A CONVENIENT PROCEDURE FOR PREPARING NALTREXONE-15,16-$^3H_2$ AND NALOXONE-15-^3H OF HIGH SPECIFIC ACTIVITY

George A. Brine and John A. Kepler*
Research Triangle Institute, Research Triangle Park, North Carolina 27709

Received on January 15, 1976
Revised on January 26, 1976

SUMMARY

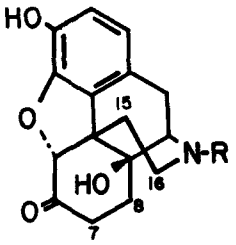
Mercury(II) oxide oxidation of naltrexone (1a) and naloxone (1b) gave 15,16-didehydronaltrexone (4a) and 15,16-didehydronaloxone (4b). Subsequent reduction of 4a with tritium gas afforded naltrexone-15,16-$^3H_2$ having a specific activity of 15.3 Ci/mmole. Subsequent equilibration of 4b with carrier free tritium oxide followed by sodium cyanoborohydride reduction yielded naloxone-15-^3H having a specific activity of 4 Ci/mmole.

Key Words: Tritium gas, Naltrexone, Tritium oxide, Naloxone

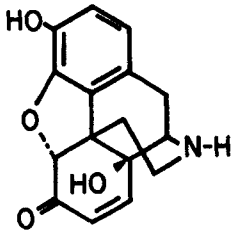
INTRODUCTION

Current studies involving the potent narcotic antagonists N-cyclopropylmethyl-7,8-dihydro-14-hydroxynormorphinone (naltrexone, 1a) and N-allyl-7,8-dihydro-14-hydroxynormorphinone (naloxone, 1b) have created a need for radiolabeled compounds of high specific activity with the label located in biologically stable positions.

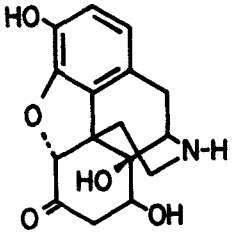
A previously reported synthesis of high specific activity naloxone-7,8-$^3H_2$ involved the reduction of 14-hydroxynormorphinone (2) with tritium gas followed by N-alkylation with allyl bromide.[1] A major drawback to this route was the multistep synthesis of 2 from thebaine which yielded a 1:1 mixture of 2 and 8,14-dihydroxydihydronormorphinone (3). Another drawback was that essentially half of the label introduced in the reduction step was labile to the conditions of the alkylation step.[2] Attempts in our laboratory to improve the synthesis of naltrexone-7,8-$^3H_2$ met with only moderate success and further indicated the need for a less complex synthesis.



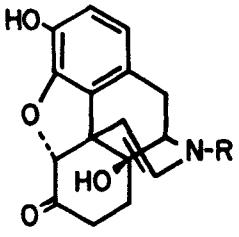
1a, R=CH_2-c-C_3H_5
b, R=$CH_2CH=CH_2$



2



3



4a, R=CH_2-c-C_3H_5
b, R=$CH_2CH=CH_2$

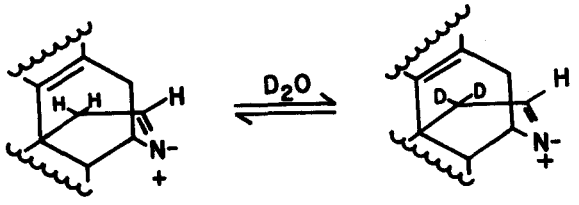
The successful synthesis of 15,16-didehydro-6,14-endo-etheno-6,7,8,14-tetrahydrooripavines[3] prompted us to investigate a route to radiolabeled 1a involving the intermediacy of 15,16-didehydronaltrexone (4a). During the course of our investigation Lewis, et al.,[4] described the synthesis of tritium labeled tetrahydrooripavines from the corresponding 15,16-didehydro-compounds. We wish now to report our synthesis of high specific activity naltrexone-15,16-$^3H_2$ and naloxone-15-^3H.

RESULTS AND DISCUSSION

Dehydrogenation of either naltrexone (1a) or naloxone (1b) with yellow mercury(II) oxide in 1.5N acetic acid[3,5] readily afforded the 15,16-didehydrocompounds 4a and 4b in moderate yield. Trace amounts of the starting material and an unidentified minor product were removed in each case by purification on ChromAR 1000. When stored in the cold and protected from light both 4a and 4b were stable indefinitely.

The ir spectrum of 4a showed bands at 1728(s) and 1621(m) cm^{-1} while

the pmr spectrum in deuterochloroform showed a doublet (J = 4 Hz) at 5.14 ppm which was assigned to H-16. No signal for H-15 was detected in the olefinic region of the pmr spectrum. However, upon deuterium oxide exchange the H-16 doublet collapsed to a singlet as expected.[4,6] In addition, a doublet (J = 12 Hz) at 2.07 ppm and a partially overlapped doublet of doublets (J = 12 Hz, 4 Hz) at 2.46 ppm disappeared from the spectrum upon deuterium oxide exchange. This pattern of exchangeable resonances was interpreted as an ABX spectrum (A at 2.07, B at 2.46, X at 5.14 ppm) with J_{AB} = 12 Hz, J_{AX} = 0, and J_{BX} = 4 Hz. The pmr spectrum of 4a in deuterobenzene was similar. These observations suggested that 4a existed in the iminium tautomeric form 5 under the conditions of the pmr experiments with the protons at C-15 being rapidly exchanged by the deuterium oxide. The fact that the iminium form 5 requires a proton source, but appears to exist in aprotic solvents even when care is taken to exclude moisture suggests that the phenolic and the 14-hydroxyl groups are the proton source and that 4a may exist as a zwitter-ion.



5

The integrity of the cyclopropylmethyl group in 4a was indicated by the cyclopropyl multiplets at 0.5 ppm (4H) and 0.9 ppm (1H) in the pmr spectrum. The chemical shifts and general appearance of these multiplets were very similar to those observed for the same moiety in naltrexone. Finally, the mass spectrum of 4a, like that of naltrexone, contained peaks derived from the loss of cyclopropyl and cyclopropylmethyl fragments.

The ir spectrum of 4b showed bands at 1727(s) and 1618(m) cm^{-1} while the pmr spectrum of deuterochloroform was very similar to that of 4a. Once again an apparent ABX pattern of deuterium oxide exchangeable reso-

nances was observed for the enamine protons (A at 2.07, B at 2.45, X at 5.02 ppm; J_{AB} = 12 Hz, J_{AX} = 0, J_{BX} = 4 Hz). These observations were again rationalized in terms of the iminium tautomeric form 5.

Reductive tritiation of 4a was carried out in absolute ethanol using carrier free tritium gas and a 10% palladium on charcoal catalyst. After chromatography naltrexone-15,16-$^{3}H_2$ with a specific activity of 15.3 Ci/mmole and a radiochemical purity of 94 ± 3% was obtained in 70.4% yield. This synthesis clearly demonstrated that compounds containing the cyclopropylmethyl group could be subjected to reductive tritiation without opening the cyclopropane ring.

The low specific activity (compared to the theoretical of 59 Ci/mmole) of the tritiated naltrexone raised questions pertaining to the position of the label. As mentioned, the pmr spectra of 4a suggested that it existed in deuterochloroform solution in the iminium form 5. The possibility therefore arises that a significant amount of 5 was present in the reduction medium. The low incorporation of isotopic hydrogen could then be explained if the iminium form is more rapidly reduced than the enamine form, thereby giving material with incorporation of isotopic hydrogen predominantly at the 16-position.

To clarify this point a sample of naltrexone-15,16-$^{2}H_2$ prepared from 4a under conditions identical to those used for the preparation of the tritium labeled material was studied.

The mass spectrum of the deuterated compound showed 30% d_0, 50% d_1, and 20% d_2 species. On the basis of the mass spectrum one might conclude that the enamine and iminium forms of 4a were being reduced simultaneously with a predominance of 16-labeled species being formed. However, the cmr spectrum of the deuterated compound clearly showed that the deuterium label was nearly equally distributed between the 15- and 16-carbon atoms, as the cmr signals assigned[7] to these carbon atoms suffered approximately equal reduction in intensity compared to the corresponding unlabeled compound. Thus, the designation of this compound as being 15,16-labeled is correct.

The synthesis of radiolabeled naloxone from 4b by reductive tritia-

tion was obviously not possible due to the presence of the allyl double bond. Moreover, the sodium borotritide reduction method[4] could not be used because of the 6-ketone. However, the above pmr data on 4a and 4b suggested that a two-step process of catalytic exchange and sodium cyanoborohydride reduction[8] might be feasible for preparing the labeled compound.

To test the above hypothesis a sample of 4b was equilibrated for ten minutes with a large excess of deuterium oxide containing a trace of concentrated hydrochloric acid. Subsequent reduction with sodium cyanoborohydride then gave a 57.4% yield of naloxone-15-$^{2}H_2$. Mass spectral analysis of the product indicated the following deuterium content: d_0, 3.9%; d_1, 28.0%; d_2, 59.2%; d_3, 6.4%; d_4, 2.4%.

For the radioactive synthesis a suspension of platinum oxide in dry tetrahydrofuran was reduced with tritium gas to get carrier free tritium oxide. This was equilibrated with 4b and the labeled iminium intermediate subsequently reduced with sodium cyanoborohydride.[9] After chromatography naloxone-15-^{3}H with a specific activity of 4 Ci/mmole[10] and a radiochemical purity of greater than 95% was obtained in 38.3% yield. This synthesis was clearly a less complex route to radiolabeled naloxone of high specific activity.

EXPERIMENTAL

Radioactive samples were counted in a Packard Tri-Carb 3375 liquid scintillation spectrometer using an Omnifluor-toluene (6 g/liter) cocktail. Developed tlc plates were scanned on a Varian Berthold Radioscanner fitted with a model LB 242 K ratemeter. Pmr spectra were recorded on a Varian HA-100 spectrometer and mass spectra were obtained on an AEI MS 902 instrument. Tritium gas was purchased from New England Nuclear, Boston, Mass.

15,16-Didehydronaltrexone (4a). Naltrexone was dehydrogenated by the method of Lewis, et al.[3] ChromAR purification of the crude product was carried out using a $CHCl_3$-CH_3OH-NH_4OH (96:4:4 drops) solvent system. Anal. Calcd. for $C_{20}H_{21}NO_4$: 339.1470. Found: 339.1477.

15,16-Didehydronaloxone (4b). Naloxone was dehydrogenated and the product purified by the same procedures used for the synthesis of 4a.

Anal. Calcd. for $C_{19}H_{19}NO_4$: 325.1310. Found: 325.1310.

Naltrexone-15,16-$^3H_2$. A mixture of 4a (19.0 mg), 10% palladium on charcoal (9.5 mg), and absolute EtOH (1.5 ml) was stirred at room temperature for 1 hr. The mixture was then filtered through a cotton plug and the filtrate evaporated to get 16.2 mg of recovered 4a. This procedure was necessary to remove trace amounts of catalyst poison from 4a.

A mixture of recovered 4a, fresh catalyst (6.0 mg) and absolute EtOH (1.0 ml) was placed in a small flask connected to a modified high vacuum microhydrogenation apparatus.[11] A break-seal ampoule containing carrier free tritium gas (5.0 Ci, 1.90 ml at STP) was also attached. The mixture was frozen in liquid nitrogen, the system evacuated, and the ampoule seal then broken. The mixture was subsequently warmed to room temperature and stirred overnight, after which time the uptake of tritium gas was 98.1% complete. The mixture was refrozen, the excess tritium gas pumped out, and the reaction flask removed from the apparatus and thawed. The catalyst was removed by filtration and the resulting solution evaporated to dryness in vacuo. The residue was chromatographed on ChromAR 1000 (20 x 20 cm sheet) using the $CHCl_3$-CH_3OH-NH_4OH (96:4:4 drops) solvent system. The zone corresponding to 1a was eluted with $CHCl_3$-CH_3OH (7:3) to get 11.4 mg (70.4%) of naltrexone-15,16-^3H with a specific activity of 15.3 Ci/mmole.

Thin layer chromatography of this material [silica gel HF_{254}, $CHCl_3$-CH_3OH-NH_4OH (90:10:4 drops)] showed a single radioactive spot with an R_f identical to that of naltrexone. Reverse isotopic dilution analysis (crystallized from CH_2Cl_2-C_6H_6) established the radiochemical purity at 94 $\pm$ 3%. Equilibration experiments with pH 7.4 phosphate buffer showed that none of the label was exchangeable.[12]

Naloxone-15-^3H. Using the modified vacuum microhydrogenation appaaratus[11] a suspension of PtO_2 (39.5 mg, 0.174 mmole) in THF (1 ml) was reduced with tritium gas (10.00 Ci, 3.80 ml at STP). After two hours the uptake of gas was essentially complete. The mixture was frozen in liquid nitrogen, the residual gas pumped out, and the reaction flask removed from

the apparatus.

To the thawed reaction mixture were added 4b (19.5 mg, 0.060 mmole) and concentrated HCl (2 µl). The resulting mixture was stirred one hour at room temperature. Then solid $NaBH_3CN$ (about 8 mg, 0.13 mmole) was added and the mixture stirred for 30 min more. It was next filtered through cotton and the removed solids washed with additional THF. The combined filtrate and washings were evaporated and the residue chromatographed on an analytical silica gel HF_{254} plate (20 x 20 cm, Brinkmann) using the $CHCl_3$-CH_3OH-NH_4OH (90:10:4 drops) solvent system. The zone corresponding to 1b was eluted with $CHCl_3$-CH_3OH (8:2) to get 7.5 mg (38.3%) of naloxone-15-^{3}H with a specific activity of 4 Ci/mmole.

Thin layer chromatography of this material [silica gel HF_{254}, $CHCl_3$-CH_3OH-NH_4OH (90:10:4 drops)] showed a single radioactive spot with an R_f identical to that of naloxone. Reverse isotopic dilution analysis (crystallized from EtOAc) established the radiochemical purity at greater than 95%.

ACKNOWLEDGMENT

This work was supported under Contract No. HSM-47-73-184 with the National Institute on Drug Abuse (formerly National Institute of Mental Health), Division of Biomedical Research, Biomedical Research Branch.

REFERENCES AND NOTES

1. Fishman, J., Cotter, M. L. and Norton, B. I., J. Med. Chem., 16, 556 (1973).
2. We observed that exchange labeled naltrexone-5β,7-$^{2}H_2$ lost essentially all of the label at the 7-position when subjected to the conditions of the alkylation reaction.[1]
3. Haddlesey, D. I., Lewis, J. W., Mayor, P. A., and Young, G. R., J. Chem. Soc., Perkin Trans. I, 872 (1972).
4. Lewis, J. W., Hance, M. J., and Young, G. C., J. Med. Chem., 17, 465 (1974).
5. Leonard, N. J., Hay, A. S., Fulmor, R. W., and Cash, D. W., J. Amer. Chem. Soc., 77, 439 (1955).

6. These observations rule out the possibility of 4a being the less likely 9,10-didehydro isomer, as one would expect the 9,10-didehydrocompound to show a one proton singlet in the olefinic region for H-10 which would disappear upon exchange with deuterium oxide.
7. Carroll, F. I., Moreland, C., Brine, G. A., and Kepler, J. A., J. Org. Chem., in press.
8. Borch, R. F., Bernstein, M. D., and Durst, H. D., J. Amer. Chem. Soc., 93, 2897 (1971).
9. A small amount of acid was used to ensure as complete exchange as possible. However, the reduction generally yielded a small amount of polar material which was characterized as a mixture of the 6-alcohols. This suggested that the pH of the reaction mixture was too low for completely selective reduction.[8] Further experimentation with the pH could improve the yield of radiolabled naloxone.
10. Use of labeled sodium cyanoborohydride[8] would yield material of even higher specific activity.
11. Glascock, R. F., and Pope, G. S., Biochem. J., 75, 328 (1960).
12. We previously observed that a considerable portion of the label in naltrexone-^{3}H (G) prepared by base-catalyzed exchange was labile in pH 7.4 phosphate buffer.