Carbon-11 labelling of an inhibitor of Acetylcholinesterase : [^{11}C]Physostigmine

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Summary

Physostigmine, an alkaloid from calabar bean is a strong inhibitor of acetylcholinesterase ($Ki = 3.33 \pm 0.26 \times 10^{-6}$M) and has been used clinically in the treatment of glaucoma, atropine intoxication, myasthenia gravis and more recently, in experimental trials in Alzheimer's disease. In order to study the AChE activity in the brain by positron emission tomography, we have undertaken the labelling of physostigmine with carbon-11. The synthesis involves the reaction of [^{11}C]methylisocyanate with eseroline. [^{11}C]Methylisocyanate was obtained by heating [^{11}C]acetylchloride with tetrabutylammonium azide in toluene. The synthesis of [^{11}C]CH_3COCl involves the carbonation of methylmagnesium bromide in THF with cyclotron produced [^{11}C]carbon dioxide and the addition of phthaloyl dichloride. The [^{11}C]methylisocyanate is distilled into a solution of eseroline in ether with a small piece of sodium. After 10 minutes at 25°C, the solution is purified by HPLC and the appropriate fraction collected.
Starting with 55.5 GBq (1.5 Ci) of [^{11}C]carbon dioxide, 0.92-1.48 GBq (25-40 mCi) of [^{11}C]Physostigmine are obtained 57 minutes after EOB.

Key words : inhibitor of AChE, [^{11}C]Methylisocyanate, [^{11}C]Physostigmine.

Introduction

Physostigmine (eserine), the principal alkaloid of calabar bean, was discovered in 1864[1], but it was not until much later that its effect on the heart was demonstrated to be due to inhibition of acetylcholinesterase[2,3] (AChE). This strong inhibitor of AChE : $Ki = 3.33 \pm 0.26 \times 10^{-6}$M[4] has been used clinically in the treatment of glaucoma[5], atropine intoxication, myasthenia gravis[6] and more recently in experimental trials in Alzheimer's disease[7-10]. We have labelled

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physostigmine with carbon-11 (a β^+ emitter $T\frac{1}{2}$ = 20 minutes) in order to visualise the AChE in the brain by positron emission tomography and to investigate the drug as a possible PET ligand for pathophysiological studies of Alzheimer's disease.
The labelling of physostigmine is based upon the reaction of [^{11}C]methylisocyanate with eseroline and therefore involves the synthesis of a new radioactive precursor : [^{11}C]methylisocyanate.

Results and discussion

The [^{11}C]methylisocyanate was produced from [^{11}C]acetylchloride.

- Preparation of [^{11}C]acetylchloride

[^{11}C]Acetylchloride is produced from [^{11}C]CO_2 by the method of S. Luthra[11] : in a typical procedure, carbon-11 carbonation of methylmagnesium bromide in ether or tetrahydrofuran is followed by direct treatment with phthaloyl dichloride and 2.6-di-t-butylpyridine.

$$N(p,\alpha)^{11}C \xrightarrow{\text{ppm } O_2} [^{11}C]CO_2$$

$$CH_3MgBr \xrightarrow[\text{ether or THF}]{[^{11}C]CO_2} CH_3COOMgBr \xrightarrow[\text{dichloride}]{\text{phthaloyl}} CH_3{}^{11}COCl$$

We have determined the optimal concentration of methylmagnesium bromide to minimise the side reaction (synthesis of [^{11}C]acetone) (table I).

Table I

Quantity of methylmagnesium bromide (μmole)	15	21	105
$\frac{[^{11}C]\text{acetanilide}}{[^{11}C]\text{acetanilide} + [^{11}C]\text{acetone}}$	73 %	95 %	49 %

The [^{11}C]acetylchloride is characterized by its reaction with aniline. The reaction goes to completion very rapidly at room temperature and produces [^{11}C]acetanilide which can be analysed by HPLC. Under these conditions, aniline cannot react with [^{11}C]acetone. HPLC separation is achieved on a semi preparative reverse-phase column (μ-bondapack C_{18}, L=25 cm,

ϕ_{int} = 0.9 cm). The eluent is made up of 80 % water and 20 % ethanol at pH 2.3 with a flow rate of 4 ml/min. The [^{11}C]acetone (R_t=3.5 min) and the [^{11}C]acetanilide (R_t=8 min) fractions are identified by U.V. absorbance at 254 nm and their radioactivities measured. These fractions are collected and the quantities of radioactivity measured.

The yields of [^{11}C]acetylchloride are the same with or without 2,6-di-t-butylpyridine.

- Preparation of [^{11}C]methylisocyanate

. Choice of labelling position. We decided to label the carbon of carbamoyl group of physostigmine because this group interacts with the esteratic subsite of AChE. The labelling of this carbon involves the synthesis of a new radioactive precursor of interest: [^{11}C]methylisocyanate.

. Advantages of labelling [^{11}C]methylisocyanate. This synthon can lead to carbamates, ureas, guanidines, amines and amides. This synthesis method can also be applied to propyl or butyl isocyanate and makes it possible to prepare derivative products of physostigmine with longer carbon chains.

. Curtius reaction. The most widely used methods for the synthesis of isocyanates are the reaction of an amine with phosgene or the Curtius rearrangment. The reaction of an amine with phosgene is very difficult to carry out under our radioactive conditions because methylamine is always in excess compared to [^{11}C]phosgene, which has a specific radioactivity between 55.5 and 74 GBq per μmole (1.5-2 Ci/μmole). The excess of methylamine leads to [^{11}C]dimethyl urea. This is why the Curtius method was selected to label methylisocyanate with carbon-11.

The Curtius reaction [12] consists of the conversion of acid azides to isocyanates and compounds derived therefrom. In its original form, as well as when using trimethylsilyl azide or diphenylphosphoryl azide, serious problems may be encountered due to hydrolysis of acetylchloride and methylisocyanate. Thus, we have used an operationally simple modification of the Curtius reaction, in which the azide ion is extracted as an ion-pair with tetrabutylammonium ion from an aqueous solution into an organic phase. The azide, which can be handled in dry form, is soluble in a number of organic solvents. The reaction of [^{11}C]acetylchloride with tetrabutylammonium azide was carried out in toluene, for 10 minutes at 80° C.

After distillation, [^{11}C]methylisocyanate is characterized by conversion to [^{11}C]methylphenyl urea with aniline by heating 5 min at 70° C or by conversion to [^{11}C]methylphenyl carbamate with phenol by heating 10 min at 80° C.

The analysis of [^{11}C]methylphenyl urea is carried out by HPLC on a C_{18} RP column (Waters L=30 cm, ϕ_{int}=0.9 cm). The eluent is water containing 20 % ethanol at pH 2.3 with a flow rate

of 4 ml/min. Chromatography of the reaction mixture shows [^{11}C]methylphenyl urea (R_t=7 min) as the major of three radioactive peaks. The two others are [^{11}C]acetanilide (R_t=8 min) and a small peak of [^{11}C]CO_2 (R_t=5 min) arising from hydrolysis, by HPLC solvent, of the [^{11}C]methylisocyanate which did not react with aniline. This peak decreases with the time of heating.

The analysis of [^{11}C]methylphenyl carbamate is carried out by HPLC on a silica column (Whatman M9, L=50 cm, ϕ_{int}=0.9 cm). The solvent is dichloromethane containing 49 % hexane and 1 % solution B (ethanol with 2.5 % water and 1.5 % ethylamine) with a flow rate of 4 ml/min. Chromatography of the reaction mixture shows three radioactive peaks. The major peak is [^{11}C]methylphenyl carbamate (R_t=9 min). The two others are [^{11}C]phenylacetate (R_t=12 min) and [^{11}C]CO_2 (R_t=3,5 min) arising from [^{11}C]methylisocyanate.

- Synthesis of [^{11}C]physostigmine

[^{11}C]Methylisocyanate is reacted with eseroline in ether for 10 minutes at 25° C. The solution is purified by HPLC on silica column (Whatman, Partisil M9, L=50 cm, ϕ_{int}=0.9 cm). The eluent is dichloromethane containing 15 % ethanol with a flow rate of 8 ml/min. As shown by the HPLC chromatogram (Fig. 1), the peak of [^{11}C]physostigmine (same retention time as standard

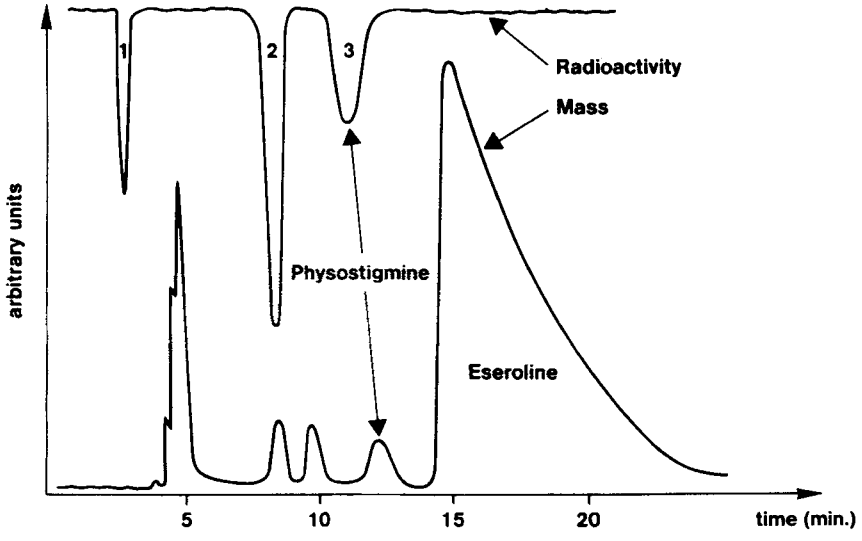


Fig. 1 : Chromatogram of [^{11}C]physostigmine

cold physostigmine (R_t=12 minutes) is well separated from the two other labelled compounds (Peak 1 and Peak 2). Peak 1 is unknown. Peak 2 is the product resulting from the action of

[^{11}C]CH_3COCl with eseroline. Under these conditions [^{11}C]physostigmine is also well separated from its cold precursor.

- Results

The results are summarized in Table II. [^{11}C]Methylisocyanate was obtained with a radiochemical yield (decay corrected) of 19-28 % (80-120 mCi at EOB+37 minutes) compared to the [^{11}C]CO_2 radioactivity.

Starting with 55.5 GBq (1.5 Ci) of [^{11}C]CO_2, 0.92-1.48 GBq (25-40 mCi) of [^{11}C]physostigmine (ready for injection to patient) were obtained 57 min after EOB with a specific radioactivity of about 11-13 GBq/μmole (300-350 mCi/μmole).

Table II

		Quantity of radioactivity	Radiochemical yield compared to $^{11}CO_2$ (decay corrected)
to	EOB	55.5 GBq (1.5 Ci)	100 %
to + 5 min	$^{11}CO_2$ trapping	46 GBq (1.25 Ci)	98 %
to + 20 min	$CH_3{}^{11}COCl$	12.2-16.7 GBq (330-450 mCi)	44-60 %
to + 37 min	$CH_3N^{11}CO$	3-4.4 GBq (80-120 mCi)	19-28 %
to + 52 min	[^{11}C]physostigmine	1.1-1.85 GBq (30-50 mCi)	12-19 %
to + 57 min	Formulation	0.92-1.48 GBq (25-40 mCi)	11-18 %

Material and methods

- Materials

Tetrahydrofuran, toluene, diethylether (SDS), butanol, chloroform were purchased from Service Developpement Scientifique. Methyl magnesium bromide, phthaloyl dichloride, physostigmine, tetrabutylammonium sulfate and tetrabutylammonium azide were purchased from Aldrich Chemical Co. Before use, all the solvents were distilled (THF from $LiAlH_4$, toluene, diethylether and butanol from sodium, chloroform was dried over $CaCl_2$ and distilled). Methylisocyanate was purchased from Fluka.

- Apparatus

The [^{11}C]methylisocyanate labelling apparatus is outlined in figure 2. The reactions are carried out in cylindroconical tubes (1.5 ml) closed by chromatographic septa (Touzart et Matignon) and

interconnected with teflon tubes (ϕ_{int}=0.8 mm) which are fixed on medical needles. The passage of gases is controlled by compressed air-operated electrovalves.

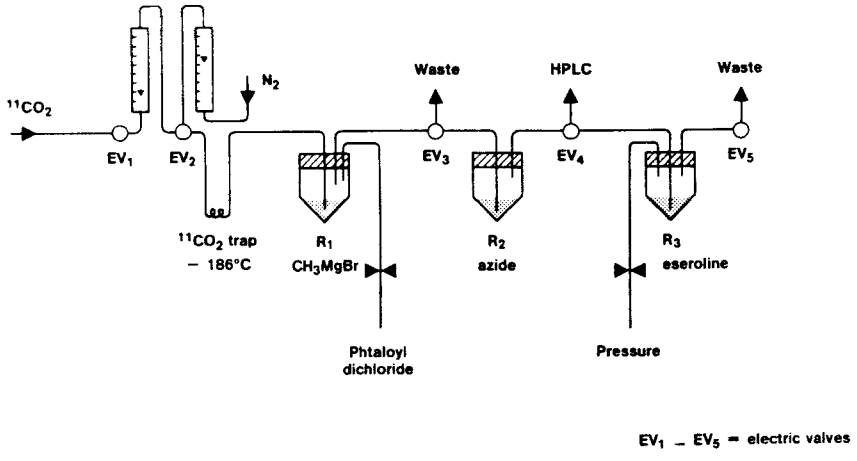


Fig. 2 : Line for labelling [^{11}C]physostigmine

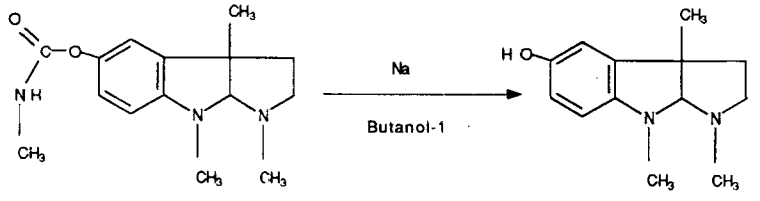
The reaction tubes are moved by pneumatic jacks from a cold bath (-10° C) to a furnace (65° C or 80° C). All this apparatus is housed in a shielded cell (5 cm lead). Pneumatic jacks and electric valves are operated from a panel located in front of the hot cell.

- Experimental

The synthesis takes place in four steps :

1 - Synthesis of eseroline

First the cold precursor, eseroline, is prepared from physostigmine by the published procedure[13] and stored as the sulfate salt : physostigmine, when refluxed in butanol-1, in the presence of sodium, gives eseroline.



PHYSOSTIGMINE ESEROLINE

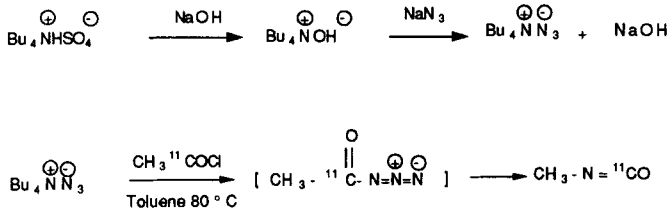
Eseroline, as a free base, is unstable and readily oxidizes to the quinone derivative, rubreserine. Its salts, however, are more stable and can be stored as a dry powder.

2 - Synthesis of [^{11}C]acetylchloride

The carbon-11 is produced by the (p,α) reaction on pure nitrogen (Air Liquide, 99.9999 % purity). By irradiating with 20 MeV protons (30 μA) for 30 minutes 55.5 GBq (1.5 Ci) [^{11}C]CO_2 are obtained. [^{11}C]Acetylchloride is initially produced from [^{11}C]CO_2 by the carbonation of methylmagnesium bromide (21 μmoles) in tetrahydrofuran (200 μl) under nitrogen at -10° C and the addition of phthaloyl dichloride (100 μl) (R1).

3 - Synthesis of [^{11}C]methylisocyanate

The [^{11}C]acetylchloride released by heating at 65° C is carried by a slow stream of nitrogen into a toluene solution (R2) of tetrabutylammonium azide (20 μmoles) which has been earlier prepared from sodium azide and tetrabutylammonium dioxide[14].

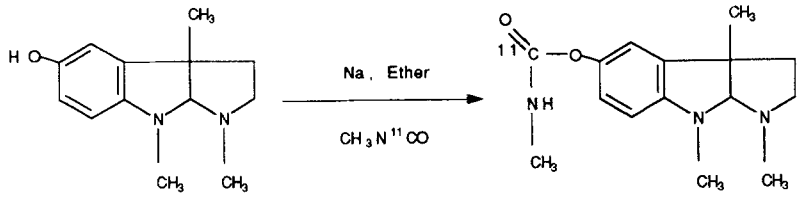


After heating 10 minutes at 80° C, the [^{11}C]methylisocyanate is separated from the solvent by distillation at 80° C into a solution of aniline in chloroform or phenol in dichloro-1,2 ethane with TEA (R3) for characterisation, [^{11}C]methyl phenyl urea or [^{11}C]methyl phenyl carbamate is formed.

Cold methyl phenyl urea and methyl phenyl carbamate are produced with methylisocyanate under the same conditions as the carbon-11 experiments. The pure products are isolated after semi-preparative HPLC and characterized by mass spectrometry and proton NMR.

4 - Synthesis of [^{11}C]physostigmine

If the [^{11}C]methylisocyanate is distilled into a vessel (R3) containing eseroline (10 μmoles) in ether (200 μl) with a small piece of sodium, [^{11}C]physostigmine is formed after heating 10 minutes at room temperature.



ESEROLINE [^{11}C] PHYSOSTIGMINE

After addition of 1 ml CH_2Cl_2, the product is injected onto the HPLC column by EV4 with pressurized nitrogen.

The radioactive peak of physostigmine ($R_t = 12$ min) was recovered, the solvent was evaporated and the [^{11}C]physostigmine was taken up with a few milliliters of saline. After filtration on a millipore filter (0.22 μm), the product is ready for injection to patient.

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