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Synthesis of 2, 4, 7-triamino-6-phenylpteridine (triamterene) labelled with tritium or carbon-14

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SUMMARY

The diuretic drug triamterene was labelled with carbon-14 by condensing the intermediate phenylacetonitrile-1-^{14}C with 2,4,6-triamino-5-nitrosopyrimidine.

The tritiated triamterene was prepared by direct synthesis from toluene-3,4-$^{3}H_{1/2}$ and toluene-3-^{3}H. The ^{3}H-toluenes were prepared in excellent radiochemical yield by hydrolyzing the dry Grignard reagent of the appropriate bromotoluene with tritium oxide.

INTRODUCTION

Metabolic studies with the diuretic triamterene required the preparation of 2,4,7-triamino-6-phenylpteridine-7-^{14}C. The ^{14}C-triamterene was prepared by the condensation of phenylacetonitrile-1-^{14}C with 2,4,6-triamino-5-nitrosopyrimidine by a modification of the procedure of Spickett and Timmis [1]. The specific activity of 1 mC/mM was satisfactory for preliminary metabolic studies.

Specific activities of greater than 25 mC/mM were subsequently required for cytological studies. The tritiation of the 6-phenyl group of triamterene appeared to be the most realistic approach. Although triamterene was stable in the exchange reagent of Yavorsky and Gorin [2, 3], it was decided that the amino groups would drastically reduce the radiochemical yield. A direct synthetic approach was used in preparing the intermediate toluene-3,4-$^{3}H_{1/2}$ [4] by the hydrolysis of the dry Grignard reagent of an equimolar mixture of *p*- and *m*-bromotoluene with 5 C/0.5 ml of tritium oxide [1]. Excellent radiochemical yields of ^{3}H-toluene were obtained by the dry Grignard reagent technique even though carrier water was used to complete the hydrolysis. The method has been previously applied to the preparation of deuterated toluenes [5]. The α-bromotoluene-3,4-$^{3}H_{1/2}$ was obtained by bromination with N-bromosuccinimide, and

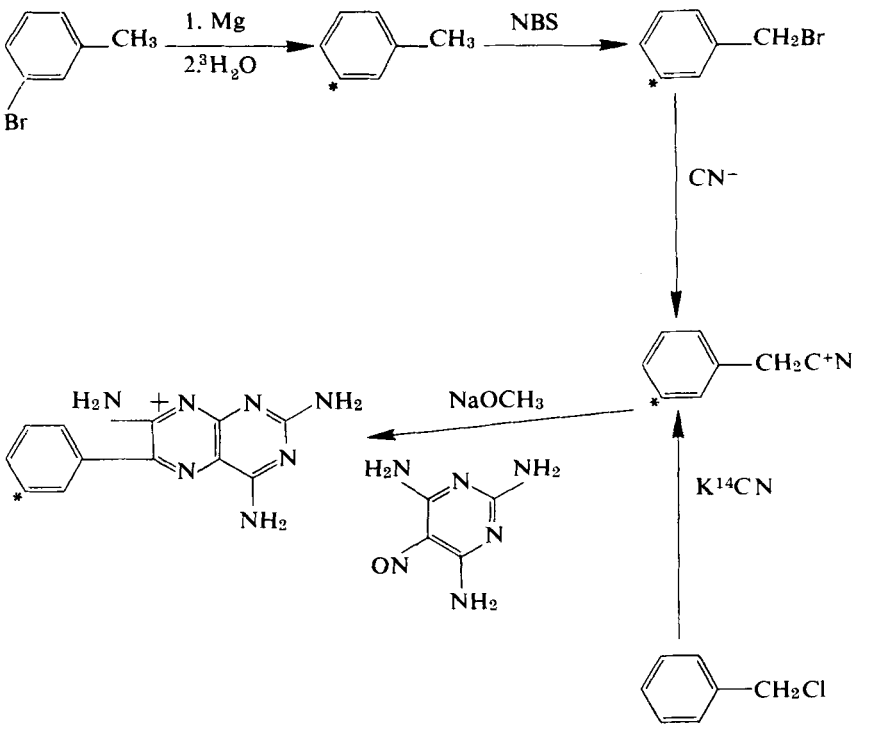
[1] Purchased from the New England Nuclear Corp., Boston, Massachusetts.

the 2,4,7-triamino-6-phenyl-3′,4′-$^{3}H_{1/2}$-pteridine was prepared by a procedure similar to that used in the synthesis of ^{14}C-triamterene. The specific activity of the ^{3}H-triamterene was 65.8 mC/mM (theoretical was 68 mC/mM). A loss of tritium activity as tritium oxide became apparent in preliminary dog studies, and a study of metabolites showed evidence of the *p*-hydroxylation of the 6-phenyl group of triamterene. The tritium was placed specifically in the meta-position of the phenyl group for subsequent double isotope studies. Since a low specific activity material was sufficient to carry out these studies, the toluene-3-^{3}H was prepared by tritiating the dry Grignard reagent of *m*-bromotoluene with 1 C/ml of tritium oxide. Although the specific activity of the resulting 2,4,7-triamino-6-phenyl-3′-^{3}H-pteridine was 4.25 mC/mM, the route is amenable to the preparation of specific activities of 100-300 mC/mM.

Experimental

Phenylacetonitrile-1-^{14}C.

Benzyl chloride was reacted with 50 millicuries of potassium cyanide-^{14}C on a 50 millimolar scale according to the procedure of Adams and Thal [6]. The yield was 4.843 g (82.7%) of phenylacetonitrile-1-^{14}C. Exploratory cold runs gave yields ranging from 82 to 87%, with quantities as low as 6 millimoles.



(* = ^{3}H; + = ^{14}C)

2,4,7-Triamino-6-phenylpteridine-7-^{14}C.

The procedure is a modification of that employed by Spickett and Timmis [1]. A suspension of 6.6 g (39.3 mM) of 2,4,6-triamino-5-nitrosopyrimidine* in 130 ml of dry dimethylformamide was heated to reflux over thirty-five minutes and a flow of nitrogen was passed over the purple solution to remove traces of water.

The heat was turned off momentarily and 2.36 g (43.6 mM) of a sodium methylate suspension in 10 ml of dimethylformamide was added to the stirred solution. The solution turned brown after a vigorous exothermic reaction had taken place. The solution was brought to reflux and 4.843 g (41.3 mM) of phenylacetonitrile-1-^{14}C in 10 ml of dimethylformamide was added over a period of ten minutes. The stirred suspension of yellow solid was refluxed thirty minutes and cooled to room temperature. The crude 2,4,7-triamino-6-phenylpteridine-7-^{14}C was filtered and washed with 75 ml of dimethylformamide and 125 ml of water. The crude pteridine was dissolved in 250 ml of hot 50% acetic acid and treated with activated charcoal. Two crops of the yellow crystalline 2,4,7-triamino-6-phenylpteridine-7-^{14}C acetate were obtained on cooling and concentrating the acetic acid solution. The acetate crops were suspended in 220 ml of water and 14.5 ml of ammonium hydroxide was added dropwise with stirring. The 2,4,7-triamino-6-phenylpteridine-7-^{14}C was filtered and washed thoroughly with water. After drying, the product weighed 6.06 g (61% yield) and had a specific acticity of 0.98 mC/mM. The purity was 100% by non-aqueous titration, ultraviolet absorption and fluorescence. No radiochemical impurities were evident upon radioscanning a paper chromatogram developed with butanol, formic acid and water (5:1:5).

Toluene-3-^{3}H.

The procedure for preparing toluene-3-^{3}H is an adaptation of the method used to prepare various deuterated toluenes [5]. A Grignard reaction flask was attached through a condenser and freeze trap to a high vacuum manifold as shown in figure 1.

The Grignard reaction flask was charged with 1.512 g (63 mM) of magnesium turnings, 7 ml of ether, a crystal of iodine and two drops of methyl iodide. The Grignard reagent of *m*-bromotoluene was formed by the dropwise addition of 7.3 ml (60 mM) of *m*-bromotoluene obtained from the center-cut of a Podbielniak fractionation. The ethereal Grignard reagent was gently refluxed for forty-five minutes to complete the reaction.

A break-seal ampoule of tritium oxide with an activity of 1 C/ml was attached to the side arm between the reflux condenser and the freeze trap. All ball and socket joints were sealed with high vacuum wax.The freeze trap was cooled with liquid nitrogen and most of the ether was removed from the Grig-

* The dry recrystallized intermediate was prepared from guanidine carbonate and potassium isonitrosomalononitrile.

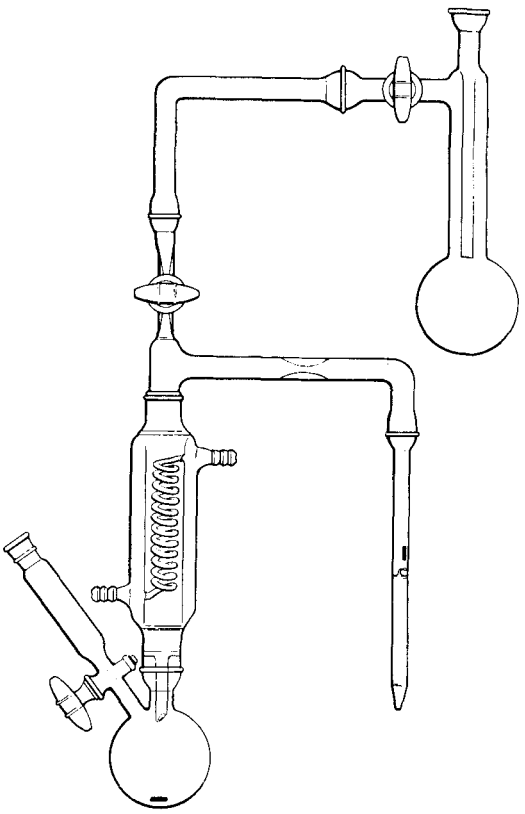


FIG. 1. — Grignard reaction equipment for the tritiation of toluene.

nard reagent by vacuum distillation until a viscous semi-solid remained in the flask. The ether was removed from the freeze trap and the entire system was evacuated to less than 1 micron, using liquid nitrogen to cool the Grignard reagent and freeze trap. The vacuum manifold and freeze trap were closed with a high vacuum stopcock, and the tritium oxide was transferred *in vacuo* to the nearly dry Grignard reagent by breaking the ampoule seal with an internal magnet. The Grignard reagent of *m*-bromotoluene was subsequently hydrolyzed by allowing the reaction mixture to warm slowly to room temperature, using a magnetic stirrer to agitate the vigorously reacting mixture. The reaction mixture was stirred overnight and 0.25 ml of water was added the following day to complete the hydrolysis. The crude toluene-3-^{3}H, ether and tritium oxide were vacuum distilled and the water was removed with calcium chloride. The filtrate was distilled at atmospheric pressure. A forerun of 3.4 ml was followed by 5 ml of toluene-3-^{3}H. The vapor phase chromatogram showed a purity of 97% for an 85% chemical yield.

α-Bromotoluene-3-^3H.

The 5 ml of toluene-3-^{3}H was diluted with 1.5 ml of toluene (61 mM total; specific activity of 7 mC/mM) and added to the bromination medium of 13.8 g (77 mM) of N-bromosuccinimide and 81 mgm of benzoyl peroxide in 50 ml of carbon tetrachloride. The reaction mixture was refluxed for four hours and cooled to room temperature. The precipitate of succinimide was removed by filtration and the carbon tetrachloride was evaporated *in vacuo*. The crude α-bromotoluene-3-^{3}H was distilled at 86 °C/28 mm. The product weighed 5.472 g (52% yield).

Yields were higher in 'cold' runs and in the preparation of α-bromotoluene--3,4-$^3H_{1/2}$ but decomposition during distillation caused an appreciable lowering of yield.

Phenyl-3-^3H-acetonitrile.

The 5.472 g of α-bromotoluene-3-^{3}H was converted to phenyl-3-^{3}H-acetonitrile according to the procedure of Adams and Thal [6]. The phenyl-3-^{3}H-acetonitrile was distilled at 86-87 °/7 mm and the yield was 2.734 g (73%). The vapor phase chromatogram showed a purity of 95%.

2,4,7-Triamino-6-phenyl-3′-^3H-pteridine.

The 2.733 g (23.4 mM) of phenyl-3-^{3}H-acetonitrile was converted to 2.825 g (65% yield) of 2,4,7-triamino-6-phenyl-3′-^{3}H-pteridine with a procedure similar to the carbon-14-tagged pteridine. The specific activity was 4.25 mC/mM and the material was chemically and radiochemically pure. No activity loss occurred during crystallization of the acetate salt and conversion to the free base.

ACKNOWLEDGEMENT

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