Acad. Sci. U.S.A., 69, 2145 (1972).
(8) A. G. Gilman, Proc. Natl. Acad. Sci. U.S.A., 67, 305 (1970).
(9) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
(10) H. Joshua, R. Gans, and K. Mislow, J. Am. Chem. Soc., 90, 4884 (1968).
(11) J. L. McNay and L. I. Goldberg, J. Pharmacol. Exp. Ther., 151, 23 (1966).
(12) L. Fieser, "Experiments in Organic Chemistry", 3rd ed, D. C. Heath, Boston, Mass., 1957, p 182.
(13) C. A. Fletscher in ref 6, p 735.
(14) J. W. Cook, J. D. Loudon, and R. K. Razdan, J. Chem. Soc., 4235 (1954).
(15) R. Pschorr and W. Buckow, Ber. Dtsch. Chem. Ges., 33, 1829 (1900).
(16) B. Chauncey and E. Gellert, Aust. J. Chem., 22, 993 (1969).
(17) E. Schlittler and J. Muller, Helv. Chim. Acta, 31, 1119 (1948).

Studies in the (+)-Morphinan Series. 5.[1] Synthesis and Biological Properties of (+)-Naloxone

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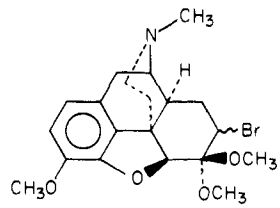
(+)-Naloxone was prepared in 26% overall yield in eight steps from (+)-7-bromodihydrocodeinone dimethyl ketal by a synthesis which excluded enantiomeric contamination. (+)-Naloxone was examined in three assay systems and found to have no more than $^1/_{1000}$–$^1/_{10000}$th the activity of (−)-naloxone; it can, thus, serve to test the stereospecificity of the biochemical and pharmacological actions of (−)-naloxone.

(−)-Naloxone (the enantiomer of **9**), prepared from natural thebaine (enantiomer of **2**), is in many assay systems a pure narcotic antagonist with no agonist activity. It is, therefore, widely used clinically to reverse opiate overdose symptoms and biochemically as a test for opiate receptor mediated phenomena. Since (−)-naloxone may exhibit pharmacological actions of its own,[2,3] reversal of a particular biological response by (−)-naloxone is not necessarily evidence that this activity is mediated by opiate receptors. Such ambiguities could be resolved by parallel experiments with the enantiomer, (+)-naloxone (**9**). This enantiomer would presumably not interact with the opiate receptor and would not share the specific actions of (−)-naloxone (enantiomer of **9**). Accordingly, we have prepared (+)-naloxone by a stereochemically controlled synthesis from (−)-sinomenine and have compared its activity to that of (−)-naloxone in several in vitro systems.



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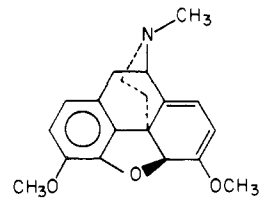

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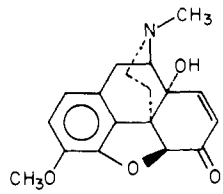

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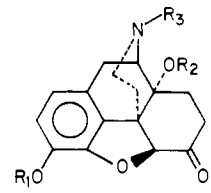
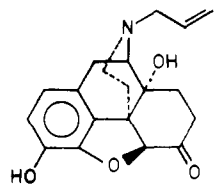


4, $R_1 = R_3 = CH_3$; $R_2 = H$
5, $R_1 = R_2 = H$; $R_3 = CH_3$
6, $R_1 = R_2 = Ac$; $R_3 = CH_3$
7, $R_1 = R_2 = Ac$; $R_3 = CN$
8, $R_1 = R_2 = R_3 = H$



9

Chemistry. Our goal could be achieved only after marked improvements had been made in the synthesis of (+)-dihydrocodeinone from natural (−)-sinomenine[1] and in the conversion of (−)-dihydrocodeinone into natural (−)-thebaine.[4] Bromo ketal **1**, prepared in five steps from sinomenine,[1] was the intermediate of choice since its (−) enantiomer had successfully been converted into (−)-thebaine,[4] starting material for the commercial synthesis of (−)-naloxone.[5] Starting with bromo ketal **1**, reactions carried out in the (+) series were as follows. Treatment of **1** with *t*-BuOK[4] in Me_2SO at 80–90 °C afforded (+)-thebaine (**2**), identical with the natural alkaloid except for its opposite optical rotation. Model experiments carried out in the (−) series suggested that oxidation of **2** with peroxide could be performed best with performic acid prepared in situ, affording the desired 14-hydroxy ketone **3** in excellent yield.[6] Catalytic hydrogenation of unsaturated ketone **3** gave saturated ketone **4** which, upon O-demethylation with BBr_3, gave phenolic hydroxy ketone **5**. Protection of the hydroxy groups by acetylation, followed by N-demethylation with cyanogen bromide in chloroform, yielded the *N*-cyano derivative **7**, via diacetoxy compound **6**. Refluxing the *N*-cyano compound **7** in 25% sulfuric acid effected deacetylation, hydrolysis, and decarboxylation and led to the desired secondary amine **8** in high yield. Since amine **8** is difficult to purify, it is a prerequisite in this synthesis that its precursors are chemically and optically pure. Synthesis of (+)-naloxone (**9**) was completed by routine N-allylation which gave the final product **9** as colorless prisms. This material was identical in every respect with a commercial sample of (−)-naloxone, except for its opposite optical rotation.[7]

Biological Results. (+)-Naloxone, prepared by a synthesis which excludes enantiomeric contamination, was examined in a brain receptor binding assay, in the guinea pig ileum assay, and in the neuroblastoma × glioma hybrid cell adenylate cyclase assay.

Rat Brain Receptor Binding Assay. Comparison of displacement of [^{3}H]-(−)-naloxone from opiate receptors

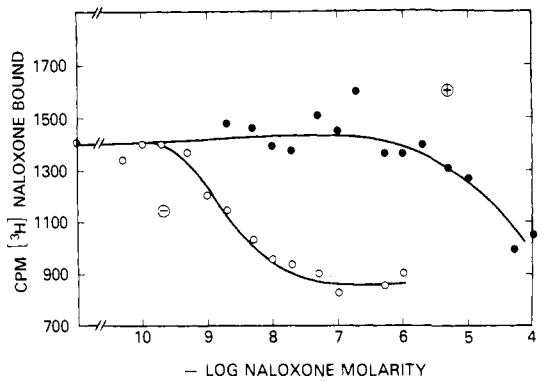


Figure 1. Displacement of [^{3}H]-(–)-naloxone from opiate receptors in rat brain membranes by (–)-naloxone (O—O) and (+)-naloxone (●—●).

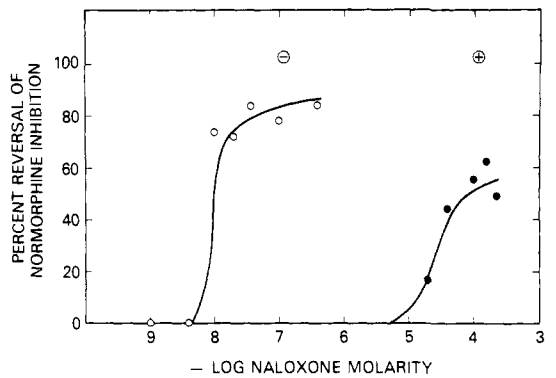


Figure 2. Reversal by (–)-naloxone (O—O) and (+)-naloxone (●—●) of the inhibition of electrically stimulated contractions of the guinea pig ileum due to (–)-normorphine (3×10^{-7} M).

in rat brain membranes[8] by (–)-naloxone and (+)-naloxone showed (+)-naloxone has, at best, only a very weak affinity for these receptors (Figure 1). (+)-Naloxone displaces [^{3}H]-(–)-naloxone only at concentrations 10 000 times higher than those of (–)-naloxone necessary to carry out the equivalent displacement. K_d values from these data are (–)-naloxone = 1 nM, (+)-naloxone = 10 000 nM.

Electrically Stimulated Guinea Pig Ileum Assay. The ileum was mounted in a 5-mL organ bath, bathed in Ringer's solution, and bubbled with a mixture of 95% oxygen and 5% CO_2 at 37 °C. Stimulation was supramaximal (60 V, 0.4-ms duration at 0.1 Hz). For each point shown in Figure 2, five successive contractions were measured and the average height was compared with that of the control and with that of the normorphine-inhibited contraction height immediately preceding the addition of naloxone.

Under these conditions, (+)-naloxone had no effect either alone or as an antagonist of (–)-normorphine at concentrations below 5×10^{-5} M. At this concentration and above it, (+)-naloxone partially reversed the inhibition of contractions caused by (–)-normorphine but had little or no effect in the absence of (–)-normorphine. In contrast, (–)-naloxone was highly effective in reversing normorphine inhibition at concentrations of 10^{-8} M (Figure 2).

Neuroblastoma × Glioma Hybrid Cell Adenylate Cyclase Assay. Assays of basal adenylate cyclase activity were performed as described previously[9] with additions to the standard assay medium of 2×10^{-5} M morphine and (–)-naloxone, 2×10^{-5} M morphine and (+)-naloxone, (–)-naloxone alone, and (+)-naloxone alone.

(+)-Naloxone had no effect upon morphine-sensitive adenylate cyclase activity of neuroblastoma × glioma hybrid, NG 108-15, cell homogenates whether in the presence or absence of added morphine. (–)-Naloxone was

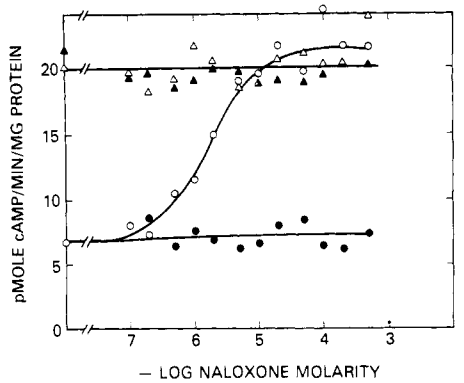


Figure 3. Effects of (–)-naloxone (O—O) and (+)-naloxone (●—●) on adenylate cyclase activity in homogenates of neuroblastoma × glioma hybrid cells with addition of 2×10^{-5} M morphine. The effect of (–)-naloxone, alone (Δ—Δ), and (+)-naloxone, alone (▲—▲), is indicated.

effective, at concentrations of 5×10^{-7} M and above, in reversing inhibition of enzyme activity due to morphine. The data (Figure 3) show that potency of (+)-naloxone as an antagonist of morphine in this assay is less than $^1/_{1000}$th that of (–)-naloxone.

Discussion

(+)-Naloxone appears to meet the necessary criteria for it to be used in parallel experiments with its enantiomer. Any pharmacological actions displayed by (–)-naloxone[2,3] can now be tested for enantiomeric specificity (e.g., action at a receptor level) because of our findings that (+)-naloxone has no more than $^1/_{1000}$–$^1/_{10\,000}$th the activity of (–)-naloxone in these three assays.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and are corrected. Elemental analyses were performed by the Section on Microanalytical Services and Instrumentation of this Laboratory. IR and mass spectra were obtained on a Perkin-Elmer 257 and Hitachi Perkin-Elmer RMU-6E, respectively. Optical rotations were measured with a Perkin-Elmer Model 141 polarimeter. Thin-layer chromatography (silica gel GF, Analtech, Newark, Del.) was used to compare enantiomeric compounds throughout the synthesis. All synthesized products had R_f values identical with those of their (–) enantiomers.

(+)-7-Bromodihydrocodeinone Dimethyl Ketal (1). (+)-7-Bromodihydrocodeinone dimethyl ketal (1) was prepared from sinomenine as described.[1]

(+)-Thebaine (2). A mixture of 1 (1.00 g, 2.36 mmol) and potassium *tert*-butoxide[4] (656 mg, 5.85 mmol) in Me_2SO (25 mL) was heated at 80–90 °C for 1.5 h, diluted with saturated NaCl solution (25 mL), and extracted with benzene, and the extracts were dried ($MgSO_4$). Removal of solvent gave crude **2**, which was recrystallized from MeOH–H_2O: 529 mg (72%); mp 193–194 °C; $[\alpha]^{20}_D$ +215.6° (*c* 0.9, EtOH) [lit.[10] (for enantiomer of **2**) mp 193 °C; $[\alpha]^{15}_D$ –219° (*c* 2.0, EtOH)]. Anal. ($C_{19}H_{21}NO_3$) C, H, N.

(+)-14-Hydroxycodeinone (3). Hydrogen peroxide (30%, 1.3 mL, 13 mmol) was added to a solution of **2** (3.11 g, 10 mmol) in a mixture of formic acid (88%, 1.3 mL) and H_2SO_4 (0.7%, 4.1 mL). The mixture was heated at 40 °C (bath temperature) for 6.5 h, cooled, diluted with water (10 mL), and made basic with concentrated NH_4OH. The precipitate was filtered, washed with H_2O, and dried ($MgSO_4$). Recrystallization from EtOH–$CHCl_3$ gave **3**: 2.70 g (86%); mp 275 °C; $[\alpha]^{20}_D$ +109.7° (*c* 1.06, 10% HOAc) [lit.[11] (for enantiomer of **3**) mp 275–276 °C; $[\alpha]^{25}_D$ –111° (*c* 0.9, 10% HOAc)]. Anal. ($C_{18}H_{19}NO_4$) C, H, N.

(+)-14-Hydroxydihydrocodeinone (4). A solution of **3** (2.00 g, 6.38 mmol) in acetic acid (10%, 40 mL) was hydrogenated [Pd–$BaSO_4$ (5%, 1.00 g)]. The catalyst was filtered and washed with H_2O, and the filtrate was made basic with concentrated NH_4OH. The solution was saturated with NaCl and extracted with $CHCl_3$. The extracts were washed with saturated NaCl

solution and dried ($MgSO_4$), and the solvent was removed. The crystalline solid 4 which was obtained was washed with Et_2O and dried: 1.91 g (95%); mp 218 °C; $[\alpha]^{20}_D$ +166.9° (*c* 1.03, 10% HOAc) [authentic sample of enantiomer of 4, mp 218 °C; $[\alpha]^{20}_D$ −160.9° (*c* 1.09, 10% HOAc)]. Anal. ($C_{18}H_{21}NO_4 \cdot 0.5H_2O$) C, H, N.

(+)-14-**Hydroxydihydromorphinone** (5). A solution of 4 (1.70 g, 5.4 mmol) in $CHCl_3$ (15 mL) was added, dropwise, to a stirred solution of BBr_3 (8.0 g, 32 mmol) in $CHCl_3$ (15 mL) at 10 °C over 10 min. The mixture was stirred for 50 min at 10–20 °C and poured into ice–H_2O (40 mL). The solution was made basic with NH_4OH, saturated with NaCl, extracted with $CHCl_3$ (8 × 50 mL), washed with saturated NaCl solution, and dried ($MgSO_4$). Removal of solvent gave solid 5, which was recrystallized from EtOH: 1.23 g (76%); mp 250 °C dec; $[\alpha]^{20}_D$ +197.5° (*c* 0.87, EtOH) (lit.[12] for enantiomer of 5, mp 244–246 °C). Anal. ($C_{17}H_{19}NO_4$) C, H, N.

(+)-3,14-**Diacetoxydihydromorphinone** (6). A mixture of 5 (1.50 g, 4.98 mmol) in acetic anhydride (15 mL) was heated at 100 °C for 1 h and evaporated under reduced pressure. Water was added to the residue and it was basified (pH 9) with concentrated NH_4OH. The precipitate was filtered, washed with cold H_2O, and dried to give 6: 1.74 g (90%); mp 218–219 °C; $[\alpha]^{20}_D$ +201.6° (*c* 1.31, $CHCl_3$) (lit.[12] for enantiomer of 6, mp 214–215 °C). Anal. ($C_{21}H_{23}NO_6$) C, H, N.

(+)-*N*-**Cyano-3,14-diacetoxydihydronormorphinone** (7). A mixture of 6 (1.60 g, 4.15 mmol) and cyanogen bromide (1.00 g, 9.44 mmol) in $CHCl_3$ (100 mL) was refluxed for 8 h. Two additional amounts of cyanogen bromide were added; the first addition (1.50 g, 14.16 mmol) was followed by a 15-h refluxing period, and the second addition (0.5 g, 4.72 mmol) was followed by refluxing for another 9 h. The solution was washed with 10% HCl and H_2O and dried ($MgSO_4$). Removal of solvent gave 7, which was recrystallized from EtOH–$CHCl_3$: 1.5 g (91%); mp 240–241 °C; $[\alpha]^{20}_D$ +220.5° (*c* 0.74, $CHCl_3$) (lit.[12] for enantiomer of 7, mp 230–233 °C). Anal. ($C_{21}H_{20}N_2O_6$) C, H, N.

(+)-14-**Hydroxydihydronormorphinone** (8). A mixture of 7 (1.66 g, 4.18 mmol) in 25% H_2SO_4 (18 mL) was refluxed for 4.5 h under N_2. After cooling, the solution was made basic with NH_4OH, and the precipitated solid 8 was filtered, washed with H_2O, EtOH, and Et_2O, and dried: 1.15 g (95%); mp 290 °C; $[\alpha]^{20}_D$ +149.8° (*c* 1.02, 10% HOAc), (lit.[12] for enantiomer of 8, mp 310–313 °C). Anal. ($C_{16}H_{17}NO_4$) C, H, N.

(+)-**Naloxone** (9). A mixture of 8 (1.13 g, 3.9 mmol), allyl bromide (520 mg, 4.3 mmol), and K_2CO_3 (594 mg, 4.3 mmol) in DMF (15 mL) was heated at 90–95 °C (bath temperature) for 3 h with stirring under N_2. The cooled mixture was diluted with H_2O, saturated with NaCl, extracted with $CHCl_3$, washed with saturated NaCl solution, and dried ($MgSO_4$). After solvent removal, the residue was purified by silica gel TLC using Et_2O–hexane (85:15) as a solvent to give 9 (970 mg, 76%), which was recrystallized from ethyl acetate as colorless prisms: mp 178–179 °C; $[\alpha]^{20}_D$ +197.4° (*c* 1.05, $CHCl_3$) [lit.[13] (for enantiomer of 9) mp 184 and 177–178 °C; $[\alpha]^{20}_D$ −194.5° (*c* 0.93, $CHCl_3$)[7]. (+)-Naloxone (9) was chromatographically and spectroscopically indistinguishable from an authentic sample of the (−) enantiomer except for its opposite optical rotation. Anal. ($C_{19}H_{21}NO_4$) C, H, N.

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References and Notes

(1) For paper 4, see I. Iijima, J. Minamikawa, K. C. Rice, A. E. Jacobson, and A. Brossi, *J. Org. Chem.*, in press.
(2) J. J. Jacob, E. C. Tremblay, and M. C. Colombel, *Psychopharmacologia*, **37**, 217 (1974).
(3) O. Sakurada, M. Shinohara, W. A. Klee, C. Kennedy, and L. Sokoloff, *Neurosci. Abstr.*, **2** (1), 613 (1976).
(4) D. D. Weller and H. Rapoport, *J. Med. Chem.*, **19**, 1171 (1976).
(5) M. J. Lewenstein and J. Fishman, U.S. Patent 3 254 088 (1966).
(6) I. Iijima, K. C. Rice, and A. Brossi, *Helv. Chim. Acta*, **60**, 2135 (1977).
(7) K. Arima, Sankyo Co., Ltd., Japan, personal communication.
(8) W. A. Klee and R. A. Streaty, *Nature (London)*, **248**, 61 (1974).
(9) S. K. Sharma, M. Nirenberg, and W. A. Klee, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 590 (1975).
(10) M. Windholz, S. Budavari, L. Y. Stroumtsos, and M. N. Fertig, Ed., "The Merck Index", 9th ed, Merck & Co., Rahway, N.J., 1976, thebaine, no. 8988.
(11) R. E. Lutz and L. Small, *J. Org. Chem.*, 4, 220 (1939).
(12) I. Seki, *Takamine Kenkyusho Nempo*, **12**, 56 (1960); *Chem. Abstr.*, **55**, 8449b (1961). For an alternate preparation of the (−) enantiomer of 8 from the (−) enantiomer of 5, see R. A. Olofson, R. C. Schnur, L. Bunes, and J. P. Pepe, *Tetrahedron Lett.*, 1567 (1977).
(13) See ref 10, (−)-naloxone, no. 6182.

Spiro[isobenzofuran-1(3*H*),4′-piperidines]. 3. Diuretic and Antihypertensive Properties of Compounds Containing a Sulfur Attached to Nitrogen

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The synthesis and antihypertensive and diuretic activity of several *N*-sulfur derivatives of 3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidine] are reported. Benzenesulfenamide 3 possessed marked, species-specific diuretic and antihypertensive activity in rats.

We recently reported the synthesis and antitetrabenazine activity of a series of 3-phenylspiro[isobenzofuran-1(3*H*),4′-piperidines] 1[1] and their corresponding derivatives 2 with an additional heteroatom attached directly to the piperidine nitrogen.[2] In both series potent antitetrabenazine activity was shown to be associated with

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