Simple Production of [1-Carbon-11]Acetate

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We report an attractive approach for the preparation of [1-^{11}C]acetate. **Methods:** The procedure involved the instantaneous hydrolysis of [1-^{11}C]acetyl chloride back to [1-^{11}C]acetic acid by simply trapping the volatile acid chloride in physiological saline. This delivered [1-^{11}C]acetate immediately in pharmaceutical quality. **Results:** An easy and quantitative gas phase separation of the radiopharmaceutical from any inorganic residue and organic contamination could be achieved. The preparation required a minimum of automation and afforded only 5 min for an amount of 15 GBq of [1-^{11}C]acetate which was yet ready for injection. Multiple preparations could be performed within 1 day. **Conclusion:** The use of [1-^{11}C]acetyl chloride as a precursor to [1-^{11}C]acetate is of considerable practical importance lending itself to automation with ease and giving the target compound directly in sterile solution without the need for further care and purification.

Key Words: [1-carbon-11]acetyl chloride; [1-carbon-11]acetate; PET; radiopharmaceutical synthesis

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The tracer compound [1-^{11}C]acetate has proved to be a useful radiopharmaceutical in nuclear cardiology investigations and clinical applications (*1*). Its use is based on the tricarboxylic acid metabolic pathway, the Krebs cycle, whose activity is assessed reliably by the [1-^{11}C]acetate kinetics through the myocardium and binding to coenzyme A. This offers the possibility of estimating the myocardial oxidative metabolic reserve along with the measurement of the myocardial oxygen utilization (*2–8*). It also provides an indirect assessment of regional myocardial perfusion and blood flow (*9,10*). A simple and efficient procedure for the preparation of [1-^{11}C]acetate is therefore, highly desirable, particularly since there is a need for multiple preparations during a daily schedule.

MATERIALS AND METHODS

Reagents

We used analytical grade chemicals and solvents. Ether was dried over sodium and distilled in an inert atmosphere immediately before use. Phthaloyl dichloride was distilled in vacuo (110°C; 2 mm) and stored under dry conditions. The Grignard compound CH_3MgCl was purchased as a 3 *M* solution in tetrahydrofuran and CH_3Li was obtained as a 1.6 *M* solution in ether.

Tetrahydrofuran was distilled off and the Grignard compound was redissolved in an equivalent amount of ether. The organometallic reagents were then partitioned into 1 ml portions into separate vials. The solutions were separated from inorganic precipitates by centrifugation immediately before use. A volume of 2 ml isotonic saline in a sterile vial containing 5 μl sodium hydrogencarbonate 8.4% was used to trap [1-^{11}C]acetyl chloride.

Radiochemistry

All labeling procedures were performed in a remote controlled apparatus consisting of two inert miniature three-way solenoid valves, one heating and one cooling bath and one flow controller for a regulated helium supply. One screw-capped vial containing the organometallic reagent and one sterile vial for collection of the radiopharmaceutical in isotonic saline were connected to the apparatus.

Radioactive [^{11}C]CO_2 was produced at the MC32 NI cyclotron of the German Cancer Research Center by the $^{14}N(p, \alpha)^{11}C$ nuclear reaction. The stainless steel target chamber contained N_2 at 30 kg · cm^{-2} and was irradiated with a 20-μA proton beam of 20 MeV. The radioactive target gas was expanded into a stainless steel capillary of 0.8 mm i.d., immersed in liquid argon. Typically 35 GBq of radioactivity were obtained within 20 min. A helium flow of 15–20 ml · min^{-1} carried [^{11}C]CO_2 into the small reaction vial which contained either 5 μmole CH_3Li or CH_3MgCl in 120 μl of ether at −15°C. Evaporation of ether required 40 sec at 80°C and left a dry salt which then reacted with 50 μl o-phthaloyl dichloride (*11,12*). The immediately formed and pure [1-^{11}C]acetyl chloride distilled off under these conditions within 2–3 min into the sterile vial. There it was trapped at a pH of 7.5 as sodium [1-^{11}C]acetate by hydrolysis with isotonic saline containing 5 μl $NaHCO_3$ 8.4%. A steady supply of helium was maintained during the whole procedure.

Analytical Procedures

A sample of the sodium [1-^{11}C]acetate was analyzed at a pH of 2.3 by HPLC on a 300 mm × 8 mm weak cation-exchange column, packed with a 9% cross-linked polystyrene sulfonate resin of high ligand density, in H^+ form. The sample eluted as [1-^{11}C]acetic acid (pK_s = 4.76) with 0.005 *M* H_2SO_4 at a flow rate of 0.8 ml · min^{-1}. Refractive index detection at 35°C by a Waters 410 differential refractometer (Waters, Eschborn) showed the acetic acid reference sample at a retention volume of 12.8 ml (16 min) which coincidenced with the radioactive peak of the preparation. Some of the preparations were quantitated in total on a Radiomatic FLOW-ONE A200 detection system (Radiomatic Instruments and Chemical Co., Tampa, FL) at about seven half-life times after the end of synthesis for specific activity determinations.

RESULTS

We demonstrated that [1-^{11}C]acetate could be obtained effectively and with great ease by hydrolytic cleavage of [1-^{11}C]acetyl chloride to the chemically uniform sodium [1-^{11}C]acetate in sterile physiological saline as presented in Figure 1. The resulting solution was of pharmaceutical quality and immediately ready for injection. The synthesis apparatus required no wash-up procedure, just the exchange of two reaction vials, and again was available for any further [1-^{11}C]acetyl chloride radiochemical synthesis. The total procedure, including ^{11}C-production and equipment set up, required 25–30 min and comfortably delivered an average of 15 GBq labeled acetate in one batch (up to 30 GBq [1-^{11}C]acetate may be produced). This corresponded to a 43% yield not corrected for decay and related to the amount of initially trapped [^{11}C]CO_2. The specific activity calculated back to the time at the end of synthesis varied between 90–200 GBq/μmole. Chemical and radiochemical purity of the product was

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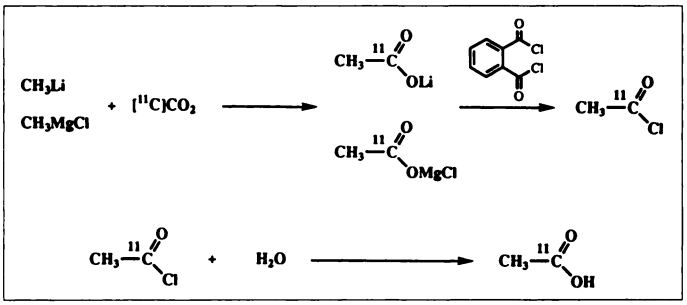

FIGURE 1. Preparation of [1-^{11}C]acetic acid using [1-^{11}C]acetyl chloride as the key intermediate.

99.5%. The preparation clearly passed the test for absence of pyrogens and contained not more than 1.5 EU/ml according to the chromogenic LAL test (calculated endotoxin limit for a 2-ml sample: 175 EU/ml).

Significant contaminations were not found in the final solution. Traces of [1-^{11}C]ethanol were detected occasionally. This occurred particularly when the initial amount of the organometallic reagent (especially Grignard reagent) was excessive, i.e., above 5 μmole. Excess Grignard reagent presumably leads to reduction of the formed [1-^{11}C]acetate during the first step of the reaction, which produces [1-^{11}C]ethanol. Surprisingly, this product distilled together with the [1-^{11}C]acetyl chloride, although one would expect condensation of these two products to ethyl acetate with elimination of hydrogen chloride. This, however, was not observed. It also was our clear impression that CH_3Li was superior to the Grignard reagent with regard to the suppression of the [1-^{11}C]ethanol formation. In any case, [1-^{11}C]ethanol contamination was below 0.5%. The expected Gilman-van-Ess synthesis in the presence of CH_3Li which usually delivered [^{11}C]acetone (*13*) was circumvented by carefully controlling the amount of CH_3Li.

DISCUSSION

Previously reported syntheses of [1-^{11}C]acetate preponderantly used direct decomposition of the mixed magnesium salt ([1-^{11}C]CH_3COO)MgX (X = Cl, Br). Solvent extraction techniques with intensive inert gas purging and its concomitant automation difficulties, and various distillation procedures or solid-phase extraction techniques with chromatographic work-up, were used to separate the [1-^{11}C]acetic acid from organic and inorganic contamination before it was finished as the parenteral [1-^{11}C]acetate solution (*14–18*). All these operations were feasible, but laborious, and always involved a significant loss of the desired labeled product. Although the [1-^{11}C]acetate production is trivial, we nevertheless tried to obtain this radiopharmaceutical through simple techniques directly from a standardized and routinely used ^{11}C-labeled precursor without additional equipment, and with the possibility of repeat daily production of the tracer compound.

[1-Carbon-11]acetyl chloride offered this possibility. Acetyl chloride may be prepared directly from any dry salt of acetic acid. This is of considerable importance only in preparative organic synthesis when acetyl chloride of high purity is required as the synthetic equivalent of acetic acid, which was the objective for a rapid labeled acetate production of high purity and quality. The hydrolysis of properly prepared [1-^{11}C]acetyl chloride in physiological saline was quantitative and it occurred on a no-carrier-added level. No other products than Cl^- and H_3O^+ would be expected beside the sodium [1-^{11}C]acetate. These ions are quite abundant in the neutral injection solution and do not interfere with the diagnostic application.

CONCLUSION

A reliable and quick route to [1-^{11}C]acetate for application in PET has been presented. The procedure eliminates the need for laborious solvent extraction and phase separation techniques which are usually incomplete and are obstacles in labeled acetate production. Chromatographic work-up of the final product is omitted. Nevertheless, the method delivered the high quality target compound and in an excellent yield, directly in sterile solution. This production technique is of considerable practical importance, allowing multiple batch preparations within 1 day and the distribution of the product at least within a 1-hr radius from the production facility. One might expect that this would contribute to widespread diagnostic use of [1-^{11}C]acetate and improve its application as the tracer of choice for the detection of viable myocardium (*19*).

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