SYNTHESIS OF ^{14}C-LABELLED NITROSAMINES. IV SYNTHESIS OF ^{14}C-METHYL-(1-ACETOXY)METHYL NITROSAMINE AND 1-^{14}C-ETHYL(ACETOXY)ETHYL NITROSAMINE.

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SUMMARY

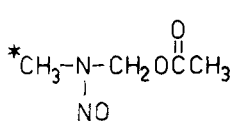
A scheme is developed for synthesizing ^{14}C labelled α-acetates of secondary α-hydroxylated nitrosamines. In this manner ^{14}C-methyl(1-acetoxy)ethyl nitrosamine and 1-^{14}C-ethyl(1-acetoxy)ethyl nitrosamine are synthesized in 16.3 and 33.3% yield, radiochemically pure.

Key-words: ^{14}C-Methyl(1-acetoxy)methyl nitrosamine, 1-^{14}C-ethyl(1-acetoxy)ethyl nitrosamine, addition of nitrosylchloride to imines

INTRODUCTION

Nitrosamines are now recognized as a group of carcinogens which are widely distributed in foods and drugs, being human hazards [1]. Their metabolism is well studied concerning biological actions [2] (alkylation)but there are only few informations about the intermediates in this metabolic scheme [3].

As an entry for studying nitrosamine metabolism we have synthesized ^{14}C labelled acetoxymethyl-methyl-nitrosamine 1 as a "stabilized" hydroxymethylmethylnitrosamine which can be formed from the former by hydrolisis by water or esterases [4]. The same synthetic scheme



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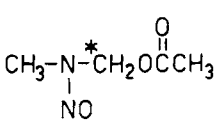

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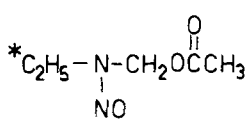

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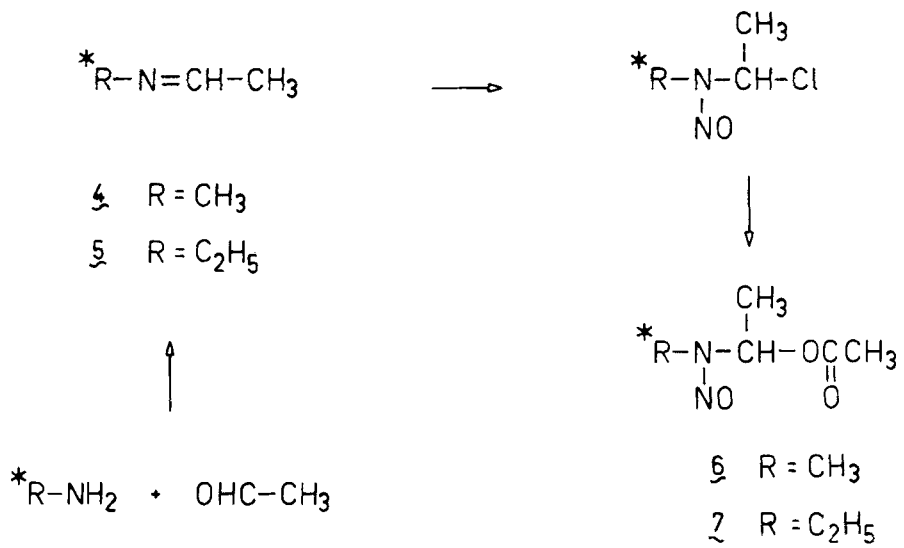
can be used for the synthesis of the corresponding ^{14}C-methylene labelled compound 2 [5] and the higher homologue 3 with the label in the ethyl($^{14}C_1$) group[6]. So the published procedure seems to be well suited for synthesizing any alkyl-acetoxy-methyl-nitrosamine, labelled in the alkyl-group or in the methylene group. However, these compounds are derived solely from primary acetates. For the synthesis of secondary acetates for instance 6 and 7 using the addition of nitrosylchloride to imines we have to start with the labelled imines 4 and 5, which are formed by the reaction of the corresponding labelled amine and acetaldehyde. The imines 4 and 5 are well known substances[7] but isolable only in poor yields and not very stable.



So we developed a synthetic method for imines 4 and 5 in avoiding their isolation. The reaction of 4 and 5 with nitrosylchloride and subsequently with silveracetate bears no further problems [8].

RESULTS AND DISCUSSION

Starting from the corresponding labelled alkylamine hydrochlorides the amine was set free in a closed apparatus by reaction with a concentrated solution of KOH. By passing through a weak stream of dry nitrogen the amine was absorbed at -80°C in dichloromethane. This solution containing the free amine was condensed with acetaldehyde in the presence of molecular sieves. The so formed imines were further reacted with nitrosylchloride and silveracetate according to the published procedure.

The ∝-acetates 6 and 7 are stable oily compounds in the absence of water, which could be purified by chromatography and distillation[9].

MATERIALS AND METHODS

^{14}C-methylaminehydrochloride (specific activity 55.5 mCi/mmole) was purchased from The Radiochemical Center, Amersham, Bucks, England and 1-^{14}C-ethylamine hydrochloride from New England Nuclear, Boston, Mass. (specific activity 8.5 mCi/mmole).

Precoated Silica gel plates (20 x 20 cm, F-254, E. MERCK, Darmstadt, F.R.G.) developed in the solvent system hexane/ether/dichloromethane 50/70/100 were used for thin layer chromatography. Silica gel (Woelm 0.063 - 0.2 mm, 230 mesh Nr. 04667) was used for column chromatography and eluted with pentane/ether 80/20. Radiochemical purity of the product was measured on thin layer chromatography by an LB 2723 thin layer scanner (Berthold, Wildbad, F.R.G.). Radioactivity was determined in a Mark III liquid scintillation counter from Nuclear Chicago. Molecular sieves, 3Å (E. MERCK, Darmstadt, F.R.G.).

PILOT SYNTHESIS

1.35 g (20 mmole) methylaminehydrochloride or 1.631 g (20 mmole) ethylaminehydrochloride dissolved in 1 ml water were placed in a 25 ml three necked flask, equipped with a dropping funnel, gas inlet tube and an outlet tube connected to a trap which contains 25 ml dichloromethane cooled to -80°C. Through the dropping funnel a solution of 5 g KOH in 5 ml H_2O was given slowly to the hydrochloride solution. At the end of this operation the flask was brought to 120°C by means of an oil bath whereas a slow stream of dry nitrogen passed through the reaction vessel. After 15 hrs 6 g molecular sieves were given to the dichloromethane solution containing the free amine and set aside for further 15 hrs at -30°C. A further 4 g molecular sieves was added and at -40°C a solution of 880 mg (20 mmole) acetaldehyde in 5 ml dichloromethane was dropped slowly to the amine. After 5 hrs at room temperature with stirring the molecular sieves were filtered off and washed three times with 5 ml absolute dichloromethane. The solution containing the imines 4 or 5 were used for further reactions.

REACTION WITH NOCl AND SILVER-ACETATE

20 ml of a one molar solution of nitrosylchloride in dichloromethane were placed in a three necked round bottomed flask (250 ml) fitted with dropping funnel and internal thermometer and brought to

-30°C by external cooling. Through the dropping funnel the dichloromethane solution from above was added slowly during one hour. After a further hour at -30°C 3.3 g (20 mmole) silver acetate was added in small portions and the reaction mixture was stirred for 15 hrs at room temperature. After filtration of the insoluble silver salts, which were washed two times with 20 ml portions of dichloromethane,

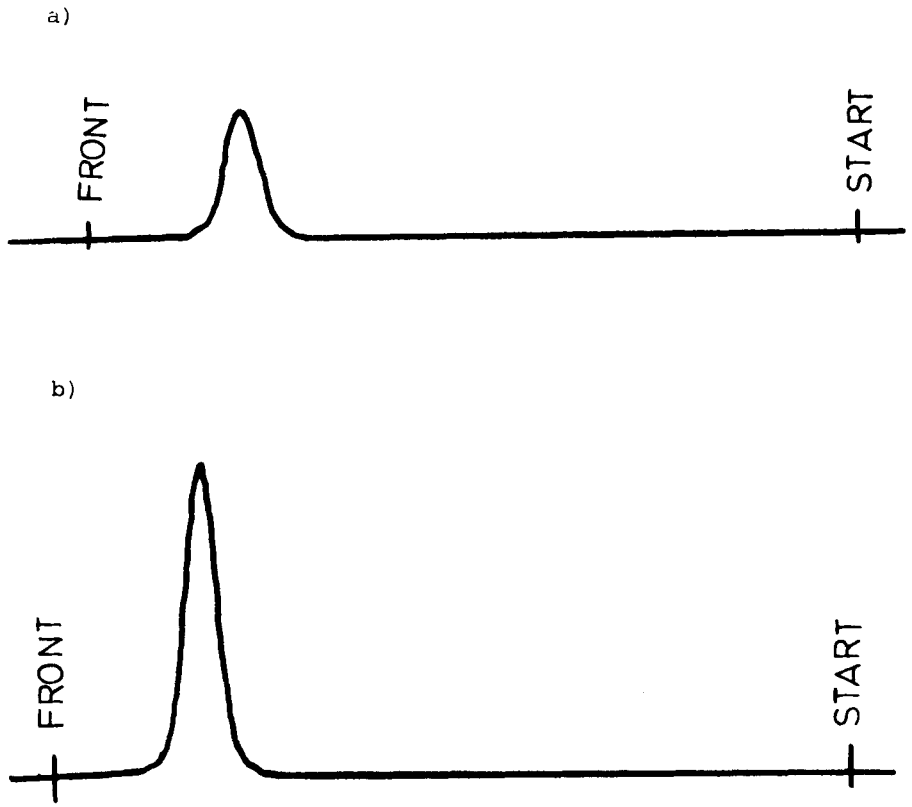


Fig. 1 Thin layer scan of ^{14}C-methyl(1-acetoxy)ethyl-nitrosamine a) and 1-^{14}C-ethyl(1-acetoxy)ethyl-nitrosamine b)

the solution containing the nitrosamines 6 and 7 was evaporated to dryness in vacuo. The residue was chromatographed on Silica gel (column 3.2 cm in diameter and filled 45 cm). The fractions containing the nitrosamine (as checked by thin layer chromatography) were collected and evaporated. The oily residues were distilled in vacuo in a little short way apparatus with a hair-drier as heating source.

(1-acetoxy)ethyl-methyl-nitrosamine 6: 513 mg (3.5 mmole) corresponding to a 17.5% yield based on methylaminehydrochloride.

(1-acetoxy)ethyl-ethyl-nitrosamine 7: 1052 mg (6.6 mmole) corresponding to a 32.9% yield based on ethylamine hydrochloride.

^{14}C-METHYL-(1-ACETOXY)ETHYLNITROSAMINE 6:

^{14}C-methylaminehydrochloride (5 mg, 4000 µCi) was mixed with 1.345 inactive methylaminehydrochloride. All other manipulations followed the procedure described in the pilot synthesis. 476 mg 6 (3.26 mmole) corresponding to 16.3% yield.

To test the radiochemical purity, a CH_2Cl_2 solution was spotted on a TLC plate, developed and scanned.

The specific activity as determined by liquid scintillation counting (corrected) was 208 µCi/mmole and agreed with 198 µCi/mmole, which was computed from the specific activity of ^{14}C-methylaminehydrochloride (figure 1a).

1-^{14}C-ETHYL-(1-ACETOXY)ETHYLNITROSAMINE 7:

1.592 g inactive ethylaminehydrochloride was mixed with 38.8 mg 1-^{14}C-ethylaminehydrochloride (4000 µCi). All other manipulations are done as described in the pilot synthesis. 1070 mg 7 (6.7 mmole) corresponding to 33.3% theoretical yield.

Radiochemical purity was measured as described above (figure 1b). The corresponding yields are 201 µCi/mmole measured by scintillation counting which agrees with 199 µCi/mmole calculated from specific activity.

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3) W. KIRMSE, - Angew. Chem. 88:273 (1976)
4) H. BRAUN and M. WIESSLER, - J.Lab.Comp.Radiopharm. 13:379 (1977)
5) Nitrosamine 2 has been synthesized starting from ^{14}C-labelled formaldehyde in 21.1% yield with a specific activity of 171 µCi/mmole measured by scintillation counting (calculated 181 µCi/mmole).
6) Nitrosamine 3 was synthesized starting from ^{14}C labelled ethylamine hydrochloride in 27.6% yield with a specific activity of 194 µCi/mmole measured by scintillation counting (calculated 200 µCi/mmole).

7) R. TIOLLAIS, - Bull. Chem. Soc. France 708 (1947)

8) M. WIESSLER, - Angew. Chem. 87: 807 (1975)
A publication with further experimental details shall be sent to Chemische Berichte.

9) 6: $Kp_{0.3}$ 53°C. 7: $Kp_{0.1}$ 42°C.