# PREPARATION OF NICOTINIC-5-$^2$H ACID FROM 5-BROMONICOTINIC ACID

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## SUMMARY

Methyl nicotinate-5-$^2$H was prepared in dry tetrahydrofuran by palladium-catalyzed deuterolysis of methyl 5-bromonicotinate. Acid hydrolysis of the crude reaction mixture resulted in a 92% yield of deuterium-labeled nicotinic acid of which 80 mole% was nicotinic-5-$^2$H acid and 12 mole% was nicotinic-5-$^2$H acid containing an additional deuterium atom elsewhere in the molecule. The structure assignment was based on the results of nuclear magnetic resonance spectroscopy, mass spectroscopy, ultraviolet spectroscopy, and elemental analysis.

Key Words: 5-Bromonicotinic Acid, Nicotinic-5-$^2$H Acid, Catalytic Reduction

## INTRODUCTION

The protons attached to carbon atoms, 2, 4, 5 and 6, of the pyridine ring of nicotinamide and nicotinic acid are non-exchangeable under physiological conditions. In $N^1$-alkylpyridinium compounds, however, the protons on carbon atoms, 2 and 6, are rendered slowly exchangeable as a result of the strong inductive effect of the adjacent, positively-charged quaternary nitrogen atom (1). Nicotinamide adenine dinucleotide ($NAD^+$) is an $N^1$-alkylpyridinium derivative that participates in many enzyme-catalyzed redox reactions in living cells. In biological oxidations involving $NAD^+$, a hydride ion is transferred from a redox substrate to carbon atom 4 of the pyridine ring of $NAD^+$ thereby generating NADH. Either the α or β proton attached

to carbon atom 4 of the pyridine ring of NADH may be enzymatically transferred, along with a pair of electrons, to a hydride-ion acceptor thereby regenerating $NAD^+$. Thus, the protons attached to carbon atoms, 2, 4 and 6, of the pyridine ring of $NAD^+$ may be exchanged in biochemical systems. The proton on carbon atom 5 remains as the only potentially non-exchangeable proton. For these reasons, a specific synthesis of nicotinic-5-$^2H$ acid was sought which could be utilized in a commercial custom synthesis of nicotinic-5-$^3H$ acid.

Alkyl and aryl halides are easily hydrogenolyzed in the presence of basic palladium catalysts to the corresponding alkanes and aromatic compounds (2). 5-Bromonicotinic acid is available commercially for which reason it was chosen as the starting material for a three step-synthesis of nicotinic-5-$^2H$ acid by the reactions in Figure 1.

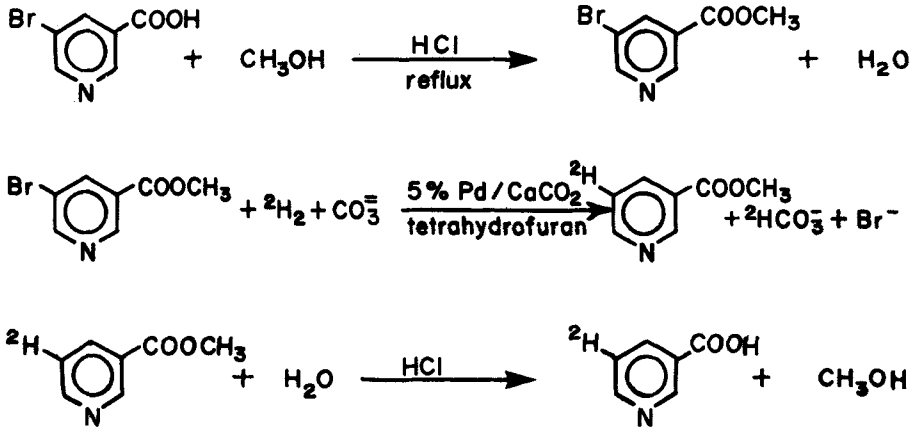


Figure 1. Reactions employed to synthesize nicotinic-5-$^2H$ acid from 5-bromonicotinic acid.

## MATERIALS AND METHODS

Materials--5-Bromonicotinic acid was purchased from Columbia Organic Chemicals, Columbia, S.C. Deuterium gas was purchased

from Liquid Carbonic, San Carlos, Calif. Nicotinic acid was a product of Matheson Coleman and Bell, Cincinnati, Ohio. The ester, methyl nicotinate, was purchased from Sigma Chemical Co., St. Louis, Missouri. Palladium (5%)/calcium carbonate was obtained from Matheson Coleman and Bell, Cincinnati, Ohio. Tetrahydrofuran, a product of Mallinckrodt Chemical Works, St. Louis, Missouri, was redistilled over lithium aluminum hydride just before use.

Preparation of Methyl 5-Bromonicotinate--Five grams of 5-bromonicotinic acid was dissolved in 200 ml dry methanol to which 2 ml acetyl chloride had previously been added at -20°. The solution was refluxed for 24 hr after which the solvent was removed in a water-aspirated rotary evaporator. The residue was dissolved in a small amount of hot water from which methyl 5-bromonicotinate crystallized upon cooling. The crystals were collected and recrystallized from water several times. The structure of the product was verified by nuclear magnetic resonance spectroscopy.

Deuterolysis of Methyl 5-Bromonicotinate--One hundred milligrams of methyl 5-bromonicotinate, 50 milligrams 5% palladium on calcium carbonate, and 100 milligrams dry barium carbonate powder were added to a 12 ml conical, screw-top centrifuge tube and 2.5 ml redistilled, dry tetrahydrofuran was added. The tube was stirred on a Vortex mixer and immediately placed in a 500 ml, thick-walled glass hydrogenation vessel and connected to a Paar hydrogenation apparatus. The hydrogenation vessel was flushed five times with dry deuterium before sealing the system which contained deuterium gas at 1 atmosphere pressure. The reaction mixture was shaken in the Paar apparatus at room temperature for 8 hr. The centrifuge tube containing the reaction mixture was then removed from the hydrogenation vessel and 10 ml tetrahydrofuran was added. The tube was topped with a Teflon-lined cap, shaken, and spun in a clinical centrifuge for 5 min. The supernatant was transferred to a 50 ml round-bottom flask and the precipitate was reextracted

with two, 5 ml volumes of tetrahydrofuran. The rinses were combined with the supernatant in the 50 ml round-bottom flask, and the total volume was evaporated to dryness at 4° in a water-aspirated rotary evaporator. Yield of crude methyl nicotinate-5-$^{2}$H: 60 mg; % yield: 94.

Hydrolysis of Deuterium-labeled Methyl Nicotinate--The crude deuterolysis product was dissolved in 2.0 ml 6N HCl and allowed to stand in the dark at room temperature for 7 days. The resultant acid hydrolysate was quantitatively transferred to a 25 ml round-bottom flask and evaporated to dryness at 50°C in a water-aspirated rotary evaporator. The residue was dissolved in distilled water, evaporated in order to remove HCl, and redissolved in distilled water.

A 10 µl aliquot was removed and diluted to 10 ml with 0.01M sodium acetate-acetic acid buffer, pH 4.7. The absorbance was measured at 262- and 285 nanometers in order to determine the yield of deuterium-labeled nicotinic acid by comparison with absorbances of standard solutions of nicotinic acid and 5-bromo-nicotinic acid. Yield of deuterium-labeled nicotinic acid: 53 mg; % yield: 92.

Purification and Characterization of Deuterium-labeled Nicotinic Acid--The distilled water solution of the acid-hydrolyzed product was lyophilized and the residue was dissolved in hot ethanol and crystallized. After recrystallization once from ethanol and once from distilled water, the crystals were dried over concentrated sulfuric acid in a vacuum desiccator and samples of the dry product were subjected to elemental analysis, mass spectroscopy, ultraviolet light absorption spectroscopy and melting point determination.

## RESULTS AND DISCUSSION

A nuclear magnetic resonance spectrum of the crude deuterolysis product does not contain the proton signal, centered at $\delta$ = 7.35,

that is associated with the proton attached to ring carbon 5 of authentic methyl nicotinate. Also, the doublet proton signals centered at $\delta = 8.25$ and $\delta = 8.75$, associated with the protons on ring carbon atoms, 4 and 6 respectively, appear as single peaks in the spectrum of the deuterolysis reaction product. The reaction was essentially quantitative since none of the signals corresponding to protons of the unreacted starting material, methyl 5-bromonicotinate, appear in the spectrum of the reaction product.

The lack of absorption at 285 nanometers in the ultraviolet absorption spectrum of acid-hydrolyzed product reveals that essentially no 5-bromonicotinic acid is present since the latter absorbs appreciably at this wavelength. Assuming that deuterium-labeled nicotinic acid has a molar absorbance equal to that of authentic nicotinic acid, the total reaction-product absorbance at 262 nanometers indicated that 92% of the starting material had been recovered in the acid hydrolysate.

The shift of all the major peaks by one molecular-weight unit in the mass spectrum of the reaction product compared to the mass spectrum of authentic nicotinic acid, verifies that the major product of deuterolysis, after acid hydrolysis, is nicotinic-5-$^2H$ acid.

The mole percentages of molecular ions with weights of 123 (no deuterium), 124 (one deuterium), and 125 (two deuteriums) were computed from the molecular ion peak intensities of the mass spectrum for deuterium-labeled nicotinic acid. Eighty mole% was nicotinic-5-$^2H$ acid, 8 mole% was unlabeled nicotinic acid which probably formed as a result of the presence of hydrogen in the deuterium gas and/or water in the reaction mixture, and 12 mole% was nicotinic-5-$^2H$ acid containing an additional deuterium atom elsewhere in the molecule.

The uncorrected melting point range of the deuterium-labeled nicotinic acid was 226-228°; the reported value for authentic

nicotinic acid is 230-232° (3).

An elemental analysis of the recrystallized deuterium-labeled nicotinic acid gave: carbon, 58.19 (theoretical = 58.04); hydrogen plus deuterium, 4.32 (theoretical = 4.88); nitrogen, 11.22 (theoretical = 11.28). The percentage of $^{1}H$ plus $^{2}H$ in the pure product was also calculated from the mass spectral data and was found to be 4.87. These data along with the nuclear magnetic resonance and mass spectra, show that the principal product of deuterolysis of methyl 5-bromonicotinate was methyl nicotinate-5-$^{2}H$.

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