# THE SYNTHESIS OF N-NITROSOHEXAMETHYLENEIMINE LABELED WITH $^{14}C$ IN THE 2-POSITION

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## SUMMARY

*A synthetic procedure for 2-$^{14}$C-N-nitrosohexamethyl-eneimine [1-aza-1-nitroso-(2-$^{14}$C)-cycloheptane] is presented. The starting material was 1-$^{14}$C-cyclohexanone which underwent ring expansion, reduction, and finally nitrosation. The synthesis resulted in a product in good yield (68% overall), which is chemically and radiochemically pure.*

## INTRODUCTION

N-nitrosamines are an important class of carcinogens because of their efficacy in many organs of many different species of animals and because of their potential as human health hazards[1,2,3]. The biological mode of action of these compounds has been the subject of many investigations[1,2]. However, the majority of the accumulated data has dealt with the simple dialkyl N-nitrosamines undoubtedly due to their commercial availability or ease of synthesis of the $^{14}$C-labeled compounds. In order to aid in the complete elucidation of their mode of action, it is necessary to also study the more complex N-nitrosamines (i.e. the cyclic N-nitrosamines).

Preliminary experiments* have shown that $^{3}H$-labeled N-nitrosohexamethyleneimine (NHX) is very unsatisfactory for biochemical studies. Hence, the synthesis of $^{14}C$-labeled NHX is a necessary requirement for further experiments. Furthermore, because of the reported importance of the 2-position in these compounds,[2,4] this is the position which we wished to label with carbon-14.

In this paper we wish to present the synthesis of 2-$^{14}C$-N-nitrosohexamethyleneimine from 1-$^{14}C$-cyclohexanone**. The synthetic route is presented in the reaction scheme (see page ). Intermediate determinations of purity have indicated that no further purifications are necessary throughout the entire procedure presented below.

## EXPERIMENTAL PART

All of the purity determinations presented below were by GLC on a Beckman GC-4. The conditions and column types that were used were: (1) Hexahydro-2H-(2-$^{14}C$)-azepin-2-one (I) - 5.6% Versamide on 80 - 100 mesh Chromasorb G at 200°C, (2) 1-aza-(2-$^{14}C$)-cycloheptane (II) - 28% Penwalt 223 • 4% KOH on Gas Chrom R at 152°C, and (3) 1-aza-1-nitroso-(2-$^{14}C$)-cycloheptane (III) - 8% DEGS on 80 - 100 mesh Chromasorb W at 140°C.

### Hexahydro-2H-(2-$^{14}C$)-azepin-2-one (I)[5]

To a solution of 175 mg (1.79 mmol) of 1-$^{14}C$)-cyclohexanone in 10 ml of concentrated hydrochloric acid was added 200 mg of sodium azide in one portion.

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*C.J. Grandjean, Unpublished Results.

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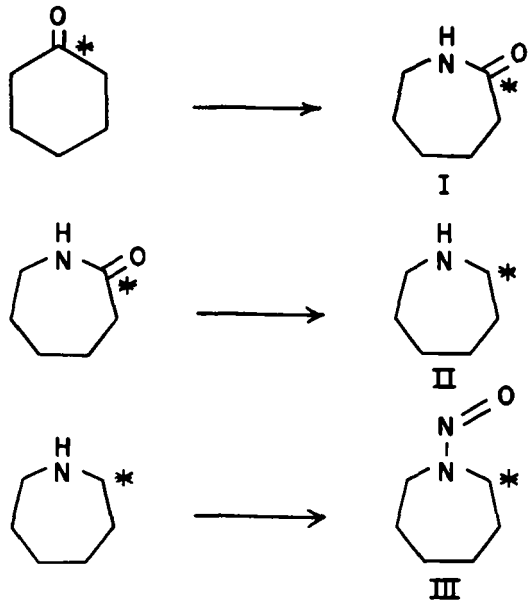
Reaction Scheme




Preparation of N-Nitrosohexamethyleneimine

The mixture was stirred for four hours at 0°C and then saturated with solid $K_2CO_3$. Compound I was isolated by extraction with three 10 ml portions of chloroform. The solvent was then removed with a stream of nitrogen. Yield 1.56 mmol (94%) as determined by GLC. No detectable GLC impurities were observed and the melting point (63 - 64°C) supported the GLC data.

1-aza-(2-$^{14}$C)-cycloheptane (II)

To a solution of 176 mg (1.56 mmol) of I in 20 ml dry benzene was added 300 mg of $LiAlH_4$. The mixture was refluxed for 44 hours at which time the complex was decomposed by adding 10 ml of water dropwise. This was followed by adding 1 ml of a 50% (w/w) solution of NaOH in water. The aqueous phase was

extracted with benzene, and the resultant benzene solution was then extracted three times with 5 ml portions of 0.5 N hydrochloric acid. The free base (Compound II) was subsequently regenerated by saturating the acidic solution with solid $K_2CO_3$ and extracted with diethyl ether. Yield 1.52 mmol (98%) as determined by GLC. Again, no impurities were detected by GLC.

1-aza-1-nitroso-(2-$^{14}$C)-cycloheptane (III) [N-nitrosohexamethyleneimine]

To a solution of 150 mg (1.52 mmol) of II in 15 ml of Na-dried diethyl ether was added 200 mg $K_2CO_3$ and a 5 ml aliquot of a solution of 500 mg (7.7 mmol) of NOCl in 25 ml Na-dried diethyl ether. The mixture was stirred at 0°C in subdued light for 24 hours and then washed three times with 3 ml aliquots of a 25 ml aqueous solution containing 200 mg of $K_2CO_3$ and saturated with NaCl (pH = 10.5). Yield 1.22 mmol (80%) as determined by ultraviolet absorption ($E^{365}_{1cm}$ = 143). GLC of the final product revealed no impurities.

RADIOCHEMICAL PURITY

The ether containing the final product III was evaporated carefully with a stream of nitrogen and the product was dissolved in 10 ml of distilled water. A small aliquot of the aqueous solution was developed by thin layer chromatography using diethyl ether:hexane (1:4 v/v). The chromatogram was subsequently fixed with iodine and scanned for radioactivity*. Only one radioactive peak was observed, and it had the same Rf value as an authentic sample of N-Nitrosohexamethyleneimine. The final product III retains this purity while being stored in distilled water at a reduced temperature. (This work was supported by contract PH43-NCI-E-68-959 from the National Cancer Institute.)

*Packard Radiochromatogram Scanner Model 7201

## LITERATURE

1. Magee, P.N. and Barnes, J.M., Advances Cancer Res., 10, 163 (1967).
2. Druckrey, H., Preussmann, R., Ivankovic, S. and Schmähl, D., Z. Krebsforsch., 69, 103 (1971).
3. Magee, P.N., Fd. Cosmet. Toxciol., 9, 207 (1971).
4. Druckrey, H., Preussmann, R. and Ivankovic, S., Ann. N.Y. Acad. Sci., 163, 676 (1969).
5. Smith, P.A., J. Amer. Chem. Soc., 70, 320 (1948).