Appl. Radiat. Isot. Vol. 39, No. 5, pp. 373–379, 1988
Int. J. Radiat. Appl. Instrum. Part A

0883-2889/88 $3.00 + 0.00

Routine Synthesis of N-[^{11}C-Methyl]Scopolamine by Phosphite Mediated Reductive Methylation with [^{11}C]Formaldehyde

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(*Received* 30 *November* 1987)

A synthesis of [^{11}C]scopolamine capable of clinical delivery of this agent in high specific activity is described. The precursor [^{11}C]formaldehyde was produced by catalytic oxidation of [^{11}C]CH_3OH over metallic silver and was used to N-^{11}C-methylate norscopolamine using aqueous neutral potassium phosphite as the reducing agent. The labeling reaction was complete after 5 min at 75–80°C and the [^{11}C]scopolamine (99% radiochemical purity) was isolated by preparative HPLC. Total synthesis time is less than 45 min. Decay corrected radiochemical yields from [^{11}C]CO_2 are presently 20–43%.

Introduction

High densities of muscarinic cholinergic receptors are present in areas of the brain and heart (Levine *et al.*, 1986; Hirschowitz *et al.*, 1984). Various diseases and disorders in these organs are thought to involve changes in the number and distribution of muscarinic receptors. High resolution *in vivo* human imaging using PET and appropriately radiolabeled muscarinic ligands could be of value in the study and possibly early diagnosis of disease processes involving the muscarinic cholinergic system.

[^{11}C]Scopolamine was chosen as a candidate for PET studies following favorable evaluations of [^{3}H]scopolamine as a ligand for central muscarinic receptors in rats (Frey *et al.*, 1985a, b, c). Scopolamine is a very potent antimuscarinic drug with a binding affinity in the low nanomolar range. At the same time it is a clinically familiar drug with a long history of use in humans. [^{11}C]Scopolamine of low specific activity (1–4 Ci/mmol) previously has been prepared and evaluated in rats (Vora *et al.*, 1983). For the human studies planned, a synthesis capable of delivering a minimum of 20 mCi of [^{11}C]scopolamine with a specific activity of greater than 300 Ci/mmol was required. The present work describes a convenient synthesis of high specific [^{11}C]scopolamine by reductive N-[^{11}C]methylation of norscopolamine with [^{11}C]CH_2O, using potassium phosphite as the reducing agent under neutral aqueous conditions. A catalytic [^{11}C]CH_2O synthesis with improved specific activity and reproducibility is also described.

Materials and Methods

(−)Norscopolamine·HCl (m.p. 220°C, lit. m.p. 203–205°C; Werner and Schickfluss, 1969) was prepared from scopolamine·HBr hydrate (Sigma) by neutral $KMnO_4$ oxidative demethylation (Schmidt *et al.*, 1965). Final norscopolamine purification by preparative HPLC was necessary to remove small traces of scopolamine which would otherwise lower the specific activity of the final radiolabeled product. KH_2PO_3, 1 M pH 6.5, was prepared by neutralization of phosphorous acid (Aldrich) with K_2CO_3 under inert atmosphere and was stored refrigerated in a multidose vial. Stock reaction solutions of norscopolamine base (10 mg/mL) in 1 M phosphite were prepared in advance and were stable for at least 3 months when stored frozen.

Analytical HPLC was performed using a 4.6 × 250 mm 5 micron C-18 column with an isocratic solvent system of 40 vol% CH_3CN, 10% CH_3OH and 50% pH 6.5 2.0 mM KH_2PO_4, at a flow of 0.7 mL/min. Eluent was monitored by u.v. detector 220 nm (ISCO V^4) in series with a γ flow detector (Beckman Model 170). In the analytical HPLC sys-

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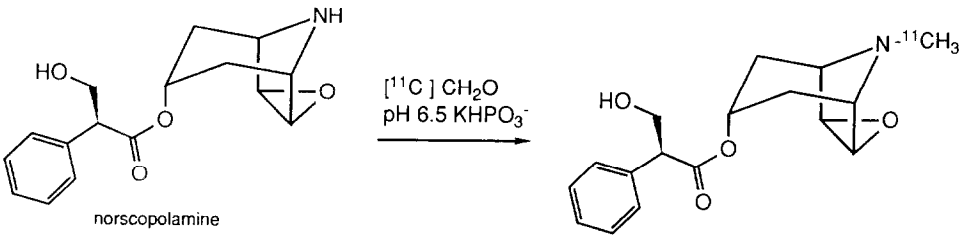

Fig. 1. [^{11}C]Scopolamine synthesis.

tem the elution time for norscopolamine was 6.6 min; for scopolamine 9.7 min. Tropic acid eluted in the void volume, and aposcopolamine at 12 min. Preparative HPLC was performed on two 9.4 × 100 mm Whatman PAC 5 micron columns in series using 90% CH_3CN, 10% isopropanol as the solvent at 3 mL/min. Ultraviolet detection of eluate was at 214 nm followed by radiation detection with an ionization chamber. Typical elution times in the preparative HPLC system were: scopolamine, 10 min; norscopolamine, 13 min. Degradation products including tropic acid and aposcopolamine eluted between 4 and 7 min. Thin layer chromatography was performed on glass backed silica plates (Merck F254) which had been stored under ambient humidity conditions, using 10:10:1:1 CH_2Cl_2:Et_2O:EtOH:Et_3N as the developing solvent: scopolamine R_f 0.65; norscopolamine R_f 0.40; aposcopolamine R_f 0.8. TLC plate radioactivity was quantitated using a Berthold Linear TLC scanner.

[^{11}C]Formaldehyde ([^{11}C]CH_2O)

In a modified version of the procedure of Berger *et al.* (1980), [^{11}C]CH_2O was produced in two steps by lithium aluminum hydride reduction of [^{11}C]CO_2 to [^{11}C]CH_3OH followed by oxidation over Ag to [^{11}C]CH_2O. No-carrier-added [^{11}C]CO_2 was produced by proton irradiation of a nitrogen gas target. The [^{11}C]CO_2 was concentrated by trapping in a glass loop cooled in liquid nitrogen. The loop was then warmed and the [^{11}C]CO_2 transferred by N_2 flow to a conical tipped glass reaction vessel containing 100 μL of a 0.1 M tetrahydrofuran solution of lithium aluminum hydride (Fluka). The tetrahydrofuran was evaporated to dryness by warming under a stream of nitrogen. Eighty five percent phosphoric acid (~100 μL) was added to hydrolyze the residue of metal [^{11}C]methoxides. The [^{11}C]CH_3OH was distilled from the reaction vessel and was carried in a nitrogen stream (44 mL/min) through a column of Porapak P (6 mm × 70 mm), which removed traces of tetrahydrofuran, then through a column of silver needles (Aldrich) at 390°C which catalytically oxidized the [^{11}C]CH_3OH to gaseous [^{11}C]CH_2O. Catalyst columns were prepared by loading 2 g of Ag needles into 4 mm i.d. pyrex tubes. They were activated prior to radiosynthesis by heating in a luminous flame (~525°C) while purging with a stream of oxygen which had been bubbled through 10% MeOH 90% H_2O. Residual CH_3OH and CH_2O were removed from the column by heating at 100–200°C under pure O_2 purge and then under N_2 purge at 390°C for 10 min preceding [^{11}C]CH_3OH oxidation.

Colorimetric formaldehyde assay

A colorimetric (Sawicki *et al.*, 1961) assay was used to help optimize formaldehyde production and identify sources of carrier carbon. Known amounts of CH_3OH vapor (0–100 nmol) diluted in nitrogen gas were injected into the N_2 carrier upstream of the heated catalyst column. Gaseous effluents from the Ag catalyst column were bubbled into tubes containing 200 μL of 0.2% aqueous 3-methyl-2-benzothiazolone hydrazone·HCl (MBTH, Aldrich) for 1 min at flow rates of 20–75 mL/min. The tubes were then placed in a 100°C heating block for 3 min. After cooling to room temperature, 125 μL of 0.4% $FeCl_3$ was added to each tube. After 5 min, samples were diluted to 1 mL total volume with acetone and absorbances were measured at 670 nm in 1 cm cuvettes. The intense blue derivatives obeys Beer's law down to 2 nmol/mL and is qualitatively detectable to the eye at a level of 3.5 nmol CH_2O/mL. Standard calibration curves over the range of 0–3 μmol CH_2O were constructed by adding known amounts of aqueous CH_2O to the MBTH reagent and processing samples as usual.

Preparation of [^{11}C]scopolamine

Gaseous high specific activity [^{11}C]CH_2O in a 44 mL/min N_2 carrier was bubbled through 150–250 μL of stock reaction solution of norscopolamine/phosphite at room temperature. After the accumulated activity reached a maximum as measured by a silicon diode detector (Computrol RAD-40), the reactor was sealed and heated in a 75–80°C oil bath for 5 min. The reaction mixture was then applied to a 3 cm × 4.6 mm C-18 cartridge pre-column which retained the [^{11}C]scopolamine while permitting aqueous salts and other H_2O soluble material to pass through to waste. After rinsing the reactor and pre-column with distilled water the pre-column was switched in line with the pumping HPLC organic mobile phase and the preparative HPLC columns by means of an electrically activated 8-port rotary injection valve. Adsorbed organics were eluted

from the pre-column onto the semiprep-columns where separation of [^{11}C]scopolamine (retention time 10 min) from unlabeled norscopolamine (13 min) was achieved. The [^{11}C]scopolamine fraction cut was evaporated to dryness and formulated in saline containing 10% ethanol. The final product was assayed by analytical HPLC and TLC.

Results and Discussion

In exploratory efforts to label scopolamine with carbon-11, [^{11}C]CH_3I was tried first as the precursor because it is generally recognized to be more easily produced and in higher specific activity than the alternative [^{11}C]CH_2O. However, we were unable to label scopolamine in satisfactory yield using [^{11}C]CH_3I in base because of rapid decomposition of the alkaloid. These results agreed with previous observations of Vora *et al.* (1983). Three points in the scopolamine molecule, the epoxide, the ester linkage and the hydroxymethyl function are known to be attacked under basic conditions by the routes shown in Fig. 3 (King, 1919; Schmidt *et al.*, 1965; Werner and Schmidt, 1967; Willstatter and Berner, 1923). The major degradation product observed in these experiments was aposcopolamine, an unsaturated derivative produced by dehydration of the hydroxymethyl group. Silyl or acyl protection of the hydroxyl function did not prevent occurrence of this side reaction. Consequently we turned attention to reductive labeling with [^{11}C]CH_2O as the precursor.

[^{11}C]Formaldehyde synthesis

For clinical production of [^{11}C]scopolamine, we required a catalyst for the oxidation of [^{11}C]methanol to [^{11}C]formaldehyde that was reliable and simple to prepare for repeated syntheses. A molybdate catalyst (Christman *et al.*, 1972) was found to introduce unacceptable levels of carrier carbon. We thus developed a modification of the procedure of Berger *et al.* (1979, 1980), which used a silver catalyst. Inexpensive silver needles rather than wire were used, allowing the catalyst to be simply poured into glass columns rather than packed. By use of the sensitive colorimetric formaldehyde assay of Sawicki *et al.*, (1961), a reliable method of catalyst activation was found which contributed less than 10 nmol of carrier formaldehyde to the synthesis and which gave a maximum formaldehyde yield at about 390°C. The Ag columns were reusable after reactivation with no loss of catalytic efficiency. The yield was relatively unaffected by small variations in temperature which allowed the catalyst temperature to be controlled by a small insulated aluminum heating block instead of an oven. The use of water to decompose the metal alkoxide formed during the reduction of [^{11}C]CO_2 by

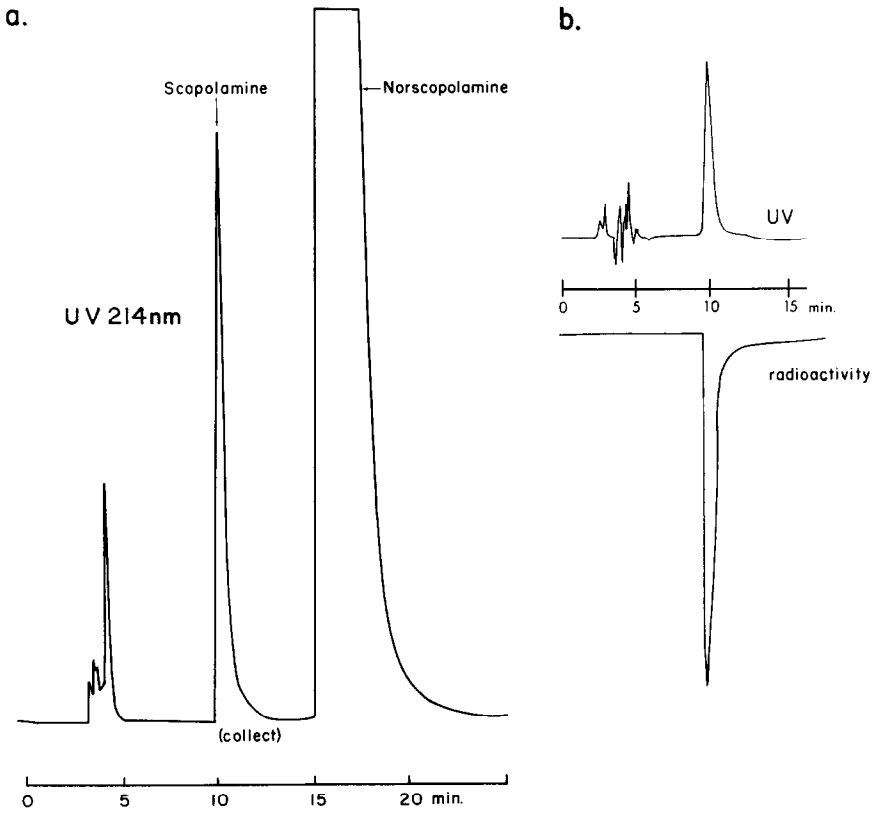

Fig. 2. Preparative (a) and analytical (b) HPLC chromatograms during typical [^{11}C]scopolamine synthesis.

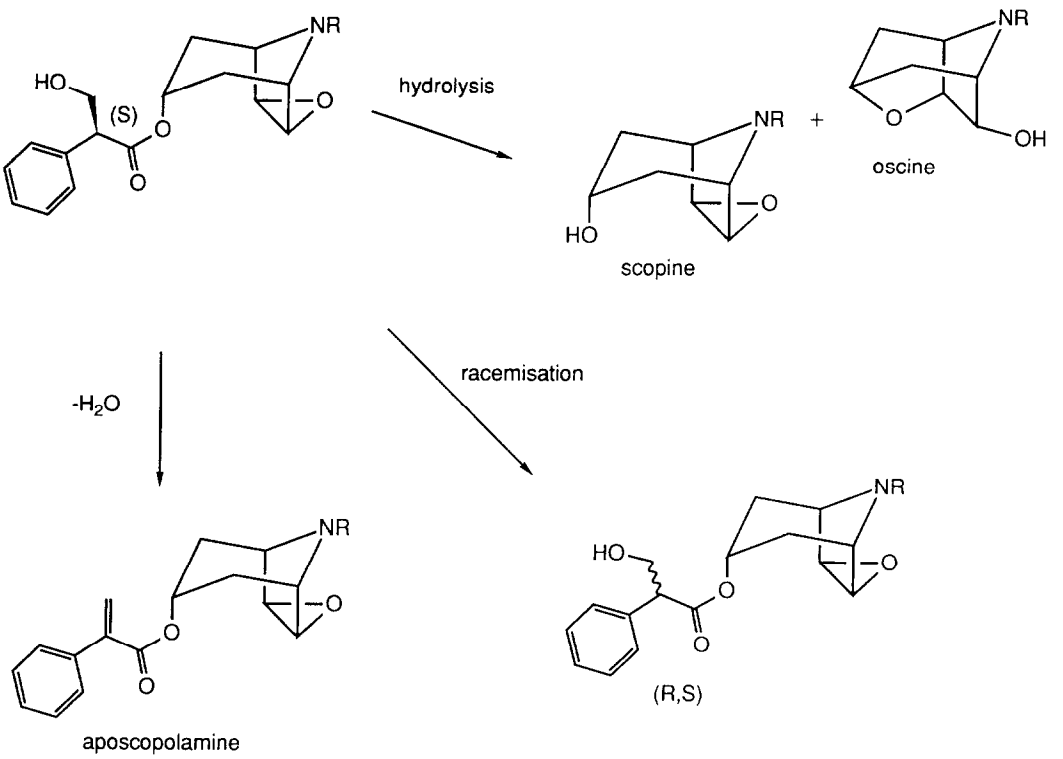

Fig. 3. Decomposition routes of (nor)scopolamine in base.

$LiAlH_4$ adversely affected the [^{11}C]formaldehyde yield both by interference with the catalytic oxidation (Madix, 1986) and also by causing condensation in the transfer lines which retained significant amounts of formaldehyde. This problem was avoided by using 85% H_3PO_4 to decompose the alkoxide.

At the 33 nmol level the above method was found to convert nonradioactive methanol to formaldehyde reliably in 60–70% yield. Because of the large number of possible variables, e.g. flow rate, temperature, nature of carrier, catalyst bed volume and geometry, catalyst activation and oxygen content, detailed optimization of all parameters was not attempted. Nevertheless, by the use of the Ag catalyst prepared as described above along with careful control of [^{11}C]CH_3OH synthesis to minimize the introduction of adventitious carbon and water, reliable yields of [^{11}C]CH_2O of specific activity 1500–3000 Ci/mmol at 8–13 min EOB were achieved. The recent detailed studies by the oxidation of methanol on single silver crystals (Madix, 1986) indicate that further improvement in yield may be obtainable by a more complete understanding and control of conditions.

Phosphite reductive [^{11}C]methylation

Reductive [^{11}C]methylation of amines with [^{11}C]CH_2O has most often been carried out using $NaBH_3CN$ as the reductant (Finn *et al.*, 1984; Berger *et al.*, 1979; Boullais *et al.*, 1985). However, use of $NaBH_3CN$ in radiosyntheses of agents intended for humans is complicated by the fact that cyanide ion is released in the course of reaction and additional steps must be taken to assure the absence of cyanide from the final product.

We sought to avoid this complication by investigating other reducing agents. Phosphite came to our attention in a report (Loibner *et al.*, 1984) showing it to be useful in reductive methylations of simple primary and secondary amines. Preliminary non-radioactive experiments using formaldehyde as

$$RR'NH + CH_2O + H_2PO_3^- \rightarrow RR'NCH_3 + H_2PO_4^-$$

the limiting reagent showed that norscopolamine was readily converted to scopolamine. Additionally, it was found this reaction could be carried out in completely aqueous solution. An examination of the effect of temperature upon the rate of reductive methylation of norscopolamine with one equivalent of formaldehyde (Fig. 4) showed that the reaction at 85°C was complete within 5 min. Unlike methyl iodide methylation, reductive methylation using neutral phosphite was very clean with only minor amounts of scopolamine degradation products formed even at elevated (90°C) reaction temperatures. The purified product was chromatographically and analytically identical to authentic scopolamine.

The ability to conduct the methylation reaction in neutral aqueous solution offered practical advantages

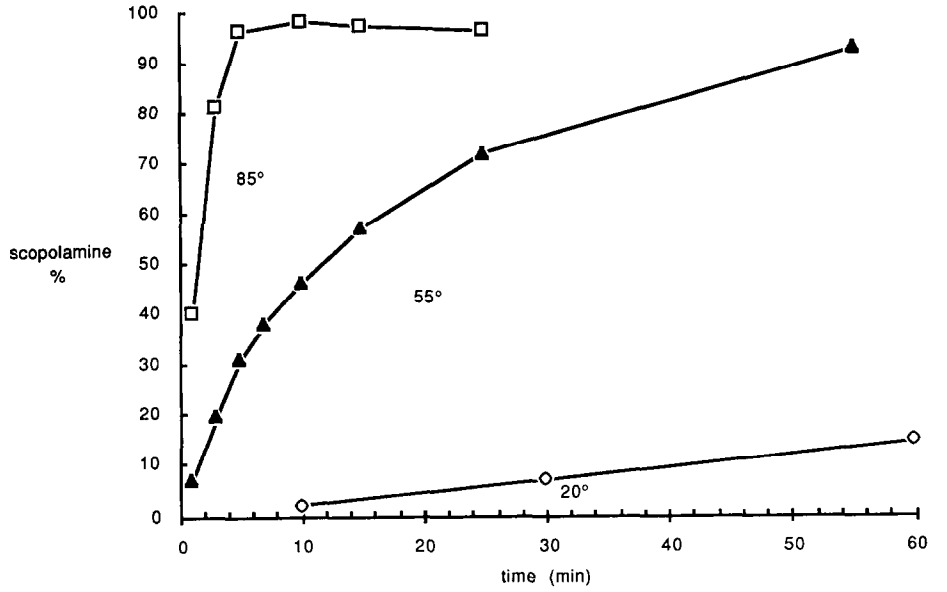

Fig. 4. Effect of temperature on rate of phosphite mediated reductive methylation of norscopolamine. Initial conditions: 0.033 M norscopolamine, 0.033 M formaldehyde, 1.0 M pH 6.5 phosphite. Products were measured by reverse phase HPLC (see Methods section for conditions).

from the standpoint of carbon-11 labeling. First, because gaseous [^{11}C]formaldehyde is readily absorbed into water (Boullais *et al.*, 1985) even from very fast gas carrier streams, rapid and complete trapping of the [^{11}C]formaldehyde in a small volume (150–250 μL) of reaction solution was possible. This resulted in faster rates of N-[^{11}C]methylation because of the high effective concentrations of reactants. Second, no neutralization step was required after completion of the methylation reaction. Rapid isolation of labeled scopolamine could be achieved simply by passing the reaction solution through a C-18 sample enrichment cartridge which retained scopolamine and norscopolamine but allowed aqueous inorganic salts to flush to waste. By configuring the C-18 cartridge with a rotary injection valve (Fig. 5), the operations of sample extraction, concentration and HPLC injection for purification were combined in a single device.

A diagram of the [^{11}C] methylation apparatus is shown in Fig. 5. Three two-position multiport rotary valves were linked together to control the operations of reagent addition and product workup with a minimum of switch throwing. Liquid transfers were effected either by syringe or with helium pressure. Utilization of standard electrically actuated HPLC type valves rendered assembly of the apparatus convenient and resulted in a compact, reliable system which was amenable to automation.

Preparative HPLC with a bonded aminocyano normal phase (Whatman PAC) was preferred over underivatized silica gel because the former packing was not adversely affected by the presence of water in the injection volume. Specific activity was determined during preparative HPLC purification and was checked again by analytical HPLC. Scopolamine has a weak u.v. chromophore and its limit of detectibility under routine conditions in our analytical HPLC was 20 ng (67 pmol). Specific activities and other data for the [^{11}C]scopolamine synthesis are summarized in Table 1.

Overall radioactivity balance for the [^{11}C]scopolamine synthesis

For a typical human dose preparation, approximately 1100 mCi of [^{11}C]CO_2 were accumulated from the target in a liquid nitrogen cooled loop at 4 min past EOB (end-of-bombardment). By 33–38 min, between 60 and 150 mCi of radiochemically pure [^{11}C]scopolamine had been collected from the preparative HPLC. This corresponds to decay corrected radiochemical yields between 20 and 43%. The balance of radioactivity could be accounted for in the following three locations. The first portion, constituting 7–20% of the total carbon-11, was in vent

Table 1. [^{11}C]Scopolamine synthesis data

Number of syntheses	19
Synthesis time[a]	40–45 min
Decay corrected radiochemical yield range[b]	20–43%
Radiopurity	>99%
Specific activity range[c]	350–1300 Ci/mmol
Mean specific activity[c]	650 Ci/mmol
End of synthesis yield range[c]	60–150 mCi

[a]Including formulation for injection.
[b]Based on [^{11}C]CO_2 at EOB plus 4 min.
[c]After HPLC purification.

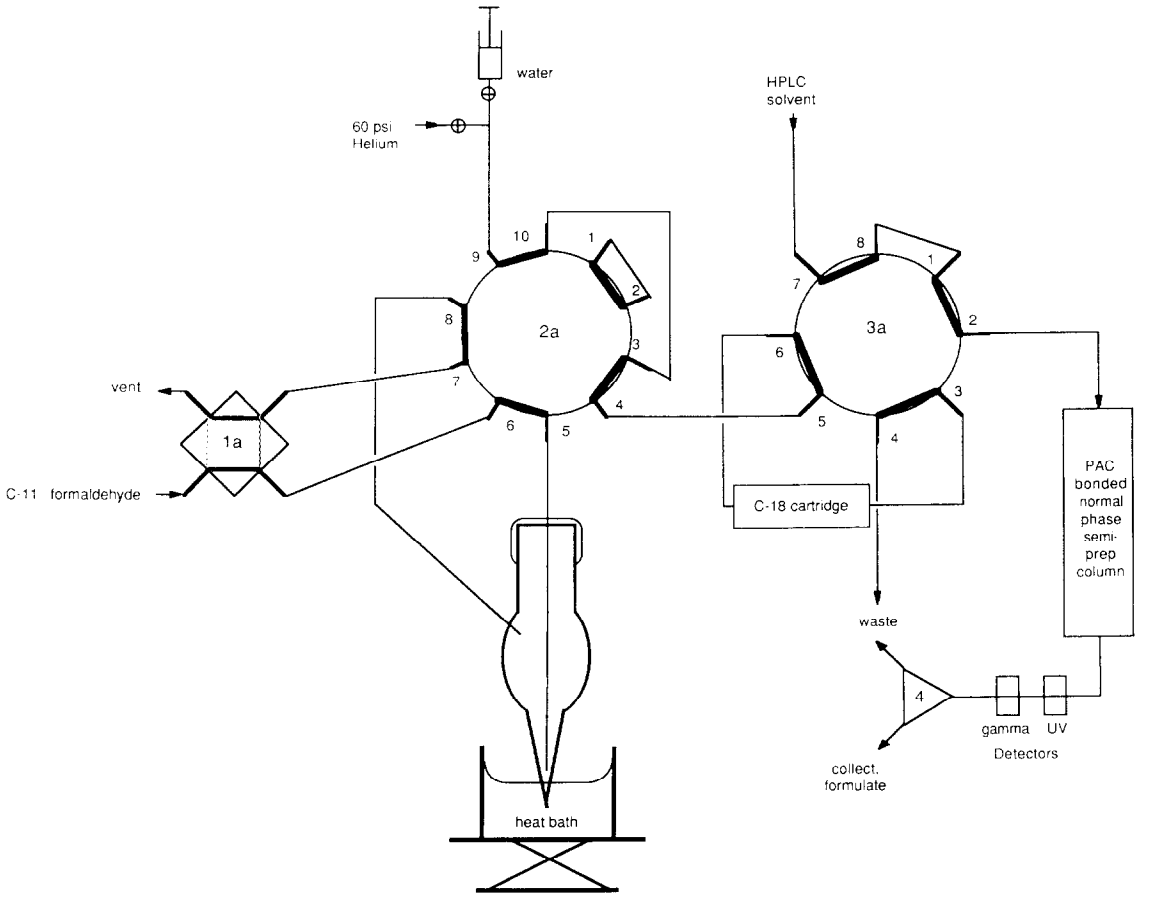

Fig. 5. [^{11}C]-Methylation apparatus. Diagram shows rotary valve settings during passage of gaseous [^{11}C]CH_2O into aqueous phosphite reaction solution. After accumulated radioactivity has reached a maximum, reactor is sealed by switching valve 1a to position 1b, and the heating bath is raised. The reactor is heated for 5 min then the reaction solution is transferred onto the C-18 cartridge by actuating valve 2a to position 2b and applying helium pressure. The reactor and C-18 cartridge are rinsed with three 1 mL portions of H_2O from the syringe. HPLC purification of the [^{11}C]scopolamine now adsorbed to the C-18 cartridge is initiated by switching rotary valve 3a to position 3b. The HPLC column eluent is monitored by u.v. (214 nm) and radioactivity. The eluting [^{11}C]scopolamine peak is collected by switching 3-way valve 4 to the "collect" position.

gases from the phosphite reduction reactor. This activity was dimedon (Berger *et al.*, 1980) negative (no [^{11}C]CH_2O present), could be trapped on soda-lime and the trapped radioactivity for the most part was liberated when the sodalime is added to dilute H_2SO_4. On the basis of this behavior it is assumed that most of the reactor vent gas radioactivity was in the chemical form of CO_2.

The second portion (10–30%) of radioactivity was found in the aqueous washings from the C-18 cartridge precolumn. Analysis with the dimedon reagent showed little free [^{11}C]CH_2O. TLC analysis of the washings showed variable but significant amounts of activity remaining at the origin, and mobile, volatile activity, most of which was [^{11}C]CH_3OH as determined by GC. No significant amount of [^{11}C]scopolamine breakthrough from the C-18 cartridge rinse was observed. The nature of the material(s) at the TLC origin requires further investigation and is uncertain at this time. It elutes near the void volume in the analytical RP-HPLC system. Possible identities are [^{11}C]methylated alkaloid fragments such as scopine or oscine (Schmidt *et al.*, 1965; Willstatter and Berner, 1923), or [^{11}C]hydroxymethylphosphonic acid $HOCHPO_3H$ which under certain conditions may be produced from phosphite and CH_2O (Akad Wissenschaft DDR, 1985).

The final balance of 0–2% of the total decay corrected ^{11}C activity from a typical [^{11}C]scopolamine synthesis appeared in the early fractions of the preparative HPLC, eluting several minutes before and cleanly separated from scopolamine. The retention behavior of this ^{11}C containing species is consistent with that of aposcopolamine.

Summary and Conclusions

Improved production of [^{11}C]CH_2O on a routine basis and its application for labeling clinical scale doses of [^{11}C]scopolamine in high specific activity has been achieved. Further refinements in [^{11}C]CH_2O production and speed of reductive methylation should be achievable which can make this approach very competitive with existing [^{11}C]CH_3I labeling procedures in terms of overall radiochemical yield. The usefulness of the phosphite mediated reductive labeling procedure was demonstrated successfully in a situation where, because of the base sensitivity of the scopolamine molecule, normal labeling attempts using [^{11}C]CH_3I had failed. A number of additional technical advantages followed from being able to conduct ^{11}C-labeling under essentially neutral, totally aqueous reaction conditions, including simplicity of reaction workup and product purification, fast synthesis turnaround time (< 1 h) and the ease of system automation. It is believed that the phosphite ^{11}C-labeling approach can be of general utility for synthesis of a wide variety of [^{11}C-methyl]amines for applications with PET.

Acknowledgements—The support of NINCDS No. 2PO1-NS15655 is gratefully acknowledged. We thank Ms Linder Markham for preparation of this manuscript and Dr Michael Kilbourn for his suggestions and careful review of this work.

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