ACID CATALYZED TRITIUM EXCHANGE OF ACTIVATED AROMATIC PROTONS

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SUMMARY

A technique is described for the acid catalyzed tritium exchange of activated aromatic protons using acetic acid-^{3}H/ tritium chloride. In the two examples reported, specific activities of 0.5 Ci/mmol were obtained for propranolol and 1.73 Ci/mmol for reserpine. The reagent mixture was found to be very effective and easily manipulated under vacuum.

Key Words: Tritium exchange, acid catalysis, activated aromatic protons, propranolol-^{3}H, reserpine-^{3}H.

INTRODUCTION

Tritium exchange reactions have become an extremely important technique for the preparation of labelled compounds which otherwise could not be prepared. A number of acid-catalyzed aromatic exchange procedures have been described for the preparation of deuterium labelled substrates (1-5) but few are readily adaptable to high level tritium labelling. In this connection, we wish to report the use of an easily manipulated reagent mixture, acetic acid-^{3}H/tritium chloride, for the exchange labelling of activated aromatic hydrogens. The preparation of tritium labelled propranolol (1) and reserpine (2) are given as examples.

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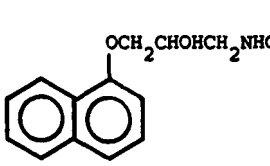

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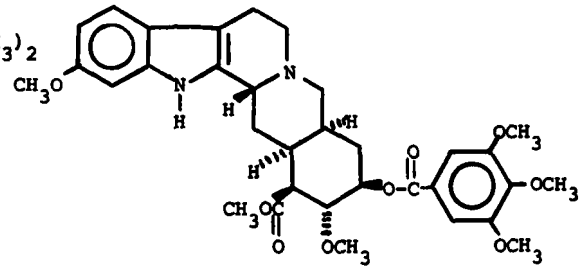

2

Tritium labelled 1 and 2 were required for radioimmunoassay studies to correlate the therapeutic efficacy of these drugs with tissue concentrations and blood levels. The presence of activated aromatic hydrogens in both 1 and 2 suggested that tritium labelling could be achieved by an acid catalyzed exchange reaction. In an attempt to find an efficient and volatile acid catalyst, it was found that a mixture of acetic acid-d_1/deuterium chloride (generated by the decomposition of acetyl chloride with deuterium oxide) was an extremely effective exchange reagent. Moreover, the reagent mixture could be generated, transferred into the reaction flask and finally removed entirely under vacuum.

Equilibration of propranolol (1) with acetic acid-d_1/deuterium chloride at room temperature overnight gave a stable labelled derivative having the following deuterium content (mass spectrum): d_o=0%, d_1=6.8%, d_2=93.2%. The nmr spectrum of the sample confirmed that the single ortho hydrogen (δ6.95, 1H, J=3 and 6HZ, doublet of doublets) had been exchanged along with one other aryl proton (presumably in the para). Equilibration of 1 in a similar manner with acetic acid-^{3}H/tritium chloride gave a radioactive labelled sample having a specific activity of 1.94 mCi/mg (0.501 Ci/mmol).

Similarly equilibration of reserpine (2) with acetic acid-d_1/deuterium chloride gave a derivative having the following deuterium content (mass spectrum): d_o=d_1=d_2=0.0, d_3=57.5%, d_4=27.3%, d_5=14.1%, d_6=1.1%. The nmr spectrum was two complex to assign protons. Subsequent tritium exchange of reserpine as described above gave a sample having a specific activity of 2.85 mCi/mg (1.73 Ci/mmol).

EXPERIMENTAL

General. 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.

Propranolol-^{3}H. (1). Acetyl chloride (314 mg, 285 µl, 4 mmol, freshly distilled) was added to a 100 ml round bottom flask (to contain the volume of gas evolved) which was then attached to a high vacuum line (10^{-4}mm). After the flask was cooled (liquid nitrogen) and evacuated and the solution degassed (freeze/thaw), tritium oxide (100 µl, 10 Ci, 2 Ci/mmol) was admitted by vacuum transfer. The flask was sealed and kept at room temperature 1 hr. The entire mixture (acetic acid-^{3}H/tritium chloride) was then vacuum transferred into a pear shaped flasked (100 ml) containing propranolol (4.5 mg, 0.017 mmol). After the reaction solution was brought back to room temperature, and the pressure within the flask adjusted to one atmosphere (by addition of dry nitrogen), the solution was stirred overnight. Vacuum transfer removal of the acetic acid-^{3}H/tritium chloride mixture gave a residue which was taken up in methanol (3x4 ml, to remove labile tritium) and reconcentrated *in vacuo*. Preparative thin layer purification (silica gel; 4% NH_4OH/ethyl acetate) of the crude product gave 2.3 mg (4.47 mCi, 51%) of 1

having a specific activity of 1.94 mCi/mg (0.501 Ci/mmol).

Reserpine-^{3}H (2). In the manner described above, reserpine (10.4 mg, 0.017 mmol) was equilibrated with acetic acid-^{3}H/tritium chloride (10 Ci). Preparative thin layer purification (silica gel; chloroform/acetic acid/cyclohexane/methanol, 32/4/4/1) of the crude product yielded 5.2 mg (14.8 mCi, 50%) of 2 having a specific activity of 2.85 mCi/mg (1.73 Ci/mmol).

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