SITE-SPECIFIC DEUTERATION OR TRITIATION OF BENZO[b]THIOPHENE AND 1-METHYLINDOLE ANALOGS OF BIOLOGICALLY ACTIVE INDOLE DERIVATIVES (1)

T.R.Bosin (2) and R.B.Rogers
Departments of Chemistry and Pharmacology
Indiana University
Bloomington, Indiana, 47401, U.S.A.

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SUMMARY

The site-specific deuteration and tritiation of 1-methylindole and benzo[b]-thiophene analogs of biologically active indole derivatives has been achieved by use of the facile metalation of these heterocycles with n-butyllithium and subsequent reaction with ^2H_2O *or* ^3H_2O*. Metalation occurs at the 2-position and site and extent of deuterium incorporation was determined by N.M.R. The use of similar procedures with* ^3H_2O *led to ready incorporation of tritium into these heterocycles.*

INTRODUCTION

The demand for specifically labeled organic molecules has resulted in the development of selective procedures for the incorporation of deuterium (3), tritium (4), and carbon-14 (5) into organic compounds. Specifically, radiolabeled molecules are extensively used in mechanistic and biomedical research (6); in addition, the application of stable isotopically labeled molecules to metabolic studies has recently been described (7).

As a result of our study of the chemical pharmacology of tryptophan and its isosteres, it became necessary to prepare suitably labeled benzo[b]thiophene and 1-methylindole analogs of biologically active indole derivatives. The method which was employed utilizes the facile metalation of benzo[b]thiophene (8) and 1-methylindole (9) with n-butyllithium and subsequent reaction with ^2H_2O or ^3H_2O. Metalation has been shown (8,9) to occur at the 2-position due to the inductive effect of the heteroatom, and is therefore the active site for introduction of the deuterium or tritium atom.

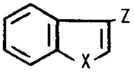
RESULTS AND DISCUSSION

A. Exchange Studies Using Deuterium

The general procedure for deuterium incorporation is as follows: The compound to be labeled was dissolved in dry, freshly distilled tetrahydrofuran (THF) and the resultant solution cooled to 0° for 1-methylindole and -78° for benzo[b]thiophene derivatives. The reaction solution was treated with n-butyllithium and allowed to stir for 30-75 minutes at the low temperature prior to reaction with ^2H_2O. The nmr spectra of 1-methylindole and benzo[b]thiophene derivatives possessed a characteristic absorption for the H-2 proton, and therefore, the decrease or loss of the H-2 absorption provided a ready means of determining the percent exchange. The compounds labeled in this study and the nmr data appear in Table I.

Table I

Compounds and Percent Exchange Based on NMR



Compound		Substituents		NMR Data	
No.	Name	X	Z	δH-2§	% H-2 Exchanged
I	3-(2-Aminoethyl)benzo[b]thiophene	S	$CH_2CH_2NH_2$	7.07	80
II	Benzo[b]thiophene-3-acetic acid	S	CH_2COOH	7.26- 7.48*	70

III	3-(2-Dimethylaminoethyl)-benzo[b]thiophene	S	$CH_2CH_2N(CH_3)_2$	7.10	100
IV	1-Methyl-3-(2-dimethylaminoethyl)indole	N-CH_3	$CH_2CH_2N(CH_3)_2$	6.71	75

§ These δH-2 values are those for the parent unexchanged compound.
* This proton is part of a multiplet.

The exchange labeling of the sulfur analog of tryptamine (I) was complicated by its extreme sensitivity to air. Preparation of the free base from its hydrochloride salt and subsequent handling under an inert atmosphere were required.

The metalation of the sulfur analog of indole-3-acetic acid (II) with n-butyllithium resulted in 70% exchange of the H-2 proton and 35% exchange of the methylene protons. Increasing the molar ratio of n-butyllithium and/or the reaction time failed to significantly alter the extent of methylene proton exchange.

In order to facilitate the exchange of the H-2 proton in the 1-methylindole derivative (IV) it was necessary to elevate the reaction temperature to 0° and extend the reaction time to 75 minutes; further elevation of the reaction temperature or increase of the reaction time led to degradation of the compound.

B. Exchange Studies Using Tritium

The use of ^3H_2O as the quenching agent for the metalated heterocycles allows for the facile preparation of specifically labeled compounds. The specific activities of the compounds so labeled appear in Table II. The chemical purity of each compound was demonstrated by thin-layer chromatography using several different solvent systems and the radiochemical purity was verified by radioscanning of the thin-layer plates.

Table II

Specific Activity of Exchanged Compounds§

Compound No.	Specific Activity (μCi/mmole)
I	518
II	729
III	431
IV	176

§ Reactions were run as per the experimental using ^3H_2O (2 mCi/mmole).

Compound II was selected for a study of the effects of the volume and the specific activity of the quenching ^3H_2O on the rate of tritium incorporation. These data appear in Table III.

The study of the effect of ^3H_2O volume used to quench 3.0 mmoles of base reveals the highest rate of tritium incorporation occurred when 1.5 mmoles of ^3H_2O and the lowest when 6.0 mmoles of ^3H_2O were used to quench the reaction. The decreased tritium incorporation observed upon utilization of excess ^3H_2O may reflect a tritium isotope effect and/or the greater availability of ^1H vs. ^3H atoms.

Table III

Effects of Volume and Specific Activity of the Quenching ^3H_2O§

Volume (μl)	mmoles ^3H_2O	^3H_2O Specific Activity (mCi/mmole)	Specific Activity of Compound II (mCi/mmole)
27	1.5	2	2.15
54	3.0	2	1.76
108	6.0	2	0.91
27	1.5	10	5.18
27	1.5	20	11.10

§ Reactions were run using 1.0 mmole of compound II and 3.0 mmoles of n-butyllithium as per the experimental.

The study of the effect of the specific activity of the quenching ^3H_2O on the rate of tritium incorporation was undertaken using 1.5 mmoles of ^3H_2O. The rate of tritium incorporation was a linear function of the specific activity of the ^3H_2O. Studies are presently in progress to develop appropriate exchange procedures for heterocycles containing NH groups, i.e., indole, imidazole, thiazole, etc., and should greatly extend the versatility of this method.

EXPERIMENTAL

Melting points were measured on a Mel-Temp capillary melting point apparatus. UV data were obtained on a Unicam SP1800 Ultraviolet Spectrophotometer and concentrations of solutions used in specific activity determinations were obtained via UV. Radioactivity was measured in a Packard Tri-Carb 4322 Liquid Scintillation System, using the scintillation solution described by Maickel, et al (10). Nmr spectra were run on a Varian Associates Model HA-100 spectrometer using deuterochloroform as the solvent and tetramethylsilane as the internal standard. Aromatic proton assignments were made by analogy to compounds previously reported (11,12). One dimensional thin-layer chromatography was carried out on Analtech silica gel G precoated plates (Analtech, Inc., Newark, Delaware). The solvent systems used in thin-layer chromatography were: A. methyl acetate-2-propanol-ammonia (45:35:20); B. chloroform-methanol-acetic acid (60:35:5); and C. methanol-ammonia (98.5:1.5). All thin-layer chromatograms were developed 12 cm and visualized with one or more of the following reagents (13): Van Urk reagent, alkaline potassium permanganate, ninhydrin, and iodine vapor. The developed thin-layer plates containing radioactive material were scanned on a Packard Model 7200 Radiochromatogram Scanner. All exchange reactions were conducted with oven-dried glassware which was assembled and placed under a continuous flow of dry nitrogen gas, prior to the introduction of any reaction component.

3-(2-Aminoethyl)benzo[b]thiophene-2-^{2}H or ^{3}H. (I). 3-(2-Aminoethyl)benzo[b]thiophene hydrochloride (0.213 g, 1.0 mmole) was dissolved in 15 ml of 1 N NaOH and extracted with 2 x 25 ml of diethyl ether. The combined ether extracts were dried over anhydrous $MgSO_4$ and the ether removed under reduced pressure. The free base was unstable in the atmosphere necessitating handling in an inert atmosphere. The free base was dissolved in 60 ml of freshly distilled (from $LiAlH_4$) THF, cooled to -78° in a CO_2/acetone bath, and treated with n-butyllithium (1.5 ml of a 2.5 M solution, 3.75 mmoles). The solution was stirred for 30 min at -78° prior to quenching with either ^2H_2O (0.1 ml, 5 mmoles) or ^3H_2O (0.1 ml, 5.0 mmoles). The quenched reaction was dried with anhydrous $MgSO_4$. The dried and filtered THF solution was treated with dry HCl gas to produce the amine hydrochloride. Recrystallization from methanol/ethyl acetate gave 0.176 g (83%) of large colorless plates, m.p. 220-221°. TLC using solvent systems A, B, and C gave R_f's 0.82, 0.59 and 0.24 respectively. Nmr of ^{2}H-exchanged I: δ 1.22-1.60 (m, 2H, NH_2), 2.97 (broad s, 4H, CH_2CH_2), 7.09 (s, 0.20H, H-2), 7.20-7.46 (m, 2H, H-5,6), 7.62-7.90 (m, 2H, H-4,7). Using ^2H_2O (99%) as the quenching agent gave 80% deuterium incorporation at the 2-position, while using ^3H_2O (2 mCi/mmole) gave a specific activity of 518 μCi/mmole.

Benzo[b]thiophene-2-^{2}H or ^{3}H-3-acetic acid. (II). Benzo[b]thiophene-3-acetic acid (0.192 g, 1.0 mmole) was dissolved in 60 ml of freshly distilled (from $LiAlH_4$) THF and the reaction vessel cooled to -78° in a CO_2/acetone bath and treated with n-butyllithium (1.28 ml of a 2.34 M solution, 3.0 mmoles). The reaction was allowed to stir for 30 min at -78° and quenched with ^2H_2O (0.108 ml, 6.0 mmoles) or ^3H_2O (0.108 ml, 6.0 mmoles). Upon removal of the THF under reduced pressure, the residue was dissolved in 20 ml of 1 N HCl and the aqueous phase extracted with 2 x 25 ml of ether.

Removal of the ether and recrystallization of the product from H_2O gave 0.155 (81%) of long colorless needles, m.p. 110-111°. TLC using solvent systems A and B gave R_f's 0.68 and 0.91 respectively. Nmr of ^2H-exchanged II: δ 3.82 (s, 1.3H, CH_2), 7.25-7.50 (m, 2.3H, H-2,5,6), 7.64-7.94 (m, 2H, H-4,7), 11.50 (broad s, 1H, OH). Using ^2H_2O (99%) as the quenching agent gave 70% deuterium incorporation at the 2-position and 35% incorporation at the methylene position. Quenching with ^3H_2O (2 mCi/mmole) gave a specific activity of 719 μCi/mmole.

3-(2-Dimethylaminoethyl)benzo[b]thiophene-2-^2H or ^3H. (III). 3-(2-Dimethylaminoethyl)benzo[b]thiophene (0.205 g, 1.0 mmole) was dissolved in 60 ml of freshly distilled (from $LiAlH_4$) THF and the reaction vessel was cooled to -78° in CO_2/acetone prior to treatment with n-butyllithium (1 ml of 2.5 M solution, 2.5 mmoles). The reaction was allowed to stir for 30 min at -78° before quenching with either ^2H_2O (0.1 ml, 5 mmoles) or ^3H_2O (0.1 ml, 5 mmoles). The THF was removed under reduced pressure, the free base was dissolved in 50 ml of diethyl ether and the ether dried over anhydrous $MgSO_4$ prior to treatment with dry HCl gas to produce the hydrochloride. Recrystallization of the hydrochloride from methanol/ethyl acetate gave 0.20 g (83%) of long colorless needles, m.p. 260-261°. TLC using solvent systems A, B, and C gave R_f's 0.92, 0.42 and 0.36 respectively. Nmr of the ^2H-exchanged III: δ 2.31 (s, 6H, $N(CH_3)_2$), 2.52-2.76 (m, 2H, CH_2N), 2.85-3.10 (m, 2H, $ArCH_2$), 7.18-7.43 (m, 2H, H-5,6), 7.63-7.86 (m, 2H, H-4,7). Using ^2H_2O (99%) as the quenching agent gave 100% deuterium incorporation at the 2-position, while using ^3H_2O (2 mCi/mmole) gave a specific activity of 431 μCi/mmole.

1-Methyl-3-(2-dimethylaminoethyl)indole-2-^2H or ^3H. (IV). 1-Methyl-3-(2-dimethylaminoethyl)indole (0.203 g, 1.0 mmole) was dissolved in 60 ml of freshly distilled (from $LiAlH_4$) THF, was cooled to 0° in an ice bath, and treated with n-butyllithium (0.8 ml of 2.5 M solution, 2.0 mmoles).

The reaction was allowed to stir for 75 min at 0° prior to quenching with either ^2H_2O (0.1 ml, 5.0 mmoles), or ^3H_2O (0.1 ml, 5.0 mmoles). The THF was removed under reduced pressure, the free base was dissolved in 50 ml of diethyl ether and the ether was dried over anhydrous $MgSO_4$ prior to conversion to the hydrochloride with dry HCl gas. Recrystallization from ethanol/diethyl ether gave 0.176 (74%) of colorless needles, m.p. 169-170°. TLC using solvent system C gave R_f 0.31. Nmr of ^2H-exchanged IV: δ 2.30 (s, 6H, $N(CH_3)_2$), 2.49-2.74 (m, 2H, CH_2N), 2.80-3.04 (m, 2H, $ArCH_2$), 3.64 (s, 3H, NCH_3), 6.80 (s, 0.25H, H-2), 6.97-7.26 (m, 3H, H-4,5,6), 7.49-7.63 (m, 1H, H-7). Using ^2H_2O (99%) as the quenching agent gave 75% deuterium incorporation at the 2-position, while using ^3H_2O (2 mCi/mmole) gave a specific activity of 176 μCi/mmole.

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