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The Preparation of 3-Aminoxy-1-amino[1,1′-$^3H_2$]propane

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Summary

3-Aminoxy-1-aminopropane (APA) has previously been shown to be a potent inhibitor of the polyamine biosynthesis enzymes ornithine decarboxylase, adenosylmethionine decarboxylase, and spermidine synthase. Little information is known, however, regarding its mechanism of action, binding site mode(s), or cellular distribution. This report presents a relatively simple three step synthesis of 3-aminoxy-1-amino[1,1′-$^3H_2$]propane via the catalytic tritiation of 3-aminoxypropionitrile hydrochloride. The latter compound has previously been described in the literature, albeit in little detail, but is included in this report for continuity.

Key Words: tritiation, 3-aminoxypropionitrile, 3-aminoxy-1-aminopropane, APA, polyamines

Introduction

First prepared as a potential inhibitor of ornithine decarboxylase (1), 3-aminoxy-1-aminopropane (APA) has since been shown to inhibit several other enzymes involved in polyamine biosynthesis, including mouse kidney ornithine decarboxylase (Ki 3.2 nM), rat liver S-adenosylmethionine decarboxylase (Ki 50 μM), and bovine brain spermidine synthase (Ki 2.3 μM) (2,3). While it is likely that APA is acting as an isosteric analog of putrescine (1,4-diaminobutane), its mode of binding to the above enzymes, structural requirements, and intracellular disposition (transport, metabolism, excretion) are not known. These observations have prompted us to investigate the preparation of [^{3}H]APA by methods applicable to the synthesis of similar analogs of biological interest.

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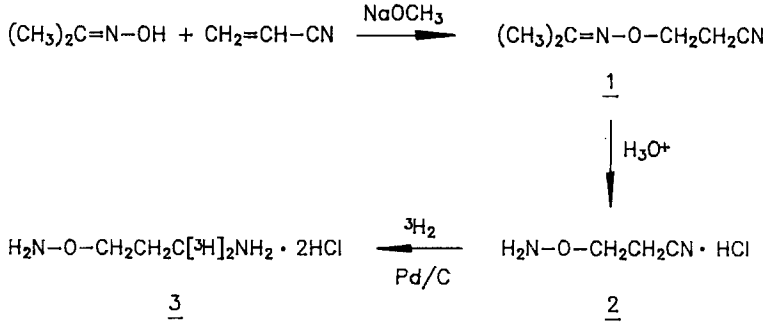
Discussion

The preparation of [^{3}H]APA, 3, is shown in Scheme I. The aminoxy group is introduced by reacting acetoxime with acrylonitrile in the presence of a catalytic amount of sodium methoxide to give the oxime ether, 1. In order to avoid reduction of the oxime to the secondary amine during catalytic tritiation, the oxime ether was hydrolyzed in dilute acid to yield 3-aminoxypropionitrile hydrochloride, 2, albeit in only 30% yield. An earlier account of the synthesis of 2 reported only a 7% yield, although few experimental details were reported (4). More rigorous conditions resulted in hydrolysis of the nitrile to the carboxylic acid, which may in part account for the low yields even under mild hydrolysis conditions. The nitrile, 2, was then subjected to catalytic tritiation using 10% Pd/C and employing 5% anhydrous HCl in ethanol as the solvent to reduce the formation of secondary amine by-products (5). This method was chosen over other reductive methods (e.g. $LiAlH_4$ or $NaBH_4/CoCl_2$) in order to achieve higher specific activities and to simplify workup of the final product after tritiation.



Scheme I. Synthesis of [^{3}H] 3-Aminoxy-1-aminopropane Dihydrochloride

Experimental

Routine NMR, IR, and MS spectra were consistent with the assigned structures. Thin layer chromatography (TLC) employed pre-coated Avicel plates developed in butanol/water/pyridine/acetic acid (6:2:1:2) and visualized with iodine vapors or ninhydrin (5% in butanol, 80°C). Radioisotopic purity was determined by TLC using pre-coated silica gel plates developed in either butanol/acetic acid/pyridine/water (6:2:1:2) as system 1 or n-propanol/HCl/water (10:2:1) as system 2, and the radiochromatograms scanned with a Bioscan 200 TLC scanner.

3-(Isopropylideneaminoxy)propionitrile, 1. Acetoxime (20.0 g, 0.27 mol) and sodium methoxide (0.74 g, 0.014 mol) were dissolved in 300 ml of freshly distilled dioxane. Acrylonitrile (18.0 ml, 0.27 mol) was added dropwise and the

solution stirred at room temperature for 24 hrs. The reaction mixture was neutralized by the addition of 1N HCl (12 ml), filtered, and concentrated in vacuo. The residue was then vacuum distilled to yield 21.8 g (66%) of 1 (bp 95-102°C at 12 mm Hg).

3-Aminoxypropionitrile hydrochloride, 2. 2.0 g (0.016 mol) of 1 was dissolved in 15 ml of 1N HCl and stirred at room temperature overnight. The solution was concentrated in vacuo and the residue dissolved in 10 ml of absolute ethanol and evaporated again. After repeating this process three times to remove residual water and HCl, the residue was recrystallized twice from absolute ethanol to yield 0.6 g (30%) of 2, homogeneous on TLC (Avicel, Rf 0.7) (mp 131-135°C, lit 135-136°C (4)).

3-Aminoxy-1-aminopropane dihydrochloride, 3. To a solution of 1.0 g (0.008 mol) of 2 dissolved in 50 ml of 5% anhydrous HCl in ethanol was added 0.5 g of 10% Pd/C and the mixture stirred under 1 atm of hydrogen for 24 hrs at room temperature. The mixture was filtered through a pad of Celite, washed with an additional 20 ml of ethanol, and the filtrate evaporated in vacuo. The residue was recrystallized from methanol/ethyl acetate to yield 1.1 g (81%) of 3, homogeneous on TLC (Avicel, Rf 0.4; silica gel, system 1, Rf 0.6; silica gel, system 2, Rf 0.4)(mp 182-185°C, lit 203-204°C (1)).

3-Aminoxy-1-amino[1,1'-$^3H_2$]propane dihydrochloride, [^{3}H]3. To a solution of 25.0 mg (0.2 mmol) of 2 dissolved in 10 ml of 5% anhydrous HCl in ethanol was added 25.0 mg of 10% Pd/C and the mixture stirred under 20 Ci of tritium gas at 1 atm pressure for 24 hrs at room temperature. The mixture was filtered through a pad of Celite, washed with an additional 20 ml of ethanol, and the filtrate evaporated in vacuo. Labile tritium was removed by repeated evaporations of ethanol. An aliquot (50 mCi) of the crude product was applied to a preparative TLC plate (Avicel, 1.0 mm thickness), developed in solvent system 2, and the product band located at Rf 0.6. The product was eluted from the absorbent with 1 M HCl and the solvent lyophilized to give [^{3}H]3 (30 mCi, approximately 200 mCi/mmol), with a radiochemical purity of >95% (silica gel, system 2), which was stored as a solution in methanol at -20°C.

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