SYNTHESIS OF ^{14}C-HYDROXYUREA WITH IMMOBILIZED ^{14}C-CYANATE.

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SUMMARY

A solution of hydroxylamine hydrochloride is passed over a column bed of Amberlite IRA-400 pre-loaded with $^{14}CNO^-$ anions. ^{14}C-hydroxyurea is formed in 50% radiochemical yield after recrystallization. The product is not contaminated with iso-hydroxyurea or other by-products usually found in the reaction of hydroxylamine hydrochloride with aqueous potassium cyanate.

Key words: ^{14}C-hydroxyurea, Polymer-bound ^{14}C-cyanate.

INTRODUCTION

The selective cytotoxic properties of hydroxyurea have been examined extensively, particularly its synergistic effects when co-administered with other chemotherapeutic agents (2,3). As a result, the need has increased for tracer labeled hydroxyurea in studies aimed at determining its distribution and metabolism in biological test systems.

A recent report by Winstead *et al.* (4) described the synthesis and preliminary scintigraphic evaluation of ^{11}C-hydroxyurea prepared from ^{11}C-cyanate by the classical method of Kofod (5). Although suitable as an imaging agent, the product obtained in this manner is contaminated

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with iso-hydroxyurea at levels which render it significantly less useful for quantitative pharmacodynamic studies. A further shortcoming of the Kofod method is the requirement for repeated manipulation of reagents and end-products in aqueous solutions under conditions encouraging hydrolytic cleavage and material losses.

This paper presents a convenient, high yield synthesis of ^{14}C-hydroxyurea from ^{14}C-cyanate immobilized on an ion exchange resin. It was adapted from an industrial patent (6) and obviates many of the disadvantages inherent to the prior work just cited.

EXPERIMENTAL

Chemical reagents and special equipment. $K^{14}CNO$ from New England Nuclear, Amberlite IRA-400 (1.1 meq/ml, wet) from Sigma, and hydroxylamine hydrochloride from Aldrich were used as received. Reaction columns were the jacketed borosilicate glass Econo-Column brand (1 cm inner diameter by 30 cm length, 24 ml nominal volume) from Bio-Rad fitted at the bottom with a Luer 2-way stopcock. The reservoir top of the column was capped with a 1.5 cm rubber septum perforated to permit passage of a Yellow Springs Instuments YSI-402 thermister probe (0.28 cm diameter by 16.51 cm length) into the lumen of the column so that reaction temperatures could be monitored on a single channel, multirange telethermometer. Connection of the column jacket compartment to a Neslab refrigerated bath circulater (isopropanol/water) provided the necessary cooling.

Chemical and radiochemical purity analyses. The identity and homogeneity of reaction products were determined by thin layer chromatography using silica gel plates (0.250 cm thickness) with 1:4 10

N NH_4OH/ethanol (A) or 1:4 10 N NH_4OH/isopropanol (B) as eluants and 6% 4-dimethylamino-benzaldehyde (PDAB) in 3N H_2SO_4 or 5% $FeCl_3$ in water as visualizing reagents. Samples of standards and urea, as an internal reference, were applied adjacent to the reaction product spot; and a Packard Model 7230 chromatogram scanner was used to determine the radioactivity associated with each analyte after elution, but prior to reagent spray visualization. Melting points were measured in capillary tubes attached to a thermometer and immersed in a heated oil bath. A Varian 360A spectrometer provided NMR spectra.

[^{14}C] hydroxyurea. A reaction column was packed with 6ml (6.6 meq) of resin in the chloride form and the thermister probe inserted into the top 3 cm of the bed. A solution of 65 mg $K^{14}CNO$ (20.5 mCi, 20.5 mCi/mmol) in 10 ml water was added to the column by syringe and allowed to percolate slowly through the column into a beaker. The effluent was recycled three times and discarded. The jacketed column then was cooled by circulating the bath fluid at a temperature of 0-2° C. To start the reaction a pre-chilled solution of hydroxylamine hydrochloride (360 mg, 5mmol) in 5 ml of water was introduced through the septum cap, again by syringe, and onto the column at such a rate as to control the resulting exothermic reaction and maintain the bed temperature below 15°C.

The effluent at this stage of the reaction was collected in a 50 ml Virtis lyophilization flask, and the column was rinsed with an additional 15 ml of distilled water. A total of 20 ml of solution was collected and freeze-dried. The residue was extracted with three 50 ml portions of boiling diethyl ether, to remove soluble by-products, and then extracted into five 10 ml portions of warm methanol (60°C). The methanolic extracts were concentrated under reduced pressure at 20°C,

and the residual 60 mg of radioactive solid admixed with 100 mg of authentic hydroxyurea. Recrystallization from ethanol afforded 120 mg of colorless rosettes, (10.3 mCi, 6.5 mCi/mmol), mp 140-141°C. ^{1}H-NMR (ppm downfield from tetramethylsilane) (pyridine-d_5): 5.25 and 5.78 (m, 2H, NH_2), 6.65 (s) and 7.63 (m, 1H, NH), 8.5 (m) and 9.15 (broad s, 1H, OH); (acetone-d_6): 5.7 (broad m, 1-2H, NH_2), 7.65 (m, 0.5H, NH), 8.1 (m, 1.5H, NH and OH).

RESULTS AND DISCUSSION

The role of polymeric supports as aids in general organic synthesis is generally accepted to be significant (7). Reaction of hydroxylamine with ^{14}C-cyanate bound to a polymeric group transfer reagent offers a case in point, since it afforded ^{14}C-hydroxyurea on a micro-scale in 50% radiochemical yield - a yield higher than those obtainable by homogeneous solution chemistry procedures (4,5).

In addition to showing a sharp melting point, undepressed by admixture with authentic unlabeled material, the end-product of this radiosynthetic effort was found to be homogeneous by thin layer chromatographic radioscans and by development with spray reagents. At least 98.7% of its radioactivity co-chromatographed with authentic hydroxyurea at an R_f value of 0.75 in solvent A and 0.37 in solvent B. The appearance of a single, brilliant yellow spot upon chromatogram visualization with PDAB spray and of a single purple spot with aqueous $FeCl_3$ further indicated the absence of iso-hydroxyurea and related by-products (8). The NMR chemical shifts of ^{14}C-hydroxyurea also coincided with published spectra (9), and its biological activity was confirmed satisfactorily in a tracer study on interactions with 1-β-D-arabinofuranosylcytosine in cell culture (10).

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