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Notes

Narcotic Antagonists. 2. Preparation and Biological Stability of Naloxone-7,8-^3H

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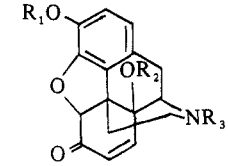
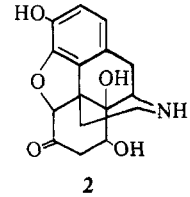
Naloxone (*N*-allylnoroxymorphone, **3b**) is a potent narcotic antagonist[1] which is in current clinical use as an antidote for narcotic overdosage† and is also a promising agent in the chemical treatment of narcotic addiction.[2] It is unique in that it is a pure antagonist devoid of any agonist activity,[3] and it is therefore of particular importance in the study of the biochemistry of narcotic action and addiction. To permit detailed studies of the metabolism and disposition of naloxone in experimental animals and in man,‡ a labeled substrate with the isotope located in biologically stable positions was required. A high specific activity of at least 10 Ci/mmol was desirable to allow for the use of the labeled material in contemplated protein binding studies.

Reduction of a double bond with tritium gas is a most effective method of achieving high specific activity labeling. Since the synthesis of naloxone from thebaine involves the reduction of an unsaturated intermediate,[5] this procedure was applicable in this instance subject to two principal considerations. One was that the tritium reduction had to be carried out prior to the introduction of the *N*-allyl group, and the other was that to ensure practical yields a minimum of chemical manipulation subsequent to the isotope incorporation be required. The above requirements were best realized by the reduction of 14-hydroxynormorphinone (**1d**) with tritium which then needed only N-allylation to convert it to naloxone. 14-Hydroxynormorphinone (**1d**) was obtained from 14-hydroxymorphinone (**1a**) by the following sequence.

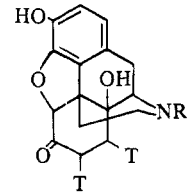
14-Hydroxymorphinone (**1a**)[6] was converted to its diacetate **1b** in refluxing acetic anhydride. Reaction of the diacetate **1b** with CNBr led to the *N*-cyano derivative **1c**. Removal of the cyano and acetate groups in **1c** by acid hydrolysis proved to be troublesome in that infrared analysis of the product revealed the presence of variable proportions of a saturated keto compound. By analogy to the 14-hydroxymorphinone case,[6] the contaminant was probably derived from hydration of the 7,8-double bond and was assigned the 8,14-dihydroxydihydronormorphinone (**2**) structure. The most satisfactory conditions for the hydrolysis of **1c** were found to be refluxing in 20% HCl for 3 hr. These conditions were sufficient to effect removal of both the cyano and acetate groups and yielded a product containing 14-hydroxynormorphinone (**1d**) and 8,14-dihydroxydihydronormorphinone (**2**) in 1:1 proportion. The mixture proved to be difficult to separate and was reduced with tritium in the presence of 10% palladized charcoal without separation. Since the 8,14-dihydroxy component **2** of the mixture contained no aliphatic unsaturation, it was unaffected by the reduction and only the 14-hydroxynormorphinone incorporated the isotope to give 14-hydroxydihydronormorphinone-7,8-^3H (**3a**), without major radiochemical impurities. The product of the tritium reduction was then allowed to react with allyl bromide to yield naloxone-7,8-^3H (**3b**) in about 40% yield. The latter when purified by preparative tlc or by gradient elution partition chromatography on Celite provided material with a specific activity of 40 Ci/mmol, which by reverse isotope dilution with inert carrier naloxone was better than 97% pure.



1a, R_1, R_2 = H; R_3 = CH_3
b, R_1, R_2 = Ac; R_3 = CH_3
c, R_1, R_2 = Ac; R_3 = CN
d, R_1, R_2 = R_3 = H



2



3a, R = H
b, R = $CH_2CH{=}CH_2$

The biological stability of the isotope in both the C-7 and C-8 positions of naloxone was confirmed by the following experiment. After the administration of 10×10^6 cpm of the above naloxone-^3H to a volunteer male subject, urine was collected for 3 days. Lyophilization of an aliquot of the combined urines yielded water with a specific activity of 1 cpm/ml. Since the total body water volume of the subject was 35 l., 35,000 cpm or only 0.35% of the dose

†"Narcan," Endo Laboratories, Inc., Garden City, N. Y.

‡The urinary metabolites of inert naloxone have been reported on in the chicken and rabbit[4a] and in man.[4b]

has been displaced from the substrate molecule indicating more than adequate biological stability of the isotopes in naloxone-7,8-^3H.

Experimental Section §

14-Hydroxymorphinone 3,14-Diacetate (1b). A solution of 1 g of 14-hydroxymorphinone (**1a**) in 15 ml of acetic anhydride was refluxed for 1 hr. The acetic anhydride was removed under vacuum and the residue was crystallized from ethanol to give 0.93 g of 14-hydroxymorphinone diacetate (**1b**): mp 248–250°; nmr δ 2.08, 2.27 (acetate methyls), 2.40 (*N*-methyl). *Anal.* ($C_{21}H_{21}NO_6$) C, H, N.

***N*-Cyano-14-hydroxynormorphinone 3,14-Diacetate (1c).** A solution of 0.53 g of the diacetate **1b** in 30 ml of $CHCl_3$ was refluxed with 1.5 g of CNBr for 4 hr. After cooling the chloroform was washed with 50 ml of 5% HCl, dried, and evaporated. The residue was crystallized from ethanol to give 0.27 g of *N*-cyano-14-hydroxynormorphinone 3,14-diacetate (**1c**): mp 243–245°; nmr δ 2.18, 2.25 (acetate methyls), no *N*-methyl absorption. *Anal.* ($C_{21}H_{18}N_2O_6$) C, H, N.

14-Hydroxynormorphinone (1d). A suspension of 100 mg of the *N*-cyano derivative **1c** in 10 ml of 20% HCl was heated on a steam bath for 3 hr with continuous stirring. The solution was then evaporated to dryness under a vacuum, and the residue was taken up in 2 ml of H_2O and adjusted to pH 8 with dilute NH_4OH. The aqueous mixture was extracted three times with 5 ml of $CHCl_3$ which was dried and evaporated. The residue showed an equal amount of unsaturated carbonyl absorption at 1680 cm^{-1} and saturated carbonyl at 1730 cm^{-1}, assigned to 14-hydroxynormorphinone and 8,14-dihydroxydihydronormorphinone, respectively. Repeated attempts at separation on various tlc systems failed to effect purification and only increased the intensity of the saturated carbonyl absorption.

Naloxone-7,8-^3H (3b). The above mixture (20 mg) dissolved in 0.5 ml of ethanol containing 20 mg of 10% palladium on charcoal was treated with 5 Ci of tritium gas overnight at room temperature. Labile tritium was removed by dissolving in a small quantity of ethanol and evaporating the solvent, repeating the procedure three times. The residue contained 225 mCi of radioactivity. An aliquot of the above material was taken up in 2 ml of ethanol to which 100 mg of $NaHCO_3$ and 0.20 ml of allyl bromide was added. After refluxing for 18 hr under efficient condensation the reaction mixture was filtered, and the filtrate was taken down to dryness. The residue, containing naloxone-7,8-^3H, was dissolved in 1 ml of ethanol, and an aliquot containing 115,000 cpm was diluted with 53.8 mg of inert naloxone. Recrystallization from ethyl acetate gave specific activities of 917, 827, and 819 cpm/mg, indicating that 39% of the radioactive product was naloxone.

The naloxone-7,8-^3H was purified by preparative tlc on silica gel in the system chloroform–methanol (9:1) containing 4 drops of NH_4OH. The area corresponding to standard naloxone which was run alongside was eluted with ethanol–methylene chloride. One portion of the eluted material was chromatographed on thin-layer silica gel in the system ethanol–acetic acid–water (60:30:10). After development the plate was scanned for radioactivity, showing only one radioactive peak at R_f 0.5 corresponding to standard naloxone which was run alongside.

Another portion of the purified naloxone-7,8-^3H containing 40,800 cpm was diluted with 29.6 mg of inert naloxone. Recrystallization from ethyl acetate gave the following successive specific activities: 1349, 1305, 1383 cpm/mg. The average specific activity of 1346 cpm/mg corresponds to a total of 39,840 cpm or 97.5% of the radioactivity present.

The naloxone-7,8-^3H could also be purified on a Celite partition column with 90% methanol–10% water as the stationary phase and 100% isooctane followed by a 25% dichloroethane gradient as the eluting solvents. The 10-ml fractions were obtained by an automatic fraction collector and aliquots were removed for counting. Naloxone was eluted with 100% isooctane as a single radioactive peak in fractions 25–44. After introduction of a 25% dichloroethane gradient at fraction 100, another radioactive peak in fractions 205–225 was obtained and was presumed to consist of 14-hydroxydihydronormorphinone-7,8-^3H which had failed to react with the allyl bromide. The naloxone-7,8-^3H purified by this procedure was also 98% pure by reverse isotope dilution.

§ Nmr spectra were obtained in deuteriochloroform on a Varian A-60 instrument. Infrared spectra were obtained in KBr solutions on a Beckman IR 13 spectrometer. Analyses are by Spang Laboratories. Counting was carried out either in toluene or in Diotol using a Packard scintillation counter.

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New 5-Substituted 1-Alkyl-2-nitroimidazoles

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During the course of a literature survey we have noticed that although there is much information on the well-known antiprotozoal activity of 2-nitroimidazoles, very little data on the antibacterial and antifungal activity are available.

According to Nakamura,[1] 2-nitroimidazole (azomycin) itself mainly inhibits the *in vitro* growth of gram-negative bacteria (*Escherichia coli*, MIC 25 μg/ml), whereas it is completely inactive against fungi. Beaman[2] reported that, at a concentration of 5 μg/ml, azomycin inhibits *Pseudomonas aeruginosa*, *E. coli*, and *Staphylococcus aureus*, 1-methylazomycin inhibits yeasts and 1-allylazomycin *E. coli* only, whereas 4,5-dimethylazomycin is completely inactive. Grunberg,[3] examining the protective effect against lethal systemic infections in mice of a series of 2-nitroimidazoles carrying in position 1 alkyl substituents with amido or hydroxy functions, revealed no activity against *P. aeruginosa* and *Proteus vulgaris*, a weak activity against *E. coli* and *Streptococcus pyogenes*, and a consistent activity against *S. aureus*. Prince[4] found that 1-(3-methoxy-2-hydroxypropyl)-2-nitroimidazole possesses a weak *in vitro* activity against *E. coli* (MIC 250 μg/ml) but it is practically ineffective against *S. aureus*, *S. pyogenes*, *P. aeruginosa*, and *P. vulgaris*.

We have tested for their *in vitro* activity on a series of gram-negative and gram-positive bacteria and fungi about 30 variously substituted 2-nitroimidazole derivatives previously examined for their antitrichomonas activity.[5] Compounds possessing antimicrobial activity below concentrations of 200 μg/ml are shown in Table I. The only correlation between antibacterial and antitrichomonas activity is for activity against *Clostridium perfringens*, as already noted by Prince[4] with similar nitroimidazoles. The most active compounds **14–16** showed no protection in experimental infection in mice against *E. coli* at 60 mg/kg.

Therefore, in order to extend the study on 2-nitroimidazole derivatives, it seemed interesting to introduce in posi-