PREPARATION OF [7,8,19,20 - ^{3}H] NALOXONE OF HIGH SPECIFIC ACTIVITY

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SUMMARY

The preparation of [7,8,19,20 - ^{3}H] naloxone (3c) is described starting from noroxymorphone 2. After saturation of the 7,8 double bond with tritium gas (using PdO catalyst) the resulting compound was propargylated under very mild conditions for a short time with propargyl bromide to give N-propargyl, [7,8-^{3}H] dihydro-noroxymorphone at 925 GBq:mmole (25,1 Ci/mmole) specific activity. This material was tritiated again in the presence of Lindlar catalyst in dimethylformamide (DMF) under carefully controlled conditions. Pure, tritiated naloxone with a specific activity of 3,09 TBq/mmole (83,7 Ci/mmole) was isolated from the reaction mixture by TLC in one dimension using a two-step developing system. Biological assays on the radiochemically homogenous compound using two *in vitro* systems (guinea pig ileum and receptor binding assays in synaptosomes prepared from rat brain stem) also confirmed the purity of the labelled naloxone (3c).
Key words : naloxone, tritium labelling, partial saturation

INTRODUCTION

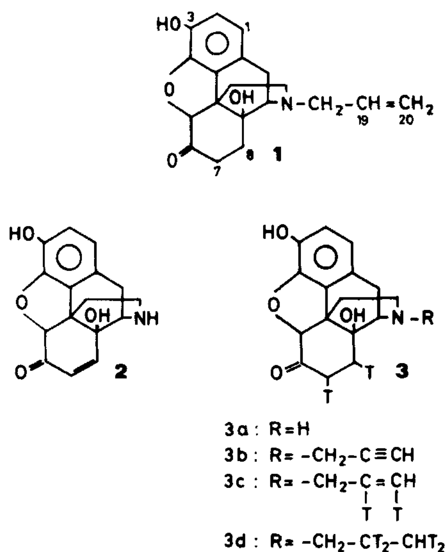
There has been a growing interest in the research of opiate receptors as indicated by the increasing number of papers published in this field (for recent reviews see 1,2). For both the *in vivo* and *in vitro* studies it is important to use small molecular weight receptor ligands (either of the alkaloid or the peptide type) labelled to high specific

radioactivity. As for the alkaloid surrogates, both agonists (morphine, dihydromorphine, etc.) and antagonists (nalorphine, naloxone) have been commercially available in radioactive (mostly tritiated) form. One of the most important in both the biochemical and pharmaceutical research is the pure antagonist, naloxone (N-allyl-noroxymorphon).

Tritiated naloxone (generally labelled) has been first prepared by catalytic tritium exchange reaction using ^3H_2O starting from the unlabelled compound (3). The specific activity of the radioactive naloxone however, was low (225,7 GBq, 6,1 Ci/mmole). Fishman (4) reported the preparation of naloxone labelled in the positions 7,8 by catalytic tritiation of the appropriate unsaturated derivative (noroxymorphone) using ethanol as solvent, followed by allylation with inactive allylbromide. The authors have claimed they obtained the compound at a specific activity of 1,48 TBq/mmole (40 Ci/mmole). However, our repeated attempts to achieve this value were unsuccessful, the maximum specific activity we could produce was only 925 GBq/mmole (25 Ci/mmole). This could be due to the observed tritium loss from position 7 in the course of allylation (5). Another way to introduce tritium into the molecule has been described by Brine and Kepler (5). Naloxone is labelled in the D ring in the position 15. However, the specific activity of this product was also rather low (148 GBq/mmole, 4 Ci/mmole).

Beside some modifications of the reaction conditions in the synthesis of [7,8-^{3}H] naloxone we also explored another possibility to introduce tritium atoms into the positions

19,20 of the molecule by means of partial reduction of the propargyl derivative (3b) with tritium gas. In combination with previous labelling in the positions 7,8, naloxone of very high specific activity was obtained.



EXPERIMENTAL

1. Materials and Methods

Tritium gas was purchased from Technabexport (USSR) and stored in the form of uranium tritide. Thebaine was kindly provided by Dr Péter Kerekes (KLTE, Debrecen, Hungary). Nonradioactive naloxone was a generous gift from Endo Inc. The catalyst PdO

was from Merck, Lindlar catalyst was from Fluka. Precoated silicagel plates were Merck DC-Fertigplatten Kieselgel-60. All the other chemical products and solvents were of the highest purity available.

Chemical concentrations of labelled compounds were determined by comparison with UV absorption of the standard solution, at 287 nm, with the use of a Beckman model DK-2A Spectrophotometer. Radioactivity was measured in a Searle Delta 500 liquid scintillation spectrometer. Radioscans of thin-layer chromatographic plates were performed with a Packard model 7201 Radiochromatogram Scanner. Autoradiography was achieved on Medifort RP X-ray film (Forte).

Hydrogenations and tritiations were performed in a glass vacuum manifold described earlier (7).

2. Preparation of the compounds

2.1. Noroxymorphone (2). The compound was synthesized from thebaine according to Iijama (6). The crude product was used for the further synthesis without purification.

2.2. [7,8-^{3}H] dihydro-noroxymorphone (3a). In a small flask to 5 mg (about 16,6 μmole) of crude noroxymorphone dissolved in 0.5 ml DMF were added to 10 mg PdO. After connecting to the vacuum manifold (7) it was evacuated and treated with about 592 GBq (16 Ci) tritium gas, under constant magnetic stirring at 25°, for 60 min. To remove catalyst, the solution was filtered through a Whatman GF/C glass fiber filter. DMF was removed from the filtrate by evaporation.

2.3. N-propargyl [7,8-^3H] dihydro-noroxymorphone (3b).
The residue obtained in the previous step (3a) was immediately dissolved in 600 μℓ DMF and to this solution 2-3 mg K_2CO_3 and 3,5 μℓ (44,7 μmole) propargyl bromide were added and the flask was connected to the manifold system. After evacuation, N_2 gas 4,9 x 10^4 Pa, (370 torr) was expanded into the reaction vessel and the mixture was slowly stirred magnetically at 25° for 20 min. The labile tritium was removed by repeated evaporations with a large excess of aqueous methanol (150 ml). The residue was dissolved in 200 μℓ methanol and 3b was separated on Silica gel TLC sheets using chloroform : methanol : conc.NH_3 (90 : 10 : 1) for development. After autoradiography the band corresponding to 3b was scraped off and the compound was eluted with 4x3ml ethanol. Total activity : 3,3 GBq (96,8 mCi), yield : 3.85 μmole, ic. specific activity 925 GBq/mmole (25,1 Ci/mmole). The material was also found to be homogenous by TLC using the following solvent system for development ; ethanol : acetic acid : H_2O (60 : 30 : 10) (4). (R_f = 0,43).

2.4. [7,8,19,20 - ^3H] naloxone (3c). To a solution of 1,3 GBq (35 mCi, 1,39 μmole) 3b in 2ml DMF, 1,60 mg Lindlar catalyst was added. After evacuation, tritium gas at 4.6 x 10^4 Pa (350 torr) about 555 GBq (15 Ci) was allowed to react with the stirred mixture. After 15 min the reaction was stopped by chilling, the catalyst filtered off and labile tritium removed, as above.

The final product (3c) was purified by TLC using a two-step developing system : ether : hexane (90 : 15) followed by drying and developing the chromatogram in chloroform : methanol (10 : 1). Autoradiography revealed that naloxone 3c clearly separated from unreacted 3b and from the propyl derivative 3d. Elution from the silica was made again with ethanol.
Total activity in 3c : 2,17 GBq (58,6 mCi), yield 0,7 μmole (50%), specific activity 3,09 TBq/mmole (83,7 Ci/mmole).
As side product 3d was also isolated (0,38 μmole, 27%), specific activity 4,32 TBq/mmole (117 Ci/mmole).
The purity and homogenity of 3c was checked by TLC using three different solvent systems : chloroform : methanol : conc.NH_3 (90 : 10 : 1), ethanol : acetic acid : H_2O (60 : 30 : 10) and by argentation chromatography (8), using chloroform : methanol (70 : 30) for development. R_f values of 3c in these systems were 0,60 , 0,40 and 0,30, respectively. In all systems tested the material proved to be homogenous. The purified compound showed a single maximum at 287 nm.

3. Biological assays. Biological activity of 3c was measured according to Kosterlitz (9) in isolated preparations of electrically stimulated longitudinal muscle strip of guinea pig ileum. The K_E value was determined against normorphine and was found to be 3.3 ± 0.3 mM, in good agreement with the value obtained for standard naloxone. Opiate receptor binding assays were performed basically by the method of Pert and Snyder (3). Synaptosomes from rat brain stem were prepared with slight modification of the method of Hajos (10). Specific

binding of the tritiated naloxone to the receptor was defined as the difference between the total binding and nonspecific binding (in the presence of 10^{-5} M unlabelled naloxone) in Tris-HCl pH 7.4 100 mM NaCl at 25°. Stereospecificity of the binding was proved by using dextrorphane. Two distinct, independent binding sites were detected with the apparent K_d's of 6 and 63 nM.

RESULTS and DISCUSSION

To improve specific activity and also to minimize side product formation we undertook several modifications on the reported reaction routes leading to [7,8-^{3}H] naloxone.
We observed that [7,8-^{3}H] dihydro-noroxymorphone (3a) could be obtained in much shorter time than described by Fishman et al. (4). Using DMF instead of ethanol, PdO catalyst and a higher excess of tritium gas over the material, already one hour tritiation resulted in, (after allylation with unlabelled allylbromide) [7,8-^{3}H] naloxone of reasonable specific activity. PdO/Al_2O_3 catalyst proved to be less efficient (532.8 GBq/mmole, 14.4 Ci/mmole). Also, reaction conditions of the allylation (likewise the propargylation) step were greatly altered as compared to those reported by others (4,6). We found that, on the mg scale, increasing the temperature resulted in many side products. However, under inert atmosphere, in DMF if 3-5 fold excess of allyl (or propargyl) bromide was reacted with the crude 3a, the reaction took place rapidly (20 min) at room temperature, and only one side product was formed as revealed by TLC (data not shown)

In the synthesis of the multiple labelled naloxone 3c the most crucial step was the partial reduction of the propargyl side chain to the allylic one and the subsequent separation of the products formed in this reaction (3b, 3c, and 3d). The saturation reaction was extremely sensitive to even minor changes in concentrations of the catalyst and the starting material, to H_2 or T_2 pressure and was also greatly influenced by the time allowed for the reaction to proceed. The occurence of over-tritiated (propyl-) derivative 3d was unavoidable if we wished to achieve an appreciably high yield of 3c. Under optimal reaction conditions 50 per cent of naloxone, and about 27 percent of the propyl derivative were produced. Separation of 3b, 3c, and 3d was made by TLC in one dimension using a two-step developing system.

Using the improved methods outlined above we succeded in preparation of pure highly tritiated naloxone (3c) at relatively good yield (related to 3b) that retained its full biological activity, as checked by two independent in vitro biological assays.

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