SYNTHESIS OF N-p-AZIDOPHENYLETHYL-7,8-DIHYDRONORMORPHINE AND ITS 7,8-DITRITIO ANALOGUE. POTENTIAL OPIATE RECEPTOR PHOTOAFFINITY LABELS

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SUMMARY

The morphine derivatives N-p-azidophenylethyl-7,8-dihydronormorphine (1) and its 7,8-ditritio analogue (1a) were synthesized from morphine. This material, a potential photoaffinity label with high specific radioactivity and with opiate agonist activity comparable to morphine, may be useful for labeling of opiate receptors.

Key Words: Azidophenylethyldihydronormorphine, ^{3}H labeled, photoaffinity label, opiate receptors

Extensive investigations have been made of the receptors[1] in animal nervous systems and smooth muscles which interact with opium alkaloids and the endogenous opiate peptides.[2] A number of workers have used the technique of affinity labeling in attempts to identify the molecular species responsible for the initial interaction with the opiates in vivo. Derivatives of morphine,[3] synthetic opiates,[4] and opiate peptides[5] incorporating chemically reactive groups such as α-chloromethylketones and mustard-type alkylating moieties, as well as photochemically activated groups such as aryl azides, azines, and α-diazoketones, have been prepared.

However, of the morphine derivatives, only the C-ring region of the molecule has been reported to be modified in this manner. In order to examine the efficacy of introducing photolabile substituents into other portions of the morphine structure, we undertook the synthesis of an N-substituted morphine derivative.

To allow incorporation of a high specific activity radiolabel, catalytic hydrogenation of the 7,8 double bond with tritium gas was chosen, as 7,8-dihydromorphine shows no loss of activity as an opiate agonist relative to morphine.[6] The plan was to prepare the appropriate amino-substituted morphine

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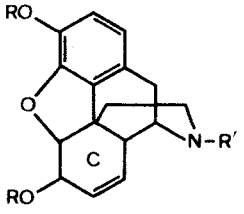
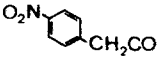
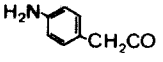
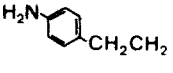
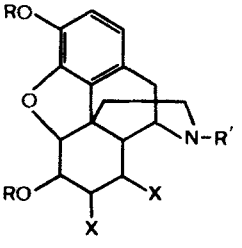
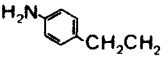
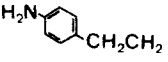
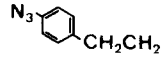
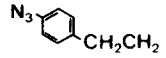
derivative, reduce with tritium, then convert the amino group to the corresponding azide. The target molecule selected was N-p-azidophenylethyl-7,8-dihydronormorphine (1). We hypothesized that this compound would show undiminished opiate bioactivity based on the known biological potency of N-phenylethylmorphine,[7] and this was indeed the case (see below).

The conversion of morphine to N-p-azidophenylethyl-7,8-dihydronormorphine was carried out via a blocked normorphine. The 3- and 6-hydroxyl groups of morphine were acetylated and the heroin N-methyl group cleaved by reaction with trichloroethylchloroformate and removal of the carbamate moiety with zinc metal.[8] The resulting norheroin (4) was acylated with p-nitrophenylacetyl chloride to yield N-p-nitrophenylacetylnorheroin (5), the nitro group was reduced with stannous chloride, and the amide was reduced with lithium aluminum hydride. This treatment also cleaved the O-acetyl groups, giving N-p-aminophenylethylnormorphine (7) in 49% yield. Catalytic reduction of the 7,8-double bond with hydrogen and PtO_2 occurred in 91% yield. Conversion to the azide 1 took place using sodium nitrite, then sodium azide in aqueous sulfuric acid under subdued light. TLC purification of the final product gave a 44% yield of the desired azide 1 in non-radioactive form. Azide 1 was found to cause 50% inhibition of guinea pig ileum contraction at 10^{-7}M, and give 50% displacement of etorphine or naloxone from a rat brain preparation at 10^{-8}M, comparable to morphine.[9] Thus azide 1 appears to have satisfactory receptor affinity.



	R	R′
2	CH_3CO	CH_3
3	CH_3CO	CCl_3CH_2OCO
4	CH_3CO	H
5	CH_3CO	
6	CH_3CO	
7	H	



	X	R	R′
8	H	H	
8a	^3H	H	
1	H	H	
1a	^3H	H	

Catalytic reduction of the double bond with tritium was carried out in dioxane to minimize the amount of tritium exchange into the solvent. Tritiation occurred smoothly on a 30 mg scale under 680 mm Hg tritium pressure to yield N-p-aminophenylethyl-7,8-dinitronormorphine (8a), which was converted to the azide and purified by preparative TLC.

This compound may be useful for photoaffinity labeling of opiate receptors, either membrane bound or in solubilized form. The high specific activity radiolabel should make identification of low concentrations of receptors feasible.

EXPERIMENTAL

General. NMR Spectra were obtained in $CDCl_3$, IR spectra were taken in $CHCl_3$, and UV spectra were recorded in C_2H_5OH. All reactions were conducted in a nitrogen atmosphere and final organic phases resulting from reaction isolations were dried over $MgSO_4$.

Heroin (2). Morphine sulfate (15.0 g, 45 mmol) was dissolved in acetic anhydride (50 mL) and triethylamine (50 mL), the solution was refluxed 16 hours. The mixture was cooled to room temperature, 1 M phosphoric acid (200 mL) and chloroform (200 mL) were added, and the aqueous phase was washed with additional chloroform, then chilled in ice and basified to pH 10 with 4M NH_4OH. Extraction with chloroform, drying and evaporating left 16.6 g, 100% yield of heroin which was used directly in the next step: mp (from ethanol) 171-173°C (lit.[10] mp 173°); NMR δ 6.8 (1H, d, J=8Hz), 6.6 (1H, d, J=8), 5.8 (1H, d, J=10), 5.5 (1H, d, J=10), 5.3 (2H, s), 3.5 (1H, m), 3.0-2.0 (7H, m), 2.6 (3H, s), 2.4 (3H, s), 2.2 (3H, s).

β,β,β-Trichloroethylcarbamylnorheroin (3). Heroin (16.6 g, 45 mmol) was dissolved in dry chloroform (200 mL, distilled from P_2O_5) and β,β,β-trichloroethylchloroformate (10.5 g, 50 mmol) was added. The mixture was refluxed 22 h, then was cooled to room temperature, and washed with 1M NaOH, then 1M phosphoric acid, then water. The organic phase was dried and evaporated yielding 23.8 g (100%) of the desired carbamate. The material was used directly in the next step without purification: NMR δ 6.8 (1H, d, J=7Hz), 6.6 (1H, d, J=7), 5.8 (1H, d, J=10), 5.5 (1H, d, J=10), 5.2 (2H, s), 5.0 (2H, s), 4.2 (1H, m), 3.4-2.4 (4H, m), 2.4 (3H, s), 2.2 (3H, s), 2.2-1.8 (2H, m); Mass Spectrum m/z 531, 529 (M^+).

Norheroin (4). The trichloroethylcarbamate (3, 23.8 g, 45 mmol) was dissolved in 90% acetic acid (50 mL) and zinc dust (29.3 g, 450 mmol) was added in portions as the mixture was stirred vigorously in a water bath at room temperature. The suspension was stirred overnight, then filtered and the unreacted zinc washed with a small portion of 90% acetic acid. The filtrate was taken up

in 1M phosphoric acid (200 mL) and chloroform (200 mL), and after separation, the aqueous phase was washed with chloroform, then cooled with ice and basified to pH 10.5 with ammonium hydroxide. This mixture was then extracted well with chloroform, which was dried and evaporated to yield a glass. Crystallization occurred readily on trituration with ethyl acetate. The yield was 8.0 g (50% from morphine). The first chloroform phase was dried and evaporated to give 3.2 g (13%) of recovered starting material. The norheroin (14) recrystallized from ethyl acetate had a melting point of 138-140°. This compound was previously characterized only as its hydrochloride.[11] NMR δ 6.8 (1H, d, J=8Hz), 6.5 (1H, d, J=8), 5.7 (1H, d, J=10), 5.4 (1H, d, J=10), 5.1 (2H, s), 3.7 (1H, m), 3.2-2.6 (5H, m), 2.2-1.8 (2H, m), 2.4 (3H, s), 2.2 (3H, s). Anal. Calcd: C, 67.6; H, 6.0; N, 3.9. Found: C, 67.6; H, 6.0; N, 4.0.

p-Nitrophenylacetylchloride. Commercial p-nitrophenylacetic acid (9.0 g, 50 mmol) was dissolved in dry benzene (50 mL), then oxalyl chloride (6.5 g, 4.4 mL, 51 mmol) was added and the mixture refluxed until gas evolution had ceased (12 h). The mixture was cooled, filtered and evaporated. The last traces of oxalyl chloride and benzene were removed under vacuum, yielding 8.1 g (81%) of the desired acid chloride which crystallized on standing: NMR δ 8.1 (2H, d, J=8Hz), 7.4 (2H, d, J=8), 4.3 (2H, s).

N-p-Nitrophenylacetylnorheroin (5). Norheroin (3.55 g, 10 mmol) was dissolved in dry DMF (5 mL, distilled from CaH_2), and dry potassium carbonate (2.06 g, 15 mmol) was added. The mixture stirred at room temperature as p-nitrophenylacetyl-chloride (2.38 g, 12 mmol) in dry DMF (5 mL) was added dropwise. After 2 h, water (30 mL) was added, and the mixture was extracted with chloroform. The organic phase was washed with 1M phosphoric acid, then water, and was dried and evaporated to yield 3.61 g (70%) of the desired amide as a glass. NMR δ 8.2 (2H, d, J=8Hz), 7.4 (2H, d, J=8), 6.8 (1H, d, J=8), 6.6 (1H, d, J=8), 5.8 (1H, d, J=10), 5.5 (1H, d, J=10), 5.2 (2H, s), 4.6 (1H, m), 4.0 (2H, m), 3.4-2.6 (5H, m), 2.4 (3H, s), 2.2 (3H, s), 2.2-1.8 (2H, m). Exact mass calcd for $C_{28}H_{26}N_2O_8$ m/z 518.1689, found 518.1698.

N-p-Aminophenylacetylnorheroin (6). The nitroamide (500 mg, 0.96 mmol) in glacial acetic acid (3 mL) plus one drop of conc. hydrochloric acid was warmed on a steam bath as stannous chloride dihydrate (1.0 g, 4.5 mmol) was added. The mixture was kept at 90° for 30 minutes, then it was cooled and basified to pH 10.5 with ice cold 4N NaOH and extracted well with chloroform. The organic phase was dried and evaporated yielding 400 mg (82%) of the desired amino compound as a glass which was used directly in the next step: NMR δ 7.2-6.4 (6H, m), 5.7, (1H, d, J=10), 5.4 (1H, d, J=10), 5.0 (2H, s), 3.7 (2H, m), 3.0-1.6 (8H, m), 2.3 (3H, s), 2.1 (3H, s). Exact mass calcd for $C_{28}H_{28}N_2O_6$ m/z 488.1947, found 488.1952.

N-p-Aminophenylethylnormorphine (7). N-p-Aminophenylacetylnorheroin (6, 490 mg, 1.0 mmol) in dry THF (10 mL) was added to a well stirred suspension of $LiAlH_4$ (133 mg, 3.5 mmol) in THF (10 mL) at room temperature. The mixture was stirred and refluxed 1.5 h, then cooled to 0° and the excess hydride decomposed with ice cold 1M phosphoric acid. The solution was filtered and basified in the cold to pH 9, extracted well with 10% methanol in chloroform, and the organic phase dried and evaporated to yield 330 mg (85%) of the desired aminophenol 7. The material was further purified by preparative TLC on silica gel with 10% methanol in chloroform, and the sulfate salt could be recrystallized from ethanol: NMR δ 7.0 (2H, d, J=8), 6.6 (2H, d, J=8), 6.5 (1H, d, J=8), 6.4 (1H, d, J=8), 5.6 (1H, d, J=10), 5.2 (1H, d, J=10), 4.8 (1H, d, J=6), 4.2 (1H, m), 3.6 (2H, m), 3.0-1.6 (10H, m). IR: 3600 (br), 3000, 1610, 1510, 1450, 1200, 1100. Anal. Calcd: C, 57.9; H, 5.9; N, 5.6 for sulfate hemihydrate. Found: C, 57.8; H, 6.2; N, 5.4.

N-p-Aminophenylethyl-7,8-dihydronormorphine (8). N-p-Aminophenylethylnormorphine (7, 330 mg, 0.85 mmol) was dissolved in methanol (40 mL) and PtO_2 (20 mg) was added. The mixture was shaken under 33 psi of hydrogen for 1.5 h, then the catalyst was filtered, and the filtrate evaporated. The residue (300 mg, 91%) appeared to be substantially pure: NMR δ 6.95 (2H, d, J=8Hz), 6.7-6.4 (4H, m), 4.6 (1H, d, J=6), 4.1 (1H, m), 3.2-2.0 (10H, m), 2.0-1.0 (6H, m); mass spectrum m/z 392 (M+).

N-p-Azidophenylethyl-7,8-dihydronormorphine (1). N-p-Aminophenylethyl-7,8-dihydronormorphine (8, 207 mg, 0.53 mmol) was dissolved in 2.0M aqueous sulfuric acid (1.5 mL), and chilled to 6° under red safelight as sodium nitrite (44 mg, 0.64 mmol) was added. After 30 minutes, the UV absorption maximum had shifted to 270 nm. Then sodium azide (52 mg, 0.80 mmol) in water (1 mL) was added. The solution was stirred 30 minutes, the UV absorption maximum shifting to 248 nm. Saturated sodium bicarbonate solution (20 mL) was added until the pH reached 8.5, and the solution was extracted well with 10% methanol in chloroform. The pH was adjusted to 9.5 and the extraction repeated. The organic phases were combined, dried and evaporated to yield 120 mg (55%) of the azide 1. The product was purified by preparative TLC on silica gel with 5% methanol in chloroform. After protecting the TLC plate with an aluminum sheet, the edge was exposed to UV light to visualize the compound. A band with R_f 0.2 was recovered to yield 53 mg (44% recovery, 24% from amine 8) of azide 1: NMR δ 7.2 (2H, d, J=9Hz), 7.0 (2H, d, J=9), 6.8 (1H, d, J=8), 6.6 (1H, d, J=8), 5.4 (2H, s), 4.8 (1H, d, J=6), 4.2 (1H, m), 3.5 (2H, m), 3.2-2.4 (8H, m), 2.0-1.4 (6H, m). IR: 3500 (br), 3000, 2900, 2050 (strong), 1610, 1500, 1450, 1300, 1100. Exact mass calcd for $C_{24}H_{26}N_4O_3$ m/z 418.2005, found 418.2000.

N-p-Aminophenylethyl-7,8-[$^3H_2$]-normorphine (8a). N-p-aminophenylnormorphine (7, 31.2 mg, 0.80 mmol) was dissolved in dry dioxane (1.0 mL) and PtO_2 (15 mg) was added. The mixture was then stirred under a carrier-free tritium gas pressure of 680 mm Hg (2.5 Curies/mL T_2) for 24 hours, the pressure dropping to 580 mm Hg. The excess tritium gas was flushed out with nitrogen and the catalyst was removed by centrifugation, and washed with methanol. The combined organic phases were lyophilized, and additional methanol (2 mL) was added and lyophilized to remove exchangeable tritium. The product was taken up in 10% methanol in benzene (10 mL), and frozen for storage.

N-p-Azidophenylethyl-7,8-[$^3H_2$]-normorphine (1a). A quarter of the previous solution containing the tritiated amine 8a was thawed and extracted with 2M aqueous sulfuric acid (0.5 mL). The aqueous solution was cooled to 0° under red safelight, then sodium nitrite solution (0.1 mL of 1.0M) was added and the mixture stirred 10 minutes. Sodium azide solution (0.1 mL of 1.0M) was added and the reaction mixture was stirred an additional 30 minutes. Then sodium carbonate solution (1.2 mL of 10% aqueous) was added, and the mixture was extracted with five portions of 10% methanol in chloroform. The organic phase was dried, the solvent was evaporated under a nitrogen stream, and the residue was taken up in absolute ethanol (2 mL) for storage. Comparison with the known extinction coefficient [UV λ_{max} 248 nm (ε 11,080)] gave the alkaloid concentration, and the specific activity was determined to be 30 Ci/mmol.

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