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A Novel One Step Approach to Small Scale Labeling of Organic Compounds with Hydrogen Isotopes

Sohail Malik*, Margaret Kenny
Departments of Laboratory Medicine and Medicine, SB-10
University of Washington, Seattle, Washington 98195
George Doss[a] and Varghese John[b]
Department of Chemistry, Stanford University, Stanford, CA 94305

SUMMARY

A rapid method to generate tritium gas for small scale labeling is described. Tritium labelled sodium borohydride is used with cobalt (II) chloride to generate isotopic hydrogen gas. The reaction was carried out in a closed vessel and three different compounds were concurrently labelled. This simple and inexpensive method can be applied to a broad range of molecules and does not require any special set up.

Key words: Hydrogen isotopes, tritium, tritium generation, methodology, microscale

INTRODUCTION

The hydrogen isotope tritium is one of the most widely used radionuclides in bio-organic research. More than 900 publications appeared in biomedical journals (Medline) involving tritium in the year 1992. The methods currently available for labeling compounds using tritium gas (1) are by no means simple, specially in case of small scale labeling . Generally such experiments require transfer of tritium gas from storage ampoule into the reaction flask, which is often performed by volume displacement with an appropriate packing fluid, eg. mercury (2), or by Toepler pump (3). Tritium gas transfer has also been achieved by liquid helium (4). These methods are cumbersome, require special care and complex set ups. Often the complications associated with the reactions involving tritium gas force independent researchers to

*Corresponding Author
[b]Current Address: Merck, Sharp and Dohme, Research Laboratories, Rahway, NJ 07065
[b]Current Address: Athena Neurosciences, 800 F, Gateway Blvd., South San Francisco, CA 94080

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send their samples to commercial laboratories. This is normally a very expensive procedure which can also cause unwanted delays.

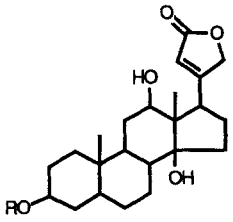
As a result of our continuing studies on labeling organic compounds with hydrogen isotopes (5) we wish to report here a rapid method to generate tritium gas and label organic compounds in one-step.

EXPERIMENTAL

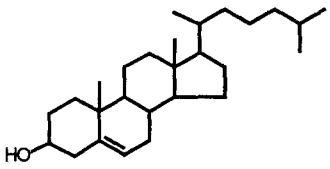
Three compounds, digoxin (1), digoxigenin (2) and cholesterol (3) were labeled with tritium by catalytic hydrogenation. Each compound, 1, 2 and 3 (1.0 mg in ethanol, 1.28 x 10^{-3}mmol; 1.15 mg in methanol, 1.61 x 10^{-3}mmol and 0.8 mg in ethyl acetate, 2.07 x 10^{-3}mmol) was placed in a small glass vial (A) with 5% Pd/C and a small magnetic stirring bar. These vials were then placed in a larger glass vial (B) containing NaB^3H_4 (20 mCi, 600 mCi/mmol). The outer vial (B) was tightly capped with a rubber septum. $CoCl_2$ (0.41 mg, 3.17 x 10^{-2}mmol) in 0.5 ml water was added at once to the bottom of the vial B with the help of a syringe. The syringe was removed immediately thereafter and the reaction mixture was stirred for 5 hrs. at room temperature. Complete hydrogenation was achieved by introducing hydrogen (H_2) gas to the reaction vial. The products were worked up in a usual manner applied for catalytic hydrogenation. The catalyst was removed by filtration and hydrogenated products of 1, 2 and 3 were purified by using small silica gel columns with mixtures of chloroform:methanol (glycosides) and hexane:ether (cholesterol). The amount of radioactivity recovered in the tritiated products of 1, 2 and 3 was 0.918 mCi/mmol, 1.202 mCi/mmol and 0.836 mCi/mmol, respectively. Water (3.0 ml) was added to the reaction vial (B) before disposal. A general set up for the reaction is presented in Figure 1.

RESULTS AND DISCUSSION

Tritium gas was generated and labeling of compounds (1,2,3) was carried out in one step, in a closed vessel. The method described here to label organic compounds with hydrogen isotopes can be performed in an ordinary laboratory and does not require any special set up. In this method tritium gas is generated by the reaction of ^3H labeled sodium borohydride with cobalt (II) chloride. The isotopic hydrogen gas thus produced, can be used to label various compounds using variety of different chemical reactions including catalytic hydrogenation described here. Deuterium gas can also be generated and used in the similar manner to label different compounds. In addition to being simple, fast and cost efficient, this method can be used to label more than one compound simultaneously specially where high specificic activity is not a major concern. In order to obtain compounds with higher specific activity, one compound at a time, with larger amounts of NaB^3H_4 would be



1 R = (digitoxose)$_3$
2 R = H



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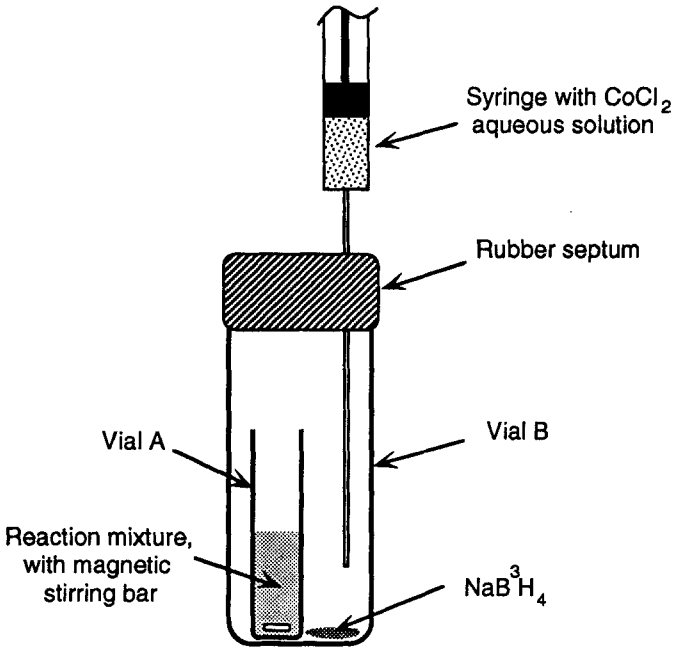

Figure 1. A general setup for the reaction

more useful. A commercial kit, based upon this method can also be developed to generate tritium gas for small scale labeling. Such a kit would further simplify the technology and can be used more commonly in the laboratories.

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