THE PREPARATION OF ^{11}C-LABELED CAFFEINE

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SUMMARY

A rapid and mild procedure for the preparation of [N-7-methyl-^{11}C] caffeine is described. Gaseous ^{11}C-methyl iodide was led into dimethyl sulphoxide (DMSO), containing theophylline (1,3-dimethylxanthine) and sodium hydride. Purification of the reaction product was achieved by High Performance Liquid Chromatography (HPLC).

Key words : ^{11}C-Caffeine, ^{11}C- Radiopharmaceuticals, ^{11}C-Methyl Iodide, HPLC.

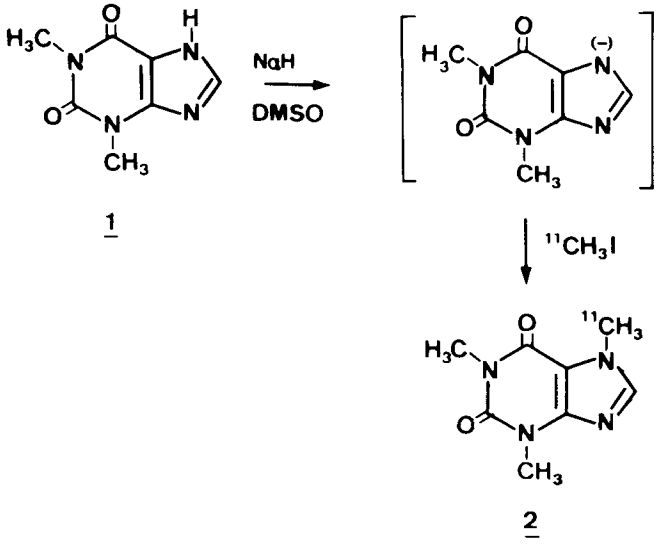
INTRODUCTION

The labeling of radiopharmaceuticals with short-lived radionuclides for use in Positron Emission Tomography requires fast and high yield methods.

Comar et al. (1) and Maziere et al. (2) described the preparation of ^{11}C-labeled caffeine (1,3,7-trimethylxanthine) using ^{11}C-methyl iodide as a precursor. Theobromine (3,7-dimethylxanthine) was used as a starting material. Sodium carbonate was added to a solution of theobromine in methanol and the mixture was subsequently heated in the presence of $^{11}CH_3I$ for 10 min.

We developed a new method for the preparation of ^{11}C-caffeine. Theophylline (1) was selected as a starting material because of its greater solubility in DMSO compared to theobromine.

Sodium hydride in DMSO is added to the solution of theophylline in DMSO so that proton abstraction at N-7 occurs. The resulting anion reacts with $^{11}CH_3I$ (Fig.1). The reaction product, ^{11}C-caffeine (2), is isolated and purified by reversed phase HPLC.



1

2

Fig. 1 Synthesis of ^{11}C-Caffeine(2) by N-methylation of theophylline (1)

DISCUSSION

This novel method allows the preparation of 60 mCi (2.22 GBq) [N-7-methyl-^{11}C] caffeine within 40 min.

Drastic conditions such as heating for several minutes are avoided by the use of a strong base (NaH) in a dipolar aprotic solvent (DMSO). It is obvious that this method is also applicable for the labeling of other molecules.

The fact that N-demethylation is a metabolic pathway for many compounds, so that the label can be lost during the in vivo experiment, may in some instances be a drawback. Caffeine is also partially metabolized by demethylation at N-1,3 and 7 (7). However this pathway is slow compared to the half-life of ^{11}C. We therefore anticipate that the process of demethylation would have no significant influence on the tomographic results.

EXPERIMENTAL

^{11}C-Methyl Iodide production

The method applied for the production of $^{11}CH_3I$ is roughly similar to the one described by Marazano et al. (3).

Protons of 18MeV are used in the [$^{14}N(p,\alpha)^{11}C$] reaction. The nitrogen pressure in the water cooled aluminium target (35 cm x 5 cm (I.D.), with an aluminium window of 475 µm) is 8.5 bar before irradiation. The target is irradiated for 20 min at a 15 µA beam intensity. The energy effectively incident on the N_2 gas is 14.8 MeV.

The resulting $^{11}CO_2$ is trapped in a three-necked flask containing 500-100 µmoles of $LiAlH_4$ in 0.5 ml of tetrahydrofuran at -80°C. The trapping is controlled with 100 ml of 0.5M NaOH, placed after the flask.

After evaporating the THF (160°C) the methanolate is hydrolysed with 0.5 ml of 10M HCl. The resulting $^{11}CH_3OH$ is swept through 1 ml hydriodic acid at 180°C. After passing through a 0.5M NaOH solution and P_2O_5, the $^{11}CH_3I$ formed is led into the vial for the ^{11}C-caffeine synthesis.

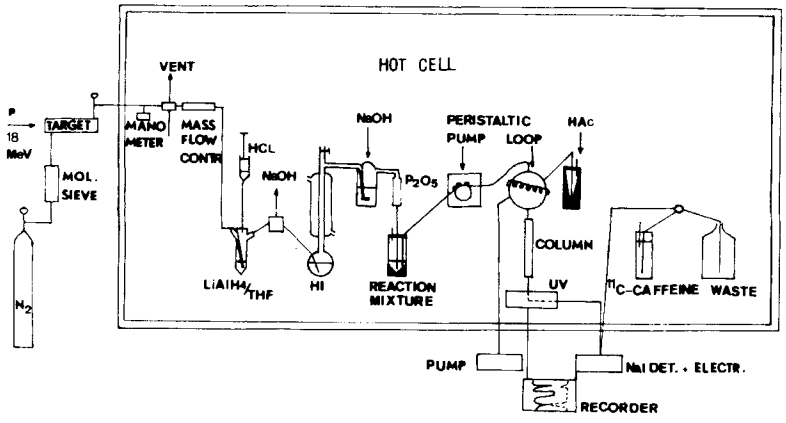


Fig. 2 : Schematic representation of the apparatus.

Synthesis of ^{11}C-caffeine

Theophylline (125 mg) (Sigma, St. Louis USA) is dissolved in 5 ml of DMSO.

Purified sodium hydride (1.2g) (Aldrich, Milwaukee USA) is added to 100 ml of anhydrous DMSO. The mixture is heated for 1h at 55°C and purged with nitrogen gas. After filtration on sintered glass the reagent is stored at -20°C. This reagent is tested before use by adding 100 µl of a solution containing 1 mg triphenylmethane in DMSO to 50 µl reagent : an immediate intense red color, due to the triphenylmethane anion, should develop (5).

In a 5-ml mini-vial with Teflon ^{R} - faced rubber liner (Alltech, Illinois USA) 75 µl NaH/DMSO reagent is added to 175 µl theophylline solution. A nitrogen stream sweeps the $^{11}CH_3I$ for 10 min through a hypodermic needle (Aldrich, gauge 18) into the reaction mixture kept at room temperature. The trapping efficiency is 100 %. The yield of incorporation of the labeled methyl group is 90 %. Sixty percent of the total amount of activity produced is found in ^{11}C-caffeine.

Purification.

The ^{11}C-caffeine is isolated by reversed phase high performance liquid chromatography (Knauer liquid chromatograph). The method of Foenander et al. (5) is modified and extended to a preparative scale.

A RP C_{18} HL 30 µm column (25 cm x 1 cm I.D.) (RSL, Eke, Belgium) is eluted with a mixture of water:ethanol, 85:15 (v,v) brought to

a pH 5.2 by adding NaH_2PO_4 (0.01 M) at a flow rate of 6 ml/min. Detection is performed simultaneously by a NaI (Tl)-scintillation detector and a UV absorption detector operated at 254 nm. After reaction the mixture is pumped with a peristaltic pump (Gilson Minipuls 2) through the sampling loop into a mini-vial (1 ml), containing 50 µl acetic acid, connected to the waste outlet of the loop (Fig.2). The acid stops the reaction and protects the column from the strong basic solution. Then the pump direction is reversed and the solution transferred into the HPLC loop (300 µl) from where it is injected. By this injection procedure more than 90 % of the reaction mixture is brought on the column. Fig.3 shows a chromatogram and Table 1 lists the characteristics of the separation. Twelve ml of eluent, containing the ^{11}C-caffeine is collected. The whole procedure takes 30 min and 40 min after the end of bombardment, 60 mCi labeled caffeine are available for pharmacological experiments. The amount of carrier is about 1 µmole so that a specific activity of 60 mCi/µmole is obtained.

Table 1 Characteristics of the separation : Rt is retention time and k' capacity factor.

Compound	Rt (min)	k'
side product	1.6	0.7
theophylline	4.6	3.8
^{11}C-caffeine	7.9	7.3

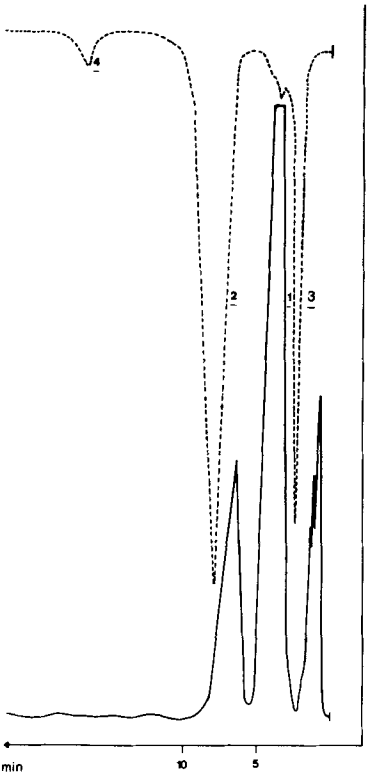


Fig. 3 Chromatogram : (1) theophylline (2) ^{11}C-caffeine (3) side product (4) ^{11}C-methyl iodide.

(---) γ-ray detection (——) UV detection.

Identification.

^{11}C-labeled caffeine had the same capacity factor (k') as a reference standard under the HPLC conditions used. Other identifications were performed using caffeine synthesized with unlabeled CH_3I under the same conditions as described above.
Thin layer chromatographic behaviour of the compound was evaluated on silica gel 60 F 254 (precoated plastic sheets, 20 x 20 cm). Rf values in two different eluent systems are given in Table 2.

Detection was performed with a UV lamp at 254 nm and subsequent spraying with 2M HCl and Dragendorff reagent which causes caffeine to develop an orange color (6).

Table 2 Rf values of theophylline (1), caffeine standard (2) and compound (3).

a = $CHCl_3$: ethanol, 90:10 (v,v)
b = $CHCl_3$: acetone : ammonia, 50:50:1 (v,v).

solvent system	1	2	3
a	0.26	0.40	0.40
b	0.08	0.28	0.29

UV spectra were recorded with a Pye Unicam SP 1800 double beam spectrophotometer equipped with 1 cm quartz cells, using HPLC eluent as a blank. Caffeine and the synthesized product were found to yield identical spectra (λmax 272 nm, λmin 244 nm).
Infrared spectra (KBr) of caffeine, theophylline and the lyophilized reaction product, collected from HPLC, were run on a Pye Unicam SP 1100 instrument. Spectra of caffeine and product matched perfectly.
Finally combined gas chromatography-mass spectrometry (GC-MS) was performed to confirm the identity of the product. The fused silica, capillary column SE 30 (0.31 mm x 25 m) was connected to a Hewlett-Packard 5992B quadrupole instrument operating at 70 eV, electron impact mode. The following GC-conditions were used : injection port 270°C and oven 200°C. The carrier gas was helium at a flow rate of $1.3 ml.min^{-1}$. Spectra of caffeine and the product were identical as confirmed by a spectra search system (molecular ion at m/z 194).

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