PREPARATION OF TRITIUM-LABELED COMPOUNDS. VI. NAFOXIDINE HYDROCHLORIDE BY CLEMMENSEN REDUCTION AND BY SOLVENT EXCHANGE OF INTERMEDIATES

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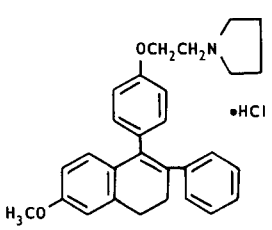
SUMMARY

Tritium-labeled nafoxidine hydrochloride, 1-{*p*-[2-(N-pyrrolidino) ethoxy]phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene hydrochloride (I), having two different intramolecular distributions of tritium, was prepared from 6-methoxy-2-phenyl-1-tetralone (IV). I was labeled in the 4 position by incorporation of IV which had been labeled in the 4 position by Clemmensen reduction of 2-phenyl-4-(*m*-methoxyphenyl)-4-ketobutyric acid (II) in the presence of tritiated water, followed by cyclization of the resulting 2-phenyl-4-(*m*-methoxyphenyl)butyric acid (III). I was labeled in the aromatic positions of its phenylnapthalene moiety at a higher specific activity by incorporation of IV which had been generally labeled by exchange with tritiated trifluoroacetic acid.

Key Words: Tritium, Nafoxidine, Clemmensen reduction, Solvent exchange

INTRODUCTION

Metabolism studies with nafoxidine hydrochloride, 1-{*p*-[2-N-pyrrolidino) ethoxy]phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene hydrochloride (I) required the preparation of a radioactive form of the drug. Tritium, rather than carbon-14, was chosen as the radioactive label in order to take advantage of established synthetic pathways.



I

0362-4803/78/0214-0215$01.00/0

EXPERIMENTAL

Radioactivity Measurements

All counting was performed with a Packard Tricarb Model 314EX2A liquid scintillation spectrometer at -8° under conditions suitable for measuring tritium. Appropriate aliquots of samples were dissolved in 15 ml of scintillation solvent [toluene-dioxane-methanol (350:350:210 by volume) containing 73 g of naphthalene, 4.6 g of 2,5-diphenyloxazole, and 0.08 g of 1,4-bis [2-(5-phenyloxazolyl)] benzene per liter]. The absolute counting efficiency for each sample was determined by recounting following addition of an internal standard of tritium-labeled toluene and results were then converted to mCi.

Thin-Layer Chromatography

Thin-Layer chromatography was carried out in the $CHCl_3$-cyclohexane (3:1 by volume) saturated with NH_4OH and 1-butanol-acetic acid-H_2O (4:1:5 by volume) systems on 0.25-mm films of silica gel GF. The Rf value for I was 0.60 in the former system and 0.50 in the latter system. The chromatograms were developed in the dark because of light-induced degradation of I when in contact with silica gel. The UV absorption of standards and products was detected by viewing the dried chromatograms under short-wavelength UV light. The zones of radioactivity were located by transferring sequential 0.5-cm segments of the developed chromatogram into individual vials and counting, using scintillation solvent containing 3% H_2O.

Synthesis

2-Phenyl-4-(m-methoxyphenyl)butyric acid-4,4-$^3H_2$(III) - A 3.0 g sample of 2-phenyl-4-(*m*-methoxyphenyl)-4-ketobutyric acid (II)* was subjected to Clemmensen reduction, as described by Lednicer, *et al* (1), using 30 g of mossy zinc. One ml of H_2O containing one Ci of tritium was added to the reaction mixture at the beginning of the reduction. The resulting 2-phenyl-4-(*m*-methoxyphenyl) butyric acid-4,4-$^3H_2$ (III), isolated as a viscous oil, was used in the next step without further purification.

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* Prepared as described in reference 1.

6-Methoxy-2-phenyl-1-tetralone-4,4-$^3H_2$ (IVA) - The crude 2-phenyl-4-(*m*-methoxyphenyl) butyric acid-4,4-$^3H_2$ (III) was cyclized in liquid HF and purified, as described by Lednicer, *et al* (1), to yield 1.09 g (41% based on II) of 6-methoxy-2-phenyl-1-tetralone-4,4-$^3H_2$ (IVA) having a specific activity of 0.21 mCi per mM; m.p. 112-116° (capillary, uncorrected). *Anal.* Calcd. for $C_{17}O_2H_{16}$: C, 80.9; H, 6.4. Found: C, 80.5; H, 6.4.

6-Methoxy-2-phenyl-1-tetralone-(G)^3H (IVB) - One ml of tritiated H_2O containing one Ci of tritium was added slowly with cooling to 7 ml of trifluoroacetic anhydride in a flask equipped with a distilling head, thermometer, and condenser. Excess trifluoroacetic anhydride was removed by distilling until the vapor temperature reached 70°. The flask containing the trifluoroacetic acid -^3H was cooled, 4.2 g of 6-methoxy-2-phenyl-1-tetralone (IVB)* was added, and the reaction mixture was heated under reflux for 24 hours. The reaction mixture was worked up as previously described to yield 3.79 g (90%) of 6-methoxy-2-phenyl-1-tetralone-(G)^3H (IVB) having a specific activity of 15.4 mCi per mM; m.p. 117-118° (capillary, uncorrected). *Anal.* Calcd. for $C_{17}O_2H_{16}$: C, 80.9, H, 6.4. Found: C, 80.6; H, 6.5.

1-{p-[2-N-Pyrrolidino) ethoxy]phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene-4,4-$^3H_2$ Hydrochloride (IA) - A Grignard solution was prepared from 0.101 g of Mg and 1.12 g of *p*-[2-(N-pyrollidino)ethoxy] bromobenzene** in 12 ml of tetrahydrofuran. Approximately one hour of reflux was required to completely consume the Mg. The solution of Grignard reagent was cooled in an ice bath and a solution of 1.05 g of 6-methoxy-2-phenyl-1-tetralone-4,4-$^3H_2$ (IVA) in 10 ml of tetrahydrofuran was added. The reaction mixture was heated under reflux overnight. After cooling to room temperature, one ml of H_2O was added to the reaction mixture, and the resulting gel was removed by filtration through Supercell® and washed well with ether. The filtrate was washed with H_2O and evaporated to dryness *in vacuo*. The residue was dissolved in benzene-ether (1:1 by volume) and the resulting solution was extracted three times

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* Prepared as described in Reference 1.

** Prepared as described in Reference 2.

with 0.5 N HCl. The aqueous phase was extracted with four portions of CH_2Cl_2. The residue from the CH_2Cl_2 extract was recrystallized three times from ethyl acetate-$CHCl_3$ to yield 0.287 g (14.9%) of 1-{*p*-[2-(N-pyrrolidino)ethoxyl] phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene-4,4-$^3H_2$ hydrochloride (IA) having a specific activity of 0.20 mCi per mM; m.p. 167-169° (capillary, uncorrected). The IR and UV spectra of the product corresponded to those of authentic standard I [UV (EtOH) 304 mμ (ε16,200), shoulder 252 mμ (ε13,550), shoulder 236 mμ (ε16,200) and IR (Nujol mull) 2590, 2550, 2470 (N-H); 1610, 1600, 1575, 1565, 1508, 1495 (C=C); 1285, 1240, 1125, 1040 (C-O/C-N); 835, 815, 765, 700 (Aromatic C-H) cm^{-1}]. Thin-layer chromatography of the product in the two systems previously described, revealed a single UV-absorbing and radioactive zone corresponding to that of authentic standard.

A considerable portion (0.65 g, 62%) of the tritium-labeled ketone (IVA) starting material was recovered from the benzene-ether phase following its extraction with HCl. The specific activity of the recovered ketone was 0.20 mCi per mM.

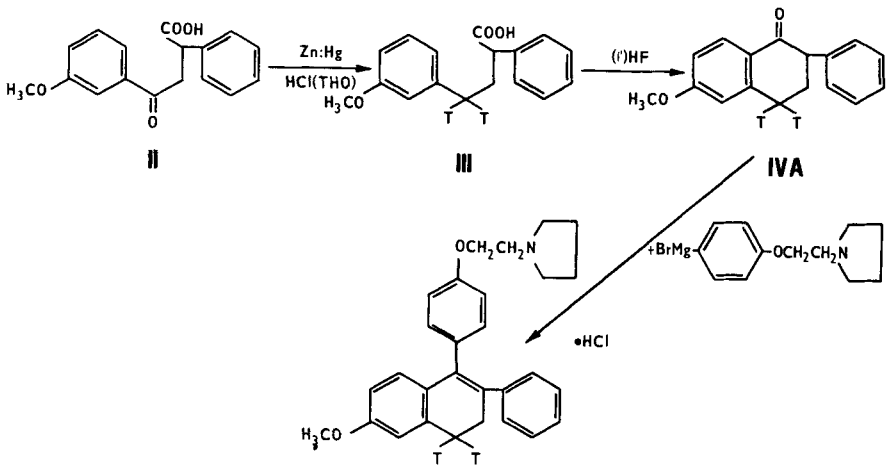
1-{p-[2-(N-Pyrrolidino)ethoxy]phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene-(G)^{3}H Hydrochloride (IB) - A 3.70 g sample of 6-methoxy-2-phenyl-1-tetralone-(G)^{3}H was treated with a Grignard reagent prepared from 0.375 g of Mg and 3.96 g of *p*-[2-(N-pyrollidino)ethoxy] bromobenzene and worked up, as previously described, to yield 0.908 g (13.4%) of 1-{*p*-[2-(N-pyrrolidino)ethoxy]phenyl}-3,4-dihydro-6-methoxy-2-phenylnaphthalene-(G)^{3}H hydrochloride (IB) having a specific activity of 10.3 mCi per mM; m.p. 168-171° (capillary, uncorrected). The IR and UV spectra of the product corresponded to those of authentic standard I as described above. Thin-layer chromatography of the product in the two systems previously described, revealed a single UV-absorbing and radioactive zone corresponding to that of authentic standard.

A portion (1.37 g, 37%) of the tritium-labeled ketone (IVB) starting material was recovered from the benzene-ether phase following its extraction with HCl. The specific activity of the recovered ketone was 10.4 mCi per mM.

RESULTS AND DISCUSSION

Tritium-labeled nafoxidine hydrochloride was prepared first by Clemmensen reduction of the intermediate keto acid (II) in a tritiated water medium as shown in Scheme 1. In this manner tritium was introduced specifically in the 4

Scheme 1

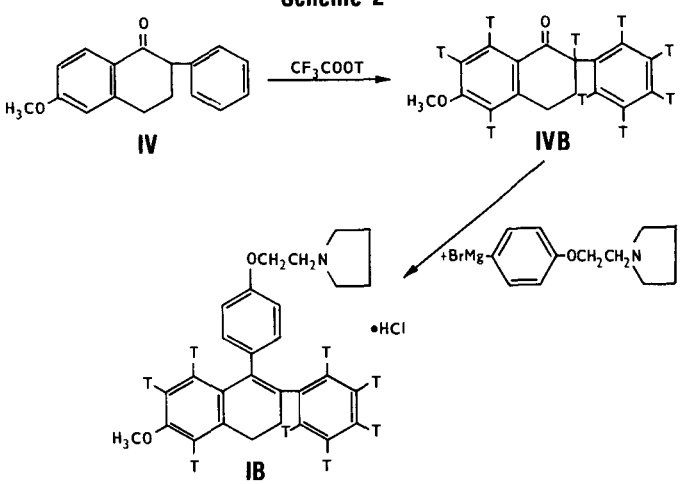


position as shown in IA. This was thought to be a desirable location for the tritium label since metabolism would likely involve one or more of the aromatic rings, not the 4 position. Indeed, less than 0.25% of the tritium was released and isolated as tritiated water in urine when this material was administered to a monkey. Unfortunately, the efficiency for introducing tritium by Clemmensen reduction was poor, resulting in a low radiochemical yield of a product having a low specific activity.

Therefore, another method for labeling nafoxidine with tritium was developed. This involved exchange labeling of the intermediate cyclic ketone (IV) by exchange with tritiated trifluoroacetic acid as shown in Scheme 2. In this manner the tritium-labeled cyclic ketone (IVB) was prepared in 250 times the radiochemical yield and 73 times the specific activity of that (IVA) prepared by the Clemmensen reduction method. Similar improvements in radiochemical yield and specific activity of the final product, tritium-labeled nafoxidine (I), also were obtained by the exchange method. Nafoxidine (IB) labeled by exchange would be expected to contain tritium in aromatic positions. Thus, some tritium would

likely be lost when such a material is metabolized. Indeed, when this material was administered to a monkey, nearly 9% of its tritium was released and recovered as tritiated water in urine. Nevertheless, the greater yield and higher specific activity of tritium-labeled nafoxidine prepared by exchange, as compared to Clemmensen reduction, made Scheme 1 the preferred route for preparing the labeled drug.

Scheme 2



The loss of tritium from the exchange-labeled ketone (IVB) during its conversion to nafoxidine (IB) gave information concerning the location of tritium in this intermediate (IVB). The specific activity of nafoxidine hydrochloride (10.3 mCi/mM), IB, was lower than that of the starting ketone (15.4 mCi/mM), IVB, but the same as that of the recovered ketone (10.4 mCi/mM). The tritium lost was likely that initially in the 2 position, alpha to the carbonyl, of the starting ketone (IVB). Tritium in this position would have been lost by spontaneous dehydration of the naphthol condensation product, thought to be an unstable intermediate, leading to IB. The lower specific activity of the recovered ketone, relative to the starting ketone (IVB), probably resulted from loss of the tritium in the 2 position by enolization of the Grignard reagent - starting ketone complex. When this recovered ketone was condensed with a different Grignard reagent the resulting analog of nafoxidine had the same specific activity (10.4 mCi/mM) as the recovered ketone. Thus, one third of the tritium

introduced into the ketone, IVB, as a result of exchange with tritiated trifluoroacetic acid, resided in the 2 position, alpha to the carbonyl group. The remainder likely was present on the aromatic rings.

ACKNOWLEDGMENTS

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REFERENCES

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2. Lednicer, D., Lyster, S. C., and Duncan, G. W.-J. Med. Chem. 10:78 (1967).