

SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF 18,19-DEHYDROBUPRENORPHINE[#]

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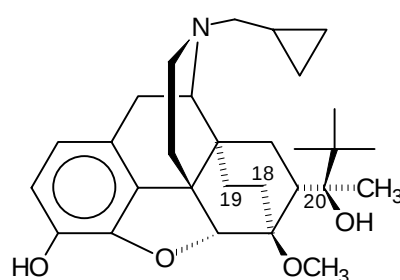
Abstract – 18,19-Dehydrobuprenorphine (**2**) was prepared in five steps starting from thevinone (**3**) which is readily available from thebaine by Diels-Alder reaction. Grignard reaction with *tert*-BuMgBr afforded *tert*-butylthevinol (**4**) which was *N*-demethylated via *N*-cyano-*tert*-butylthevinol (**5**) using BrCN. Alkali treatment gave *N*-nor-*tert*-butylthevinol (**6**) which was alkylated with cyclopropylmethyl bromide to give *N*-cyclopropylmethyl-*tert*-butylthevinol (**7**), followed by ether cleavage with thiolate to yield **2**. 18,19-Dehydrobuprenorphine displayed in opioid receptor binding studies very high affinity for μ receptors, while the affinity for κ and δ receptors was lower. In the tail-flick test in mice, compound **2** was 25 times more potent than morphine and *ca.* 2.5 times as potent as buprenorphine.

Buprenorphine (**1**) is a strong and widely used analgesic with marked opioid agonist potency.¹ It is a partial agonist at μ opioid receptors and an antagonist at κ receptors.² As a partial agonist, buprenorphine produces limited opioid side effects and, thus, overdose is rare. Because of its affinity for μ receptors, buprenorphine effectively prevents the effects of other opioids, thus reducing the likelihood of heroin

[#] This paper is dedicated to Prof. Dr. Sho Ito on the occasion of his 77th birthday and to Prof. Dr. Sándor Makleit on the occasion of his 70th birthday.

abuse.³ As well as being a maintenance agent, buprenorphine has been prescribed for rapid detoxification due to its reduced tendency to cause withdrawal effects and its ability to block the effects of other opioids.⁴ A recent study demonstrated that buprenorphine was able to suppress ethanol intake in rats.⁵ It was of interest to prepare the 18,19-dehydro analogue (**2**) of buprenorphine and to compare it biologically and pharmacologically with buprenorphine.

Figure



1 Buprenorphine

2 18,19-Dehydrobuprenorphine: $\Delta^{18,19}$

RESULTS AND DISCUSSION

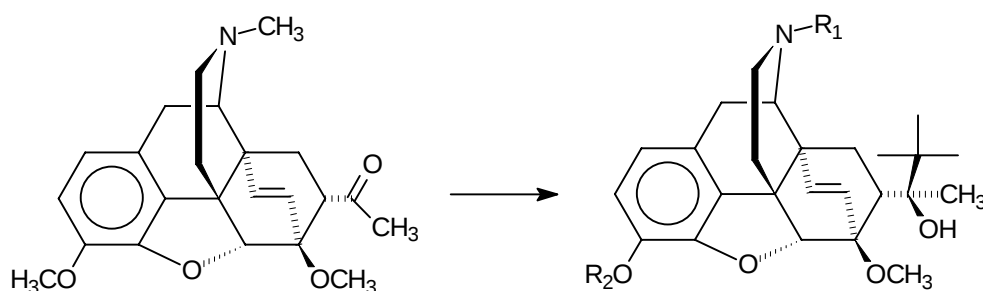
Synthesis

The synthesis of 18,19-dehydrobuprenorphine (**2**) has been disclosed earlier in two patent applications without any proof of the structure.^{6,7} We prepared compound (**2**) by a partly different route compared to the routes described (outlined below). For structure elucidation of **2**, NMR, IR, MS, elemental analysis and X-Ray diffraction analysis (performed with intermediate (**4**)) were used.

Thevinone (**3**), which was readily available from thebaine by Diels-Alder reaction,⁸ underwent Grignard reaction with *tert*-BuMgBr in *tert*-butylmethyl ether to yield *tert*-butylthevinol (**4**).⁹ The absolute configuration of **4** was established by X-Ray diffraction analysis. *N*-Demethylation was accomplished by using BrCN in CHCl₃ to give cyanamide (**5**),⁶ which was treated with KOH in diethylene glycol at 170°C to afford secondary amine (**6**).⁶ *N*-Alkylation with cyclopropylmethyl bromide gave compound (**7**),⁶ which was 3-*O*-demethylated using sodium ethanethiolate in DMF to yield 18,19-dehydrobuprenorphine (**2**).^{6,7} In ref. [6] the secondary amine (**6**) was acylated with cyclopropylcarbonyl chloride prior to reduction of the resulting amide to amine (**7**). In ref. [7], cyanamide (**5**) was treated with KOH in

diethylene glycol at 200°C, the resulting 3-*O*-demethylated secondary amine acylated with cyclopropylcarbonyl chloride and the corresponding amide reduced to 18,19-dehydrobuprenorphine (**2**) with lithium aluminium hydride.

Scheme



3 Thevinone

2 $R_1 = \text{CPM}$, $R_2 = \text{H}$

4 $R_1 = R_2 = \text{CH}_3$

5 $R_1 = \text{CN}$, $R_2 = \text{CH}_3$

6 $R_1 = \text{H}$, $R_2 = \text{CH}_3$

7 $R_1 = \text{CPM}$, $R_2 = \text{CH}_3$

CPM = cyclopropylmethyl

Pharmacological Evaluation

18,19-Dehydrobuprenorphine (**2**) was evaluated in comparison to buprenorphine (**1**) and morphine in opioid receptor binding studies employing [³H]DAMGO (μ agonist), [³H]deltorphin II (δ agonist) and [³H]U69593 (κ agonist) as radioligands (Table). Compound (**2**) displayed *ca.* three fold higher affinity for μ opioid receptors than buprenorphine (**1**) but lower affinities for κ and δ receptors, thus exhibiting clear preference for μ receptors. The μ/δ and μ/κ selectivity of compound (**2**) is about one order of magnitude higher than that of buprenorphine (**1**).

Compound (**2**) produced dose-related antinociceptive effects in mice¹⁰ after subcutaneous administration (Table). Compound (**2**) was *ca.* twice as potent as buprenorphine (**1**) and 25 times more potent than morphine. The antinociceptive effect was antagonized by naltrexone (5 mg/kg, s.c.).

Table

Opioid Receptor Binding Assay (ORBA) and Antinociceptive Potencies in the Tail-flick Assay (TFA) of Compounds 1, 2 and Morphine

Compound	ORBA (K_i^a , nM)			TFA
	μ	κ	δ	AD ₅₀ ^{b)} (μ mol/kg)
(1)	2.0	8.5	2.5	0.21
(2)	0.57	32.0	8.5	0.13
morphine	11.0	500	-	3.2

a) K_i = inhibition constant

b) AD₅₀ = median antinociceptive dose

EXPERIMENTAL

General Details

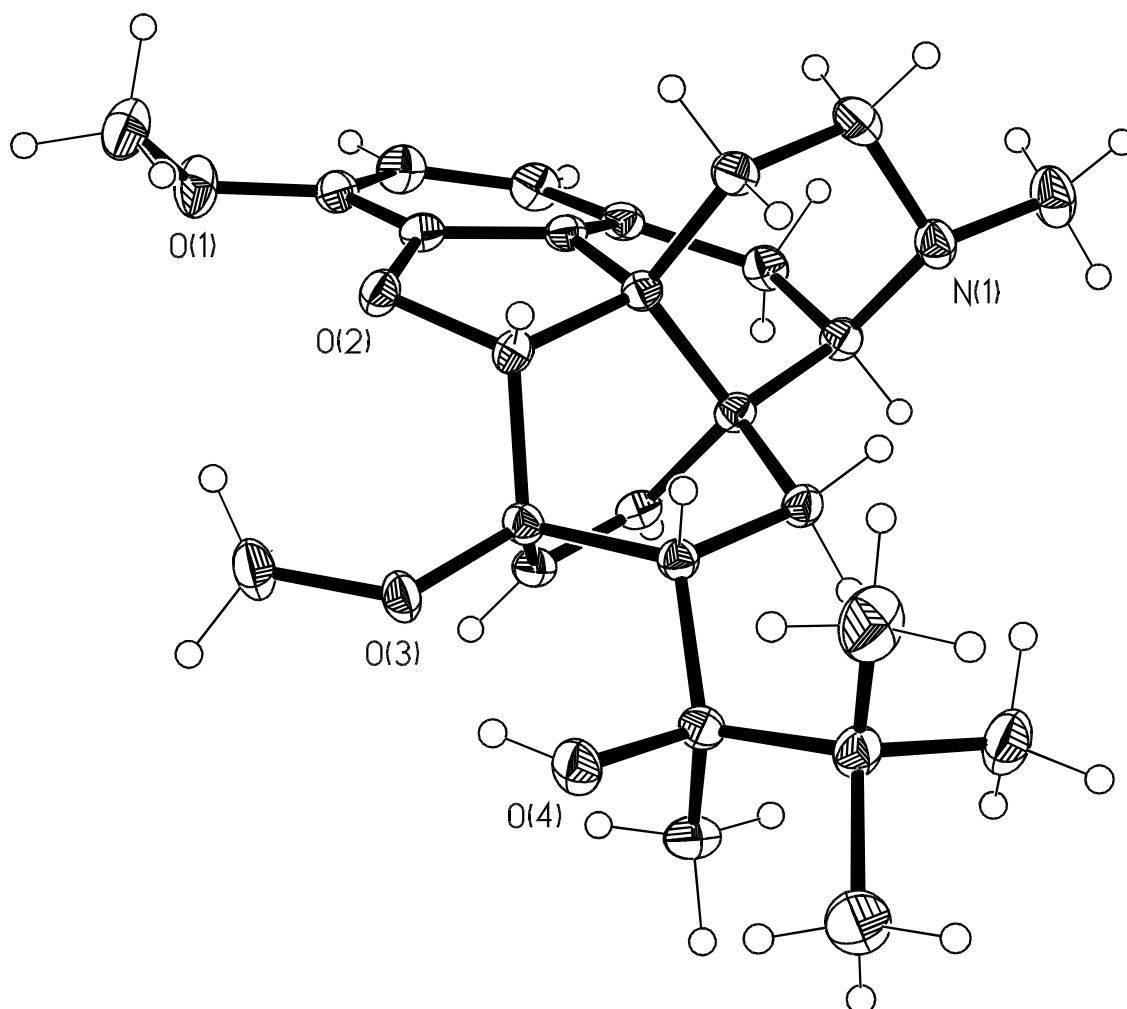
Melting points: *Kofler* melting-point microscope; uncorrected. NMR Spectra: *Varian Gemini 200* spectrometer; δ in ppm rel. to SiMe₄ as internal reference, *J* in Hz. IR Spectra: *Mattson Galaxy Series FTIR 3000*. Mass Spectra: *Varian MAT 44 S* apparatus. Elemental Analyses were performed at the Institute of Physical Chemistry of the University of Vienna.

***tert*-Butylthevinol (= 4,5 α -Epoxy-6 α ,14 α -etheno-7 α -(1-hydroxy-1,2,2-trimethylpropyl)-**

3,6 β -dimethoxy-17-methylmorphinan; 4). 100 mL of 2 M *tert*-BuMgCl solution in diethyl ether was evaporated in a 1 L three necked flask, 100 mL of *tert*-butyl methyl ether added and again evaporated. This operation was repeated with 50 mL of *tert*-butyl methyl ether to give a colorless solid which was dissolved in 350 mL *tert*-butyl methyl ether and refluxed under N₂. A Soxhlet apparatus containing 15.0 g of thevinone ((**3**); 39.32 mmol) was placed onto the three necked flask and thevinone was slowly dissolved by the refluxing *tert*-butyl methyl ether (3 h). After cooling to rt, the mixture was poured on 400 mL of saturated NH₄Cl solution. The organic phase was separated and the H₂O phase extracted with Et₂O (2 x 80 mL, 2 x 50 mL). The combined organic layers were washed with H₂O (100 mL) and brine (100 mL), dried (Na₂SO₄) and evaporated to give 16.49 g of a slightly yellow solid which was treated

with boiling MeOH (20 mL) to yield 10.03 g (58%) of **4** which contains a little amount of thevinone (TLC). This material was used for the next step without further purification. An analytical sample was obtained the following way: 1.58 g of **4** were recrystallized three times from MeOH/CH₂Cl₂ (6:1) to give 1.08 g of **4** which contained very little thevinone (TLC). Flash chromatography of this material (silica gel; elution with CH₂Cl₂/MeOH/conc.NH₄OH 250:2:0.5) afforded 790 mg of crystalline **4** which was treated with 3 mL of MeOH to yield 720 mg of pure **4**. mp 220-222°C (lit.,⁹ 216°C (EtOH)). IR (KBr): 3438 (OH). ¹H-NMR (CDCl₃): 6.62 (*d*, *J* = 8.1, 1 arom. H); 6.51 (*d*, *J* = 8.1, 1 arom. H); 5.99 (*d*, *J* = 8.7, 1 olef. H); 5.62 (*s*, OH-C(20)); 5.43 (*d*, *J* = 8.7, 1 olef. H); 4.56 (*s*, H-C(5)); 3.82 (*s*, CH₃O-C(3)); 3.77 (*s*, CH₃O-C(6)); 2.36 (*s*, CH₃N); 1.01 (*s*, (CH₃)₃C-C(20)); 0.99 (*s*, CH₃-C(20)). ¹³C-NMR (CDCl₃): 148.73 (arom. C); 142.38 (arom. C); 135.96 (olef. CH(18)); 135.19 (arom. C); 129.14 (arom. C); 125.52 (olef. CH(19)); 119.91 (arom. CH(1)); 114.49 (arom. CH(2)); 99.47 (CH(5)); 85.02 (C(6) or C(20)); 79.02 (C(6) or C(20)); 60.60 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 57.47 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 55.84 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 47.01 (quart. C); 46.49 (CH); 46.10 (CH₂); 44.18 (N-CH₃); 43.79 (quart. C); 40.32 (quart. C); 34.40 (CH₂); 32.68 (CH₂); 27.28 ((CH₃)₃C-C(20)); 22.95 (CH₂); 20.30 (CH₃-C(20)). EI-MS: 439 (*M*⁺). Anal. Calcd for C₂₇H₃₇NO₄ · 0.3 MeOH: C 73.00, H 8.57, N 3.12. Found: C 72.96, H 8.43, N 3.11.

Crystal Structure Data of 4: C₂₇H₃₃NO₄, *M* 439.58; orthorhombic, P2₁2₁2₁, *a* = 10.693(2), *b* = 12.094(2), *c* = 17.999(4) Å, *V* = 2327.7(7) Å³ (*λ* 0.71073), *Z* = 4, *D*_{calc} = 1.254 g/cm³, *F*(000) = 952; *μ* = 0.083 mm⁻¹, crystal size 0.9 x 0.7 x 0.35 mm. Data were collected at 213(2) K in the *θ* range 2.5 - 24.0° on a Bruker P4 diffractometer. Data were measured via *ω*-scans and corrected for Lorentz and polarisation effects, but not for absorption. The structure was solved by direct methods (SHELXS-86) and refined by full matrix least-squares against *F*² (SHELXL-93). All non-hydrogen atoms were refined with anisotropic displacement parameters. All hydrogen atoms were located by difference Fourier methods and refined at the hydroxyl group, the others were generated geometrically and refined with isotropic displacement parameters 1.2 and 1.5 times higher than *U*(eq) of the attached C atoms. In the final least-squares refinement cycles the model converged at *R*₁ = 0.0306, *wR*₂ = 0.0773, and GOF = 1.067 for 3464 reflections with *F*_o ≥ 4σ(*F*_o) and 301 parameters. Copies of the crystallographic data are available on application to the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44(1223)336033; e-mail: deposit@ccdc.cam.ac.uk) citing the deposition No CCDC-135525.



ORTEP plot of the molecular structure of **4** (with 30% probability ellipsoids)

***N*-Cyano-*tert*-Butylthevinol (= 17-Cyano-4,5 α -epoxy-6 α ,14 α -etheno-7 α -(1-hydroxy-1,2,2-trimethylpropyl)-3,6 β -dimethoxymorphinan; **5**).** A mixture of **4** (10.05 g, 22.86 mmol), BrCN (4.70 g, 44.37 mmol) and CHCl₃ (45 mL) was refluxed for 18 h and then evaporated. The crystalline residue (9.61 g) was recrystallized from 30 mL of EtOH to afford 9.06 g (89%) of slightly impure **5** which was not further purified for the next step. Flash chromatography of 2.0 g of this slightly impure **5** (see above) gave 840 mg of crystalline **5** which was treated with 5 mL of MeOH to yield 600 mg of pure **5**. mp 265-267°C (lit.,⁶ 254-258°C (EtOH)). IR (KBr): 3457, 2202 (OH, NCN). ¹H-NMR (CDCl₃): 6.67 (*d*, *J* = 8.2, 1 arom. H); 6.56 (*d*, *J* = 8.2, 1 arom. H); 6.09 (*d*, *J* = 8.9, 1 olef. H); 5.47 (*s*, OH-C(20)); 5.37 (*d*, *J* = 8.9, 1 olef. H); 4.56 (*d*, *J* = 1.0, H-C(5)); 3.83 (*s*, CH₃O-C(3)); 3.77 (*s*, CH₃O-C(6)); 1.01 (*s*, (CH₃)₃C-C(20)); 0.99 (*s*, CH₃-C(20)). ¹³C-NMR (CDCl₃): 148.93 (arom. C); 143.03 (arom. C); 133.38 (olef. CH(18)); 133.01 (arom. C); 127.07 (olef. CH(19)); 126.19 (arom. C); 120.52 (arom. CH(1)); 118.61 (NCN); 115.26 (arom CH(2)); 98.95 (CH(5)); 84.56 (C(6) or C(20)); 78.77 (C(6) or C(20)); 59.02 (CH(9))

or CH₃O-C(3) or CH₃O-C(6)); 57.37 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 55.96 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 46.47 (quart. C); 46.42 (CH); 42.61 (CH₂); 42.51 (quart. C); 40.32 (quart. C); 32.43 (CH₂); 27.23 ((CH₃)₃C-C(20)); 20.18 (CH₃-C(20)). EI-MS: 450 (*M*⁺). Anal. Calcd for C₂₇H₃₄N₂O₄ · 0.1 MeOH: C 71.73, H 7.64, N 6.17. Found: C 71.78, H 7.73, N 6.14.

***N*-nor-*tert*-Butylthevinol (= 4,5 α -Epoxy-6 α ,14 α -etheno-7 α -(1-hydroxy-1,2,2-trimethylpropyl)-**

3,6 β -dimethoxymorphinan; 6). A mixture of KOH (10.0 g, 178.22 mmol), **5** (10.0 g, 22.19 mmol) and diethylene glycol (70 mL) was stirred at 165-175°C (bath temperature) for 80 min, cooled to rt and poured on 550 mL of ice water. This mixture was extracted with CH₂Cl₂ (1 x 150 mL, 3 x 50 mL), the combined organic layers washed with H₂O (100 mL) and brine (100 mL), dried (Na₂SO₄) and evaporated to give 8.22 g of a slightly yellow crystalline residue. Treatment with boiling MeOH (20 mL) yielded 7.07 g (75%) of slightly impure **6** which was not further purified for the next step. Flash chromatography of 1.87 g of this slightly impure **6** (see above) gave 1.63 g of crystalline **6** which was treated with 5 mL of MeOH to yield 1.31 g of pure **6**. mp 204-206°C (lit.,⁶ 186-190°C). IR (KBr): 3382, 3316 (OH, NH). ¹H-NMR (CDCl₃): 6.64 (*d*, *J* = 8.3, 1 arom. H); 6.52 (*d*, *J* = 8.3, 1 arom. H); 6.03 (*d*, *J* = 9.1, 1 olef. H); 5.59 (*s*, OH-C(20)); 5.41 (*d*, *J* = 9.1, 1 olef. H); 4.55 (*d*, *J* = 1.4, H-C(5)); 3.83 (*s*, CH₃O-C(3)); 3.78 (*s*, CH₃O-C(6)); 1.02 (*s*, (CH₃)₃C-C(20)); 1.00 (*s*, CH₃-C(20)). ¹³C-NMR (CDCl₃): 148.78 (arom. C); 142.53 (arom. C); 135.50 (olef. CH(18)); 134.85 (arom. C); 128.68 (arom. C); 126.12 (olef. CH(19)); 120.01 (arom. CH(1)); 114.67 (arom. CH(2)); 99.81 (CH(5)); 84.81 (C(6) or C(20)); 78.95 (C(6) or C(20)); 57.45 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 55.88 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 53.54 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 47.75 (quart. C); 46.59 (CH); 42.85 (quart. C); 40.34 (quart. C); 37.88 (CH₂); 34.70 (CH₂); 33.82 (CH₂); 32.59 (CH₂); 27.29 ((CH₃)₃C-C(20)); 20.27 (CH₃-C(20)). EI-MS: 425 (*M*⁺). Anal. Calcd for C₂₆H₃₅NO₄ · 0.1 MeOH: C 73.11, H 8.32, N 3.27. Found: C 73.06, H 8.35, N 3.25.

***N*-Cyclopropylmethyl-*tert*-Butylthevinol (= 17-Cyclopropylmethyl-4,5 α -epoxy-6 α ,14 α -etheno-**

7 α -(1-hydroxy-1,2,2-trimethylpropyl)-3,6 β -dimethoxymorphinan; 7). A mixture of **6** (10.0 g, 23.5 mmol), NaHCO₃ (12.0 g, 142.84 mmol), cyclopropylmethyl bromide (3.0 mL, 30.0 mmol) and anhydrous DMF (75 mL) was stirred at 80° (bath temperature) for 50 min, cooled to rt and filtered. The filtrate was diluted with H₂O (500 mL) and extracted with CH₂Cl₂ (2 x 100 mL, 3 x 50 mL). The combined organic layers were washed with H₂O (3 x 200 mL) and brine (150 mL), dried (Na₂SO₄) and evaporated to give 11.12 g of a crystalline solid which was treated with boiling MeOH (20 mL) to afford 10.23 g (91%) of slightly impure **7** which was not further purified for the next step. Flash chromatography

of 1.8 g of this slightly impure **7** (see above) gave 1.7 g of an oil which was crystallized from a mixture of MeOH (3 mL) and CH₂Cl₂ (1 mL) to yield 1.53 g of **7**. Recrystallization from a mixture of MeOH (5 mL) and CH₂Cl₂ (1 mL) afforded 910 mg of pure **7**. mp 165-168°C (lit.,⁶ 145-153°C). IR (KBr): 3463 (OH). ¹H-NMR (CDCl₃): 6.61 (*d*, *J* = 8.2, 1 arom. H); 6.48 (*d*, *J* = 8.2, 1 arom. H); 5.99 (*d*, *J* = 8.9, 1 olef. H); 5.63 (*s*, OH-C(20)); 5.44 (*d*, *J* = 8.9, 1 olef. H); 4.56 (*d*, *J* = 1.2, H-C(5)); 3.82 (*s*, CH₃O-C(3)); 3.77 (*s*, CH₃O-C(6)); 1.01 (*s*, (CH₃)₃C-C(20), CH₃-C(20)); 0.51 (*m*, CH₂(cp)); 0.15 (*m*, CH₂(cp)). ¹³C-NMR (CDCl₃): 148.74 (arom. C); 142.33 (arom. C); 136.18 (olef. CH(18)); 135.41 (arom. C); 129.15 (arom. C); 125.44 (olef. CH(19)); 119.89 (arom. CH(1)); 114.44 (arom. CH(2)); 99.62 (CH(5)); 85.13 (C(6) or C(20)); 79.01 (C(6) or C(20)); 60.17 (N-CH₂-cp); 57.46 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 55.84 (CH(9) or CH₃O-C(3) or CH₃O-C(6)); 47.73 (quart. C); 46.48 (CH); 44.72 (CH₂); 43.71 (quart. C); 40.32 (quart. C); 34.61 (CH₂); 32.81 (CH₂); 27.28 ((CH₃)₃C-C(20)); 23.76 (CH₂); 20.25 (CH₃-C(20)); 10.13 (CH(cp)); 4.94 (CH₂(cp)); 3.82 (CH₂(cp)). EI-MS: 479 (*M*⁺). Anal. Calcd for C₃₀H₄₁NO₄ · 0.8 MeOH: C 73.21, H 8.82, N 2.77. Found: C 73.17, H 8.70, N 2.76.

18,19-Dehydrobuprenorphine (= 17-Cyclopropylmethyl-4,5α-epoxy-6α,14αetheno-7α-(1-hydroxy-1,2,2-trimethylpropyl)-6β-methoxymorphinan-3-ol; 2). A mixture of **7** (9.5 g, 19.8 mmol), sodium ethanethiolate (6.66 g, 79.18 mmol) and anhydrous DMF (150 mL) was stirred under N₂ at 130° (bath temperature) for 3.5 h, cooled to rt and poured on saturated NH₄Cl solution (700 mL). The mixture was alkalized with conc. NH₄OH, extracted with CH₂Cl₂ (3 x 100 mL, 3 x 50 mL), the combined organic layers washed with H₂O (3 x 200 mL) and brine (100 mL), dried (Na₂SO₄) and evaporated to yield 10.4 g of a yellowish crystalline solid which was recrystallized from MeOH (20 mL) to give 6.89 g (75%) of slightly impure **2**. Flash chromatography of 850 mg of this slightly impure **2** (see above) gave 650 mg colorless crystals of **2** which was recrystallized from MeOH to yield 530 mg pure **2**. mp 245-247°C (lit.,⁶ 238-240°C (MeOH), lit.,⁷ 237.5-239° (MeOH)). IR (KBr): 3423, 3280 (OH-C(20), OH-C(3)). ¹H-NMR (CDCl₃): 6.59 (*d*, *J* = 8.1, 1 arom. H); 6.45 (*d*, *J* = 8.1, 1 arom. H); 5.96 (*d*, *J* = 8.9, 1 olef. H); 5.58 (*s*, OH-C(20)); 5.43 (*d*, *J* = 8.9, 1 olef. H); 4.59 (*d*, *J* = 1.4, H-C(5)); 3.76 (*s*, CH₃O-C(6)); 1.01 (*s*, (CH₃)₃C-C(20), CH₃-C(20)); 0.52 (*m*, CH₂(cp)); 0.15 (*m*, CH₂(cp)). ¹³C-NMR (CDCl₃): 147.30 (arom. C); 138.06 (arom. C); 136.35 (olef. CH(18)); 135.16 (arom. C); 128.56 (arom. C); 125.12 (olef. CH(19)); 120.39 (arom. CH(1)); 116.83 (arom. CH(2)); 99.98 (CH(5)); 85.19 (C(6) or C(20)); 79.32 (C(6) or C(20)); 60.20 (N-CH₂-cp); 57.48 (CH(9) or CH₃O-C(6)); 55.85 (CH(9) or CH₃O-C(6)); 48.07 (quart. C); 46.44 (CH); 44.77 (CH₂); 43.81 (quart. C); 40.34 (quart. C); 34.56 (CH₂); 32.81 (CH₂); 27.30 ((CH₃)₃C-C(20)); 23.84 (CH₂); 20.29 (CH₃-C(20)); 10.13 (CH(cp)); 4.95 (CH₂(cp)); 3.84 (CH₂(cp)). EI-MS: 465 (*M*⁺). Anal. Calcd for C₂₉H₃₉NO₄ · 0.1 MeOH: C 74.55, H 8.47, N 2.99. Found: C 74.55, H 8.72, N 2.97.

Opioid receptor binding assay

Binding to μ and δ opioid receptors was assayed on crude membrane preparations from adult male rat brain (Wistar, 250-300 g) and binding to κ opioid receptors was assayed on crude membrane preparations from adult male guinea-pig brain (450 g) as previously described.¹¹ The inhibition constant (K_i) was calculated from competitive binding curves with the computer program Ligand.¹² Data obtained from four independent measurements are presented as the arithmetic mean.

Tail-flick assay

Male Swiss CD-1 mice (30-40 g) were used for the experiments. They were housed at 22 ± 2 °C, with food and water ad libitum. A standard light/dark cycle was maintained with a time-regulated light period from 6 h to 18 h. The IASP guidelines on ethical standards for investigations of experimental pain in animals were followed. Compounds were dissolved in 10% DMSO and administered subcutaneously (s.c.) to the mice in a volume of $5 \mu\text{L g}^{-1}$ of body weight. Control mice received vehicle at $5 \mu\text{L g}^{-1}$. Each animal received one injection only. Every dose of each compound was evaluated in groups of 6-8 animals. Antinociception was assessed by exposing the mouse tail to radiant heat¹⁰ and the latency to a rapid tail-flick was recorded with the baseline cutoff and the maximal possible latencies set at 4 sec and 12 sec, respectively. The degree of antinociception was expressed as percentage maximum possible effect (% MPE) according to the equation: $\% \text{ MPE} = [(\text{test latency} - \text{control latency}) / (12 - \text{control latency})] \times 100$. The tail-flick latency was measured before drug treatment (control) and every 15 min after drug injections, during the first hour, and every 30 min thereafter, until antinociception disappeared. A computer program (PRISM, GraphPad, CA, USA) was used to calculate the mean peak effect for each dose. Doses that produced peak effects between 20% and 80% MPE were plotted into a log dose-response curve and AD₅₀ values were calculated as doses that produced 50% MPE.¹³

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