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SYNTHESES OF N- METHYL- ^{14}C BENZYL AMINE AND N- METHYL- ^{14}C BENZYL NITROSAMINE

Guo Zili (郭子丽), Zhu Jingrong (朱京荣), Wei Ziping (魏紫萍),
Zhou Ruiju (周瑞菊) and Zhang Yulong (张雨龙)
(Shanghai Institute of Nuclear Research, Academia Sinica, Shanghai 201800)

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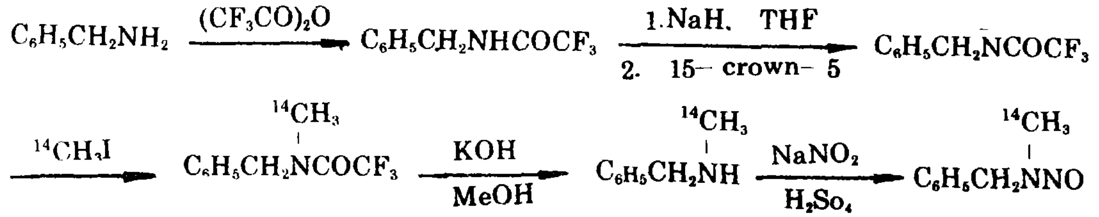
ABSTRACT

The syntheses of N – Methyl– ^{14}C benzyl amine and N – Methyl– ^{14}C benzyl nitrosamine are reported. Specific activities were approximately 920 MBq/mmol. Chemical and radiochemical purity checked by HPLC were more than 95%.

Key words: N – Methyl– ^{14}C benzyl amine N – Methyl – ^{14} benzyl nitrosamine Trifluoroacetamide ^{14}C – Labelled compounds

I . INTRODUCTION

Methyl benzyl nitrosamine is a potent esophageal carcinogen. In order to study its metabolism and distribution in rat tissues and to investigate the influence of some antitumor substances on the methylation of DNA by this carcinogen, N – Methyl– ^{14}C benzyl nitrosamine with high specific activity was needed. Its radiolabelled compound was unavailable commercially either from abroad or domestic, so we undertook the syntheses described in this paper. A specific activity of more than 740 MBq/mmol was necessary for the experiments and this was to be achieved with carbon – 14 as label on methyl group. The synthesis of alkylbenzyl nitrosamine is primarily an exercise in secondary amine synthesis. The subsequent nitrosation presents no real challenge. We consider trifluoroacetyl amine is an attractive intermediate for the transformation of primary to secondary amine through a sequence of acylation, ionization, alkylation and hydrolysis. Overalkylation is precluded, that is extremely important so as to get good radiochemical yield. Besides, the trifluoroacetyl group is readily introduced[1] and later, ease of hydrolysis[2]. Such reactions have in fact been carried out by several authors[3,4,5]. but no convenient and efficient procedure for the radiosynthesis with high radioactivity has been reported. The following scheme outlines the process of this work:



$$\mathrm{C_6H_5CH_2NH_2} \xrightarrow{\mathrm{(CF_3CO)_2O}} \mathrm{C_6H_5CH_2NHCOCF_3} \xrightarrow[\text{2. 15-- crown-- 5}]{\text{1.NaH, THF}} \mathrm{C_6H_5CH_2\overset{-}{N}COCF_3} \xrightarrow{\mathrm{^{14}CH_3I}} \mathrm{C_6H_5CH_2\overset{^{14}CH_3}{N}COCF_3} \xrightarrow[\mathrm{MeOH}]{\mathrm{KOH}} \mathrm{C_6H_5CH_2\overset{^{14}CH_3}{N}H} \xrightarrow[\mathrm{H_2So_4}]{\mathrm{NaNO_2}} \mathrm{C_6H_5CH_2\overset{^{14}CH_3}{N}NO}$$

Trifluoroacetyl benzyl amine was prepared by treating benzyl amine with trifluoro–

acetic anhydride. The trifluoroacetyl amine was methylated in tetrahydrofuran using sodium hydride as base and 15- crown- 5 as alkylation catalyst. ^{14}C- Methylation was completed in vaccum system by stirring the mixture for two days at room temperature. Deacylation was easily conducted by treatment with KOH in methanol for 5 hours at 35°C. The product had specific activity of 920 MBq/mmol and radiochemical purity of greater than 95%. The yield based on $^{14}CH_3I$ was 57%. N - Methyl- ^{14}C benzyl nitrosamine was produced in yield 80% by treating aqueous N - Methyl- ^{14}C benzyl amine with NaNO and H_2SO_4 for half an hour in an ice bath. The radiochemical purity determined by radio- HPLC and specific activity of the product was 99% and 960 MBq/mmol respectively. Compared with the authentic samples, ^{1}H NMR and UV spectra of these two products were pertinent.

We believe that this method is suitable for synthesis of small quantities with high radioactivity. In addition, it provides very pure products.

II. EXPERIMENT

1. Trifluoroacetyl benzyl amine

A solution of trifluoroacetic anhydride (12 ml) in 15 ml of dry ethyl ether was slowly added at 0°C to a solution of benzyl amine (6.6 ml, 0.06 mol) in dry ether. The mixture was stirred at room temperature for half an hour and then evaporated under diminished pressure. Several portions of carbon tetrachloride was added successively to, and distilled under diminished pressure from, the residue. Finally, the product was recrystallized from light petroleum (b.p. 60—90°C) to give trifluoroacetyl benzyl amine (10.1 g, 84%) m.p. 71—73.5°C (There is 73.5—75°C in Ref.[1])

2. N- Methyl- ^{14}C benzyl amine

51 mg (1.7 mmol) of sodium hydride was put in a small flask and freed from protective mineral oil by 1 ml hexane washing. After removing the hexane, 1 ml of anhydrous tetrahydrofuran was added. 0.34 g (1.7mmol) of trifluoroacetyl benzyl amine in 1 ml THF was slowly added at 0°C to the magnetically stirred suspension, it was attended by vigorous gas (H_2) evolution. After 5 mins. two drops of 15- crown- 5 was added. The flask was connected to a vacuum system. Then ^{14}C- methyl iodide (1.2 GBq, 1.4 mmol) was vacuum- transfered into the flask and the reaction mixture was stirred at room temperature for 53 h. Most of the THF was removed by distillation at about 30 mm, whereupon 15 ml of ether was added to the residue followed by 4 ml of 0.1 nmol/l KOH solution. The mixture was transfered to a special separatory apparatus and shaken to separate the layers, and the aqueous layer extracted with two 15 ml portions of ether. The combined ether solution was evaporated under diminished pressure. 5 ml of methanol and 1 ml of 2 nmol/l KOH were added into the resulting residue. The trifluoroacetyl group was completely removed by treatment

with KOH in methanol at 35°C for 5 h. 25 ml of ether and 4 ml of water were poured into the hydrolyzed product. The contents of the flask were vigorously stirred, the ether layer was taken, and the aqueous phase was extracted twice with ether. The combined ether phase was dried over anhydrous $MgSO_4$, filtered and concentrated. The total activity of the product was 690 MBq (57% of starting activity) as determined by liquid scintillation counting. The yield (90 mg, 0.75 mmol) was determined by HPLC comparison with a standard. Thus a specific activity of 920 MBq/mmol was obtained for N-Methyl- ^{14}C benzyl amine. The product was identical in ^1H NMR and UV spectra with that of standard compound. The chemical and radiochemical purity of the product checked simultaneously by the HPLC were more than 95%. (Fig.1)

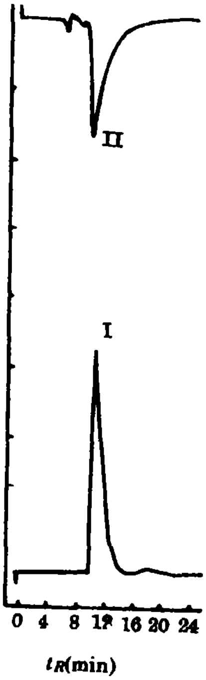


Fig.1 HPLC chromatogram of N- methyl - ^{14}C benzyl amine

I Checked by liquid scintillation counter

II Checked by UV detector

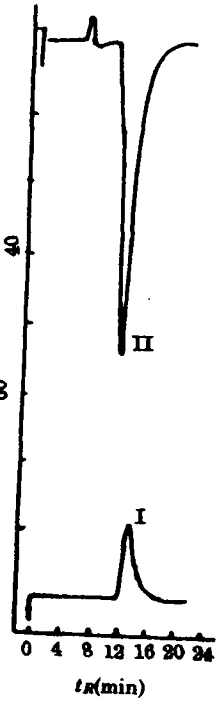


Fig.2 HPLC chromatogram of N- methyl- ^{14}C benzyl nitrosamine

I Checked by radioactive detector

II Checked by UV detector

3. N- Methyl- ^{14}C benzyl nitrosamine

^{14}C- methyl benzyl amine (160 MBq, 0.17mmol),40 mg of $NaNO_2$ and 0.3 ml of water was successively added into a small flask. While the temperature was kept at 0°C several drops of 2.5 mol/l H_2SO_4 was added to the flask with vigorous stirring until pH value of the reaction mixture was about 2. The flask was kept in ice- bath with stirring for half an hour. The contents of the flask were transferred to a small special separatory apparatus using 5 ml of ether and 1 ml of water. The product was extracted by ether and the ether layer was concentrated as much as possible. The amount of the product determined by UV was 20 mg (0.135 mmol) with a total activity of 130 MBq (80% of the activity of amine). Proton nuclear magnetic resonance spectrum and ultraviolet absorption scanning of the product were the same as those of the authentic compound. HPLC showed that the N- Methyl- ^{14}C benzyl nitrosamine was chemical (98%) and radiochemical pure (99%). (See Fig.2)

Ⅲ. DISCUSSION

Before radiosynthesis we had done some inactive experiments to find the optimum reaction conditions. The effects of crown- ether and methylation reaction time on the yield of methyl benzyl amine were shown in Table 1—2. The influence of the pH value of the aqueous phase for extracting the disubstituted trifluoroacetamide was also studied (Table 3). We found 15- crown- 5 was better than 18- crown- 6 when sodium hydride was used and methylation reaction in a vacuum system reached completion in more than 2 days. Besides, pH value of solution for extracting had some influence on the yield. Basic was better than acidic and the neutral water was the worst.

Table 1

Effect of crown ether on the yield of methyl benzyl amine^{a}

Crown-ether	18- crown- 6	15- crown- 5
Yield	24.5%	36%

Table 2

Effect of methylation reaction time on the yield of methyl benzyl amine^{a}

Vacuum reaction time (h)	7	21	48	53
Yield	30%	40%	56%	57%

Table 3

Effect of pH value on the yield of methyl benzyl amine^{a}

Aqueous phase^{b}	0.1 mol/l KOH	1% HCl	H_2O
Yield	34%	31%	26%

a, all the other reaction conditions were the same

b, aqueous phase for extracting the disubstituted trifluoroacetamide by ether

Separation of starting material, relative intermediates and the products was investigated by reversed phase HPLC on Waters' μ BONDPAK- C_{18} Column. A suitable eluant (0.05 mol/l KH_2PO_4(pH 6): methanol = 1:1 *V/V*) was found which can be used to separate the mixture of five compounds, i.e. benzyl amine, N- benzyl trifluoroacetamide, N-methyl N-benzyl trifluoroacetamide, N-methyl benzyl amine, and N– methyl benzyl nitrosamine (Fig.3). Using UV and radioactive detectrors, the peaks of UV and radionuclide were simultaneously determined.

Our preliminary experience with the radiolabelled amine and nitrosamine indicates that they are somewhat photosensitive and should be stored in the dark and at low temperature.

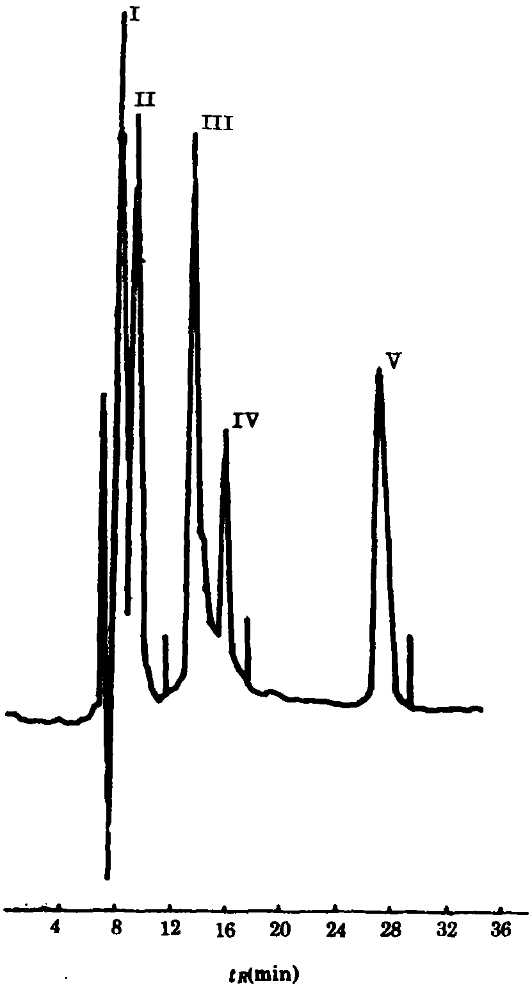


Fig.3 HPLC chromatogram

Ⅰ: Benzyl amine
Ⅱ: N– methyl benzyl amine
Ⅲ: N– Methyl benzyl nitrosamine
Ⅳ: N– Benzyl trifluoroacetamide
Ⅴ: N– methyl N– benzyl trifluoroacetamide

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