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SYNTHESIS OF [^{14}C] LABELLED N-NITRO METHYLAMINE AND N-NITRO DIMETHYLAMINE.

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Dedicated to Prof. Dr. R. Preussmann, on the occasion of his 60th birthday

SUMMARY

N-nitro [^{14}C]methylamine was synthesized by the alkaline cleavage of N-nitro[methyl-^{14}C] methylurethane obtained by nitration of [N-methyl-^{14}C] methylurethane. N-nitro[N-methyl-^{14}C]dimethylamine could be obtained by methylation of monomethylnitramine with [^{14}C] diazomethane.

KEY WORDS

[N-methyl-^{14}C]methylurethane, N-nitro[^{14}C]methylamine, [^{14}C]diazomethane, N-nitro[N-methyl-^{14}C] dimethylamine.

INTRODUCTION

N-nitrodialkylamines, oxidation products of the highly carcinogenic N-nitrosodialkylamines are not as powerful carcinogens as the nitroso compounds. Nevertheless, N-nitrodimethylamine given in drinking water was found to be hepatocarcinogenic in rats by different authors (1,2,3,4). In all these investigations the absence of traces of N-nitrosodimethylamine in the crystalline N-nitrodimethylamine was checked by gas-chromatography. Our own experiments revealed that purified N-nitrodimethylamine given by gavage produces neurogenic tumors of the nasal cavity classified as esthesioneuroepithelioma (5). Metabolic demethylation of N-nitrodimethyl-amine, in analogy to N-nitrosodimethylamine, results in N-nitromonomethylamine (6). This compound was also carcinogenic itself and induced neurogenic tumors of the

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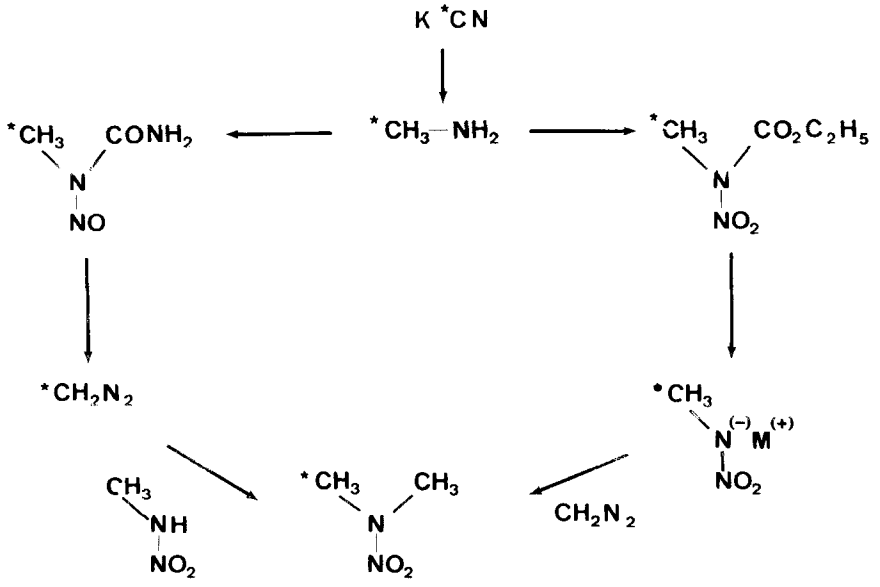
spinal cord (5). Esthesioneuroepithelioma were observed after inhalation of N-nitrosodimethylamine (1) as well as after administration of the nitrosamine by gavage. Tumors of the spinal cord were not seen. For metabolic and DNA-methylation studies, the two N-nitrocompounds were synthesized ^{14}C labelled from $K^{14}CN$. Principally two reaction pathways can be drawn as shown in Scheme 1. Pathway B involves N-nitro[^{14}C]methylamine isolated as the ammonium salt. Since this salt reacts very slowly with diazomethane, pathway A is more suitable since crystalline nitrosamine-free monomethylnitramine can be reacted with [^{14}C]diazomethane.



Scheme 1: Reaction scheme for the synthesis of N-nitro mono- and N-nitro di-methylamine.

In both cases the last step is the critical one, since N-nitromethylamine is ambident as a nucleophile producing N and O methylation products. In contrast to the literature (7) the N/O ratio was not influenced by the solvent polarity in our hands (8). Unfortunately, this N/O ratio is 1 : 2, producing the desired compound only as a minor product.

The oxidation of nitrosamines to nitramines by H_2O_2 under acidic conditions reported in the literature (9) was not considered, since purification of N-nitrodimethylamine obtained by this method from traces of the starting nitrosamine by HPLC methods seems to be difficult. N,N-dimethylnitramine crystallizes very well but is extremely volatile. These same arguments hold for the nitration of [^{14}C]dimethylamine (10).

We decided to synthesize N-nitromethylamine by alkaline cleavage of N-nitro- N-methylurethane (pathway B) (11) and N-nitro dimethylamine by pathway A.

MATERIALS AND METHODS

The synthetic intermediates were characterized in trial experiments using unlabelled material. Reaction products in the labelled synthesis were monitored by TLC or HPLC and compared to authentic unlabelled material. All solvents used were of analytical grade. HPLC measurements were done on a Hewlett Packard HP 1090. Radio activity was determined using a Mark III liquid scintillation counter from Nuklear Chicago. $K^{14}CN$ (specific activity 54.7 mCi/mmol) was purchased from Hoechst AG, Frankfurt, FRG.

[N-methyl-^{14}C]Methylurethane:

$K^{14}CN$ (52.0mg, 0.8mmol, 43.8mCi, spec. activity 54.7 mCi/mmol) was mixed with KCN (13mg, 0.2 mmol) and hydrogenated in acidic solution according to published procedures (12). The consumption of H_2 was followed until completion of the reaction. After evaporation, the crude methylamine HCl (as a mixture with salts) was dissolved in 3 ml H_2O, cooled to 5°C, ethylchloroformate (123 μ, 1mmol) was added by syringe and finally 1 ml of 2N NaOH. After stirring for 30 min at room temperature, the solution was extracted five times with 10ml diethylether and the combined organic phase was dried over Na_2SO_4. The ether was removed by column distillation.

N-nitro[N-methyl-^{14}C]Methylurethane:

The crude N-methylurethane was dissolved in 0.2 ml acetic anhydride, cooled to 5°C, and 75 μl nitric acid (100%) were added by syringe. After 30 min at room temperature crushed ice was added and the solution was extracted four times with ether (10 ml). The combined ether extracts were washed with saturated K_2CO_3 solution (3 times), finally with H_2O (2 times), and dried over $MgSO_4$. Purification was achieved by column chromatography (Si60) eluting with pentane/ether 4:1, and the chemical purity was checked by HPLC (Si60-5μ, 250 x4.6 mm, n-hexane/ethylacetate 97/3, flow 1.0ml, detection: UV 254 nm). N-nitro-N-methylurethane: 12.44 min; N-nitroso-N-methylurethane: 7.71 min). Yield 58 mg corresponding to 39% based on $K^{14}CN$.

N-nitro[^{14}C]methylamine, potassium salt:

N-nitro[N-methyl-^{14}C]methylurethane (58 mg, 0.39 mmol) were dissolved in diethylether (5 ml) and a slow stream of dry ammonia was passed through the solution for 30 min. After 12 hours at room temperature the diethylether was removed by a slight vacuum, the residue dissolved in 2ml of water and 250 mg Dowex H^+ form were added. After 20 min. at room temperature, Dowex H^+ was removed by filtration and the solution treated with 250 mg Dowex K^+ for 20 min. After

filtration, the water was removed and the residue dried over P_2O_5. N-nitro[^{14}C]methylamine potassium salt (33 mg) was obtained in a 29% yield based on K^{14}CN. Analytical purity was checked by HPLC (Fig. 1).

N-nitro[methyl-^{14}C]dimethylamine:

N-nitroso[N-methyl-^{14}C]methylurea was synthesized by conventional methods starting with K^{14}CN (12). Crystalline N-nitroso [N-methyl-^{14}C] methylurea could be isolated in 48% yield based on K^{14}CN and was used without further purification for the generation of [^{14}C]diazomethane.

N-nitroso[N-methyl^{14}C]methylurea (20.6 mg, 0.2 mmol) were placed together with pentane (5 ml) in a 25ml round-bottomed flask, connected with a distillation apparatus, which contained N-nitromethylamine (14.1 mg, 0.8 mmol) dissolved in pentane (1 ml) in a receiver cooled to -80^{o}C. Potassium hydroxide (1 ml, 50% aqueous solution) was added to the nitrosourea and this was stirred for 10 min. at room temperature. The flask was then heated in a water bath (50^{o}C) and the diazomethane distilled together with the pentane into the receiver. When the pentane was completely distilled, the receiver was closed carefully and the temperature raised to 0^{o}C. At this temperature the reaction of [^{14}C]diazomethane and N-nitromethylamine took place. After 24 hours the flask was opened and the solution was placed on a column filled with 5g Al_2O_3 (activity V), and this was eluted with diethylether. The fractions which contained N-nitrodimethylamine (checked by TLC) were combined and the solvents were removed by column distillation as far as possible. Traces of solvent were removed using a gentle stream of N_2. N-nitro[methyl-^{14}C]dimethylamine (3.6 mg, 0.04 mmol) was obtained corresponding to 20% yield based on [^{14}C]diazomethane. Radiochemical purity was checked by HPLC (Fig. 2).

RESULTS AND DISCUSSION

N-nitromethylurethane, the intermediate for the synthesis of N-nitromethylamine, is contaminated up to 1% with N-nitrosomethylurethane, as checked by HPLC. In spite of repeated chromatography on Si60 the N-nitromethylurethane could not be obtained free of nitrosamine. Since the alkaline cleavage of N-nitrosomethylurethane produces only unstable methyldiazotate, the obtained N-nitromethylamine was free of nitroso compounds.

The alkylation of N-nitromethylamine by diazomethane produces N and O methylation products since N-nitromethylamine behaves as an ambident nucleophile. In pentane as solvent O-methylation was twice the amount of N-methylation, a ratio which is independent of solvent. Since the O-methylation product is so extremely volatile and much more unpolar than N-nitrodimethylamine, separation is easy to achieve. To assure that the [^{14}C]diazomethane had completely reacted, N-

nitromethylamine was used in excess. Separation of N-nitromethylamine and N-nitrodimethylamine was achieved by chromatography on basic aluminium oxide since N-nitromethylamine was not eluted under these conditions.

The nitration of [^{14}C]dimethylamine, which is the method of choice for the synthesis of N-nitrodimethylamine on a preparative scale, cannot be used in the radioactive synthesis. N-nitrodimethylamine can be purified from traces of N-nitrosodimethylamine by consecutive crystallizations, a method which is not suitable for a μmolar scale. Separation of traces of N-nitrosodimethylamine from N-nitrodimethylamine was not possible by chromatographic methods. Since the absence of N-nitrosodimethylamine is necessary for the interpretation of DNA alkylation studies with N-nitrodimethylamine, the reaction of nitroso free N-nitromethylamine with diazomethane was choosen.

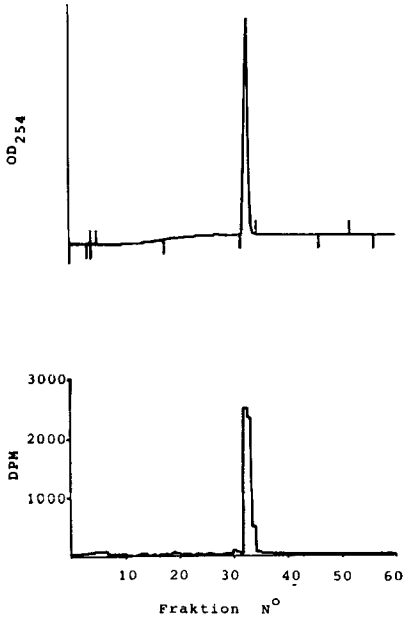


Fig. 1

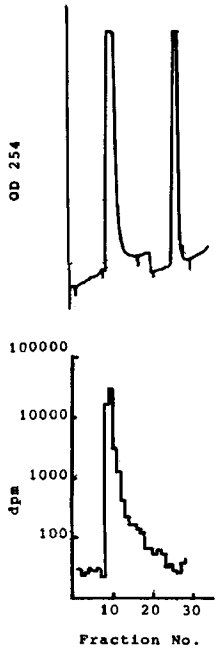


Fig. 2

HPLC chromatograms of N-nitro[^{14}C]methylamine (Fig. 1) and N-nitro[methyl-^{14}C]dimethylamine (Fig. 2) spiked with inactive standards. Monomethylnitramine is also added in Fig. 2. (RP 18, 250 x 4.6, 10 mM tetrabutyl-formiate pH 7.0, 1 ml/min, UV 254.4).

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