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B. M. Tolbert and D. M. Hughes

May 16, 1950

Berkeley, California

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AN IMPROVED SYNTHESIS OF GLYCINE-1-C^{14} AND GLYCINE-2-C^{14} FROM C^{14}-LABELED ACETIC ACID

By

B. M. Tolbert and D. M. Hughes

Radiation Laboratory and Department of Chemistry, University of California(*)

ABSTRACT

May 16, 1950

A modified procedure for the synthesis of labeled glycine from acetic acid is presented which gives a high purity product and is reliable in routine use.

(*) The work described in this paper was sponsored by the Atomic Energy Commission.

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AN IMPROVED SYNTHESIS OF GLYCINE-1-C^{14} AND GLYCINE-2-C^{14} FROM C^{14}-LABELED ACETIC ACID

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(*) The work described in this paper was sponsored by the Atomic Energy Commission.

In a recent paper (1) the preparation of labeled glycine from

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(1) R. Ostwald, J. Biol. Chem., 173, 207 (1948); see also BC-56.

---

acetic acid by chlorination and ammonalysis is described. Although the yields are excellent the method is not particularly suitable to routine use, and the product sometimes contains ammonium salts and other impurities.

In an attempt to simplify this procedure, to make it more reliable for routine use and to increase the yield, the following modifications were developed: (1) An improved method of generating the acetic acid from the sodium acetate; (2) The use of acetyl chloride to remove small amounts of water from the acetic acid before chlorination; and (3) Ion exchange columns and high vacuum sublimation purification which eliminated all ammonia salts in the product. The reactions are as follows:

$$\overset{*}{CH_3CO_2}Na + HCl \longrightarrow \overset{*}{CH_3CO_2}H + NaCl$$

$$\overset{*}{CH_3}COOH + Cl_2 \xrightarrow[\text{Acetylchloride}]{P, I_2, PCl_5} \overset{*}{CH_2}ClCOOH + HCl$$

$$2NH_4OH + \overset{*}{CH_2}ClCOOH + \xrightarrow[(NH_4)_2CO_3]{NH_4OH} \overset{*}{CH_2}NH_2COOH + NH_4^+ + Cl^- + 2H_2O$$

Experimental

Chloroacetic acid. - Anhydrous acetic acid was prepared by heating 0.6 to 1.0 g. of dry sodium acetate with dry HCl (dried by shaking with phosphorus pentoxide) in a gas solid reactor (Fig. 1.). The excess HCl was removed under vacuum in a dry ice-chilled trap. The acetic acid was then chlorinated at 100° C. for 2-1/2 hours with a stream of Cl_2 (Fig. 2). Catalysts for this reaction were 0.04 g. phosphorus, 0.02 g. iodine, 0.01 g. phosphorus pentachloride and 0.4 cc. acetyl-chloride. These reactions are described in detail elsewhere (2).

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(2) UCRL-256, D. M. Hughes and B. M. Tolbert "Preparation of Calcium Glycolate-1-C^{14} and Calcium Glycolate-2-C^{14}".

The products of the chlorination were distilled from the condensers into the reactor tube (Fig. 1) by closing all stopcocks, freezing the lower tube in liquid nitrogen and warming the two condensers with

hot water. This transfer was also done on the high vacuum manifold with appropriate back distillations. The condenser was then removed and replaced by a glass stopper. Two cc. of water was measured out for the hydrolysis of the acid, chlorination and transfer. One-half cc. of water was carefully added to the still frozen material. An immediate evolution of the frozen Cl_2 followed and was of no significance. However, as the material was carefully brought up to room temperature, HCl was evolved due to the hydrolysis of the acid chloride to the free acid. It is important that the material not be permitted to heat to more than 35-40° C (estimated and cooling is necessary to control the hydrolysis. The Cl_2 bubbling tube was then washed down with a minimum amount of water (about three drops) and the outside was scrubbed with several one drop portions. The washings were added to the hydrolized acid.

Glycine. - The apparatus for the amination consisted of a 60 cc. two-necked flask, fitted with a water condenser and a dropping funnel. The flask was set in an oil bath thermostatted at 60-65° C. Fifteen cc. of ammonium hydroxide and 7.5 g. of freshly ground ammonium carbonate was placed in the flask and permitted to come to temperature. (Not all of the ammonium carbonate dissolves).

The product of the chlorination was then transferred by pipet to the dropping funnel with a drip tip. The chlorination reaction vessel was not rinsed at this point as it was essential to keep the water down to a minimum. The chloroacetic acid was added to the ammonium carbonate ammonium hydroxide solution dropwise over a period of about one-half hour. The chlorination tube (including the bubbling tube) was then rinsed three times with the remaining transfer water which was

used each time to rinse the dropping funnel. The amination mixture was maintained at 60-65° for 8-12 hours.

The condenser and dropping funnel were removed from the amination flask and replaced with a ground glass stopper and a vacuum distillation head and condenser. Aspirator vacuum was applied, and all of the volatile materials evaporated with heating to a final maximum bath temperature of 115°. No capillary tube was necessary as the carbon dioxide evolved from the ammonium carbonate prevented bumping. The last traces of moisture were removed under high vacuum when the material appeared dry.

Purification of Glycine.- Six ion exchange columns (20 cm. x 15 cm. I.D.). containing 60 cc. each of Dowex 50 resin (20-40 mesh) were prepared by recycling the resin alternatively with 1.5 N sodium hydroxide and 1.5 N hydrochloric acid, ending with hydrochloric acid. (The columns were washed thoroughly with water after each acid or base treatment). Each column had a working capacity not exceeding 0.8 g. of the crude glycine salt mixture, and it was found preferable not to exceed 0.4 g. per column.

The salt was weighed and transferred to a 10 cc. graduated cylinder using 6-8 cc. of water to make the transfer. The columns were opened at the top, excess water removed, and the solution added directly to the resin. If the weight was more than 0.5 g. solids per column, one-half of the material was set aside and put through the column later after the regeneration. The graduated cylinder was rinsed several times with a total of 3-4 cc. of water and this was added to the columns. (A small glass wool plug was used to prevent spray from carbon dioxide

evolved).

Through each column 500 cc. of distilled water was passed dropwise, requiring about four hours. This water (called the effluate) contained the negative ions I^-, Cl^-, PO_4^{---}, any unreacted acetic acid etc. This material contained about 25% of the initial activity; a paper chromatogram indicated almost no glycine was in this cut, so it was evaporated to dryness and burned for isotope recovery. The glycine was next eluted from the columns with 250 cc. of 1.5 N ammonium hydroxide followed by a wash with 250 cc of water(**). The rate of drip

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(**) Dowex 50 is normally eluted with an acid such as HCl. However, by using excess ammonium hydroxide, the NH_4^+ saturates the column and shifts the equilibrium of the other positive ions so they are washed out of the resin column. Thus, an eluate is obtained which contains only glycine and ammonia. The latter may be easily driven off by boiling to leave relatively pure glycine. If hydrochloric acid had been used, ammonium chloride would have been present in the glycine, and these materials can only be separated with great difficulty on a micro scale in good yields. Before using the columns again, they must be regenerated with 250 cc. 1.5 N hydrochloric acid each, followed by thorough rinsing, at least 500 cc. each, or until water tests neutral to pH paper.

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was the same as before, This cut contained the glycine. It was evaporated to dryness, transferred to a 400 cc. beaker using about 25-40 cc. of water. In order to remove the last traces of ammonium hydroxide

the solution was adjusted to pH 11.5 with dilute sodium hydroxide solution, covered with a loose fitting watch glass and boiled vigorously on a hot plate to half its original volumn. Water was added to the original level and the pH was again adjusted to 11.5 and the boiling procedure repeated. (Glass beads were used to prevent bumping).

The solution was then adjusted with dilute hydrochloric acid solution to a pH of about 5, slightly more acid than the isoelectric point. (Note: If adjusted to the isoelectric point (6.1), the residue following sublimation when dissolved had a pH of 8.) The solution was evaporated to a small volume, transferred to the bottom of a sublimation apparatus (Fig. 1), evaporated to dryness and dried thoroughly at 80° and 10^{-4} mm. Hg. pressure. The sublimation finger was then filled with liquid nitrogen and the material sublimed three hours at 150° C and two hours at 200° C. The pressure in the sublimation apparatus was 10^{-4} to 10^{-5} mm. Hg. and the cold finger was 1 cm. from the glycine which was being sublimed.

The sublimate was washed and scraped wet (*) into a 9 cm. petri dish, then transferred to the bottom of a second sublimation apparatus. The non-sublimed residue was dissolved in water, the pH readjusted to 5, re-evaporated to dryness and similarly resublimed. This second crop was combined with the first and resublimed. The yield was 50-60%, but

(*) The freshly sublimed dry glycine was very light and flaky and blew easily. Also, it tended to become electrostatically charged.

occasionally as high as 60-70%. (See Table I)

The isotopic purity and identity of this product material was checked by two-dimensional paper chromatography (3). Radio-

(3) A. A. Benson, J. A. Bassham, M. Calvin, V. A. Haas, T. C. Goodale and W. Stepka, J. Am. Chem. Soc., 72, 1710 (1950).

autograms of these paper chromatograms were then prepared, and finally the papers were sprayed with ninhydrin solution to test for α-amino acids. The papers showed only one radioactive spot and only one ninhydrin spot, and these coincided exactly. The position of these spots was correct for the glycine. This data confirmed the fact that only one radioactive product was present after purification and that it was glycine.

Discussion

In a preparation using C^{14} in large amounts it is desirable to achieve several things including (1) high yield, (2) small-scale reaction, (3) high purity of product and (4) reliability of procedure. The method of synthesis of glycine just outlined has been designed to do this, but some sacrifices have been made on yield to insure the purity of the product and the reliability of the procedure.

As indicated, the yield varied from 50-70%. In Table I (page 11) radio material balances are presented for four high specific activity runs. It will be noted the recoveries of radiocarbon were never quantitative, but a number of known losses were not analyzed. Thus, 2-5% of

Table I

Material Balance of High Specific Activity Preparations

	Column Effluate		Non-sublimable Residues		Glycine (Product)		Total % Recovery
	Dis./min.	%	Dis./min.	%	Dis./min.	%	
Run DH-46-5							
Glycine-2-C^{14}	1.10×10^{10}	24.5	8.38×10^{4}	19.2	2.25×10^{10}	51.5	95.2
Run DH-46-7							
Glycine-1-C^{14}	4.88×10^{9}	22.2	3.63×10^{9}	15.6	1.22×10^{10}	56.9	95.1
Run DH-46-28							
Glycine-2-C^{14}	5.80×10^{9}	22.0	2.91×10^{9}	11.0	1.34×10^{10}	50.7	83.7
Run DH-46-30							
Glycine-2-C^{14}	4.73×10^{9} (1)	13.4 (1)	3.42×10^{9}	9.7	2.40×10^{10}	68.2	91.3

(1) These figures include 4.3×10^{8} dis./min. (1.2%) from wash of chlorination apparatus and 2.6×10^{8} dis./min. (0.7%) from distillation of ammonium carbonate following ammination.

the activity may be left in the unit used to convert the sodium acetate to acetic acid. Some activity may be swept out by the Cl_2 gas stream and the vacuum line probably holds back a few percent of the initial activity.

Attempts to reduce the two major losses, the column effluate and

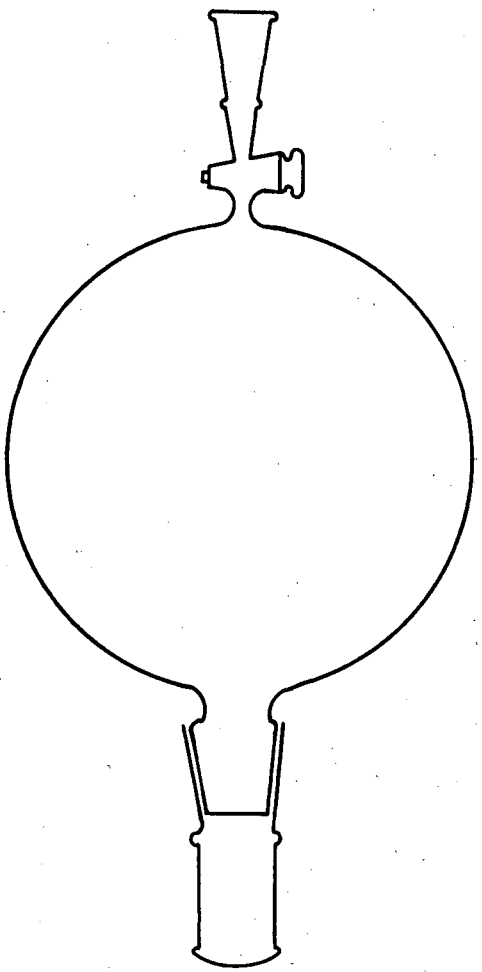
the non-sublimable residues, were not too successful. It is thought that the column effluate material is probably mostly acetic and glycolic acid; as indicated it contained little glycine. On the other hand, paper chromatograms and radioautographs of the non-sublimable residues gave a strong glycine test and a number of lesser spots, which may be disubstituted amines and various polymers.

Acknowledgment

We would like to thank Prof. M. Calvin for his continued help and encouragement in this work and Dr. Rosemarie Ostwald for her extremely valuable aid and advice.

Summary

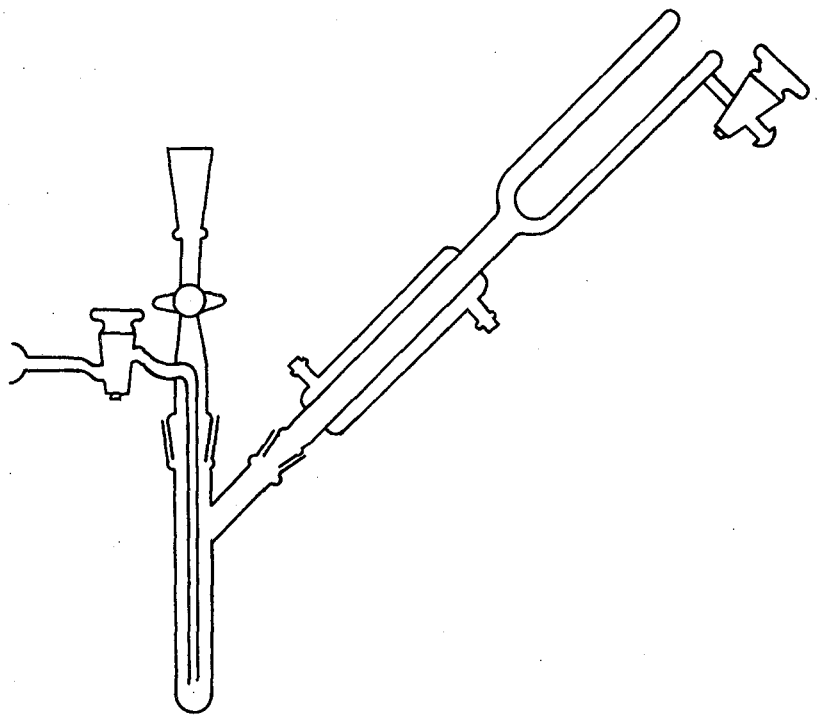
A modified procedure for the synthesis of labeled glycine from acetic acid is presented which gives a high purity product and is reliable in routine use.



GAS SOLID REACTOR
(CAPACITY = 2L)

MU 310

FIG. 1



CHLORINATION APPARATUS

MU 311

FIG. 2