A CONVENIENT SYNTHESIS OF ^{13}N-BCNU

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SUMMARY

A simple procedure is described for the preparation of millicurie quantities of the cancer chemotherapeutic agent, BCNU, labeled in the nitroso group with ^{13}N.

Key Words: Nitrogen-13, BCNU, Brain Tumor, Nitrous Acid

INTRODUCTION

We wish to report a simple, rapid and reliable method for the production of bis(2-chloroethyl)nitrosourea (BCNU) labeled in the nitroso group with the short-lived radionuclide, ^{13}N. Our interest in preparing this material with a positron-emitting radionuclide arises from the fact that BCNU is currently being effectively used in cancer chemotherapy programs for patients with brain tumors (1,2) and Hodgkin's disease (3) and yet the precise mode of action of the drug remains unknown. BCNU has been labeled with ^{14}C in the chloroethyl groups and in the carbonyl group in order to determine in vivo distribution of the drug and its metabolic fragments (4). The fate of the nitroso group remains unknown (5).

The preparation of ^{13}N-CCNU, a related drug, has been previously reported (6). However, specific activities of the material were too low for in vivo scintigraphy. The method described involved the oxidation of $^{13}NH_3$ produced

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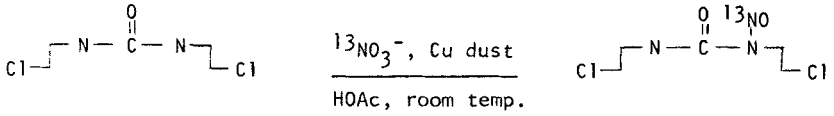
from the (d,n) reaction on methane gas to a mixture of ^{13}NO and $^{13}NO_2$. These gases in aqueous solution produced ^{13}N- nitrous acid which reacted with the parent urea to give the labeled nitroso derivative. An alternate method has been found which shortens the synthesis time, simplifies the chemical procedure and increases the specific activity of the product. Further the material can be prepared radiochemically pure.

EXPERIMENTAL

Our method involves the cyclotron production of $^{13}NO_3^-$ from the (p,α) reaction on water (7). Approximately 250 mCi of $^{13}NO_3^-$ is obtained by bombardment at 25 μ amps for 10 min. Following evaporation of the aqueous solution on a hot plate which requires about 20 min, the activity is removed from the beaker with 1.0 ml of a solution of acetic acid containing a small amount of the carrier HNO_3 and 5 mg of bis(2-chloroethyl)urea, prepared by the method of Bestian (8). The contents of the beaker are then poured onto 100-200 mg of copper dust in a centrifuge tube. The contents of the tube are vortex mixed and following a 4-5 min reaction period, the blue solution is diluted to 5-6 ml with water and immediately extracted with 1 ml of chloroform. The chloroform layer is then removed and extracted with 1-2 ml of saturated Na_2CO_3 solution to remove any acetic acid. Finally the chloroform layer is pipetted into a Pasteur pipet partially filled with anhydrous granular Na_2SO_4. A small amount of chloroform can then be used to rinse the column. Evaporation of the chloroform under a stream of N_2 affords the labeled drug.



The NO_3^- carrier solution is prepared by dilution of 1 ml of 8M HNO_3 to 160 ml with glacial acetic acid. The NO_3^- concentration of this solution is approximately 5×10^{-5} mole/ml (or 5×10^{-2}M), a slight excess of the molar amount of urea. Although the theory involved in this successful application of the reduction of nitric acid to nitrous acid is not completely understood, a partial explanation has been recounted by Travnicek and

Weber (9). In the oxidation of copper by nitric acid the production of nitrous acid has been postulated as the initial mechanistic step.

Typically radiochemical yields of 20-40% (corrected for decay) were obtained in a synthesis time of 15-20 min. Following evaporation of the chloroform layer radiochemical purity was ascertained by TLC on silica gel (Eastman) in chloroform. BCNU has a R_f = 0.40 in this solvent and could be detected under UV light. Authentic BCNU obtained from the National Cancer Institute migrated with the same R_f when it was mixed with freshly prepared samples of the labeled material.

	Radiochem. Results			TLC Results[c]		
Sample	Actual Yield[a]	% Yield[b]	Syn. Time	Origin	BCNU Spot	Solvent Front
1	2.5 mCi	37	18 min	200	4000	B.G.[d]
2	1.3 mCi	27	14 min	B.G.	1000	B.G.
3	3.4 mCi	19	23 min	150	22000	B.G.

a. activity of residue (BCNU) following evaporation of $CHCl_3$

b. based on activity of initial reaction mixture; corrected for decay

c. in cpm at surface of G-M counter

d. background activity range was 100-150 cpm

The preparation of ^{13}N-BCNU affords medical researchers a tool for investigation of its biological distribution and may aid in the understanding of its physiological action and its chemotherapeutic success.

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