A Simple Apparatus for the Carbonation of Grignard Reagents with Carbon-^{14}C Dioxide

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The standard apparatus for the carbonation of Grignard reagents with carbon-^{14}C dioxide, as described in several textbooks [1-3], is rather complicated in its construction and operational use, especially for those who only rarely meet with the problem of synthesizing ^{14}C-labelled compounds. It is generally not satisfactory to carry out the reaction in an open vessel by bubbling the carbon-^{14}C dioxide through the Grignard solution, since this process is very likely to cause clogging in the gas-inlet tube and also involves a certain loss of radioactive material.

This paper describes a simple, closed carbonation apparatus, which is easily constructed from commercially available glass equipment at a minimum cost of glass-blowing. It does not require the use of a high-vacuum system, liquid nitrogen temperatures, or a special gas-tight stirrer.

Construction. The apparatus (see Fig. 1) principally consists of a gas evolution vessel A which is in communication with the reaction chamber B through two holes in the top of the drying tube C. A capillary tube through C, connected to the dropping funnel D, allows for the addition of mineral acid to A. E is a high-vacuum stopcock and stirring is effected by means of a Teflon sealed magnet F. The ground glass joints G (B 14*), H (B 34) and I (B 19) permit the apparatus to be easily mounted and dismounted.

Operation. Barium carbonate-^{14}C is weighed into A, and A is fitted to the drying tube C by joint G, which is held together by a spiral spring. C is filled with Drierite which is kept in place by two loose plugs of dry cotton wool. The reaction vessel B is then attached *via* joint H, whereafter the whole system is evacuated and filled with dry nitrogen.

B is removed for a few seconds during which an appropriate amount of the previously prepared Grignard solution is introduced by means of a nitrogen-flushed syringe. Stirring is started and the Grignard reagent is cooled to about −70° in a dry ice-acetone bath. The system is evacuated through E by means of a conventional oil pump whereby the ether solution in B boils up when the pressure has been reduced to about 1 mm Hg. E is closed and the temperature of B is rised to about −15°. Perchloric acid (35 %) is cautiously added from the dropping funnel D, whereby the evolution of carbon-^{14}C dioxide starts. Occasionally, the perchloric acid may collect in the cavity above the capillary tube at the begin of the reaction, but this is easily overcome by cooling at B, which results in a pressure drop in the system and the perchloric acid is sucked into A. When all of the gas has been evolved (a clear solution then remains in A), B is frozen to −80° in order to draw any remaining carbon-^{14}C dioxide into the reaction mixture. The temperature is kept at −80° for about one hour after which time it is allowed to reach room temperature. The reaction mixture is worked up according to conventional procedures.

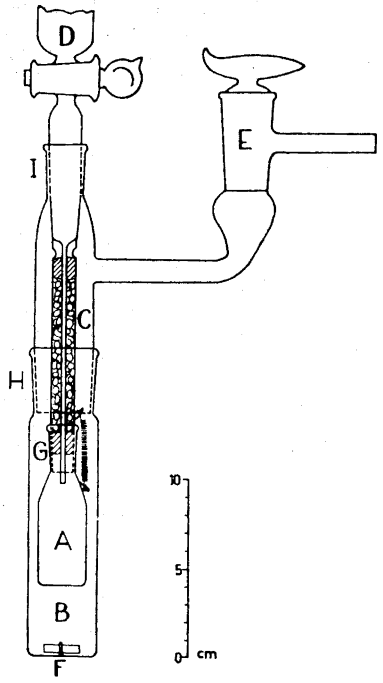

Fig. 1.

* Size designation according to "Quickfit".

When phenyl magnesium bromide or benzyl magnesium chloride was carbonated with inactive carbon dioxide according to this method at the 1.0 mmole level, almost quantitative yields of benzoic and phenylacetic acid were obtained. Phenylacetic-1-^{14}C acid was prepared in 98 % yield (crude product, m.p. 69—74°) from 1.00 mmole of barium carbonate^{14}C.

1. Murray, III, A. and Williams, D. L. *Organic Syntheses with Isotopes, Part I*, Interscience, New York 1958, p. 86.
2. Ronzio, A. R. in Weissberger, A., Ed., *Technique of Organic Chemistry, Volume VI, Micro and Semimicro Methods*, Interscience, New York 1954, p. 391.
3. Weygand, F. and Simon, H. in *Methoden der Organischen Chemie (Houben-Weyl), Band IV, Teil 2, Allgemeine Chemische Methoden*, Thieme, Stuttgart 1955, p. 643.

Received March 6, 1962.

Microdetermination of Protein with Radiocopper and Gel Filtration

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In order to make possible the determination of minute amounts of protein we have developed the following "radiobiuret" techniques:

Sephadex (G-25, coarse) is suspended and allowed to swell in 0.75 N NaOH and packed in the form of a column (diameter up to 1 cm, length about 20 cm). The column is washed overnight with NaOH to remove traces of protein. It is arranged that the protein to be determined is contained in 0.75 N NaOH in a convenient volume (*e.g.* 1—5 ml). To this is added an excess amount (up to 0.5 mg) of radioactive copper with as high specific activity as possible, *e.g.* 1 ml of an aqueous 1:25 dilution of the commercial $^{64}CuCl_2$ solution. All dilutions are made with redistilled water and observing meticulous cleanliness. The mixture is incubated 90 min at room temperature, a measured volume is introduced onto the column followed by NaOH until 25 ml of eluate has been collected. The radioactivity of the eluate is then determined with a scintillation counter. Between samples, the column is washed with NaOH. Known amounts of a standard protein and blanks are run in the same way before and after the unknown. The amount of protein in the unknown sample can be read from the resulting standard curve.

The unbound copper will be retained as the hydroxide in the upper parts of the column, but owing to the low concentration of copper in the original reaction mixture no precipitation will occur there. The determination can also be performed with larger amounts of protein by reading the emerging biuret colour photometrically. However, the free copper will then precipitate, but this can be prevented by complexing the copper ion with citrate as is done in the classic biuret method. When such a mixture is introduced into the column, copper hydroxide will be formed and retained in the upper parts (amino acid complexes are also dissociated). A straight-line relationship *amount of protein/eluted radioactivity* (corrected for radioactive decay) is observable down to at least 1 μg of protein applied onto the column in 5 ml volume, but owing to the obvious advantages of the following method we have made no attempts to reduce further the lower limit of this method.

The reaction may also be performed in another way which enables it to be used for automatic analysis, *e.g.* in continuous registration of protein eluted from chromatographic columns. The chromatographic eluent must first be alkalinized either by the continuous addition of NaOH with a pump or by automatic pH adjustment using a titrator. The mixture is then led to a Sephadex column containing an upper layer of a mixture of radiocopper hydroxide and Sephadex. This can be prepared in a beaker by mixing $^{64}CuCl_2$ with a citrate-carbonate solution (proportions as in Benedict's reagent), pouring the mixture on moist alkaline Sephadex and by shaking until all the copper is adsorbed. The slurry is poured onto the top of a short Sephadex column and the column is then washed with a large volume of NaOH. If desired, the copper hydroxide level can be raised by sucking up acid from below. When the desired level has been reached, NaOH is run in the other direction.

The eluate from the Sephadex column is passed through a glass tube spiral kept in the well of a scintillation crystal fixed in a