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SYNTHESIS OF HIGH SPECIFIC ACTIVITY [^{14}CO] METHYL ISOCYANATE AND ITS USE IN THE PREPARATION OF [^{14}CO] ESERINE, [^{14}CO] CARBARYL AND [^{14}CO] IPMU

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SUMMARY

The preparation of [^{14}CO] methyl isocyanate from [1-^{14}C] acetyl chloride and activated sodium azide through the Curtius rearrangement was optimized (50% yield/[1-^{14}C] sodium acetate- 50 mCi/mmol) and the reagent immediately used for the synthesis of [^{14}CO] eserine, [^{14}CO] carbaryl and [^{14}CO] IPMU.

The condensation of methyl isocyanate with eseroline in the presence of triethylamine does not give rise to eserine but to the isomeric urea derivative 3.

Key words: [^{14}CO] methyl isocyanate , [^{14}CO] eserine , [^{14}CO] carbaryl , [^{14}CO] IPMU

INTRODUCTION

Methyl isocyanate is a classical and important precursor of many ureas or carbamates (1) but as a product is sensitive to polymerization. It is reasonable to expect that a carbon 14 labelled version would be even more so (2). Labelling of the carbonyl group with carbon 14 has often been described at low specific activity (3, 4, 5), but no high specific activity synthesis has been reported in the literature.

Several synthetic routes lead to methyl isocyanate of which the Curtius rearrangement method is the most familiar. This route involves the creation of an acylazide, thermally rearranged into an acylisocyanate. Among the azides used, we note tetramethylguadinium azide (6), tributylstanide

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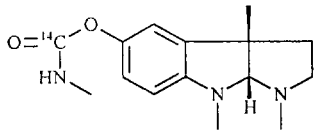
azide (7), trimethylsilyl azide (8), tetrabutylammonium azide (9). Nevertheless the most widely used method to obtain an acylazide is the reaction between an acyl chloride and sodium azide (10). The reaction between phosgene and methylamine is another route to synthesize methyl isocyanate (11).

Of the other preparations, less frequently used, we note the rearrangement of Lossen, the reaction of phosgene with trimethylsilylamine and the pyrolysis of isocyanurates (12). The carbonatation of trimethylsilylamine (13) is another attractive possibility. However, we did not explore this method as we hadpreviously studied it in this laboratory with no success (14).

In this paper, we describe the synthesis of [^{14}CO] methyl isocyanate with a specific activity higher than 50 mCi/mmol by an easy route and without the use of sophisticated apparatus (as for the synthesis of phosgene).

[^{14}CO] methyl isocyanate was used to synthesize three well known products-Eserine (or Physostigmine)-(3aS-cis)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-b]indol-5-ol methylcarbamate- 1, « Carbaryl »: 1-Naphthyl-N-methyl-carbamate-2 and IPMU: N-methyl-N'-(4-isopropyl)-urea- 3, which were obtained in satisfactory yields with high specific activity and high radiochemical and chemical purities

Eserine, an alkaloid isolated from the seeds of the Calabars beans (physostigma venenosum) is a well known potent inhibitor of acetylcholinesterase (AChE) activity. It has been used clinically in the treatment of glaucoma (15), atropine intoxication and myasthenia gravis (16). For a number of years, eserine has been used for the treatment of Alzheimers disease (17,18). To study more precisely the action of eserine on the AChE activity, we synthesized [^{14}CO] eserine

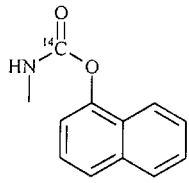


Eserine 1

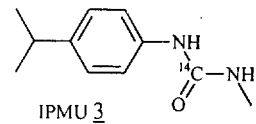
Labelling of the carbonyl group ensures that the carbon will be covalently bound to the active site of AchE. Evidence from the literature (19) supports the view that, after hydrolysis from the

AchE binding site, the carbamyl group leads to the formation of methylcarbamic acid which, in vivo, is finally metabolised to CO_2 and removed from the tissue by ventilation.

These studies required high specific radioactivity (> 50 mCi/mmol) material. For such labelling, the most convenient precursor was [^{14}CO] methyl isocyanate.



Carbaryl 2



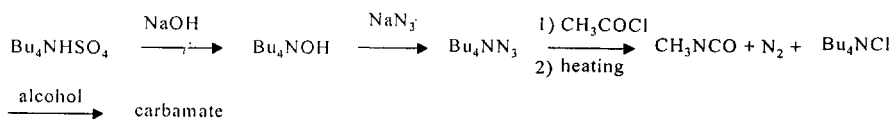
IPMU 3

Carbaryl 2 is a well known insecticide synthesized as early as 1957 (20). It has been used as a contact insecticide and as a parasiticide in veterinary therapy (21). IPMU 3 is a pesticide developed by Rhone Poulenc Agrochimie in 1971 (22). These two products were labelled on the carbonyl group and were obtained from the corresponding naphthol or aniline and [^{14}CO] methyl isocyanate.

RESULTS AND DISCUSSION

Due to its high reactivity methyl isocyanate is very difficult to analyse. After synthesis it was used immediately and the overall yield was measured on the final product. To avoid the preparation of unstable eseroline 4, the cold precursor of 1, we used β-naphthol for the preliminary experiments. The reaction of methyl isocyanate with this phenol afforded a product we called β-carbaryl.

The first trials were done with trimethylsilylazide for the production of methyl isocyanate. The results were unsatisfactory. We tried another precursor : tetrabutylammonium azide. Among the few syntheses of isocyanates using this reagent, [^{11}CO] methyl isocyanate was described (23). This product was obtained according to the scheme 1(9) and stored under a nitrogen atmosphere.



Scheme 1

Following this route, we once synthesized non-radioactive β-carbaryl in 54% yield and eserine in 25% yield. However we were unable to reproduce these results, particularly for the radioactive work (2.5< A.S. < 25 mCi/mmol).

Further experiments involving the tetrabutylammonium azide stability, the heating time or the strict control of the anhydrous conditions were to no avail. Therefore this precursor was abandoned.

At last, in spite of its insolubility in organic solvents, we used sodium azide. The product was regenerated for each batch according to the method of Nelles (24) and was kept under a nitrogen atmosphere.

$$NaN_3 \xrightarrow{\text{hydrazine}} \text{regenerated } NaN_3 \xrightarrow[\text{2) heating}]{\text{1) } CH_3{}^{14}COCl} CH_3N^{14}CO$$

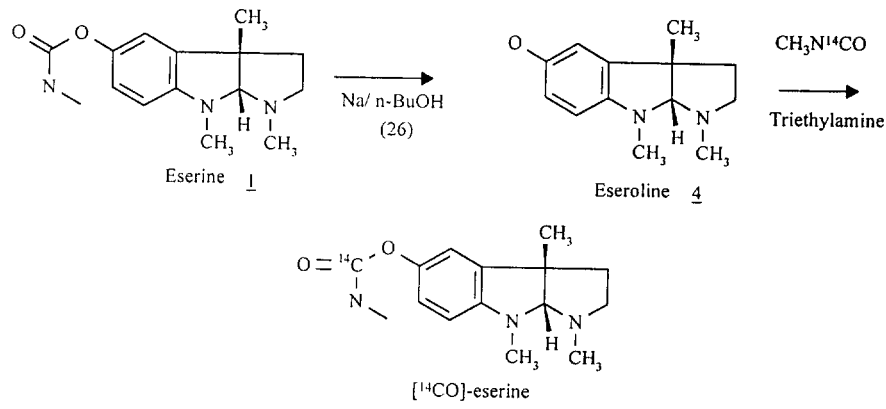
[1-^{14}C] acetyl chloride was obtained from [1-^{14}C] sodium acetate in 80% yield (25).

The experimental conditions for the preparation of methyl isocyanate with subsequent analysis of the reaction product with β-naphthol were optimized. Preliminary non-radioactive experiments with commercial acetyl chloride gave the results summarized in Table 1.

Heating time (h.)	1	3	4
Yield (%) (ß-carbaryl)	38	42	73

Table 1

The overall yield from sodium acetate reached 50 %. According to the best results obtained with β-naphthol, we attempted to synthesize [^{14} C-carbonyl] eserine (Scheme 2). Eseroline was obtained by decarbamylation of eserine(26), stored as the fumarate salt at -20 °C under an argon atmosphere. The required quantity of the free base for each experiment was made just before use by treatment of the fumarate salt with sodium bicarbonate. However, the same experimental conditions, successful for the preparation of ß-carbaryl, did not give the expected eserine. Table 2 gives the major analytical differences between analytical data for the two compounds.



Scheme 2

	MP (°C)	MS (DCI-NH_3) p+1	TLC Rf SiO_2 MeOH1 $CH_2Cl_2$9	^{1}H NMR :δ (ppm) (CD_2Cl_2)	^{13}C NMR :δ (ppm) (CD_2Cl_2 + CD_3OD)
authentic eserine	106.5	276	0.3	1.9 (m,2H,-C-$\underline{CH_2}$) 2.55-2.65(m,2H,-$\underline{CH_2}$-N-CH_3)	54: quaternary cyclic carbon 39;37: N-$\underline{CH_3}$ 28: $\underline{CH_3}$ carbamate and cyclic $\underline{CH_3}$
unexpected product	180	276	0.65	1.6;2.1(2m,2H,-C-$\underline{CH_2}$) 2.85-2.95(2m,2H,-$\underline{CH_2}$-N-CH_3)	46: quaternary carbon 41;38;35;27: $\underline{CH_3}$

(N.B.:due to very small crystals, X ray structure was not possible.)

Table 2

We checked the origin of the eseroline (batch, salt for storage (fumarate, sulphate or hemisulphate)), and that of the commercial methyl isocyanate and obtained the same results. Finally, we found that triethylamine (TEA) was responsible for the problem. Two parallel syntheses were performed (with similar reagents, solvents, concentrations) one with triethylamine and the other with sodium. With TEA, the unexpected product was solely obtained in good yield, with sodium, eserine was the only reaction product. In due course, [^{14}C-carbonyl] eserine was obtained according to this method using sodium. According to the same route, but

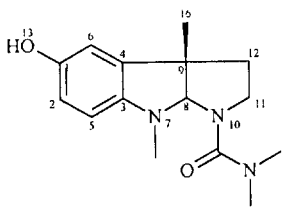
using TEA, we synthesized [^{14}C-carbonyl] Carbaryl and [^{14}C-carbonyl] IPMU. The lower yield of the radioactive syntheses is probably due to partial polymerization of [1-^{14}C] methyl isocyanate.

Product	Yield / $CH_3{}^{14}CO_2Na$ (%)	HPLC purity (%)	Specific activity (mCi/mmol)	Basis
[^{14}C-carbonyl] eserine	37	> 99	53	Na
[^{14}C-carbonyl] carbaryl	25	> 99	54	TEA
[^{14}C-carbonyl] IPMU	36	> 99 *	56	TEA

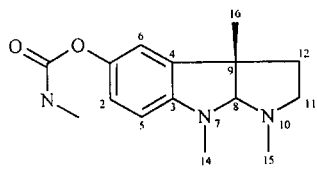
*: TLC purity

The molecular structure of the unknown product was determined by ^{1}H NMR.

A NOE-COSY experiment indicated that a methyl linked to an amido group had a spatial interaction with the proton 8. Molecular modelling of eserine showed that it was not possible with a carbamate stucture. So, we thought that the structure of the product might be the urea derivative 5.

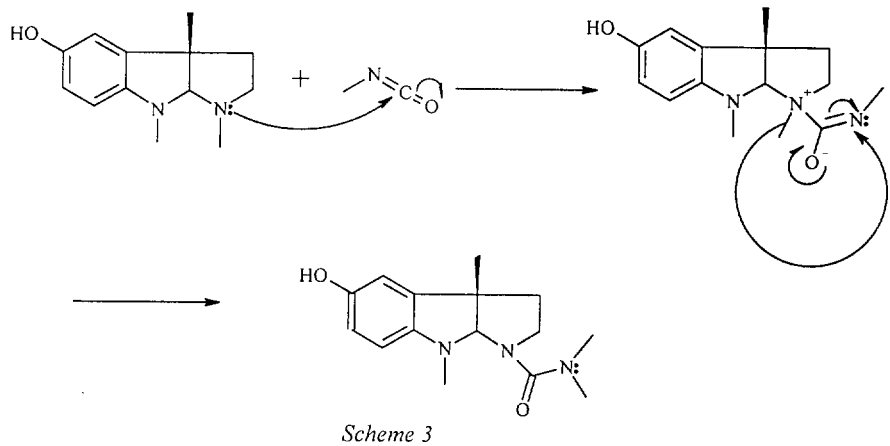


unexpected product 5



eserine 1

The carbonyl reacts with the free electrons of the nitrogen 10 and the urea is formed after rearrangement (Scheme 3).



Scheme 3

This structure is in agreement with the analyses. The mass spectra are identical; the ^{1}H NMR signals of the protons 11 could be split into two signals. It is also well known that ureas have higher melting points than carbamates.

In order to prove that this reaction does take place, we carried out the following experiment : in an NMR tube, we mixed eserine and methyl isocyanate. We observed after a few min. the appearance of the split signals of the protons on 11 and, at the same time, the dissapearance of the signal of the same methylene in eserine. After 2 days (without stirring), the reaction was complete. So, the addition of the isocyanate on the nitrogen 10 is possible.

In conclusion, the rate of addition of methyl isocyanate on the nitrogen is faster than the formation of the phenoxide ion with TEA. The isocyanate is consumed before reacting with the hydroxylate ion. However, when the base is sodium, the formation of the anion is very fast and methyl isocyanate reacts with the hydroxylate to form eserine.

EXPERIMENTAL

Reagents and analytical materials.

[^{14}C] Barium carbonate was obtained from Slavia. Reagents and eserine were purchased from Aldrich. Solvents were distilled with appropriate desiccant reagents and kept over molecular sieves. HPLC analyses were carried out on a Merck L 6200 system. Radioactive monitors were

models LB 505 and LB 503 from Berthold. The UV detector was a Merck system, model L 4250. ^{1}H NMR spectra were recorded on a Bruker AM 400 at 300 MHz. Radioactive TLC were recorded on Berthold system, model LB 2821. Specific activities were determined on a Finnigan mass spectrometer, model 4600. For the synthesis of eserine, all the solvents were degassed with helium.

Activation of sodium azide (24)

18 G of commercial sodium azide were triturated with 1 mL of hydrazine hydrate (85%). This mixture was left overnight. Then, hydrazine was dissolved with 50 mL of warm water (about 70°C) and 200 mL of cold acetone were added. Sodium azide precipitated. The solution was filtered and the precipitate was washed with cold acetone. Activated sodium azide was dried under vacuum and kept under nitrogen atmosphere.

[1-^{14}C] Acetyl chloride (25)

30 MCi (0.6 mmol) of [1-^{14}C]-sodium acetate were dried under vacuum at 130° C for one night. Under a nitrogen atmosphere, 2 mL of freshly distilled phthaloyl chloride were added and the mixture was heated under vacuum at 180°C for 2 h. Acetyl chloride was vacuum transferred to a 50 mL balloon flask and kept under vacuum at -60°C.

[^{14}CO] Methyl isocyanate.

In a dried 30 mL flask, 55 mg (0.84 mmol) of activated sodium azide and 0.3 mL of freshly distilled benzene were placed. The flask was connected to a vacuum manifold and evacuated. Then acetyl chloride was transferred onto this mixture. The flask was kept at atmospheric pressure with nitrogen. The reaction mixture was isolated from the vacuum manifold. The heterogeneous solution was heated at 100°C for 3.5 h. Then, [^{14}CO] methyl isocyanate was vacuum transferred on to the appropriate reagent in order to obtain urea or carbamate.

[^{14}CO] Eserine.

Three small pieces of sodium were added to a solution of freshly prepared eseroline (113 mg 0.52 mmol) in 18 mL of freshly distilled di-isopropyl ether. The solution was stirred

magnetically in darkness for 15 min. when the solution turned orange in color. The flask was connected to the vacuum manifold and [^{14}CO] methyl isocyanate was transferred onto this mixture. Nitrogen atmospheric pressure was established and the solution was stirred in darkness for 1 h..

TLC monitoring (Silica gel ; eluent : methanol 10 methylene chloride 90) showed 67% of the expected product. Sodium cuttings were filtered under an argon atmosphere and the orange solution was diluted with 200 mL of acetonitrile. [^{14}CO] Eserine was purified on a 40-63 μm silica gel column and eluted with ethanol-methylene chloride (15:85 v/v).

Results and analyses.

Yield /sodium acetate = 37 %. Specific activity : 53 mCi/mmol (M.S.). Radiochemical purity HPLC >99% (column Zorbax C18 eluent : methanol-water-triethylamine.(50 : 50 : 0.1 v/v)). R_t=11.1 min. Radiochemical purity TLC > 99%(Silica gel eluent : methylene chloride-ethanol (85 : 15 v/v)) R_f = 0.26

The ^{1}H NMR spectrum is in agreement with the NMR of an authentic sample spectrum.

^{1}H NMR Labelled product (CD_2Cl_2):

1.35(s,3H,-C-$\underline{CH_3}$) ; 1.9(m,2H,-C-$\underline{CH_2}$-) ; 2.45(s,3H,-CH_2-N-$\underline{CH_3}$) ; 2.55-2.65(m,2H,-$\underline{CH_2}$-N-CH_3) ; 2.8(d,3H, -NH-$\underline{CH_3}$) ; 2.9(s,3H,φ-N-$\underline{CH_3}$) ; 4.1(s,1H,$\underline{CH}$) ; 4.95(m,1H,NH) ; 6.35(d,1H,-N(CH_3)-C-$\underline{CH}$- (aromatic)) ; 6.7(m,2H aromatics)

The mass spectrum is in agreement with that obtained from an authentic compound spectrum (DCI/NH3 p+1=278).

1-Naphthyl-N-methyl-[^{14}C]carbamate: [^{14}CO] CARBARYL

Under a nitrogen atmosphere, 0.26 mmol (37 mg) of α-naphthol were dissolved in 0.15 mL of freshly distilled di-isopropyl ether. Two drops of triethylamine were added. The reaction flask was connected to the vacuum manifold and [^{14}CO] methyl isocyanate, prepared from 15 mCi of [1-^{14}C] sodium acetate, was transferred onto this solution. The nitrogen atmosphere was restored

and the mixture was gradually heated up to 80°C in 30 min. Conversion was checked by TLC (silica gel eluent: ethyl acetate-cyclohexane (20: 80 v/v)) and showed 76% of [^{14}CO] Carbaryl.

2 ML of methylene chloride were added and this solution was injected onto a small column (silica gel 40-63µm eluent: ethyl acetate-cyclohexane (3 : 7 v/v)) in order to eliminate triethylamine. The product was purified by liquid chromatography (silica gel 15-40µm eluent: cyclohexane-ethyl acetate (80 : 20 v/v)).

Results and analysis

Yield / sodium acetate : 23%. Specific activity : 54 mCi/mmol (M.S.). Radiochemical purity TLC >99% (silica gel cyclohexane-ethyl acetate(80 : 20 v/v)). R_f = 0.25. Radiochemical purity HPLC >99% (column Zorbax ODS eluent : methanol-water (40 : 60 v/v)). R_t = 16.3 min.

The ^{1}H NMR spectrum was in agreement with the NMR of an authentic sample.

^{1}H NMR labelled product (CD_2Cl_2):

2.9(d,3H,NH-$\underline{CH_3}$) ; 5.75(m,1H,NH) ; 7.25(d,1H,aromatic) ; 7.5(m,3H,aromatics) ; 7.7(d,1H,aromatic) ; 7.9(m,2H,aromatics)

N-Methyl-N'-(4-isopropylphenyl)-[^{14}C] urea : [^{14}CO] IPMU

Under a nitrogen atmosphere, 0.6 mmol (83µL) of triethylamine were added to 0.3 mmol (41 µL) of 4-isopropylaniline in 0.6 mL of methylene chloride contained in a round bottom flask which was then connected to the vacuum manifold. Freshly prepared [^{14}CO] methyl isocyanate from 15 mCi of sodium acetate (0.3 mmol)) was transferred onto this solution. The mixture was stirred for 2 h. at atmospheric pressure and room temperature. Formation of [^{14}CO] IPMU was followed by TLC (silica gel eluent : hexane-ethyl acetate (50 : 50 v/v)).

73 % of product was present in the mixture after 2 h.. [^{14}CO] IPMU was injected on to a small column (silica gel 40-63 µm eluent : hexane-ethyl acetate (50 : 50 v/v)) in order to eliminate triethylamine. The final purification was achieved on a 40-63µm silica gel column, eluted with hexane-ethyl acetate (70 : 30 v/v).

Results and analyses

Yield/sodium acetate : 36%. Specific activity : 56 mCi/mmol (M.S.). Radiochemical purity TLC > 99% (silica gel eluent : hexane-ethyl acetate (50 : 50 v/v)). $R_f = 0.19$. The ^{1}H NMR spectrum was in agreement with the spectrum of the unlabelled product.

^{1}H NMR Labelled product:(CD_2Cl_2).

1.2(d,6H,-CH-($\underline{CH_3}$)$_2$) ; 1.6(m,1H,-$\underline{CH}$-(CH_3)$_2$) ; 2.75(d,3H,-CO-NH-$\underline{CH_3}$) ; 4.85(m,1H,-$\underline{NH}$-CH_3) ; 6.5(m,1H,-φ-$\underline{NH}$-CO) ; 7.2(m,4H,aromatics).

The mass spectrum was in agreement with the spectrum of the unlabelled product (DCI/NH_3 p+1=212).

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