NE[c])
5	1.02	–	–	NE[c])
6	0.48	–	–	NE[c])
Naloxone (**1**)	0.08	–	–	1.12
Naltrexone (**2**)	0.05	–	–	0.06

[a]) The AD_{50} value (95% confidence limit) is defined as the dose at which the antinociceptive effect of the agonist was antagonized in 50% of the animals.

[b]) AD_{50} values in mg/kg (s.c.).

[c]) NE = no observable effect; no shift in the dose-effect curve of the agonist could be obtained.

In *Table 4*, the ED_{50} values of morphine, fentanyl, and sufentanil after different doses of compound **8** and the ED_{50} values without the antagonist are listed. Increasing the amount of **8** results in a dramatic increase of the ED_{50} values of fentanyl and sufentanil,

[2]) The tests were carried out for us at the *Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center*, Indianapolis, IN 46285, USA, through the courtesy of Dr. *J. D. Leander*.

Table 4. *AcOH-Writhing Test of Various Agonists Against Compound* **8**

	Morphine		Fentanyl		Sufentanil	
	ED_{50}[a)b)]	Dose ratio[c)]	ED_{50}[a)b)]	Dose ratio[c)]	ED_{50}[a)b)]	Dose ratio[c)]
Alone	0.60		0.018		0.00082	
Dose of **8**:						
0.04 mg/kg (s.c.)	1.31	2.18	0.0033	1.83	0.0025	3.05
0.32 mg/kg (s.c.)	2.33	3.88	0.095	5.28	0.0040	4.88
2.5 mg/kg (s.c.)	1.36	2.27	0.29	16.11	0.018	21.95

[a)] The ED_{50} value (95% confidence limit) is defined as the dose required for 50% reduction in frequency of writhing.

[b)] ED_{50} values in mg/kg (s.c.)

[c)] Factor by which the ED_{50} value of the agonist was multiplied.

while the ED_{50} values of morphine remained almost unchanged. These findings may suggest interaction of compound **8** with a μ-receptor subtype or subsite on the same receptor protein [13]. Further studies are being planned to clarify the pharmacological effects and receptor interactions of **8**.

In conclusion, introduction of a 14-EtO group into the partial agonists **5** and **6** resulted in compounds **7** and **8**, respectively, which show *in vivo* (AcOH-writhing test) only antagonism, while *in vitro* (GPI) also little agonism was found. Compound **8** exhibits selectivity in the sense that it does not antagonize morphine-induced antinociception but very well antagonizes fentanyl- and sufentanil-induced antinociception. To our knowledge, this type of selectivity has not been reported before.

Experimental Part

General. M.p.: *Kofler* melting-point microscope, uncorrected. Optical rotations (concentration (g/100 ml), solvent): *Perkin-Elmer-141* polarimeter. IR spectra: in cm^{-1}; *Beckman Accu Lab 2* apparatus. ^{1}H-NMR spectra: *Jeol-JNM-PMX-60* spectrometer; δ in ppm rel. to TMS as internal reference, J in Hz. Elemental analyses were performed at the Analytical Department of *F. Hoffmann-La Roche AG*, Basel.

(−)-4,5α-Epoxy-14β-ethoxy-3-methoxy-5β-methylmorphinan-6-one Hydrochloride (**11**·HCl). A mixture of **9** [1] (2.46 g, 6.8 mmol), $NaHCO_3$ (2.9 g, 34.5 mmol), 1-chloroethyl chloroformate (3.7 ml, 33.9 mmol), and 50 ml of $ClCH_2CH_2Cl$ was stirred at 60° (bath temp.) for 16 h. After filtration and evaporation of the filtrate, a slightly brown oily residue (3.4 g of **10**) was obtained. This material was pure by TLC and was used for further transformations without purification. After refluxing of **10** for 1 h in MeOH (40 ml), the soln. was evaporated to give 2.76 g of a nearly colorless crystalline residue which was recrystallized from MeOH/Et_2O to yield 2.0 g (81%) of **11**·HCl. An anal. sample was prepared by recrystallization of a small portion from MeOH/Et_2O. M.p. 195–198° (dec.). $[\alpha]_D^{20} = -141.0$ ($c = 0.99$, $CHCl_3$). IR (KBr): 3430 ($^+NH_2$), 1720 (CO). ^{1}H-NMR ((D_6)DMSO): 6.68 (*s*, 2 arom. H); 3.75 (*s*, MeO); 1.47 (*s*, CH_3–C(5)); 1.28 (*t*, $J = 6$, CH_3CH_2O). Anal. calc. for $C_{20}H_{25}NO_4$·HCl (379.88): C 63.28, H 6.90, N 3.69, Cl 9.33; found: C 62.91, H 6.97, N 3.64, Cl 9.22.

(−)-17-Allyl-4,5α-epoxy-14β-ethoxy-3-methoxy-5β-methylmorphinan-6-one (**12**). A mixture of **11**·HCl (2.0 g, 5.8 mmol), allyl bromide (0.54 ml, 6.2 mmol), K_2CO_3 (2.0 g, 14.5 mmol), and 10 ml of anh. DMF was stirred at r.t. for 3 h. After addition of H_2O (100 ml), extractions with Et_2O (3 × 30 ml), washings of the combined org. layers with H_2O (3 × 50 ml), and evaporation, 1.7 g of a colorless crystalline residue was obtained. Recrystallization from MeOH (2 ml) gave 1.7 g (85%) of **12**. A portion was recrystallized from MeOH for analysis. M.p. 104–105°. $[\alpha]_D^{20} = -191.8$ ($c = 1.05$, $CHCl_3$). IR (KBr): 1720 (CO). ^{1}H-NMR ($CDCl_3$): 6.50 (*dd*, $J = 8, 8$, 2 arom. H); 5.72 (*m*, 1 olef. H); 5.15 (*m*, 2 olef. H); 3.81 (*s*, MeO); 1.62 (*s*, CH_3–C(5)); 1.26 (*t*, $J = 6$, CH_3CH_2O). Anal. calc. for $C_{23}H_{29}NO_4$ (383.49): C 72.04, H 7.62, N 3.65; found: C 71.95, H 7.81, N 3.63.

(−)-17-Allyl-4,5α-epoxy-14β-ethoxy-3-hydroxy-5β-methylmorphinan-6-one Hydrobromide (**7**·HBr). A soln. of **12** (1.3 g, 3.4 mmol) in 5 ml of 48% HBr soln. was refluxed for 15 min and then evaporated. The residue was dissolved in 5 ml of MeOH and evaporated (this operation was repeated twice). The resulting slightly pink crystalline solid (1.35 g) was recrystallized from MeOH to yield 1.09 g (83%) of **7**·HBr. For analysis, a small sample was recrystallized from MeOH. M.p. > 230° (dec.). $[\alpha]_D^{20} = -132.6$ ($c = 0.97$, EtOH). IR (KBr): 3400, 3260 ($^+$NH, OH). ^{1}H-NMR ((D$_6$)DMSO): 8.55 (br. *s*, $^+$NH, OH); 6.59 (*s*, 2 arom. H); 5.65 (*m*, 3 olef. H); 1.47 (*s*, CH_3–C(5)); 1.32 (*t*, $J = 6$, CH_3CH_2O). Anal. calc. for $C_{22}H_{27}NO_4 \cdot HBr \cdot 0.5$ MeOH (466.39): C 57.78, H 6.58, N 3.00, Br 17.09; found: C 57.94, H 6.48, N 3.00, Br 17.13.

(−)-17-(Cyclopropylmethyl)-4,5α-epoxy-14β-ethoxy-3-methoxy-5β-methylmorphinan-6-one (**13**). A mixture of **11**·HCl (2.47 g, 6.5 mmol), K_2CO_3 (3.0 g, 21.8 mmol), cyclopropylmethyl chloride (0.77 ml, 8.33 mmol), and 20 ml of anh. DMF was stirred at 80° (bath temp.) for 20 h. After addition of 100 ml of H_2O, exctractions with Et_2O (3 × 30 ml), washings of the combined Et_2O layers with H_2O (3 × 40 ml), the org. layer was dried and evaporated to give 2.48 g of a slightly brown crystalline residue which was recrystallized from 2 ml of MeOH to yield 1.92 g (78%) of **13** (M.p. 124–128°). A portion of this material was recrystallized from MeOH for analysis. M.p. 128–130°. $[\alpha]_D^{20} = -188.3$ ($c = 1.00$, $CHCl_3$). IR (KBr): 1720 (CO). ^{1}H-NMR ($CDCl_3$): 6.50 (*dd*, $J = 8, 8$, 2 arom. H); 3.79 (*s*, MeO); 1.58 (*s*, CH_3–C(5)); 1.44 (*t*, $J = 6$, CH_3CH_2O). Anal. calc. for $C_{24}H_{31}NO_4 \cdot 0.2$ MeOH (397.52): C 72.03, H 7.84, N 3.47; found: C 72.04, H 8.03, N 3.50.

(−)-17-(Cyclopropylmethyl)-4,5α-epoxy-14β-ethoxy-3-hydroxy-5β-methylmorphinan-6-one (**8**). A soln. of **13** (1.0 g, 2.6 mmol) in 5 ml of 48% HBr soln. was refluxed for 15 min and then evaporated. The soln. was cooled, alkalized with conc. NH_4OH soln., and extracted with a mixture of $CHCl_3$/EtOH (3:1; 1 × 40 ml, 2 × 20 ml). The combined org. layers were dried, evaporated, and the residue (0.93 g slightly brown crystals) was recrystallized from MeOH to give 740 mg (74%; M.p. 167–170°) of **8**. Recrystallization of a small portion from MeOH afforded anal. pure material. M.p. 172–174°. $[\alpha]_D^{20} = -162.7$ ($c = 0.98$, $CHCl_3$). IR (KBr): 3400 (OH), 1715 (CO). ^{1}H-NMR ((D$_6$)DMSO: 8.70 (br. *s*, OH); 6.48 (*s*, 2 arom. H); 1.23 (*t*, $J = 6$, CH_3CH_2O). Anal. calc. for $C_{23}H_{29}NO_4$ (383.49): C 72.04, H 7.62, N 3.65; found: C 71.71, H 7.88, N 3.68.

Pharmacology. For AcOH-writhing tests, see [4] [14] [15]. For GPI and MVD, see [13] [16]. Opioid-receptor-binding assays were performed using homogenates of guinea-pig brain in *Tris*-HCl buffer (50 mM, pH 7.4), for 40 min at 25° as described in [16].

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