SYNTHESIS OF DEUTERIUM AND TRITIUM LABELED 2-ACETAMIDO-5,6-DIMETHYLBENZIMIDAZOLE

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SUMMARY

Synthesis of 2-acetamido-5,6-dimethylbenzimidazole, labeled with deuterium and tritium at the 4- and 7-positions of the benzimidazole ring system, is described. 4,5-Dimethyl-*o*-phenylenediamine is deuterated and tritiated in the 3- and 6-positions by means of exchange with deuterated and tritiated water. These relatively labile labels are rendered stable after formation of the benzimidazole ring system. The stability of the labels in 2-acetamido-5,6-dimethyl[4,7-^{3}H]benzimidazole is ascertained.

Key Words: Synthesis, tritium, deuterium, exchange, stability, 2-acetamido-5,6-dimethylbenzimidazole

INTRODUCTION

2-Acetamido-5,6-dimethylbenzimidazole is a member of a series of substituted benzimidazoles which exhibit hypotensive activity in test animals (1). Its different biological activity profiles in various animal species, especially in rats and dogs, prompted us to prepare its tritium labeled form to provide radioactive material for carrying out metabolism studies in these species.

DISCUSSION AND RESULTS

In the course of investigating various methods of preparing substituted 2-aminobenzimidazoles, we found that 2-acetamido-5,6-dimethylbenzimidazole (*3*) was obtained in good yield by the facile condensation of 4,5-dimethyl-*o*-phenylenediamine (*1*) with the hydriodic salt of N-acetyl-S-methylpseudothiourea (*2*), which was readily prepared by means of S-methylation of N-acetylthiourea with methyl iodide (2). The 4- and 7-positions of the

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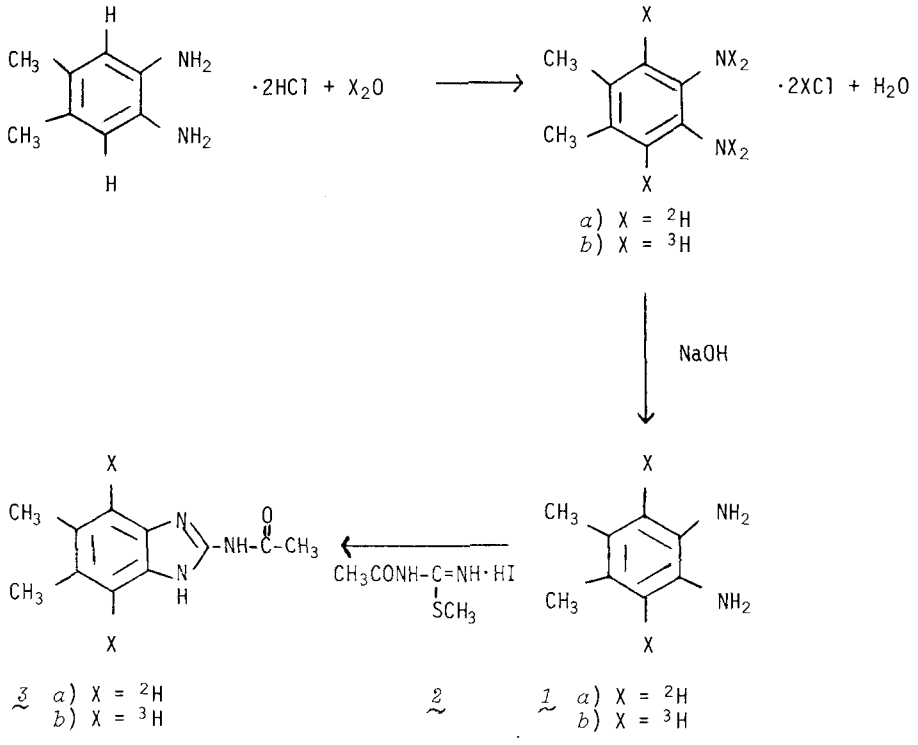
benzimidazole 3, or more expediently, the 3- and 6-positions of its precursor, the diamine 1, were recognized as potential sites for incorporation of tritium, since each of these positions is *ortho* to an aromatic amino group.



Ingold *et al.* (3) have shown that hydrogen exchange between proton or deuterium donors and the aromatic nucleus of a substituted benzene has the characteristics of an electrophilic substitution process, and is facilitated or retarded, and oriented in the same manner as are other typical aromatic electrophilic substitutions. Best and Wilson (4) demonstrated that in deuterated water, only the *ortho* and *para* positions in aniline hydrochloride participated in the exchange reaction. Although there are numerous examples in the literature (5) concerning deuterium exchange in substituted anilines and other monobasic

aromatic amines, little appears to be known about the incorporation of deuterium into *o*-phenylenediamines. A series of experiments was therefore conducted to study the deuteration of 4,5-dimethyl-*o*-phenylenediamine in order to define suitable exchange conditions for application to the analogous tritium incorporation.

The extremely simple proton magnetic resonance (PMR) spectrum of the diamine *1*, consisting of only two singlets attributable to carbon-borne protons, one for the methyl protons and the other for the aromatic protons, provided a convenient *in situ* method of following the progress of the exchange reaction. Introduction of deuterium into the benzene nucleus resulted in a decrease in the aromatic proton signal relative to the alkyl proton signal, which remained constant and therefore served as an internal standard for quantification of the extent of deuteration. The exchange reaction occurred only at the positions *ortho* to the amino groups, and the reaction was found to take place when the dihydrochloride salt of *1* was treated with deuterated water at elevated temperatures, with or without a second water-miscible solvent such as dimethylformamide, and either in the presence or absence of 10% palladium on charcoal as a catalyst. Subsequently the reaction was carried out preparatively by heating a solution of the salt in deuterated water at 90° C. The fact that both the 3- and 6-positions of *1* were deuterated was demonstrated by the complete disappearance of the aromatic proton signal in PMR spectrum of the product after repeated exhaustive treatment with deuterated water. The decline of the aromatic proton signal was plotted against time. The linearity of the plot on semi-log scale (Figure 1) indicated that the reaction was pseudo-first order with respect to *1* and the reaction half-time was 30 minutes. This provided the guidelines for carrying out the analogous tritiation reaction for eight half-times (*i.e.*, four hours) in order for the compound to reach equilibrium with the tritiated solvent.

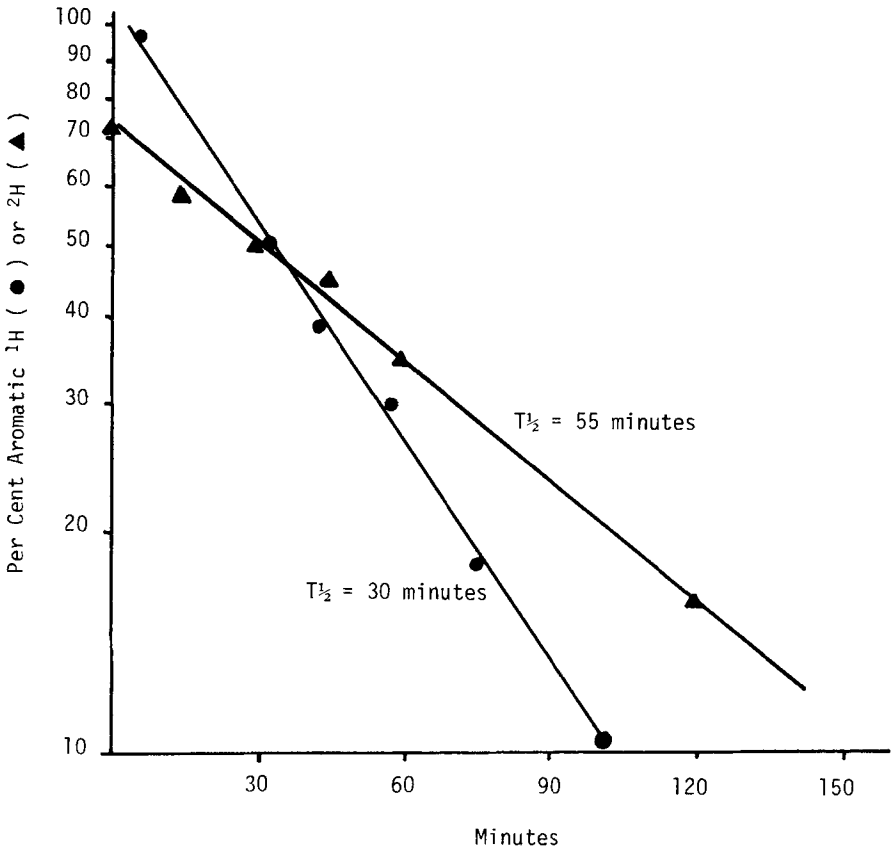
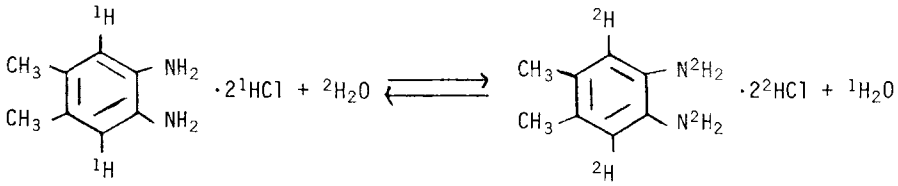


Figure 1. Aromatic ^{1}H - ^{2}H Exchange between Water and 4,5-Dimethyl-o-phenylenediamine Dihydrochloride at 90° C



The reverse reaction of the above exchange, *i.e.*, the removal of deuterium from 4,5-dimethyl-*o*-[N,N',3,6-^{2}H]phenylenediamine dideuterochloride, also occurred readily in water, but at a slower rate, the half-time for this reaction being 55 minutes. However, the deuterium labels in the free base *1a* proved to be sufficiently stable under the reaction conditions chosen for the conversion of *1a* to 2-acetamido-5,6-dimethyl[4,7-^{2}H]benzimidazole (*3a*). No detectible difference in deuterium content was found between *1a* and *3a*. Likewise, 4,5-dimethyl-*o*-[3,6-^{3}H]phenylenediamine (*1b*) and the 2-acetamido-5,6-dimethyl[4,7-^{3}H]benzimidazole (*3b*) derived therefrom were found to have the same molar specific activity, indicating there was no loss of tritium during the conversion.

Since *3b* was to be utilized in metabolism studies *via* oral administration, where it would be important that the radioisotope labels should remain in the molecule as the compound underwent absorption and subsequent distribution, the stability of the deuterium labels in *3a* was examined under some selected conditions. At 37° C and pH 1.5 in aqueous medium, an environment such as might be encountered by the compound in the stomach, *3a* proved to be both chemically and isotopically stable. Indeed the material was found to be capable of withstanding more rigorous conditions. After 50 hours at pH 0.65, it experienced no chemical change or deuterium loss. At 95° C in 3N sodium hydroxide or 3N hydrochloric acid, the compound was observed to undergo hydrolysis with the loss of the acetyl side chain. However, the resulting 2-amino-5,6-dimethylbenzimidazole still retained all of the deuterium labels. By analogy, the tritium labeled *3b* can be expected to possess similar chemical and radiochemical stability. Thus the relatively labile labels in *1a* and *1b* were rendered highly stable by the incorporation of the benzene ring into the benzimidazole ring system. This strategy of introducing tritium into readily accessible aromatic ring positions, and then "locking in" the labels by transforming these positions into more stable ones upon further elaboration

of the molecule *en route* to the desired product, has been employed successfully in other projects in our laboratories (6 - 8). The general adaptability of this method to modeling with deuterium, the ability to utilize an economical tritium source such as water, or other readily available tritiated protic solvents, to achieve a wide range of product specific activity, and the possibility of devising synthetic routes with introduction of radioactivity at a late stage, make this method an attractive approach for radiosyntheses of tritium labeled compounds.

EXPERIMENTAL METHODS

Radioactivity determinations were carried out with a Packard Tri-Carb Model 2002 liquid scintillation spectrometer by means of the internal standard method. Diotol was used as the scintillation cocktail. PMR measurements were made with a Varian Model A60-A spectrometer. Melting points were determined in capillary tubes and are uncorrected.

Exchange Reactions

A) Deuteration at 90° C

A solution of 125 mg of 4,5-dimethyl-*o*-phenylenediamine dihydrochloride (0.6 mmole) in 0.7 ml of deuterated water (Stohler Isotope Chemicals, 32 mmoles, 99.8% D) in a PMR cell was placed in the probe preheated and maintained at 90°C. Spectra were obtained at selected time intervals. Integrals for the singlet methyl and aromatic proton signals were measured and the amount of aromatic protons (δ 7.35) remaining at a given time was calculated, using the integral for the methyl proton singlet at δ 2.23 as the standard for six protons. A semi-log plot of the percentage of remaining aromatic protons *vs.* time gave a straight line (Figure 1), from which a half-time of 30 minutes was calculated for the pseudo-first order deuteration reaction.

B) Preparation of *1a*

A solution of 2.50 g of 4,5-dimethyl-*o*-phenylenediamine dihydrochloride (12 mmoles) in 10 ml of deuterated water (455 mmoles) was heated at 90°C for 3.5 hours. The solution was lyophilized to give 2.54 g of 4,5-dimethyl-*o*-[3,6,N,N'-^{2}H]-

phenylenediamine dideuterochloride, 73% deuterated in the benzene ring as determined by PMR. In another experiment, heating a mixture of 1.46 g of the salt and 6 ml of deuterated water at 170° C in a sealed glass tube for six hours afforded 1.33 g of crude solids. This material was dissolved in 30 ml of water, decolorized with activated charcoal, and the solution basified to produce 0.800 g of *1a*, 92.6% deuterated in the benzene ring.

C) Removal of Deuterium at 90° C

A solution of 1.50 g of 4,5-dimethyl-*o*-[3,6,N,N'-^{2}H]phenylenediamine dideuterochloride (6.9 mmoles) in water was heated at 90°C under nitrogen. Aliquots of 1 ml of this solution were withdrawn at selected time intervals and lyophilized. The residue was redissolved in D_2O and its PMR spectrum was obtained at room temperature. A semilog plot of remaining percentage of deuterium *vs.* time gave a reaction half-time of 55 minutes (see Figure 1).

D) Preparation of *1b*

A mixture of 525 mg of 4,5-dimethyl-*o*-phenylenediamine dihydrochloride (2.5 mmoles) and 1 ml of tritiated water containing 25 Ci* of tritium was heated at 100° C for four hours (approximately eight deuteration half-times). The mixture was lyophilized, and the residue was redissolved in 5 ml of water, and the solution again lyophilized to remove labile tritium and afford 4,5-dimethyl-*o*-[3,6-^{3}H]phenylenediamine dihydrochloride. This material was combined with 2.100 g (10 mmoles) of nonlabeled material and dissolved in 20 ml of cold water. The solution was decolorized with activated charcoal and basified with 6N sodium hydroxide. The precipitates were collected, washed with water and dried to give 1.572 g of *1b*, 92% yield, sp. act. 533 μCi/mg or 72.6 mCi/mmole.

N-Acetyl-S-methylpseudothiourea (*2*)

To a solution of 74.7 g of N-acetylthiourea (0.632 mole) in 1 liter of ethyl acetate was added 135 g of methyl iodide (0.951 mole). The mixture was stirred

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* Tritium exchange was carried out by New England Nuclear Corp., Boston, Massachusetts, U.S.A.

under reflux for 0.5 hour and cooled to room temperature. The resulting crystals were filtered and dried to give 137.5 g, 84% yield, m.p. 162-163°C [lit. (2) m.p. 148-149°C, 161-164°C]; anal.-calculated for $C_4H_8N_2OS.HI$ (MW 260.10): C, 18.47; H, 3.49; N, 10.77; found: C, 18.74; H, 3.63; N, 10.58.

2-Acetamido-5,6-dimethyl[4,7-^{2}H]benzimidazole (*3a*)

A solution of 340 mg of *1a* (2.5 mmoles, 92.6% deuterated) and 650 mg of *2* (2.5 mmoles) in 6 ml of methanol was refluxed with stirring under nitrogen for 30 minutes. The mixture was cooled and the crystalline precipitates were collected and dried, 389 mg, 76% yield; m.p. 306-307° C; PMR (in DMSO-d_6, TMS) δ: 2.18 (S, 3H, CH_3-CO-), 2.30 (S, 6H, arom. CH_3), 7.25 (S, 0.16H, arom. H), therefore 92% deuterated in benzene ring, 11.61 (S, 2H, N-H). This material was used in the stability studies discussed earlier.

2-Acetamido-5,6-dimethyl[4,7-^{3}H] benzimidazole (*3b*)

Similarly as above, from 1.362 g of *1b* (10.0 mmoles), sp. act. 72.6 mCi/mmole, and 2.601 g of *2* (10.0 mmoles), there was obtained 1.556 g of *3b*, 77% yield; m.p. 306-307° C; sp. act. 355 μCi/mg or 72.1 mCi/mmole; ultraviolet spectrum λmax (EtOH) nm(ε): 235 (9,750), 244 (10,000), 253 (10,100), 292 (16,500), 301 (17,250); infrared spectrum (Nujol mull) νmax cm^{-1}: 3270, 3200, 3140, 3040 (NH^+), 1725 (C=O), 1640, 1565 (C=N/NH def.); anal.-calculated for $C_{11}H_{13}N_3O$ (MW 203.24): C, 65.00; H, 6.45; N, 20.68; found: C, 64.67; H, 6.59; N, 20.99.

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