# ALUMINA CATALYZED TRITIUM EXCHANGE OF ENOLIZABLE HYDROGENS

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## SUMMARY

A technique for the alumina catalyzed tritium exchange of enolizable hydrogens is described. In the two examples reported, the labelling of α-bromo-p-azidoacetophenone and haloperidol, specific activities of 0.96 and 0.315 Ci/mmol, respectively, were obtained. The procedure was found to be extremely mild and required no purification techniques.

Key Words: Tritium exchange, enolizable hydrogens, alumina catalysis, haloperidol-$^{3}H$, α-bromo-p-azidoacetophenone-$^{3}H$.

## INTRODUCTION

The use of high level radioactive compounds for radioimmunoassay studies has created a need for a variety of tritium exchange techniques. While radioimmunoassay requirements do not dictate specificity in labelling, methods which lead to specific labels, by their nature, give higher levels of isotope incorporation. In this connection, we wish to report the development of a preparative alumina catalyzed exchange technique for the tritium labelling of enolizable hydrogens. In particular, the labelling of α-bromo-p-azidoacetophenone (1) and haloperidol (2) are described.

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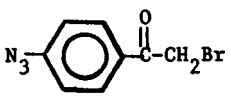
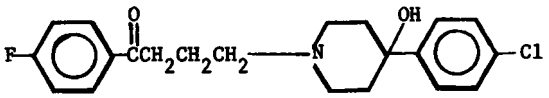


1



2

Tritium labelled 1 was required with high specific activity for affinity labelling studies of transfer RNS binding sites in several macromolecular systems. Attempts to label 1 under various neutral, (1) acidic, (2,3) or basic (4,5) exchange conditions (using deuterium) resulted either in minimal isotope incorporation or in decomposition of the molecule. In an attempt to find milder and more subtle exchange conditions, it was discovered that 1 smoothly incorporated deuterium after a single pass over neutral alumina deactivated to activity II with deuterium oxide. While this technique has found general applicability with deuterium, (6,7) and has been applied to tritium labelling (8,9), the general utility of this method for preparative tritium exchange has not been developed apparently due to the difficulty in handling alumina containing high levels of tritium. By vacuum transferring tritium oxide into a side arm bulb (prefilled with alumina) directly attached to a short chromatography column, it was found that the radioactive alumina could be completely contained. Elution of the compound with benzene through such an apparatus (see experimental) containing neutral alumina-$T_2O$ gave 1 with a specific activity of 0.96 Ci/mmol (4 mCi/mg). Spectral data on a deuterium labelled sample of 1 showed the absence of the enolizable hydrogens in the nmr spectrum ($\delta$4.38, singlet) and gave the following deuterium content in the mass spectrum: $d_o$=3.1%, $d_1$=29.1%, and $d_2$=67.8%.

Tritium labelled haloperidol (2) was required for radioimmunoassay studies to correlate therapeutic efficacy of the drug with tissue concentrations and blood levels. Similar neutral alumina-$D_2O$ exchange treatment of 2 resulted in very poor isotope incorporation presumably due to the decreased inductive effect (no α-bromine) and increased steric hindrance about the enolizable positions. A satisfactory level of incorporation was attained when the compound was stirred overnight in a slurry of the neutral alumina-$D_2O$ in chloroform in the side arm bulb, then filtered through the glass frit of the chromatography column, When this exchange was carried out using neutral alumina-$T_2O$, 2 was obtained with a specific activity of 0.315 Ci/mmol (840 μCi/mg). Spectral data on a deuterium labelled sample of 2 again showed loss of the enolizable hydrogens in the nmr spectrum (δ 2.94, triplet, J=7Hz) and gave the following deuterium content in the mass spectrum: $d_o$=15%, $d_1$=37% and $d_3$=48%.

## EXPERIMENTAL

General. All solvents were distilled. Spectra were recorded on standard instruments by the staff of the Physical Chemistry Department of Hoffmann-La Roche Inc. Radiochemical purity was determined on thin-layer chromatograms with a Packard Model 7201 Radiochromatogram Scanner System and radioactivity was measured by the liquid scintillation technique with a Packard Tricarb Model 2010 spectrometer.

α-Bromo-p-azidoacetophenone-α-$^3H$ (1). Alumina (2 g, neutral, activity super I) was added to the side arm bulb of a chromatography column and the entire apparatus was attached in a horizontal position to a conventional "T" connected to a high vacuum line (0.1 micron). After the apparatus was evacuated, the side arm bulb was immersed in a liquid nitrogen bath and tritium oxide (100 μl,10 Ci, 2 Ci/mmol ) was introduced by vacuum transfer.

Upon removal of the bath, the column was filled with dry nitrogen and the alumina was allowed to equilibrate for 2 hrs at room temperature (with occasional shaking). After being placed in a vertical position, the column was filled with dry benzene and the alumina-$T_2O$ slowly tapped in. After flushing the column with a few ml of benzene, a solution of 1 (25 mg in 2 ml of benzene) was applied and eluted with 25 ml of benzene. Vacuum transfer removal of the benzene yielded a tan powder which was redissolved in 10 ml of methanol (to remove labile activity). Concentration of the solution again by vacuum transfer gave 1 as a tan solid (18.5 mg, 75 mCi) having a specific activity of 0.96 Ci/mmol (4 mCi/mg) and a radiochemical purity of >99% as determined by tlc (silica gel, 50% ethyl acetate in benzene).

Haloperidol-$^3$H(2). Neutral alumina-$T_2O$ was prepared as described above. To the equilibrated catalyst in the side arm bulb was added a solution of 2 (10 mg in 15 ml of chloroform) and a small magnetic stirring bar. Under a nitrogen atmosphere, the slurry was vigorously stirred overnight. After the column was placed in a vertical position, the slurry was filtered through a glass frit and further eluted with 30 ml of chloroform. After vacuum transfer removal of the chloroform, the residue was taken up in 15 ml of methanol (to remove labile activity) and again concentrated. The residue (5.8 mg, 4.87 mCi) had a specific activity of 0.315 Ci/mmol (840 μCi/mg) and a purity of >99% as determined by tlc (silica gel: chloroform/acetic acid/benzene/methanol, 16:4:2:1).

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