Preparation of Oxygen-15 Butanol for Positron Tomography

Marc S. Berridge, Mark P. Franceschini, Timothy J. Tewson, and K. Lance Gould

Division of Cardiology, University of Texas Health Science Center, Houston, Texas

Butanol was labeled with ^{15}O using the reaction of tri-n-butyl borane with oxygen gas. The carrier-added synthesis and purification was accomplished in 4 min from the end of bombardment. The efficiency of radiolabel incorporation was 50%. The procedure described will produce [^{15}O]butanol in an amount and quality sufficient for positron tomographic use. This compound is immediately useful for blood flow measurement based upon previous validation of butanol labeled with other radionuclides for that purpose.

J Nucl Med 27:834–837, 1986

An important function of positron tomography is the noninvasive assessment of regional cerebral blood flow (CBF). There are presently several methods for making this assessment, all of which rely on mathematic modeling of the distribution and/or kinetics of a particular highly retained or freely diffusible tracer. These methods are well documented and remain a subject of current research (*1–6*).

One of the more commonly used tracers for CBF measurements is oxygen-15- (^{15}O) labeled water. It has been suggested, however, that water is not the optimum tracer material since butanol may be much closer to the ideal for this purpose (*7–10*). The greater lipid solubility of butanol results in nearly complete free exchange into brain tissue.

A difficulty that has prohibited the use of butanol, or any other alcohol, labeled with ^{15}O is that the methods available for synthetic incorporation of ^{15}O have not been sufficiently rapid or efficient. The 2-min half-life of ^{15}O has, therefore, prevented production by current methods of a quantity of tracer useful for positron tomography. A further constraint is that the tracer, once synthesized, must be amenable to rapid purification. The only available alternative has been to use the carbon-11- (^{11}C) labeled compound (*4–10*). Although convenient for synthesis, the longer half-life of ^{11}C (20 min) is inconvenient in common medical imaging procedures that use several sequential measurements of flow and measurements involving other radiopharmaceuticals.

Received Sept. 24, 1985; revision accepted Jan. 21, 1986.

For reprints contact: Marc S. Berridge, PhD, Dept. of Radiology, Case Western Reserve University School of Medicine, University Hospitals, 2074 Abington Rd., Cleveland, OH 44106.

Recently, a communication by Kabalka (*11*) described an organoborane reaction that allows the synthesis of butanol in good yield from oxygen gas that would be suitable for use with ^{15}O. Further communications (*12,13*) established the expected result that no change in the reaction is observed when the oxygen is labeled with ^{15}O at low specific activity. Because of the very low quantity and specific activity of the oxygen gas used, and because of the lack of a suitable purification, the method as reported (*12,13*) is not suitable for synthesis of large quantities of butanol for positron tomography. However, the reported 50% radiochemical incorporation into pure labeled butanol was encouraging for further development. We report here reaction conditions, further observations, and purification methods for the use of this reaction for large scale sequential production of ^{15}O-labeled butanol for positron tomography studies of human subjects.

MATERIALS AND METHODS

Tri-n-butyl borane (1*M*) in tetrahydrofuran (THF) was obtained commercially* and used without further purification. Argon gas (extra dry grade) was passed over dried 4A molecular sieves immediately before contact with the reagent. Reverse-phase SEP-PAKs were used according to manufacturer's instructions.

The $^{14}N(d,n)^{15}O$ reaction on nitrogen gas containing 1% oxygen was used for production of ^{15}O-labeled oxygen. The target was 5 cm in diam and 10 cm long, pressurized to 4 atm. A bombardment of 10 μA of 8 MeV deuterons for 7 min typically produced 100 mCi of useful ^{15}O measured at end of bombardment.

Preparation of [^{15}O]butanol was performed using the

published reaction (*11*):

$$B\,(n\text{-}C_4H_9)_3 \xrightarrow[2.\ H_2O]{1.\ O_2} n\text{-}C_4H_9OH.$$

The tri-n-butyl borane was prepared by evaporation of solvent from 1 ml of a 1*M* solution in THF. This was done in a dried glass reaction vessel (4 ml) sealed with a teflon-backed silicone septum. Disposable needles (25G) were used to purge the vessel with argon before introduction of the reagent. A vacuum pump (1 mmHg) equipped with a liquid nitrogen cold trap was then attached and a slow argon flow through the vessel was continued during the evaporation.

After 20 min under vacuum, the vessel was pressurized to 2 psig with argon and the needles were removed. The reagent prepared in this manner was usable for several days, and could therefore be prepared in advance. Any leakage of the seal and spoilage of the reagent was readily apparent from increasing cloudiness of the clear liquid reagent.

The prepared tri-n-butyl borane in the reaction vessel was inserted in the system shown in Fig. 1. Disposable needles were used for entrance and exit lines. Two needles placed at the bottom of the vessel served to introduce labeled oxygen and to remove product. The system was purged in advance with helium gas, and helium gas flow was continued at ~5 ml/min. The vessel was cooled in an ice bath.

The target gas was then introduced to the vessel at a flow rate of ~300 ml/min. The amount of gas used is limited to the target volume (200 ml) at 4 atm of pressure (0.3 mmol O_2). Under the conditions used, the target was emptied in ~2 min.

After introduction of the target gas, the boron complex was hydrolyzed by addition of 1 ml sterile water and the solution was passed through a reverse phase SEP-PAK cartridge. The cartridge was then rinsed with 1.5 ml sterile water and eluted with 5 ml of 10% ethanol in sterile saline. For in vivo use the eluent was passed into a sterile syringe through a 0.22-μm sterile filter.

The product was analyzed by gas chromatography and high performance liquid chromatography (HPLC). Gas chromatographic conditions used were: 3% AN600 on Anakrom A 2 meter column at 60°C. A flame ionization detector was used. Retention times were: butane 1.1 min, THF 1.5 min, butanol 2.0 min, boron compounds 4.5 to 5.3 min. Liquid chromatography conditions were: 15% acetonitrile in 0.01*M* ammonium acetate at 2 ml/min through a 25 × 0.5 cm analytic C18 reverse-phase column. Refractive index and radioactivity detectors were used. Retention times of radioactive products were: water 1.6 min, butanol 2.5 min. The only material present in sufficient quantity to give a mass peak on HPLC was butanol.

RESULTS

The reaction of oxygen with tri-n-butyl borane was found to be extremely rapid. As a result, the high flow rate through the reaction vessel did not significantly reduce the quantity of radioactivity that could be collected. Experiments were performed in which measured amounts of oxygen were passed through the vessel and measurements were subsequently made of the quantity of butanol produced. They showed that the reaction efficiency under the conditions used was >80%.

Initial studies of the reaction mixture after hydrolysis demonstrated the presence of residual THF, butanol, boron compounds, and small amounts of butane from unreacted reagent. No butyraldehyde or butyric acid was observed.

Two boron compounds were present in the mixture. The predominant material was a volatile white solid which converted on standing to a nonvolatile solid. Proton nuclear magnetic resonance (NMR) of the volatile material showed multiplets at 0.9, 1.3, and 2.1 in a pattern consistent with a B-Bu linkage. Quantitation was suggestive of B-Bu$(OH)_2$. The nonvolatile material gave no proton NMR. Boron-11 spectra of the volatile compound showed an absorption at 33 ppm from bo-

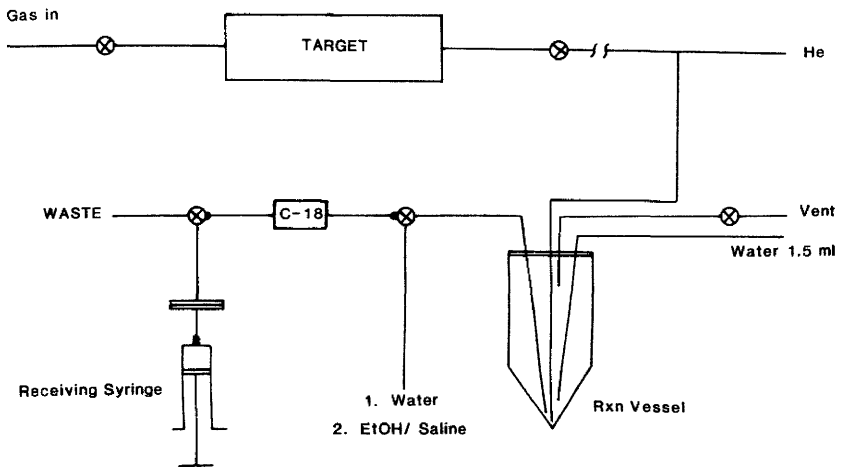

FIGURE 1
Apparatus for butanol synthesis. Crossed circles denote valves with closed circles on common side. C-18 denotes reverse phase cartridge

ron trifluoride etherate and the nonvolatile compound absorbed at 19 ppm. This result is consistent with identification of the volatile compound as $B(OH)_2Bu$ and the nonvolatile compound as $B(OH)_3$.

From the standpoint of in vivo use, small amounts of butane posed no real problem. Due to the 0.67 mmol of available oxygen, the maximum amount of butanol which could be produced was 1.2 mmol. In the final solution, this would give 5 ml of 0.24*M* butanol for a total dose of 89 mg of butanol. However, THF and boron compounds must be removed before use. It was found that a 2-g portion of any ion exchange resin could satisfactorily remove these impurities, leaving only butanol in the solution. Application of this method for labeling, however, revealed a radiolabeled impurity which was not identified in previous work. This material was almost certainly ^{15}O-labeled water. It was volatile upon distillation but not upon standing, eluted on the void volume of the HPLC in all solvent systems, and was not measurably retained by any amount of ion exchange resin. Neither butanol nor oxygen gas showed any signs of exchange with solvent water. When extraordinary measures (described below) were not taken to eliminate water from the system, this labeled impurity contained 50% of the radioactivity that was recovered.

Several approaches were taken to avoid the co-production of water with the butanol. These included hydrolysis by freshly dried and distilled ethanol or methane sulfonic acid, and hydrolysis only after heating to 150°C in order to complete any rearrangement of boron intermediates. The latter approach had no effect, while the former ones, especially dry distilled methane sulfonic acid, were successful in producing radiochemically pure butanol on several occasions. Unfortunately, this result was not consistently reproducible due to the difficulty of keeping the apparatus, solvents, and reagents completely dry.

Because water production could not be reliably eliminated, a method was sought to remove it from the butanol. Passage of the reaction mix through a reverse-phase cartridge resulted in retention of >90% of the butanol, while the labeled water passed off the cartridge. Subsequent washing with 10% ethanol in normal saline eluted the butanol with no detectable amounts of boron salts or THF, and completely free of ^{15}O water. The purification procedure using a reverse-phase cartridge required <1 min. The product was eluted directly into a syringe through a sterile 0.22-m filter.

The radiochemical incorporation of ^{15}O into butanol was 50%. The radiochemical yield (uncorrected) of purified butanol was 10% at 4 min after the end of bombardment. The maximum activity obtained routinely to date is 50 mCi ready for injection.

DISCUSSION

Ion exchange resin was useful for removal of boron compounds and residual THF from the butanol samples. The amount of resin that was required was, in fact, less than the quantity calculated from the measured capacity of the resin for ion exchange. This implies that the mechanism involved in removal of impurities is hydrophobic absorption to the resin, similar to reverse-phase chromatography. Ion exchange resin, however, does not retain butanol.

The presence of labeled water in the product mix is not easily explained. Water is not formed by passage of oxygen gas through water, nor does exchange of labeled butanol with water occur. Therefore, exchange of labeled oxygen in the intermediate boron compounds with water added for hydrolysis must occur. Although definitive proof does not exist, the accepted mechanism for the labeling reaction is:

$$R_3B + O_2 \rightarrow R_2B\text{-}O\text{-}O\text{-}R \rightarrow$$

$$RB(OR)_2 \rightarrow RB(OH)_2 + 2\ ROH.$$

The net reaction resulting from this mechanism is:

$$R_3B + O_2 + 2H_2O \rightarrow 2ROH + RB(OH)_2.$$

One explanation for oxygen exchange is that the hydrolysis may take place by attack of OH either on boron or carbon. This is supported by the observation that heating the complex to promote complete rearrangement before hydrolysis occurs has no effect on water production. However, hydrolysis with dry methane sulfonic acid or, to a lesser extent, ethanol resulted in radiochemically pure butanol. The difficulty with this approach is that the production system is not easily kept sufficiently dry. Therefore, the purity of the product is not reliable.

It was found that a reverse-phase cartridge could be used to hold not only the hydrophobic impurities, but also the butanol itself. Labeled water of course was not retained. Less than 10% of the butanol produced was eluted with the water wash of 1.5 ml, while all of the labeled water was removed. Subsequent elution with 5 ml of 10% ethanol in normal saline removed the butanol, but allowed any previously uneluted impurities to remain on the cartridge. Passage of the final cartridge elution through a sterile 0.22-μm filter resulted in a sterile, pyrogen free injectable solution.

The entire synthesis and purification requires 4 min from the end of bombardment, and is performed remotely in a shielded hood.

The technique of using butanol for obtaining blood flow information has been validated using carbon-11-labeled butanol (*4,5,10*). This method of producing the same tracer labeled with ^{15}O will allow the same studies to be performed with the advantage that sequential studies may begin within 10 min after injection of butanol.

FOOTNOTES

* Aldrich Chemical Company, Milwaukee, WI.

† Waters Associates, Milford, MA.

ACKNOWLEDGMENTS

The authors thank Dr. George Kabalka for boron-11 NMR spectra and several useful discussions.

This work was supported in part by the Clayton Foundation for Research.

REFERENCES

1. Raichle ME, Martin WRW, Herscovitch P, et al: Brain blood flow measured with intravenous O-15 water. II. Implementation and validation. *J Nucl Med* 24:790–798, 1983
2. Huang SC, Carson RE, Hoffman EJ, et al: Quantitative measurement of local cerebral blood flow in humans by positron computed tomography and O-15 water. *J Cereb Blood Flow Metab* 3:141–153, 1983
3. Koeppe RA, Holden JE, Ip WR: Performance comparison of parameter estimation techniques for the quantitation of local cerebral blood flow by dynamic positron computed tomography *J Cereb Blood Flow Metab* 5:224–234, 1985
4. Hack SN, Bergmann SR, Eichling JO, et al: Quantification of regional myocardial perfusion by exponential infusion of [C-11]-butanol. *IEEE Trans Biomed Eng* 30:716–722, 1983
5. Knapp WH, Helus F, Oberdorfer F, et al: ^{11}C-butanol for imaging of the blood flow distribution in tumor bearing animals. *Eur J Nucl Med* 10:540–548, 1985
6. Eklof B, Lassen NA, Nilsson L, et al: Regional cerebral blood flow in the rat measured by tissue sampling technique: A critical evaluation using four indicators, ^{14}C-antipyrine, ^{14}C-ethanol, ^{3}H-water, and ^{133}Xe. *Acta Physiol Scand* 91:1–10, 1974
7. Raichle ME, Eichling JO, Straatmann MG, et al.: Blood-brain barrier permeability of C-11 labeled alcohols and O-15 labeled water. *Am J Physiol* 2:543–552, 1976
8. Dischino DD, Welch MJ, Kilbourn MR, et al: Relationship between lipophilicity and brain extraction of C-11 labeled radiopharmaceuticals. *J Nucl Med* 24:1030–1038, 1983
9. Oberdorfer F, Helus F, Maier-Borst W, et al: The synthesis of (1-^{11}C)-butanol. *Radiochem Radioanal Lett* 53:237–252, 1982
10. Kothari PJ, Finn RD, Vora MM, et al: 1-[C-11]Butanol: Synthesis and development as a radiopharmaceutical for blood flow measurements. *Int J Appl Radiat Isot* 36:412–413, 1985
11. Kabalka GW: Incorporation of stable and radioactive isotopes via organoborane chemistry. *Accts Chem Res* 17:215–221, 1984
12. Kabalka GW, McCollum GW, Fabirkiewicz AS, et al: Synthesis of O-15 labeled butanol via organoborane chemistry: Preliminary report. *J Lab Comp Radiopharm* 21:11–12, 1184, 1984 (abstr)
13. Kabalka GW, Lambrecht RM, Sajjad M, et al: Synthesis of O-15 labeled butanol via organoborane chemistry. *Int J Appl Radiat Isot* 36:853–855, 1985