PRODUCTION OF 1-^{11}C-ACETOACETIC ACID.

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Interest in labeled compounds which can be studied as brain metabolites and can be detected in vivo has led to production of carbon-11 labeled acetoacetic acid (1-^{11}C-acetoacetic acid). This synthesis, which was designed specifically for use with short-lived isotopes, utilizes a solution of the stable enolate anion of acetone, generated by the reaction of CH_3Li and isopropenyl acetate, to which the $^{11}CO_2$ is added to produce the labeled acetoacetic acid in ∿55% yield with a synthesis time of only 40 minutes from the end of bombardment.

INTRODUCTION

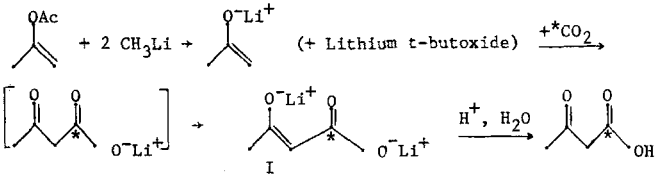
Cerebral blood flow and metabolism studies using compounds labeled with short-lived cyclotron produced isotopes have been of principal interest in our laboratory over the past few years. Metabolites currently used include oxygen-15 labeled compounds (1-4), nitrogen-13 labeled ammonia and amino acids (5,6) and carbon-11 labeled glucose (7,8).

In conjunction with these investigations, there is interest in studying the brain metabolism of secondary metabolites. Based on the report of Owen et al. (9) that in patients on starvation diets β-hydroxybutyrate and acetoacetate replaced glucose as the predominant substrate for brain metabolism, we have been interested in synthesizing these compounds labeled with carbon-11. Because production of β-hydroxybutyrate would result in a racemic mixture (the body only uses the D-form) due to its optically active center, acetoacetate was chosen as the first compound to synthesize.

Conventional synthesis of ^{14}C-acetoacetate involves a Grignard reaction for incorporation of $^{14}CO_2$ into acetate, esterification and self-condensation, and several subsequent hydrolysis, distillation, and purification steps to produce the labeled acetoacetate (10). The production time is on the order of several hours. As this time is prohibitive in syntheses using 20-minute half-lived carbon-11, it was necessary to devise an approach that is not so time-consuming.

A synthesis was therefore developed which takes advantage of a general preparation of solutions of enolate anions reported by House and co-workers

(11,12) in which a lithium enolate anion is generated by the reaction of the enol acetate of a ketone with methyl lithium in a non-protic solvent. The anion generated from isopropenyl acetate in this manner serves to trap $^{11}CO_2$ resulting in the formation of the desired carbon-11 labeled acetoacetate as 1-^{11}C-acetoacetate. The following reaction sequence illustrates the synthesis:



It should be noted that if compound I was treated with one equivalent of acid and then reduced, a racemic mixture of β-hydroxybutyrate would be produced.

EXPERIMENTAL

Preparation of the Enolate Anion of Acetone

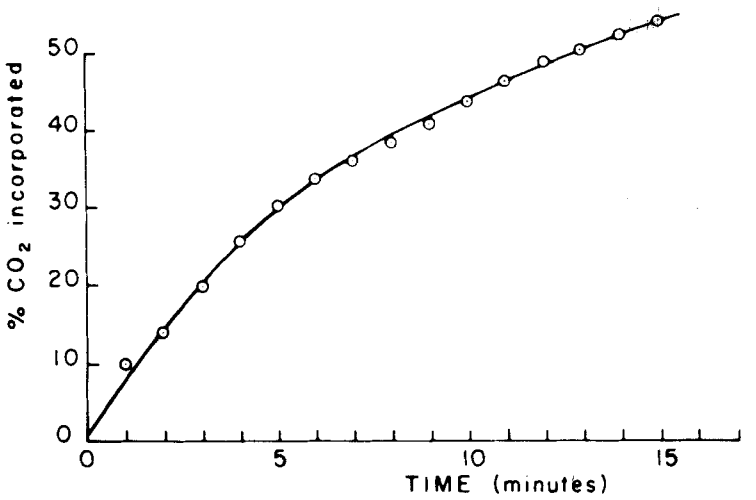
The initial reaction was carried out in an ice-cooled, 3-neck, 100 ml round-bottom flask. One neck was fitted with a bubbler to allow flushing with nitrogen to maintain an inert atmosphere. Reactants were added by syringe through silicone rubber septa (Burrell Corporation, Pittsburgh, Pa.) inserted into receptors on stoppers in the remaining two necks of the flask. To the flask containing a few crystals of α,α-dipyridyl as indicator was added 10 ml of 2M methyl lithium in ether (Alfa Inorganics). Although in the general preparation of enolate anions in this manner House and Trost (11) have suggested 1,2-dimethoxyethane as a solvent due to the insolubility of some enolate anions in ether, we found ether to be satisfactory for the enolate anion of acetone. Isopropenyl acetate (1 ml) was added dropwise until the red indicator color was just gone. (Any excess of methyl lithium is a problem in this application as it leads to acetic acid as a final product. It is therefore desirable to add the starting material to the CH_3Li.) The mixture was allowed to stir for about 45 minutes. Because of its stability, the solution of the enolate anion of acetone can be prepared in this manner several hours in advance of the isotope preparation.

Addition of CO_2

In initial experiments the rate of carrier carbon dioxide addition was studied at room temperature to determine a reasonable reaction time. A weighed amount of dry ice was added to the prepared solution of enolate anion in a flask connected to an oil manometer. The CO_2 was allowed to warm up and the rate of disappearance of CO_2 was followed during the course of the reaction as shown in Figure 1. In 20 minutes 52% of the available CO_2 was incorporated corresponding to one-half of the theoretical amount of the carbanion present. Beyond this time, no appreciable uptake of CO_2 was noted.

Production of $^{11}CO_2$ and Incorporation of Label

The $^{11}CO_2$ was prepared using the 7-MeV deuteron beam of the Washington University Medical School cyclotron by bombarding boric oxide in an atmosphere of helium containing 3% oxygen. The carbon-11 formed by the $^{10}B(d,n)^{11}C$ nuclear reaction reacts with oxygen in the boric oxide and is released as



carbon monoxide, which undergoes radiolytic oxidation to carbon dioxide (13). At the end of a 20-minute irradiation a 5 cc gas sample of carrier-free $^{11}CO_2$ (about 8 mCi) in helium was withdrawn for use in the synthesis. The $^{11}CO_2$ was injected through a septum with the syringe needle under the level of the ether solution and allowed to react with the enolate anion for 20 minutes in the closed flask with constant stirring. For syntheses requiring large amounts of activity the total $^{11}CO_2$ produced was trapped out at liquid nitrogen temperature, the solution of enolate anion was added to the trap, and the mixture was then shaken for 20 minutes to incorporate the $^{11}CO_2$.

Isolation of ^{11}C-acetoacetic Acid

The liberation of the acetoacetic acid formed was effected by addition of acid. The reaction mixture in ether was treated with 10 ml of saturated NaCl and 2 ml of conc. HCl. The ether was then extracted with 3 ml of 3N NaOH and the aqueous layer was passed down a 3 cc ion exchange column of Amberlite CG-4B and rinsed with distilled water. The 1-^{11}C-acetoacetic acid was eluted from the column in the second 1 ml fraction at pH 7.0. The sample was then passed through a 0.45μ millipore filter prior to use.

ANALYSIS

The final radiochemical yield of acetoacetate calculated as percent of activity added to the reaction intermediate was approximately 55%, corrected for decay. This is in very good agreement with the yield found using carrier carbon dioxide.

To insure that the final labeled product was ^{11}C-acetoacetic acid, four methods of analysis were performed on the product from unlabeled CO_2, the product using $^{11}CO_2$ with carrier CO_2 added, and authentic acetoacetate (Sigma Chemical Company, St. Louis, Mo.).

The first method was a general test for a keto-enol pair. When the sample was added to 3 ml of ethanol containing 2-3 drops of a 40% W/V aqueous ferric chloride solution, a deep violet-red coloration appeared. When the solution was diluted to 100 ml with distilled H_2O, subsequent dropwise addition of bromine water caused the solution to become colorless as the bromo-keto product was formed. The violet color then reappeared as the keto form of the bromination product slowly changed to the enol (14).

Further identification was achieved by liquid chromatography. The sample was adsorbed on a 10 cc neutral alumina column and eluted with a pH 4 acetate buffer (0.2M). The retention time of the two product samples agreed with the retention time of authentic acetoacetate. It was also found that use of an excess of methyl lithium in the generation of the enolate anion led to labeled ^{11}C-acetic acid as determined by alumina chromatography. Acetic acid was eluted with distilled water in an elution volume of 8 ml. Acetoacetic acid was, however, retained and a gradient change to the pH 4 buffer was necessary for its elution. Therefore the column was routinely eluted with 10 ml of distilled water before changing to the acetate buffer in this identification method. Production of acetic acid could be avoided by careful addition of isopropenyl acetate to the methyl lithium in the generation of the enolate anion. However, any labeled acetic acid is held up on the Amberlite CG-4B column used in the final purification.

A further test which confirmed the presence of 1-^{11}C-acetoacetate and the location of the carbon-11 label involved decarboxylation. The sample was heated in an extremely basic solution. The result was formation of unlabeled acetone in solution with evolution of carbon dioxide having all the activity.

The final test carried out on the three samples was a specific one for acetoacetate discussed by Walker (15). Acetoacetate reacts with a diazo reagent prepared by addition of 3.0 ml $NaNO_2$ (0.5%, W/V) to 20 ml of p-nitroaniline (0.05%, W/V in 0.05N HCl) to form a colored compound (N,N-bis-p-nitrophenyl-C-acetyl formazan). To 0.5 ml of the neutral acetoacetic acid solution was added 0.5 ml of acetate buffer (pH 5.2) and 3.0 ml of freshly prepared diazo reagent. The final pH was 4.9-5.0. After standing at room temperature for 30 minutes for color development, the reaction was stopped by adding 1 ml of 5N HCl and the product was extracted with 4 ml of ethyl acetate. The optical density of the ethyl acetate was read in the visible region against a reagent blank using water in place of the acetoacetate solution. The spectra of the product samples under these conditions were compared to that of authentic acetoacetate, confirming the presence of 1-^{11}C-acetoacetate.

CONCLUSION

We have developed a synthesis for ^{11}C-acetoacetic acid, a metabolically interesting compound, that is specifically designed for use with short-lived radioisotopes. Total preparation time is about 40 minutes from the end of bombardment, which is quite reasonable for incorporation of 20 minute half-lived carbon-11. The reaction intermediate can be formed well in advance of production of the radioisotope as it is a stable enol carbanion. Preparation is a simple procedure with a reasonably high yield of 55% based on radioactivity incorporated. Allowing for decay of the isotope, the final yield of 1-^{11}C-acetoacetic acid is ∿15% of the original $^{11}CO_2$ activity added to the reaction intermediate, so that ∿1 mCi of 1-^{11}C-acetoacetate can be prepared from 8 mCi of $^{11}CO_2$.

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