(9) R. Reiner and P. Zeller, *Helv. Chim. Acta*, **51**, 1905 (1968).
(10) D. B. R. Johnston, S. M. Schmitt, R. A. Firestone, and B. G. Christensen, *Tetrahedron Lett.*, 4917 (1972).
(11) G. H. Rasmusson, G. F. Reynolds, and G. E. Arth, *Tetrahedron Lett.*, 145 (1973).
(12) R. A. Firestone and B. G. Christensen, *J. Org. Chem.*, **38**, 1436 (1973).
(13) J. Hoogmartens, P. Claes, and H. Vanderhaeghe, *J. Med. Chem.*, **17**, 389 (1974).
(14) D. H. Barton, F. Comer, D. G. T. Greig, P. Sammes, C. M. Cooper, G. Hewitt, and W. G. E. Unterwood, *J. Chem. Soc. C*, 3540 (1971); D. O. Spry, *J. Chem. Soc., Chem. Commun.*, 259 (1973).
(15) A. K. Bose, G. Spiegelman, and M. S. Manhas, *J. Chem. Soc. C*, 2468 (1971).
(16) A. K. Bose, J. L. Fahey, and M. S. Manhas, *J. Heterocycl. Chem.*, **10**, 791 (1973).
(17) J. C. Sheehan, H. W. Hill, Jr., and E. L. Buhle, *J. Am. Chem. Soc.*, **73**, 4373 (1951); J. C. Sheehan and G. D. Laubach, *ibid.*, **73**, 4376 (1951).
(18) A. K. Bose, G. Spiegelman, and M. S. Manhas, *J. Am. Chem. Soc.*, **90**, 4506 (1968).
(19) A. Vlietinck, E. Roets, P. Claes, G. Janssen, and H. Vanderhaeghe, *J. Chem. Soc., Perkin Trans. 1*, 937 (1973).
(20) A. K. Bose and B. Anjaneyulu, *Chem. Ind. (London)*, 903 (1966).
(21) P. Claes, A. Vlietinck, E. Roets, H. Vanderhaeghe, and S. Toppet, *J. Chem. Soc., Perkin Trans. 1*, 932 (1973).
(22) A. Vlietinck, E. Roets, H. Vanderhaeghe, and S. Toppet, *J. Org. Chem.*, **39**, 441 (1974).
(23) E. H. Flynn, Ed., "Cephalosporins and Penicillins, Chemistry and Biology", Academic Press, New York, N.Y., 1972, p 356.
(24) G. A. Koppel, *Tetrahedron Lett.*, 4233 (1973).
(25) K. D. Barrow and T. M. Spotswood, *Tetrahedron Lett.*, 3325 (1965).
(26) M. S. Manhas, J. S. Chib, and A. K. Bose, *J. Org. Chem.*, **38**, 1238 (1973).
(27) A. Pinner, *Ber.*, **16**, 1654 (1883).
(28) T. Wieland and H. J. Hennig, *Chem. Ber.*, **93**, 1236 (1960).
(29) H. Wenker, *J. Am. Chem. Soc.*, **57**, 1079 (1935).

Stereospecific Synthesis of the 6β-Hydroxy Metabolites of Naltrexone and Naloxone

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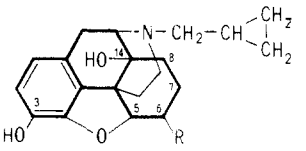
The narcotic antagonists naltrexone (1a) and naloxone (**2a**) were stereospecifically reduced to the corresponding 6β-hydroxy epimers 1**b** and **2b**, respectively, with formamidinesulfinic acid in an aqueous alkaline medium. The reaction products were obtained with no detectable quantity of the 6α epimers 1c and **2c**. The products 1**b** and **2b** were formed in yields of 88.5 and 40%, respectively, and characterized by spectral methods. Compared to 1a and **2a**, the stereospecific reduction products 1**b** and **2b** and their 6α epimers 1c and **2c** are all significantly less potent as narcotic antagonists in mice. Only 1c and **2c** also possess antinociceptive activity.

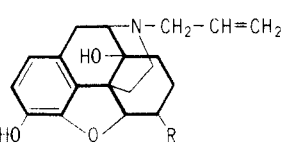
The ability of narcotic antagonists such as naltrexone (**1a**) and naloxone (**2a**) to block the euphorigenic and dependence producing effects of narcotics forms the pharmacologic basis for the use of these drugs in the treatment of opiate dependence. Compared to naloxone, naltrexone has been found to be more potent and to have a longer duration of antagonist action in laboratory rodents[1,2] and man.[3] In addition, naltrexone is an effective antagonist in man at oral doses of 30--50 mg/day, while an equieffective oral dose of naloxone would be much larger (up to 3000 mg/day).[3,4]

In man the major metabolite of naloxone is the 3-glucuronide,[5] whereas the 6-keto reduction product, a 6β-hydroxy derivative (**1b**), is the major metabolite of naltrexone.[6,7] Comparative studies of the biotransformation of both 1**a** and **2a** have revealed species variation in the stereochemistry of the alcohol resulting from reduction of the 6-keto group.[8] Our interest in both the role of biotransformation in the relatively long duration of action of **1a**, and observed differences in biotransformation of **1a** and **2a**, necessitated a quantity of the appropriate 6β epimeric alcohol metabolites **1b** and **2b** for use as analytical standards and for pharmacologic characterization.

Chemical methods are readily available for the synthesis of 6α-hydroxy epimers **1c** and **2c**. These compounds are known as N-substituted 14-hydroxydihydronormorphines and are accessible through hydride reduction of the N-substituted 14-hydroxydihydronormorphinones.[9] No claim was found in the literature of a stereospecific chemical reduction of 6-keto compounds (having the morphine nucleus) to yield the 6β-hydroxy epimers. Although attempts have been made,[6,10] no successful chemical synthesis of **1b** and **2b** has been reported. The purpose of this report is to describe a method for the synthesis of **1b** and **2b** by a stereospecific reduction of the respective 6-keto compounds **1a** and **2a**.



1 a, R = =O
b, R = H, OH
c, R = H, HO



2 a, R = =O
b, R = H, OH
c, R = H, HO

Initial attempts by us to synthesize **1b** included a reduction procedure involving lithium tri-*sec*-butylborohydride[11] and **1a**. However, this reagent[12] yielded solely the 6α-hydroxy epimer **1c**. This course was therefore abandoned in view of the state of knowledge concerning hydride reductions of compounds of this class.[6,10,13] Our objective of a successful synthesis of **1b** and **2b** was, however, achieved in a procedure using the reaction of formamidinesulfinic acid,[14,15] in alkaline solution, with naltrexone (**1a**) and naloxone (**2a**). This procedure was a modification of that of Nakagawa and Minami in the reduction of various ketones.[16] The reduction of **1a** yielded the 6β-hydroxy derivative **1b** in a yield of 88.5%, with no indication of the

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6α epimer. Definitive evidence for the assignment of 6β-hydroxy orientation was obtained from the proton nuclear magnetic resonance spectrum of the compound. The spectrum exhibited a doublet centered at δ 4.54 (J = 6 Hz) due to the 5β proton and a multiplet due to the 6α proton in the region δ 3.68–3.45. These chemical shift values are characteristic of the upfield shifts of the corresponding proton resonances of dihydroisocodeine (6β epimer) in comparison with dihydrocodeine.[17] This relationship in chemical shift values holds even though these comparison compounds lack the 14-hydroxy group.[7] Our stereochemical assignment is also based on the fact that the chemical shift values of the relevant protons (5β and 6α) are in harmony with those found in an NMR spectrum[7] of an authentic sample of dihydrohydroxycodeine C,[18] which is a compound of established 6β-hydroxy orientation,[19] and one of skeletal structure similar to **1b**. Since the NMR data[7] showed the doublets due to the 5β protons of **1b** and **1c** could be resolved, it was possible to identify each epimer in the presence of the other. The data also showed, in the case of **1b**, no detectable 6α-hydroxy epimer in the product. One might speculate that the observed stereospecificity of this reaction is due to the reduction of the enol[20] of **1a**. A free-radical mechanism, however, cannot be excluded. The compound **1b** was converted to its hydrochloride which was found to crystallize as its monohydrate.

Similar arguments hold in the case of the naloxone reduction product **2b**. This compound was isolated in a yield of 40%. There was no indication of the presence of the 6α epimer in the product. The NMR and mass spectral data clearly revealed that the allyl group had not undergone reduction in the reaction. This compound was also converted to its hydrochloride, which crystallized as a monohydrate.

The stereospecificity of this reduction procedure may be placed in perspective by considering chemical reductions of related morphine derivatives. Elad and Ginsburg found that a dihydrothebainone derivative (a compound lacking the 4,5-epoxy bridge) gave, on attempted stereospecific hydride reduction, mixtures of 6α- and 6β-hydroxy epimers.[21] In contrast, Sargent and coworkers[22] obtained, on hydride reduction of 14-hydroxycodeinone (4,5-epoxy bridge intact), 14-hydroxycodeine (6α epimer) as the sole product. Similarly, Gates obtained codeine on hydride reduction of codeinone.[23] These observations reveal that 6-keto morphine derivatives, with an intact oxygen bridge and unsaturated 7,8 positions, yield mainly the 6α-hydroxy derivatives by chemical modes of reduction. However, 7,8-dihydro-14-hydroxymorphinone derivatives, upon reduction, yield either pure 6α-hydroxy alcohols[13] or only a mixture of 6α and 6β epimers.[19] Recently a sodium amalgam reduction of a 6-keto derivative in the morphinan series was reported to yield predominantly a 6β-hydroxy epimer.[24] This reaction would not be expected to produce a 6β-hydroxy derivative with an intact oxygen bridge.

In view of these observations, the reduction with formamidinesulfinic acid should be useful for obtaining 6β epimeric alcohols from other oxymorphone derivatives.

Pharmacology. The results of pharmacologic evaluation in mice of the 6-hydroxy derivatives of **1a** and **2a** are given in Table I. Narcotic antagonist activity was measured by the method of Blumberg and Dayton.[25] Stereospecific reduction of either **1a** or **2a** to the corresponding 6-hydroxy derivative results in either α or β epimers both of which show significantly decreased potency as narcotic antagonists. In other studies **1b** and **2b** have also been found to be less potent than **1a** and **2a**, respectively, in the ability to induce jumping (narcotic antagonist activity) in morphine-dependent mice.[26]

Antinociceptive activity was determined by use of a phenylquinone writhing test.[27] The 6β-hydroxy epimers **1b** and **2b** were found, like their corresponding parent compounds **1a** and **2a**, to be practically devoid of antinociceptive activity, such that a reliable ED_{50} value could not be calculated (Table I). In contrast, the 6α-hydroxy epimers **1c** and **2c** both show significant antinociceptive activity. For example, the ED_{50} value of **1c** is in the range of that reported for nalorphine, while **2c** has an ED_{50} value close to that reported for pentazocine.[27]

Table I. Pharmacologic Activity in Mice (Subcutaneous)

Compd[a]	ED_{50}, mg/kg ± SE: Narcotic antagonist act.	ED_{50}, mg/kg ± SE: Antinociceptive act.
1a	0.020 ± 0.008	>320
1b	1.70 ± 0.35	>320
1c	0.25 ± 0.04	0.34 ± 0.06
2a	0.071 ± 0.009	>320
2b	0.33 ± 0.03	>320
2c	0.61 ± 0.03	4.5 ± 0.7

[a] As the HCl salt.

These preliminary studies suggest that reduction of **1a** or **2a** can produce either 6β epimers which are compounds with reduced narcotic antagonist activity or 6α epimers which are compounds with nalorphine-like properties (i.e., narcotic agonist–antagonist activity).

Since the α- and β-6-hydroxy epimers are species-dependent metabolites,[8] species variation in the routes of biotransformation of **1a** and **2a** may result not only in quantitative, but also qualitative, differences in the pharmacologic response seen following the administration of the parent drugs (**1a** or **2a**) chronically.

Also, the implications of these findings with respect to the stereospecificity of the receptor or receptors mediating narcotic agonist and antagonist effects will require the synthesis and pharmacologic investigation of other 6-hydroxy derivatives of the N-substituted noroxymorphones. These studies are in progress.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were obtained on a Perkin-Elmer 257 grating infrared spectrophotometer. Mass spectra were obtained on a Varian M-66 double-focusing cycloidal pass mass spectrometer. Proton nuclear magnetic spectra (NMR) were recorded on a Varian XL-100 spectrometer (Me_4Si), using $CDCl_3$ as solvent. Thin-layer chromatography (tlc) was performed on Analtech silica gel plates using a solvent system, ethyl acetate–hexane–ethanol–ammonia (60:25:14:1). Visualization was accomplished with Dragendorff's solution. Naltrexone hydrochloride and naloxone hydrochloride were manufactured products from Endo Laboratories, Inc., Garden City, N.Y. Formamidinesulfinic acid was obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis. Microanalyses for elements indicated were within 0.4% of the theoretical values.

Reduction of Naltrexone. Preparation of 1b. A solution of 754 mg (2 mmol) of naltrexone hydrochloride in H_2O (50 ml) was treated with the minimum of NaOH solution (640 mg in 50 ml of H_2O) until the mixture turned alkaline. The alkaline mixture was treated with 864 mg (8 mmol) of formamidinesulfinic acid dissolved in the remaining aqueous NaOH solution. The reaction mixture was stirred magnetically on a water bath under a current of N_2 at 80–85°. After 1 hr, the reaction mixture was allowed to cool, and the pH was brought down to about 9.8 by the addition of a few drops of 6 N HCl solution and a bicarbonate–carbonate buffer.[28] A copious white precipitate was filtered, washed with cold H_2O, and allowed to dry in a vacuum desiccator over NaOH. The dried compound **1b** weighed 607 mg (88.5%): mp 188–190°; TLC R_f 0.55; NMR δ 4.54 (d, 1, J = 6 Hz, 5β-H), 3.68–3.45 (m, 1, 6α-H).

The compound 1b was converted into its hydrochloride by dissolving it in an equal volume of EtOH and CH_3COCH_3 and treating with 6 N HCl. Recrystallization (95% EtOH–CH_3COCH_3) gave crystals of 1b hydrochloride: mp 205–210° dec; ir (KBr disk) 3500–3100 cm^{-1} (broad); $[\alpha]^{25}D$ −133.8° (c 1, H_2O); mass spectrum (70 eV) m/e 343 (100%). Anal. ($C_{20}H_{26}ClNO_4 \cdot H_2O$) C, H, N, Cl.

Reduction of Naloxone. Preparation of 2b. A solution of 1.48 g (4 mmol) of naloxone hydrochloride in the minimum volume of H_2O was treated with part of a solution of aqueous NaOH (2.22 g in 130 ml of H_2O) until the mixture turned clear and alkaline. Formamidinesulfinic acid (1.85 g, 10 mmol) was dissolved in the remaining NaOH solution and added to the reaction mixture. The final aqueous volume was made up to 200 ml. Experimental conditions were similar to the previous reaction; however, a 3-hr period was necessary for this reaction to go to completion. On work-up, as in the previous experiment, a white precipitate of 2b was obtained. This, on drying, weighed 0.52 g (40%): mp 107–110°; TLC R_f 0.70; NMR δ 4.52 (d, 1, J = 6 Hz, 5β-H), 3.68–3.40 (m, 1, 6α-H), 5.94–5.60 (m, 1, vinylic H), 5.26–5.10 (t, 2, gem vinylic H). The compound 2b was converted to its hydrochloride and recrystallized (95% EtOH–CH_3COCH_3) as in the previous case: mp of 2b hydrochloride 205–207° dec; $[\alpha]^{25}D$ −158.3° (c 0.7, H_2O); mass spectrum (70 eV) m/e 329 (100%). Anal. ($C_{19}H_{24}ClNO_4 \cdot H_2O$) C, H, N, Cl.

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References and Notes

(1) H. Blumberg, H. B. Dayton, and P. S. Wolf, *Toxicol. Appl. Pharmacol.*, 10, 406 (1967).
(2) H. Blumberg and H. B. Dayton, Abstracts of Volunteer Papers, 5th International Congress on Pharmacology, July 1972, p 23.
(3) W. R. Martin, D. R. Jasinski, and P. A. Mansky, *Arch. Gen. Psychiatry*, 28, 784 (1973).
(4) A. Zaks, T. Jones, M. Fink, and A. M. Freedman, *J. Am. Med. Assoc.*, 215, 2108 (1971).
(5) J. M. Fujimoto, *Proc. Soc. Exp. Biol. Med.*, 133, 317 (1970).
(6) E. J. Cone, *Tetrahedron Lett.*, 2607 (1973).
(7) N. Chatterjie, J. M. Fujimoto, C. E. Inturrisi, S. Roerig, R. I. H. Wang, D. V. Bowen, F. H. Field, and D. D. Clarke, *Drug Metab. Dispos.*, 2, 401 (1974).
(8) N. Chatterjie, C. E. Inturrisi, J. M. Fujimoto, and S. Roerig, *Pharmacologist*, 16, 226 (1974).
(9) I. J. Pachter and Z. Matossian, U.S. Patent 3,393,197 (1968); *Chem. Abstr.*, 69, 87282q (1968).
(10) E. J. Cone, C. W. Gorodetzky, and S. Y. Yeh, *Pharmacologist*, 16, 225 (1974).
(11) H. C. Brown and S. Krishnamurthy, *J. Am. Chem. Soc.*, 94, 7159 (1972).
(12) L-Selectride, a product obtained from Aldrich Chemical Co., Inc., Milwaukee, Wis.
(13) U. Weiss and S. J. Daum, *J. Med. Chem.*, 8, 123 (1965).
(14) E. D. B. Barnett, *J. Chem. Soc.*, 97, 63 (1910).
(15) J. Boeseken, *Proc. Acad. Sci. Amsterdam*, 39, 717 (1936); *Chem. Abstr.*, 30, 6331[6] (1936).
(16) V. Nakagawa and K. Minami, *Tetrahedron Lett.*, 343 (1972).
(17) S. Okuda, S. Yamaguchi, Y. Kawazoe, and K. Tsuda, *Chem. Pharm. Bull.*, 12, 104 (1964).
(18) R. E. Lutz and L. F. Small, *J. Org. Chem.*, 4, 220 (1939).
(19) A. C. Currie, J. Gillon, G. F. Newbold, and F. S. Spring, *J. Chem. Soc.*, 773 (1960).
(20) J. E. Herz and L. A. De Marquez, *J. Chem. Soc., Perkin Trans. 1*, 2633 (1973).
(21) D. Elad and D. Ginsburg, *J. Am. Chem. Soc.*, 76, 312 (1954).
(22) L. J. Sargent, L. H. Schwartzman, and L. F. Small, *J. Org. Chem.*, 23, 1247 (1958).
(23) M. Gates, *J. Am. Chem. Soc.*, 75, 4340 (1953).
(24) J. F. Blount, E. Mohacsi, F. M. Vane, and G. J. Mannering, *J. Med. Chem.*, 16, 352 (1973).
(25) H. Blumberg and H. B. Dayton, *Adv. Biochem. Psychopharmacol.*, 8, 33 (1973).
(26) J. Fujimoto, S. Roerig, R. I. H. Wang, N. Chatterjie, and C. E. Inturrisi, *Proc. Soc. Exp. Biol. Med.*, in press.
(27) H. Blumberg, P. S. Wolf, and H. B. Dayton, *Proc. Soc. Exp. Biol. Med.*, 118, 763 (1965).
(28) G. E. Delory and E. J. King, *Biochem. J.*, 39, 245 (1945).

Synthesis of N^{10}-Methyl-4-thiofolic Acid and Related Compounds

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Compound 21 (N^{10}-methyl-4-thiofolic acid) and related compounds were prepared as potential inhibitors of the cofactor forms of tetrahydrofolate. The preparation of 2-acetylamino-4-(benzylthio)-6-chloro-5-nitropyrimidine (4) provided an intermediate that was allowed to react with methyl p-[(3-aminoacetonyl)methylamino]benzoate oxime (16). The oxime function of the resulting 6-substituted aminopyrimidine 6 was hydrolyzed to give the corresponding acetonylaminopyrimidine 7, which on reductive cyclization gave methyl p-[[[2-amino-4-(benzylthio)-7,8-dihydro-6-pteridinyl]methyl]methylamino]benzoate (9). This dihydropteridine was oxidized with potassium permanganate, and the product was treated successively with sodium hydrosulfide to replace the benzylthio group and with aqueous sodium hydroxide to hydrolyze the ester function to give p-[[(2-amino-3,4-dihydro-4-thioxo-6-pteridinyl)methyl]methylamino]benzoic acid (N^{10}-methyl-4-thiopteroic acid, 12). Another route to 12 involved the interaction of 2,5-diamino-4,6-dichloropyrimidine (15) with 16 to give methyl p-[[(2-amino-4-chloro-7,8-dihydro-6-pteridinyl)methyl]methylamino]benzoate (13). Displacement of the chloro group of 13 with sodium hydrosulfide followed by the simultaneous air oxidation of the dihydropteridine ring and saponification of the ester group gave 12. After protection of the 2-amino and 4-thioxo moieties of 12, the resulting intermediate benzoic acid was coupled with diethyl L-glutamate. The product of this reaction was deblocked to give 21. Methylation of 21 gave the corresponding 4-(methylthio) derivative 22, which on reaction with hydrazine gave the 4-hydrazino analog 23 of methotrexate. Reduction of 12 and 21 with sodium hydrosulfite gave the dihydropteridines 24 and 25, respectively. The title compound was an excellent inhibitor of the growth of *Streptococcus faecium* ATCC 8043. However, this and related compounds were ineffective inhibitors of dihydrofolic reductase and showed no significant activity in either the KB cell culture screen or against L1210 leukemia cells in mice.

The 4-amino-4-deoxy derivatives of folic acid and its N^{10}-methyl derivative, aminopterin and methotrexate, are among the most active anticancer agents in use today. Both compounds interact with dihydrofolic reductase to give complexes with low dissociation constants (pseudo-irreversible) that inhibit the function of this enzyme. In con-