2,5-Dimethyl-2′-hydroxy-9α- and 9β-(3-Methylbutyl)-6,7-benzomorphans and *N*-Substituted Compounds in the 9α-(3-Methylbutyl) Series: Chemistry, Pharmacology, and Biochemistry

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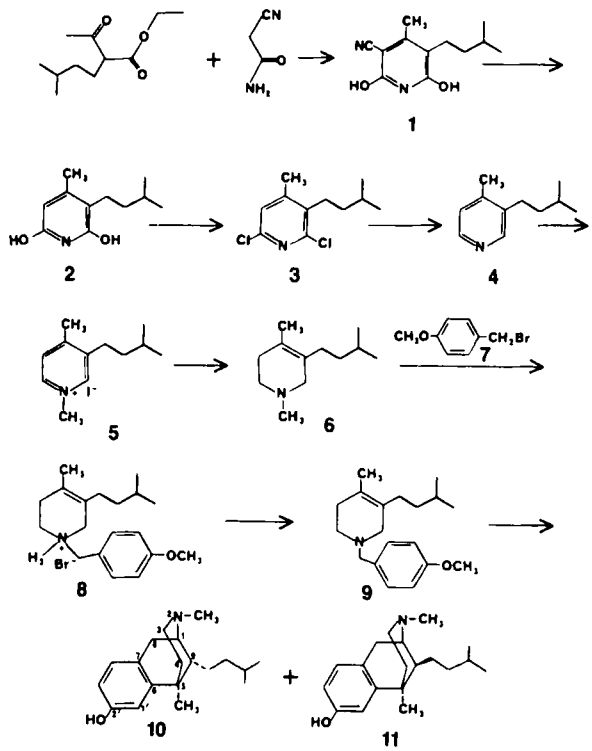
Abstract □ 2,5-Dimethyl-2′-hydroxy-9α-(3-methylbutyl)-6,7-benzomorphan, the 9β-analogue, and 9α-*N*-substituted (*N*-ethyl, propyl, butyl, pentyl, hexyl, phenylethyl, allyl, and cyclopropylmethyl) compounds were synthesized and evaluated biochemically and pharmacologically. The 9β *N*-methyl compound was found to be as potent as morphine in the mouse hot plate assay and had one-seventh the affinity of morphine for the opioid receptor. The *N*-alkyl and *N*-phenethyl 9α-substituted compounds were either inactive or relatively ineffective as antinociceptive agents. None of the examined compounds substituted for morphine in single-dose suppression studies in the rhesus monkey. The *N*-cyclopropylmethyl compound in the 9α series had half the narcotic antagonist potency of nalorphine and one-eighth of its affinity for the opioid receptor. The 9α-(3-methylbutyl) moiety, unlike bulky substituents in the 9β position of 6,7-benzomorphans, generally lowers affinity for the μ opioid receptor and diminishes their in vivo activity as agonists or antagonists.

The 6,7-benzomorphan class of analgesics was originated[1] and explored by May and Murphy,[2] and later by investigators worldwide,[3] because of their potential medical usefulness and their biochemical importance. For example, the prototypic κ-(2′-hydroxy-2-cyclopropylmethyl-5-ethyl-2-keto-9α-methyl-6,7-benzomorphan, ethyl-ketocyclazocine), and σ-(*N*-allylnormetazocine) opioid receptor ligands were found among this analgesic class by Martin et al.[4]

The SAR of *N*-alkyl substituted 6,7-benzomorphans generally display biphasic agonist (antinociceptive) activity in the mouse hot plate assay. In the 5,9α-dimethyl (*N*-normetazocine) series, the *N*-methyl, *N*-pentyl, and/or *N*-hexyl compounds have maximal antinociceptive effect.[5] The intermediate length *N*-propyl-substituted 6,7-benzomorphans generally possess potent narcotic antagonist properties and have little antinociceptive effect. *N*-Ethyl and *N*-butyl substituents are much less effective as agonists; they have weak, if any, narcotic antagonist activity in vivo. Alkyl substituents in the C-5 and C-9α positions of 6,7-benzomorphans have been generally found to quantitatively aid or detract from the potency of the compound as an agonist or antagonist, but their qualitative nature (agonist versus antagonist) appeared to be dependent on the *N* substituent. There have been at least two exceptions to that general rule with 6,7-benzomorphans. We have previously found that a C-9α substituent could qualitatively alter the pharmacological effect of the 6,7-benzomorphan.[6,7] In that study, we found that 2′-hydroxy-5-methyl-9α-propyl-2-pentyl-6,7-benzomorphan was not an agonist; instead it acted as a long-acting antagonist in precipitated withdrawal studies in rhesus monkeys. The bulky 9α-propyl group was presumed to be responsible for that qualitative change. Long-chain, bulky substituents in the 9β position of the 6,7-benzomorphan have been found to exert similar effects.[8] In order to further explore the effect of bulky substituents on activity and opioid receptor binding affinity in the C-9 position of 6,7-benzomorphans, we have synthesized and evaluated an *N*-methyl-9β-(3-methylbutyl)-6,7-benzomorphan and a series of *N*-substituted 9α-(3-methylbutyl) compounds.

Results and Discussion

The synthesis of **10** and **11** was accomplished using the route previously described for the corresponding 9α- and 9β-propyl derivatives (Scheme I).[6] *N*-Demethylation of **10** was accomplished using the phenyl chloroformate–hydrazinolysis procedure.[9] Methods A and B used in this study for the preparation of **13–20** (Scheme II) were described earlier.[7] The purification of **9** via the fumarate from a five-component mixture containing <40% of **9** is noteworthy. Of the ~10 salts prepared, satisfactory results could only be obtained with this acid which gave almost completely pure salt by a



Scheme I

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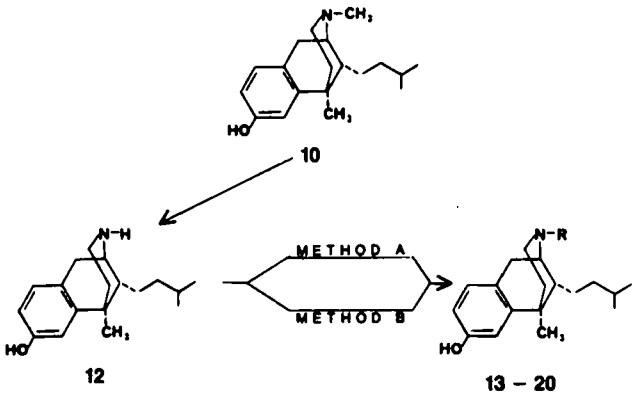

Scheme II

simple crystallization. It is relatively simple and rapid to prepare and find the solubility properties of amine salts on a micro scale. Large scale chromatography, on the other hand, is much more labor-intensive and requires considerably more material (solvent, silica, etc.) to separate more than 100 g of a five-component mixture. These observations speak strongly for "finding the right salt" as an alternate to large scale chromatography.

Although the *N*-methyl analogue in the 9β (11) series was morphine-like in potency in the hot plate[10] assay (mice, s.c. injection), the *N*-methyl analogue in the 9α series (10) was only half as potent as codeine in the hot plate assay, and even less potent in the phenylquinone assay[11] (Table I). None of the other *N*-substituted compounds (the *N*-ethyl, propyl, butyl, pentyl, hexyl, 2-phenyethyl, allyl, and cyclopropylmethyl analogues) in the 9α series had any antinociceptive activity in the hot plate or tail flick[12] assays, although a few showed a little activity in the phenylquinone assay. The *N*-propyl, *N*-allyl, and *N*-cyclopropylmethyl analogues did show some narcotic antagonist activity in the tail flick assay versus morphine, the latter compound having half the potency of nalorphine. None of the 9α compounds acted as opioid-like agonists in monkeys in single-dose substitution studies.[13,14] In receptor binding studies, the *N*-cyclopropylmethyl compound had one-eighth of the affinity of morphine, the *N*-methyl compound in the 9β series (11) had one-seventh of its affinity, and the remaining compounds had even less affinity for the μ-opioid receptor (Table I). The two compounds with the highest in vitro affinity, 11 and 18, were the most potent in vivo as an agonist or antagonist, respectively. Compounds with K_i values between 107 and 235 nM displayed some in vivo activity. The remaining compounds, with K_i's >250 nM, were inactive in vivo. The relatively high in vitro affinity of the *N*-phenethyl analog 19 was anomalous. It had no detectable in vivo activity. There was reasonable correlation between in vivo activity in antinociceptive assays for compounds 10, 14, 15, 20, and 11, or narcotic anatagonist activity (for 18), and in vitro affinity for the μ-opioid receptor.

Dreiding molecular models clearly indicate that rotation around the single bonds in the 9α chain can cause the end methyl groups to reside under the aromatic ring (on the face opposite that of the piperidine ring), which could well hinder the approach of the aromatic ring to the receptor macromolecule and interfere with receptor interaction. When that group is removed from the vicinity of the aromatic ring, as it is in the 9β series, the *N*-methyl analogue has antinociceptive potency equivalent to many benzomorphans with methyl or ethyl groups in the C9 position, and binds with reasonable affinity to the receptor. Thus it is probably not the bulk of the 3-methylbutyl group per se which causes inactivity, but rather its position in space relative to the aromatic ring. This is in accord with other known 9β-substituted benzomorphans with long side-chains.[8]

Experimental Section

Melting points were determined in open capillary tubes using a Thomas-Hoover apparatus and are corrected. Boiling points are uncorrected. Microanalyses were performed by the Section on Microanalytical Services and Instrumentation, LC, NIDDK, and are within ±0.4% of the calculated values. Results from IR (Perkin-Elmer 21), NMR (Varian A-60 or HR-220), and MS (Hitachi Perkin-Elmer RMU-6E for electron impact ionization [EIMS], or a Finnigan 1050D with model 6000 data collection system for chemical ionization [CIMS]) were consistent with the assigned structures.

3-Cyano-2,6-dihydroxy-4-methyl-3-(3-methyl)butylpyridine (1)—Analogous to the procedure for the 5-propyl derivative,[6] treat-

Table I—Pharmacological and In Vitro μ-Opioid Receptor Binding Data on *N*-Substituted 2'-Hydroxy-5-methyl-9-(3-methylbutyl)-6,7-benzomorphans

N-Substituent	HP[a]	TF[a]	PPQ[a]	TFA[a]	SDS[a]	K_i[b]
9α-Series						
Methyl (10)	14.1 (11.7–17.0)	I[a]	13.8 (4.6–41.4)	I	I (5.6–10.0)	112
Ethyl (13)	I	I	I	I	I (5.6–17.0)	630
Propyl (14)	I	I	7.0 (3.1–16.1)	14.8 (9.1–24.0)	I[c] (1.0–5.0)	137
Butyl (15)	I	I	27.3 (13.3–56.0)	I	I (5.6–17.0)	235
Pentyl (16)	I	I	I	I	I (4.0–16.0)	399
Hexyl (17)	I	—	—	—	—	255
CPM[d] (18)	I	I	14.4 (10.9–19.1)	5.5 (3.2–9.5)	I[e] (3.0)	17
Phenylethyl (19)	I	I	I	I	I (5.6–10.0)	22
Allyl (20)	I	I	10.3 (5.5–19.9)	11.2 (4.4–29.0)	I[c] (1.7–5.6)	107
9β-Compound						
Methyl (11)	1.5 (1.2–2.0)	—	—	—	—	15
Morphine	1.3 (0.9–1.6)	5.8 (5.7–5.9)	0.23 (0.20–0.26)	I	S[f]	2
Nalorphine	13.8 (9.0–21.3)	I	0.6 (0.3–1.4)	2.6 (0.7–9.8)	I (0.1–1.0)[g]	2
Codeine	6.8 (4.5–10.2)	14.5 (8.1–26)	1.1 (0.5–2.5)	I	S[h]	660

[a] In mice, sc injection (mg/kg); numbers in parentheses are the 95% confidence limits obtained by probit analysis; I = inactive (no dose–response or insufficient response for probit analysis). Highest dose tested: HP—100 mg/kg except for 15, 80 mg/kg, and 18, 50 mg/kg; TF, PPQ, and TFA—30mg/kg. Antinociceptive assays: HP = hot plate, TF = tail flick, PPQ = phenylquinone; narcotic antagonist assay: TFA = tail flick antagonism versus morphine; SDS = single dose substitution test in the morphine dependent rhesus monkey (numbers in parentheses are the doses used in mg/kg). [b] Displacement of [^{3}H]DAGO from rat brain membranes; K_i = calculated inhibition constant (nM). [c] Did not precipitate withdrawal in nonwithdrawn monkeys. [d] Cyclopropylmethyl. [e] Slight exacerbation of withdrawal noted in nonwithdrawn monkeys. [f] Substituted at 3 mg/kg. [g] Precipitates withdrawal (0.5 mg/kg) in nonwithdrawn monkeys. [h] Substituted for morphine at 4 and 8 mg/kg.

ment of 277.0 g (1.47 mol) of ethyl 2-(3-methyl)butylacetoacetate and 232.9 g (2.77 mol) of cyanoacetamide in 2085 mL of MeOH, with 91.7 g (1.4 mol) of 85% KOH in 975 mL of MeOH, yielded 176.8 g of a solid. Treatment of this material with 70 mL of 37% HCl in 1600 mL of 6.25% EtOH in H_2O (v/v) gave 146.7 g (48%) of nearly pure, off-white **1**. This material and that from a similar run were combined (310.0 g) and recrystallized from isopropyl alcohol (filtered hot through celite) to give 254.6 g (82% recovery) of pure **1**, mp 242–244 °C.

Anal.—Calc. for $C_{12}H_{16}N_2O_2$: C, 65.43; H, 7.32; N, 12.72. Found: C, 65.30; H, 7.67; N, 12.34.

2,6-Dihydroxy-4-methyl-3-(3-methyl)butylpyridine (2)—A stirred mixture of 129.6 g (0.59 mol) of **1**, 1040 mL of 48% HBr, and 150 mL of AcOH were stirred and refluxed for ~72 h. The reaction mixture was diluted with 3.0 L of H_2O, cooled to 5 °C, and filtered to give 103.3 g (90%) of beige **2**. Recrystallization from EtOH gave pure **2**, mp 161–162 °C.

Anal.—Calc. for $C_{11}H_{17}NO_2$: C, 67.66; H, 8.78; N, 7.17. Found: C, 67.66, H, 8.51; N, 7.01.

2,6-Dichloro-4-methyl-3-(3-methyl)butylpyridine (3)—A mixture of 67.0 g (0.34 mol) of **2** and 250 g (150 mL, 1.63 mol) of $POCl_3$ were placed in a 330-mL steel bomb and capped. The bomb was placed in an oil bath at 180–200 °C for 4 h. The cooled contents of the bomb were poured over crushed ice and the mixture was extracted with hexane. The hexane was dried ($MgSO_4$) and evaporated, and the residue was distilled to afford 64.9 g (80%) of **3**, bp 118–120 °C/0.5 mm.

Anal.—Calc. for $C_{11}H_{15}NCl_2$: C, 56.91; H, 6.51; N, 6.03. Found: C, 56.73; H, 6.37; N, 6.02.

4-Methyl-3-(3-methyl)butylpyridine (4)—Reduction of 46.5 g (0.2 mol) of **3** (analogous procedure to that used for the synthesis of 4-methyl-5-propylpyridine) gave 31.1 g (95%) of pure **4**, bp 108–110 °C/10 mm. The picrate was prepared in acetone, mp 151–152 °C.

Anal.—Calc. for $C_{17}H_{20}N_4O_7$: C, 52.04; H, 5.14; N, 14.28. Found: C, 52.31; H, 5.38; N, 14.56.

4-Methyl-3-(3-methyl)butylpyridine Methiodide (5)—A stirred solution of 100 g (0.61 mol) of **4** in 1100 mL of acetone was treated with 176.0 g (1.24 mol) of CH_3I (exothermic to 47 °C). After the mixture was allowed to stand overnight, it was seeded and slowly diluted with 1100 mL of Et_2O. The solid was filtered, washed with Et_2O, and dried under reduced pressure to give 176.8 g (94%) of **5**, mp 111–112 °C.

Anal.—Calc. for $C_{12}H_{20}NI$: C, 47.23; H, 6.61; N, 4.59. Found: C, 47.56; H, 6.77; N, 4.63.

1,4-Dimethyl-1-(*p*-methoxybenzyl)-3-(3-methyl)butyl-1,2,5,6-tetrahydropyridinium Bromide (8)—In a manner analogous to that used for the synthesis of the corresponding 5-propyl derivative,[6] 159.5 g (0.52 mol) of **5** afforded the corresponding crude **6** quantitatively. The oily, relatively unstable **6** was characterized as the crystalline quaternary salt **8**, by treatment with 120.0 g of 4-methoxybenzyl bromide (**7**) in 330 mL of Et_2O:acetone (100:5 v/v) to give 147.2 g (73%) of **8**. An analytical sample was recrystallized from CH_3CN:EtOAc, mp 157–159 °C.

Anal.—Calc. for $C_{20}H_{32}NOBr$: C, 62.82; H, 8.44; N, 3.66. Found: C, 62.54; H, 8.69; N, 3.43.

1-(4-Methoxybenzyl)-3-(3-methyl)butyl-4-methyl-1,2,4,6-tetrahydropyridine (9)—A slurry of 147.2 g (0.38 mol) of **8** in 480 mL of Et_2O was treated with 460 mL (1.2 mol) of 2.6 M BuLi in hexane. After the brisk reflux subsided, the mixture was stirred for 15 min, poured onto crushed ice, rendered strongly alkaline with 30% NaOH, and extracted with Et_2O. The extract was washed with H_2O, dried (Na_2SO_4), and evaporated to a dark oil. This oil was treated with a solution of 44.1 g of fumaric acid (0.38 mol) in 230 mL of CH_3CN to afford 45.6 g (32%) of essentially pure **9**·fumarate. Recrystallization from CH_3CN gave an analytical sample, mp 154–155 °C.

Anal.—Calc. for $C_{24}H_{34}NO_5$: C, 69.21; H, 8.23; N, 3.36. Found: C, 69.28; H, 8.25; N, 3.22.

(±)-2,5-Dimethyl-2′-hydroxy-9α-(3-methylbutyl)-6,7-benzomorphan (10)—Treatment of 120.0 g (0.29 mol) of **9**·fumarate with excess 1N NaOH, extraction with Et_2O, and evaporation of the solvent afforded 9 base which was refluxed for 24 h with 1200 mL of 48% HBr. The mixture was cooled, poured onto crushed ice, rendered strongly alkaline with 28% aqueous NH_3, and extracted with $CHCl_3$. The extract was washed with H_2O, dried (Na_2SO_4), treated with activated charcoal (Norite), filtered, and evaporated. Crystallization from Et_2O afforded 43.5 g (52%) of essentially pure **10** in three crops. Recrystallization from 5% EtOH in EtOAc afforded an analytical sample, mp 194–195 °C.

Anal.—Calc. for $C_{19}H_{29}NO$: C, 79.39; H, 10.17; N, 4.87. Found: C, 79.46; H, 9.90; N, 4.59.

Treatment of **10** with an equivalent of CH_3SO_3H in acetone: EtOAc gave **10**·CH_3SO_3H, mp 140–142 °C.

Anal.—Calc. for $C_{20}H_{33}NO_4S$: C, 62.63; H, 8.67; N, 3.65. Found: C, 62.69; H, 8.48; N, 3.85.

(±)-2,5-Dimethyl-2′-hydroxy-9β-(3-methyl)butyl-6,7-benzomorphan (11)—The filtrate and washings from the third crop of **10** above were evaporated and crystallized from a small volume of MeOH to give 9.0 g (11%) of **11**. Recrystallization gave an analytical sample, mp 147–148 °C.

Anal.—Calc. for $C_{19}H_{29}NO$: C, 79.39; H, 10.17; N, 4.87. Found: C, 79.28; H, 10.05; N, 4.85.

The **11**·HCl·H_2O was crystallized from H_2O and air-dried for analysis, mp 162–164.5 °C.

Anal.—Calc. for $C_{19}H_{32}NO_2Cl$: C, 66.75; H, 9.44; N, 4.10. Found: C, 66.84; H, 9.55; N, 3.97.

(±)-2′-Hydroxy-5-methyl-9α-(3-methyl)butyl-6,7-benzomorphan (12)—A solution of **10** (4.5 g, 15.7 mmol) in $CHCl_3$ (300 mL) was stirred at reflux with phenyl chloroformate (19.6 g, 125 mmol) and $KHCO_3$ (26.7 g, 267 mmol). After 18 h, the mixture was cooled to 20 °C, filtered, and evaporated to an oil. The oil was taken up in MeOH (120 mL), combined with a solution of $KHCO_3$ (15.7 g, 157 mmol) and 85% KOH (10.3 g, 157 mmol) in H_2O (120 mL), and stirred at 20 °C for 2.5 h. The solution was then acidified to pH 1 with 37% HCl and extracted with CH_2Cl_2 (2 × 200 mL). Evaporation of the solvent left a syrup which was distilled under reduced pressure to remove phenol, yielding an undistilled residue which solidified to a brown glass upon cooling. The glass was dissolved in warm CH_2Cl_2 (50 mL) and passed through a 7 × 3.5-cm diameter silica column using CH_2Cl_2 (600 mL), giving a light yellow syrup (5.86 g). The syrup was stirred at 130 °C for 3 d with 80% aqueous hydrazine (40 mL). The product crystallized from the reaction mixture and was collected by filtration, washed with H_2O, and dried yielding pure **1** as white crystals (2.55 g, 60%), mp 209–211 °C; CIMS (NH_3) *m/e* 274 (M + 1).

Anal.—Calc. for $C_{18}H_{27}NO·¼H_2O$: C, 77.79; H, 9.97; N, 5.04. Found: C, 77.61; H, 9.99; N, 4.72.

Procedure for Synthesis of Compounds 13–19 (Method A)—The following experimental procedure (method A), leading to **13**, is representative of the general procedure used to synthesize compounds **14–19**.

(±)-2-Ethyl-2′-hydroxy-5-methyl-9α-(3-methyl)butyl-6,7-benzomorphan **(13)**—A suspension of **12** (640 mg, 2.34 mmol) in dry pyridine (15 mL) was stirred at 20 °C while a solution of acetyl chloride (760 mg, 9.38 mmol) in $CHCl_3$ (1 mL) was added. The resulting mixture was diluted with $CHCl_3$ (4 mL) to give a homogeneous solution. After being stirred overnight, the solvent was evaporated and the residue was partitioned between $CHCl_3$ (100 mL) and successive washes of 1N HCl (2 × 100 mL), saturated aqueous $NaHCO_3$ (1 × 100 mL), and brine (1 × 100 mL). Evaporation of the solvent gave a syrup which was taken up in tetrahydrofuran (50 mL) and refluxed overnight with $LiAlH_4$ (800 mg, 21.1 mmol). Excess hydride was destroyed by cautious addition of 9 M NH_4OH (5 mL). The mixture was filtered, evaporated, and purified by silica gel flash chromatography (11 × 3.5-cm diameter column using CH_2Cl_2 with increasing MeOH, from 0–20%, in 1.2 L of eluent). The resulting syrup (637 mg) was dissolved in acetone and diluted with a solution of oxalic acid·$2H_2O$ (370 mg, 2.94 mmol) in acetone to yield pure **13** as an oxalate salt (670 mg, 73%), mp 181–183 °C; CIMS (NH_3) *m/e* 302 (M + 1).

Anal.—Calc. for $C_{20}H_{31}NO·C_2H_2O_4·½H_2O$: C, 65.98; H, 8.56; N, 3.27. Found: C, 65.65; H, 8.19; N, 3.29.

(±)-2′-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-n-propyl-6,7-benzomorphan **(14)**—Compound **14** was prepared by method A; purification by chromatography was unnecessary. Compound **14** was recrystallized from methanol:acetone in 61% yield as an oxalate salt, mp 221–223 °C.

Anal.—Calc. for $C_{21}H_{33}NO·C_2H_2O_4$: C, 68.12; H, 8.70; N, 3.45. Found: C, 68.13; H, 8.76; N, 3.20.

(±)-2-n-Butyl-2′-hydroxy-5-methyl-9α-(3-methyl)butyl-6,7-benzomorphan **(15)**—Compound **15** was prepared by method A; purification by chromatography was unnecessary. Compound **15** was recrystallized from methanol:acetone in 48% yield as an oxalate salt, mp 225–227 °C.

Anal.—Calc. for $C_{22}H_{35}NO·C_2H_2O_4·½H_2O$: C, 67.26; H, 8.94; N,

3.27. Found: C, 67.51; H, 9.04; N, 3.33.

(±)-2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-n-pentyl-6,7-benzomorphan (16)—Compound 16 was prepared by method A. Compound 15 was recrystallized from acetone in 66% yield as an oxalate salt, mp 189–190 °C.

Anal.—Calc. for $C_{23}H_{37}NO{\cdot}C_2H_2O_4$: C, 69.26; H, 9.07; N, 3.23. Found: C, 69.01; H, 8.75; N, 3.16.

(±)-2-n-Hexyl-2'-hydroxy-5-methyl-9α-(3-methyl)butyl-6,7-benzomorphan (17)—Compound 17 was prepared by method A; purification by chromatography was unnecessary. Compound 17 was recrystallized from acetone:methanol in 39% yield as an oxalate salt, mp 126–128 °C.

Anal.—Calc. for $C_{22}H_{33}NO{\cdot}C_2H_2O_4{\cdot}\frac{1}{2}H_2O$: C, 67.76; H, 8.51; N, 3.28. Found: C, 67.65; H, 8.21; N, 3.04.

(±)-2-Cyclopropylmethyl-2'-hydroxy-5-methyl-9α-(3-methyl)butyl-6,7-benzomorphan (18)—Compound 18 was prepared by method A; purification by chromatography was unnecessary. Compound 18 was recrystallized from acetone in 39% yield as an oxalate salt, mp 190–192 °C.

Anal.—Calc. for $C_{26}H_{35}NO{\cdot}C_2H_2O_4$: C, 71.92; H, 7.98; N, 3.00. Found: C, 71.89; H, 7.92; N, 2.95.

(±)-2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-phenylethyl-6,7-benzomorphan (19)—Compound 19 was prepared by method A. Compound 19 was recrystallized from acetone in 30% yield as an oxalate salt, mp 185–187 °C.

Anal.—Calc. for $C_{21}H_{31}NO{\cdot}C_2H_2O_4{\cdot}\frac{1}{2}H_2O$: C, 66.97; H, 8.31; N, 3.40. Found: C, 67.24; H, 8.37; N, 3.31.

(±)-2'-Hydroxy-5-methyl-9α-(3-methyl)butyl-2-(2-propenyl)-6,7-benzomorphan (20)—Compound 20 was prepared by Method B as follows. A solution of 12 (500 mg, 1.83 mmol) in dimethylformamide (10 mL) was stirred at 100 °C with powdered K_2CO_3 (505 mg, 3.66 mmol) and allyl bromide (244 mg, 2.0 mmol). After 4.5 h, the mixture was cooled to 20 °C and filtered, and the filter cake was washed with $CHCl_3$ (2 × 20 mL). Evaporation of the solvent gave an oil which was partitioned between H_2O (20 mL) and $CHCl_3$ (2 × 40 mL), dried ($MgSO_4$), and evaporated to a light yellow oil (708 mg). The oil was dissolved in acetone (5 mL) and diluted with a solution of oxalic acid (2.01 mmol) in acetone (5 mL). The white crystalline solid which came out of solution was collected by filtration to yield 20 as the oxalate salt (533 mg, 72%), mp 170–172 °C; EIMS *m/e* 313 (M^+).

Anal.—Calc. for $C_{21}H_{31}NO_4{\cdot}\frac{1}{2}H_2O$: C, 66.97; H, 8.31; N, 3.40. Found: C, 67.24; H, 8.37; N, 3.31.

In Vivo Study—*Mouse Antinociception Tests*—Male mice weighing 20–30 g were used. All drugs were administered as salts, dissolved in distilled water, and administered subcutaneously in a volume of 0.1 mL/10 g of body weight. At least three doses per curve were tested and 6–10 animals per dose were used. The ED_{50} values were calculated using computerized probit analysis.

Tail Flick (TF) Tests—The procedure and modification[10] have been described. Briefly, the mouse tail was placed in a groove which contained a slit under which was a photoelectric cell. When the heat source or noxious stimulus was turned on, it focused on the tail and the animal responded by flicking its tail out of the groove. Thus, light passed through the slit and activated the photocell which, in turn, stopped the recording timer. The apparatus was calibrated so that control mice would flick their tails in 2–4 s. Mice were injected subcutaneously with drug and tested 20 min later. Vehicle controls were also tested.

In the antagonism experiments (tail flick versus morphine), the antagonists were administered subcutaneously 10 min before morphine, and the animals were tested 20 min later.

Phenylquinone (PPQ) Abdominal Stretching Tests[11]—The mice were injected subcutaneously with drugs, and 10 min later they received 2 mg/kg of a fresh *p*-phenylquinone solution intraperitoneally. The mice were then placed in cages in groups of two. At 10 min after the phenylquinone injection, the total number of stretches per group was counted within a 1-min period. A stretch was characterized by an elongation of the body, development of tension in the muscles in the abdominal region, and extension of the forelimbs. The antinociceptive response was expressed as the percent inhibition of the phenylquinone-induced stretching response. Appropriate controls were used.

Hot Plate (HP) Assay[12]—The hot plate was held at a constant temperature (55 °C) via a refluxing 1:1 mixture of ethyl formate and acetone. Mice were placed on the hot plate, and antinociceptive activity was noted as a delay of 5 s, but no more than 30 s, beyond the control time for the movement of the hind limb of the mouse, over at least two consecutive time periods. The mice were tested at 5, 10, 20, 30, and 60 min, and longer if necessary, until responses returned to control levels.

Dependent Rhesus Monkey—In the single-dose substitution (SDS) test, for the most part, the recommendations of Seevers[13] and Seevers and Deneau[14] were utilized. A brief description of the procedure, including modifications,[15] is as follows. Male and female rhesus monkeys (*Macaca mulatta*) weighing 2.5–7.5 kg were used. The animals were housed in pens, four or five to a group, and received 3.0 mg/kg of morphine (sc) every 6 h. This dose schedule was reported to produce maximal physical dependence. All the animals had received morphine for >3 months. A minimal, 2-week wash-out and recuperation period was allowed for each animal between tests. The SDS test was initiated by the subcutaneous injection of the test drug or control substances (morphine and vehicle water, respectively) into the animals in a group that had not received morphine for 14–15 h and showed definite signs of withdrawal. Each animal was randomly allocated to one of four or five treatments: (*a*) two or three dose levels of the compound under investigation; (*b*) the morphine control, 3.0 mg/kg; or (*c*) the vehicle control, 1 mL/kg. The animals were scored for suppression of withdrawal signs during a 2.5-h observation period. The observer was "blind" regarding the allocation of treatments. At the end of the study, the data were grouped according to dose and drug, and the results were analyzed using the Mann–Whitney U-test.[16]

Precipitated Withdrawal (PPTW) Test in Rhesus Monkeys—The PPTW test was performed under the conditions described above for the SDS test, except that the animals of a group were challenged 2 or 3 h after the last dose of morphine. Naloxone at a dose of 0.05 mg/kg (sc) served as the positive control.

In Vitro Tests—*Assay for μ-Opioid Receptor Binding*—The binding assay was adapted from the procedure of Zajac and Roques,[17] with the following modifications. In the tissue preparation, the whole brain minus the cerebellum from Sprague-Dawley rats was disrupted with 25 vol of 20 mM potassium phosphate buffer (pH 7.4) with a Brinkman polytron (setting 6, 20 s). The homogenates were centrifuged at 27 000 × *g* for 15 min at 5 °C. The pellet was resuspended in fresh buffer and centrifuged again for a total of three times. The final suspension was in 25 vol of fresh buffer and kept on ice until needed. Binding to homogenates was determined in a 1-mL incubation volume, consisting of 500 μL of tissue (containing 1.0 mg of protein by Lowry analysis), 100 μL of 2 nM [^{3}H]DAGO (60 Ci/mmol; New England Nuclear) and 300/400 μL of buffer (100 μL of naloxone was used, for a final concentration of 10 μM naloxone, for the determination of nonspecific binding). After a 1-h incubation at 25 °C, the reaction was terminated by rapid filtration (Brandel Cell Harvester, Gaithersburg, MD) through Schleicher and Schuell No. 32 filters. The filters were washed with three 5-mL aliquots of ice-cold buffer and placed in counting vials with 4 mL of Hydrofluor scintillation cocktail (National Diagnostics). Vials were allowed to stand overnight prior to counting. The inhibition constant (K_i), for absolute determination of the affinity of a substance for a receptor, was calculated using the Cheng–Prusoff equation.[18]

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