SYNTHESIS OF ^{14}C-LABELLED COMPOUNDS. I. SYNTHESIS OF 2 (^{14}C)-DINITROSO-HEXAHYDROPYRIMIDINE.

Horst Braun, Barbara Bertram, and Manfred Wiessler
Institute for Toxicology and Chemotherapy, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, F.R.G.
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SUMMARY

A convenient procedure to synthesize 2-^{14}C-dinitroso-hexahydropyrimidine (DNHP) is described. The synthesis begins with the condensation of ^{14}C-formaldehyde and propylenediamine-1,3 in benzene with azeotropic removal of water. The resulting bases are isolated from the reaction mixture as hydrochlorides and are nitrosated with sodium nitrite in aqueous medium at a constant pH of 4 to 5. After column chromatography on Silica gel and recrystallisation from ether 2-^{14}C-dinitroso-hexahydropyrimidine (DNHP) is isolated in 30.7% yield based on ^{14}C-formaldehyde.

Key-Words: ^{14}C-Hexahydropyrimidine, Nitrosation, ^{14}C-Dinitroso-hexahydropyrimidine

INTRODUCTION

In spite of the wide-spread occurrence of dialkylnitrosamines in foods and drugs, the metabolism of these compounds has not yet been extensively investigated. More effort should be made to study the metabolism of cyclic nitrosamines, because all attempts to isolate any of their reaction products with DNA or RNA have been unsuccessful until now (1). This is in contrast to results with open chained compounds, like dimethyl- or diethylnitrosamine, where alkylated reaction products with DNA and RNA are well documented (2).

Recently the metabolism of 2,5-^{14}C-dinitrosopiperazine (DNP) in rats was investigated and published in detail (3). One of the isolated and identified metabolites was 3-hydroxy-N-nitrosopyrrolidine. Its formation was explained by the excision of one N-nitroso moiety leading to a ring contraction (Figure 1).

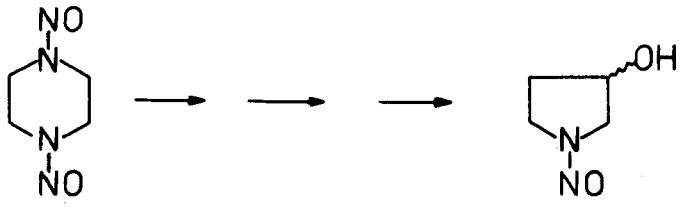

Fig. 1. Ring-contraction of Di-nitrosopiperazine

In order to prove whether this reaction also takes place in other cyclic dinitrosamines, we wanted to investigate the metabolism of dinitroso-hexahydropyrimidine (DNHP) which is an isomer of DNP. Radioactive substrates are necessary for such investigations. Therefore we first had to find a way to synthesize the radioactive compound.

This paper will describe the synthesis of 2-^{14}C-DNHP.

RESULTS AND DISCUSSION

The synthesis of hexahydropyrimidine described by Evans (4) (condensation of propylenediamine-1,3 and formaldehyde) leads,after a complicated purification procedure including a fractionated distillation, to very low yields of the pure compound. We therefore modified this method and converted the crude base mixture into the corresponding hydrochlorides which could be isolated as solids. The paper chromatogram reveals that,besides propylenediamine-dihydrochloride,there is at least one other hydrochloride. This mixture was analyzed as containing 30.71% C, 7.79% H, 15.19% N and 42.65% Cl. The calculated values for hexahydropyrimidine-dihydrochloride are 30.19% C, 7.55% H, 17.61% N and 44.65% Cl, and the corresponding values for propylenediaminedihydrochloride are 24.50% C, 8.23% H, 19.05% N and 48.22% Cl.

Since DNHP is a solid which can be purified by recrystallisation, the mixture of hydrochlorides could be nitrosated without purification.

MATERIALS AND METHODS

General: ^{14}C-formaldehyde (specific activity 22 mCi/mmole + 96% radiochemical purity) was purchased from Radiochemical Center, Amersham, Bucks. England.

Precoated Silica gel plates (20 x 20 cm, F-254, E. MERCK, Darmstadt, F.R.G), predeveloped in the solvent system hexan/ether/dichloromethane 50/70/100 were used for thin-layer chromatography. Silica gel (Woelm 0.063 - 0.2 mm, 70 - 230 mesh Nr. 04667) was used for column chromatography and eluted with the same solvent system. For paper chromatography (Schleicher and Schüll Nr. 20436) we used an upper phase of butanol/ether/water 40:10:50 (descending, two days). Radiochemical purity of the product was measured on thin-layer chromatograms by an LB 2723 thin-layer scanner (Berthold, Wildbad, F.R.G.). Activity was determined in a Mark III liquid scintillation counter from Nuclear Chicago.

PILOT SYNTHESIS:

740 mg of trimethylenediamine (10 mmole) in 150 ml of benzene are added to a solution of 300 mg formaldehyde (10 mmole) in 9 ml of water. This mixture was stirred in a closed vessel for 70 hrs at room temperature. The water was removed by azeotropic distillation with a Dean-Stark tube. The resulting benzene solution was dried over solid potassium hydroxide (10 grams) overnight. The drying agent was separated by filtration and washed twice with 30 ml portions of

benzene. Then dry hydrogenchloride was bubbled through the benzene solution. The resulting hydrochlorides separate in the solution as white crystals. After 1 hr the crystals were collected by careful filtration and dried to yield 1.4 gr of the hydrochloride mixture.

These hydrochlorides were placed in a 100 ml Erlenmeyer flask, dissolved in 5 ml of water and covered with dichloromethane (50 ml). The flask was cooled in an ice water bath. Then a solution of 1.38 g(20 mmole) sodium nitrite in 2 ml of water was added from a dropping funnel for a period of 1 hr. During this time the pH was controlled by a pH-meter and adjusted to pH 4.5 by addition of 0.02 n-hydrochloric acid, when necessary. After further stirring for 30 minutes the dichloromethane was removed in a separatory funnel and the water phase was extracted twice with 50 ml portions of dichloromethane. The combined organic phases were dried over sodium sulfate and evaporated in vacuo at 20°C to yield the crude DNHP (1.1 g). The nitrosamine was purified by column chromatography (50 x 4 cm). The fractions containing the nitrosamine (as checked by thin-layer chromatography) were collected and the crystalline material was recrystallized twice from absolute ether. The recovery was 462 mg, corresponding to a 32% yield based on formaldehyde.

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^{14}C-formaldehyde (6.8 mg in a 3% water solution, 5000 µCi) was diluted to 10 ml (300 mg, 10 mmole) with a 3% water solution of inactive material. All other manipulations followed the procedure described in the pilot synthesis. The yield of the twice recrystallized 2-^{14}C-dinitroso-hexahydropyrimidine was 442 mg (3.07 mmole), 30.7% of the theoretical value based on formaldehyde.

To test the radiochemical purity, a CH_2Cl_2 solution was spotted on a TLC plate, developed and scanned (Figure 2)

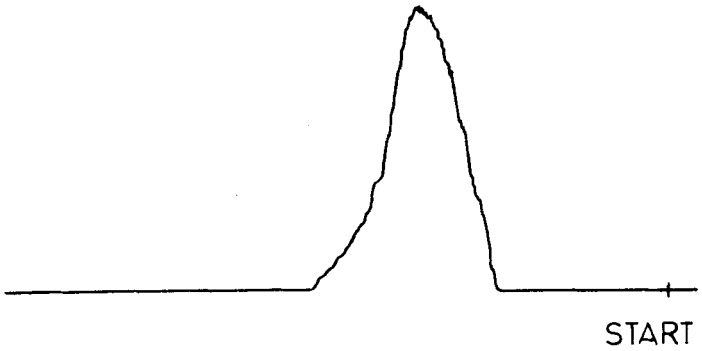

Fig. 2. Thin-layer scan of 2-^{14}C-Dinitrosohexahydropyrimidine.

The specific activity as determined by liquid scintillation counting (corrected) was 470 µCi/mmole and agreed with 480 µCi/mmole, which was computed from the specific activity of ^{14}C-formaldehyde and corrected for radiochemical purity.

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