NOTE

SYNTHESIS AND PURIFICATION OF ^{14}C N-2-HYDROXYETHYL-N-NITROSOUREA

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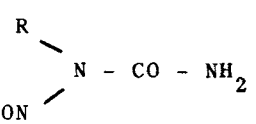
SUMMARY

^{14}C N-2-hydroxyethyl-N-nitrosourea was prepared at a specific activity of 30 mCi/mmol and 1.29 mCi/mmol by a two-step synthetic sequence using ^{14}C ethanolamine as the labelled precursor. Its purification was performed by HPLC using a Lichrosorb-DIOL column eluted by ethyl ether.The overall radiochemical yield was 10%.

Key words: Carbon-14, N-2-hydroxyethyl-N-nitrosourea, HPLC.

INTRODUCTION

Alkylating agents of the general structure



are potent carcinogens which do not require metabolic activation. Chronic carcinogenicity studies have been carried out predominantly with N-methyl-N-nitrosourea and N-ethyl-N-nitrosourea (1,2). The reactions of N-n-butyl-N--nitrosourea with DNA in vitro were studied by Ortlieb and

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Kleihues (3). The investigated N-alkyl-N-nitrosourea solutions are unstable at neutral pH and in 35-37°C range of temperature (4); therefore their significant degradation can be expected in the blood. Such a decomposition leads to the alkylation of the various cell constituents.

The pattern of products resulting from the reaction of alkylating agents with DNA varies considerably and depends on the chemical reactivity of the respective compound (5,6). In order to study another reactive nitrosourea we prepared ^{14}C labelled N-2-hydroxyethyl-N-nitrosourea, following the synthesis for "cold" material reported in the literature (7). A particular HPLC procedure was used for the purification of the product in order to avoid its decomposition.

EXPERIMENTAL

$\left[2\text{-}^{14}C\right]$ -ethan-1-ol-2-amine was purchased from Radiochemical Centre, Amersham, England, at a nominal specific activity of 30 mCi/mmol. The pH of the sample, that was furnished in 2.5 mL of aqueous solution, was adjusted to 2 by addition of 10 N HCl. 100 μL of a potassium cyanate solution (1.64 mol L^{-1}) were then added and the mixture was allowed to reflux for half an hour.

After cooling in an ice-bath, the solution was acidified with 20 μL of 5N sulphuric acid and 100 μL of a sodium nitrite solution (0.96 mol L^{-1}) were slowly added. The mixure was stored for one hour at 0°C and then was extracted four times with 3 mL of ethyl acetate. The extracts were put together, washed with water (1 mL), and dried on anhydrous magnesium sulphate.

The radioactive extract was analyzed by isocratic HPLC in a chromatographic system (System 1) constituted by a home-packed 10 μ Lichrosorb-DIOL column (l=25 cm.; i.d.=4 mm.) eluted at 1.0 mL min^{-1} by a mixture of ethyl ether- n-hexane (60:40). Since the chromatogram showed many radioactive impurities, the sample

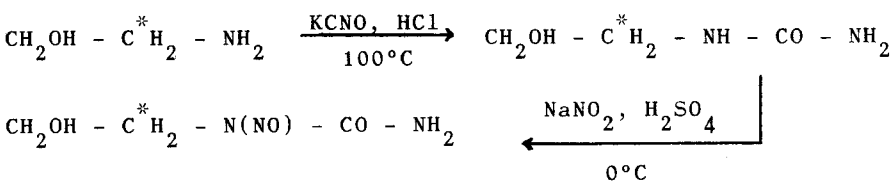
was purified by HPLC, injecting 0.5 mL portions of the ethyl acetate solution on the same column eluted by ethyl ether (System 2), and collecting the peak at an elution volume of about 12 mL.

The chemical purity of the collected sample was tested by HPLC (System 1), injecting 250 μL of the collected effluent. Its radioactive purity (98%) was confirmed by measuring the activity of the fractionated chromatographic effluent with a liquid scintillation spectrometer.

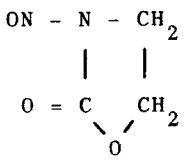
In order to complete the extraction, 1.12 mg of "cold" N-2-hydroxyethyl-N-nitrosourea, prepared as reported in the literature (7) and identified by ^{1}H nmr, were added to the aqueous solution which was extracted and purified by the same procedures described above.

RESULTS AND DISCUSSION

^{14}C N-2-hydroxyethyl-N-nitrosourea was prepared by the two-step reaction:



As reported in the literature (7), nitroso-oxazolidone is



formed in the same reaction in a comparable yield. Therefore, particular efforts were devoted to enhance the yield of nitrosourea compared to the cyclic product and to their separation.

The reaction conditions, reported in the experimental

section, ensured a yield of nitrosourea of 35-40%, as calculated in the cold syntheses we carried out.

As observed in inactive runs, this nitrosourea shows a high reactivity. Attempts to concentrate the ethyl acetate solution failed because the transformation of the product into an impurity which seems to act as a catalyst for such a reaction. We did not investigate its nature, but the observation that it is present as a by-product in the reaction mixture and that it is the predominant product contained in the methylene chloride extract, when we prepared the standard hydroxyethyl-nitrosourea following the method reported by Lijnsky and Reuber, suggests that it might be the nitroso-oxazolidone mentioned. Particular conditions of extraction were then selected in order to avoid a large volume of ethyl acetate which slowly reacts with the compound and at the same time to obtain a yield of the product as large as possible. The selected conditions, reported in the experimental part, ensure a yield of extraction of 79%.

Owing to the reactivity of hydroxyethyl-nitrosourea, unreactive solvents must be used for the HPLC separation. As recently suggested (8), ethyl ether and n-hexane do not react with fluoroethyl-nitrosoureas, but our attempts to separate the ethyl acetate extract on a silica gel column using such solvents failed: the analysis of the collected hydroxyethyl-nitrosourea showed the presence of the mentioned impurity. Therefore we utilized a polar bonded phase: Lichrosorb -DIOL.

The chromatographic pattern of the ethyl acetate extract analyzed in System 1 shows the presence of some more or less retained impurities, sharply separated from the N-2-hydroxyethyl-N-nitrosourea peak. Unfortunately, since our product is scarcely soluble in n-hexane, for a preparative purpose ethyl ether, which gives a poorer separation, must be used as the eluent. Under such conditions we collected only a part of the injected compound (61%).

The overall yield we obtained was 10% with respect to the

nominal activity of the starting ethanolamine.

The addition of the inactive carrier to the water solution allows a recovery of another 1 % of the radioactivity.

Starting from 1 mg of ethanolamine at a specific activity of 30 mCi $mmol^{-1}$ we obtained 46.015 μCi of product at the same specific activity and 5.277 μCi whose specific activity was 1.29 mCi/mmol.

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