SYNTHESIS OF ^{14}C-LABELLED COMPOUNDS. II. SYNTHESIS OF ^{14}C-METHYLACETOXY METHYLNITROSAMINE

Horst Braun and Manfred Wiessler
Institute for Toxicology and Chemotherapy, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, F.R.G.
Received July 12, 1976

SUMMARY

1,3,5-^{14}C-trimethylhexahydro-1,3,5-triazine was prepared by condensing ^{14}C-methylamine-hydrochloride and formaldehyde in a concentrated aqueous solution. The so formed triazine reacts with Nitrosylchloride at 0°C to ^{14}C-methylchloromethylnitrosamine which was not isolated and reacted further with silver acetate. After column chromatography on Silica gel and distillation ^{14}C-methylacetoxymethylnitrosamine was obtained in a 24.1% yield based on ^{14}C-methylamine-hydrochloride.

Key-Words: ^{14}C-1,3,5-Trimethyl-hexahydrotriazine, Nitrosylchloride, ^{14}-Chlormethylnitrosamine, ^{14}C-Dimethylnitrosamine-α-acetate

INTRODUCTION

Nitrosamines are well recognized carcinogens and their widespread occurrence directs our special attention to the metabolism of this class of compounds. The proposed metabolism (1) of dimethylnitrosamine is shown in Figure 1. According to pathway A hydroxylation by drug metabolizing enzymes (2) should lead to methyl-hydroxymethylnitrosamine which is transformed to methyl-diazohydroxide and formaldehyde. The original compound itself or its breakdown products serve as the alkylating agents in methylation of DNA or RNA (1).

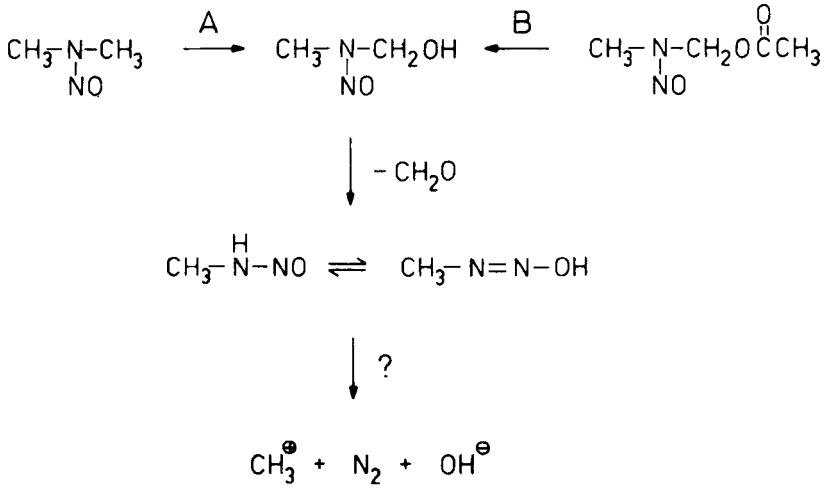

Fig. 1. Proposed metabolism of dimethylnitrosamine in relation to methyl-acetoxymethyl-nitrosamine.

© 1977 by John Wiley & Sons, Ltd.

Methyl-acetoxymethyl-nitrosamine was synthesized in 1975 on different routes by two groups (3). According to pathway B this compound gives, after hydrolysis by water (4) or esterase (3a, 5), the same methyl-hydroxy-methylnitrosamine which also results from enzymatic activation in pathway A. When assuming that the proposed mechanism is correct, this ester could serve as a pool for the ultimate carcinogen of dimethylnitrosamine. Our prediction that this compound is a locally active carcinogen was confirmed experimentally (6). Chronic oral application in water to rats produces carcinomas of the forestomach in a 90% yield. Testing for mutagenicity was also positive (7). These findings confirmed the conclusions drawn above.

For a more detailed study of this compound's metabolism and alkylating ability it is necessary to have it ^{14}C-labelled. In this paper we will describe the synthesis of ^{14}C-methylacetoxymethylnitrosamine.

RESULTS AND DISCUSSION

Imines react at -30°C with nitrosylchloride in dichloromethane to α-chloronitrosamines which on further reaction with silver acetate give α-acetates in moderate to good yields (8). The N-methyleneamines are not stable in the monomeric form but trimerize to 1,3,5-trialkyl-hexahydrotriazines-1,3,5. (Figure 2).

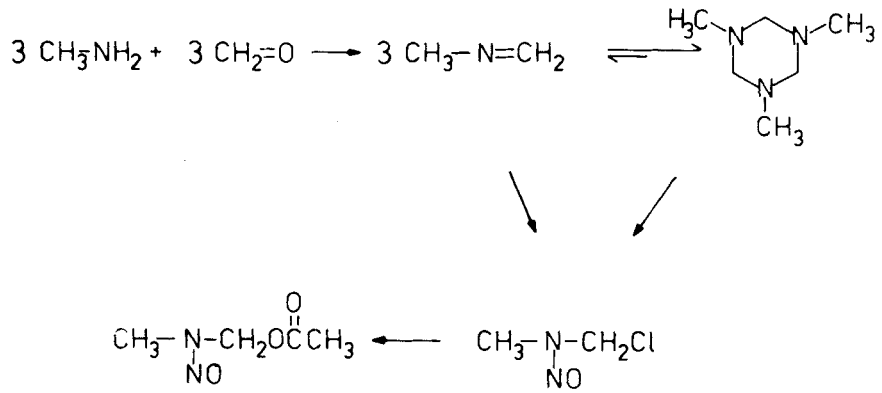

Fig. 2. Scheme for synthesis of methyl-acetoxymethyl-nitrosamine.

An equilibrium between monomers and trimers exists, but in the case of N-methylenamine, an intermediate for our synthesis, there is no indication of such an equilibrium (9). In spite of this disadvantage 1,3,5-trimethyl-hexahydrotriazine-1,3,5 (which is formed by the reaction of methylamine with formaldehyde) reacts with nitrosylchloride at 0°C to give methyl-chloromethylnitrosamine (which can be isolated) (10). Then, after the addition of silver acetate the desired compound can be isolated in a 24% yield (Figure 2). At least two byproducts were formed. They were separated by column chromatography. The structure of one of these byproducts has been determined (11) and it gives some indication that

nitrosylchloride attacks the hexahydro-triazine directly. The formation of methyl-chloromethylnitrosamine probably also results from a direct attack at the trimeric molecule and not only from reaction of nitrosylchloride with the monomeric form.

MATERIALS AND METHODS

General: ^{14}C-methylamine hydrochloride, specific activity 591 µCi/mg (99% pure) was purchased from Amersham Buchler, Bucks., England. Radioactivity was measured in a Nuclear Chicago Mark III scintillation counter.

The radiochemical purity of the product was measured on thin-layer chromatograms by an LB 2723 thin-layer scanner II (Berthold, Wildbad, F.R.G.). Precoated Silica gel plates (5 x 20, F-254, E. MERCK, Darmstadt, F.R.G.) (predeveloped in the solvent system hexane/ether 4:1) were used for thin-layer chromatography. Silica gel (Woelm, 0.063 - 0.2 mm, 70 - 230 mesh, Nr. 04667) was used for column chromatography. Nitrosylchloride was synthesized (12) and purified by rectification.

PILOT SYNTHESIS OF INACTIVE ACETATE

1) Preparation of 1,3,5-trimethyl-hexahydro-triazine-1,3,5

To 1.35 g (20 mmole) methylamine-hydrochloride in a 25 ml three necked flask fitted with a reflux-condenser (cooled with icecold water) and dropping funnel 1.62 ml (20 mmole) of formaline (37% aqueous solution) was added. While cooling with ice/water, a solution of 4 g (100 mmole) sodiumhydroxide in 10 ml of water was slowly added through the dropping-funnel. The cooling bath was removed and the solution stirred for two hours at room temperature. The solution was transferred to a liquid-liquid extractor (the flask was rinsed with 1 ml of water) and extracted with ether overnight (14 hrs). The ether solution was separated and dried over 10 g of potassium hydroxide overnight. After filtration and washing of the potassium hydroxide with 30 ml of ether, the solution was concentrated to 8 ml on a rotating evaporator at 40°C without vacuum. The resulting solution was used without further purification.

2) Preparation of methyl-acetoxymethyl-nitrosamine.

For this reaction we used a 100 ml three necked round bottomed flask fitted with an internal thermometer and a dropping funnel. 16 ml of a 1 molar solution of nitrosylchloride in dichloromethane was placed in the flask, cooled in an ice/water bath and stirred with a magnetic stirrer. Through the dropping funnel 1,3,5-trimethylhexahydrotriazine-1,3,5 dissolved in 20 ml of dichloromethane was gradually added during the course of 1.5 hrs and the temperature was maintained below +5°C. After the dark brown colour of the nitrosylchloride had faded to a bright yellow, 3.35 g of silver acetate (20 mmole) were added in small portions in order to keep the temperature from raising over +5°C. The resulting suspension was stirred overnight in the dark. After filatration and se-

veral washings of the residue with dichloromethane, the obtained solution was concentrated in a rotating evaporator at 30°C and a vacuum of 200 Torr. The residue was placed on a Silica gel column (50 cm high and 1.5 cm in diameter) and eluted with hexane/ether 4:1. 30 ml fractions were collected, plated on TLC plates and searched for the α-acetate, which was eluted as the first compound. The fractions containing the compound were collected and evaporated to dryness at 30°C and 100 Torr vacuum. In a small shortway distillation apparatus the α-acetate was distilled at 10^{-1} Torr, with a hair dryer as heating source (13). 623 mg (4.7 mmole) of methyl-acetoxy-methylnitrosamine were obtained analytically pure in 23.6% yield based on methylaminehydrochloride.

SYNTHESIS OF ^{14}-C-METHYL-ACETOXYMETHYLNITROSAMINE

6.76 mg ^{14}C-methylamine-hydrochloride (3995 µCi) was dissolved in water, diluted with 1.343 g inactive compound, transferred to a 25 ml three necked flask and evaporated to dryness. All other manipulations followed the procedure described in the pilot synthesis. The yield of distilled ^{14}C-methyl-acetoxymethyl-nitrosamine was 635 mg (4.82 mmole) (24.1% of the theoretical value). To test the radiochemical purity, a CH_2Cl_2 solution was spotted on a TLC plate, developed and scanned (Figure 3)

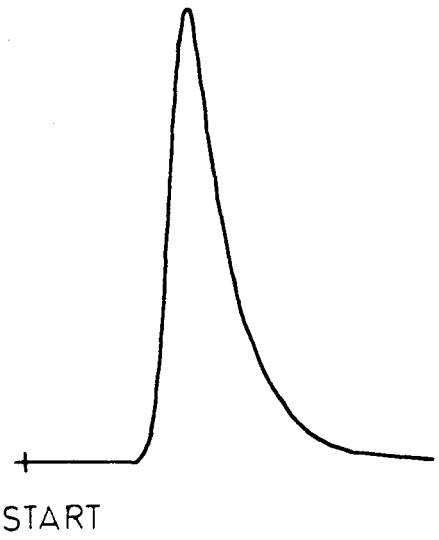

Fig. 3. Thin layer scan of ^{14}C-Methyl-Acetoxymethyl-nitrosamine.

The specific activity determined by liquid scintillation counting (corrected) was 196 μCi/mmole and agreed closely with 201 μCi/mmole which was computed from specific activity of ^{14}C-methyl-aminehydrochloride.

References:

1) P.N. MAGEE, J.W. NICOLL, A.E. PEGG and P.F. SWANN, - Biochem. Soc. Transactions 3: 62 (1975)
P.N. MAGEE and J.M. BARNES, - Advances Cancer Res. 10: 163-246 (1967)
P. BROOKES, - Life Sciences 16: 331 (1975)
See also R. SCHOENTAL, - Brit. J. Cancer 28: 436 (1973)

2) B.G. LAKE, M.J. MINSKI, J.C. PHILIPPS, S.D. GANGOLLI and A.G. LLOYD, - Life-Sciences 17: 1599 (1975)
C.N. FRANTZ and H.V. MALLING, - Cancer Research 35: 2307 (1975)

3a) P.R. ROLLER, D.R. SHIMP and L.K. KEEFER. - THL 2065-8 (1975)

b) M. WIESSLER, - THL 2575-8 (1975)

4) N. FRANK and M. WIESSLER, - to be published in Chem. Ber.

5) The estimated half-life of Ethylacetate in rats is in the range of 5 to 10 minutes. See E.J. GALLAHER and T.A. LOOMIS, - Tox. appl. Pharm. 34: 309 (1975)

6) M. WIESSLER and D. SCHMÄHL, - Z. f. Krebsforschung 85: 47 (1976)

7) O.G. FAHMY, M.J. FAHMY and M. WIESSLER, - Biochem. Pharmacol. 24: 1145 (1975)

8) M. WIESSLER, - Angew. Chem. 86: 817 (1974)
Angew. Chem. Int. Edition 13: 743 (1974)

9) D.G. JONES, - Chem. Ind. 705 (1975)

10) After distillation 25-30% yield $Kp_{0.1}$ 24°C [3b] unpublished results.

11) From mass-spectroscopic data and NMR-data the structure of one byproduct was determined as $CH_3-\underset{NO}{N}-CH_2-\underset{NO}{N}-CH_3$. This could result from hexahydro-s-triazine by direct attack only.

12) Nitrosylchloride was synthesized from Nitrosylhydrogensulfate and Sodiumchloride according to Brauer, Handbuch der präparativen anorganischen Chemie. Volume 1, F. ENKE VERLAG, STUTTGART, 1975.

13) The reported boiling points are Kp_{32} 113°C [3a] and $Kp_{0.5}$ 36°C [3b]