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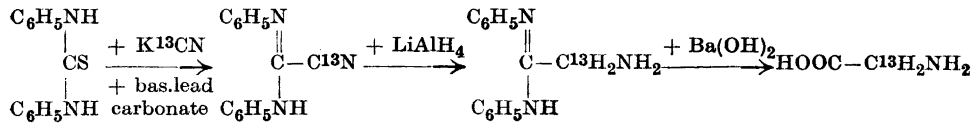
An Easy Route to Methylene-labelled Glycine

G. EHRENSVÄRD and R. STJERNHOLM

Department of Physiological Chemistry, Wenner-Gren Institute, University of Stockholm, Sweden

The preparation of glycine, labelled with C^{13} or C^{14} in the methylene group, is at present a rather tedious process, involving preparation of methyl-labelled acetic acid, subsequent halogenation, and exchange of the halogen atom against NH_2 [1, 2]. Using $KC^{13}N$ as the starting material we got an overall yield of round 12 % by the above route, included the preparation of methyl-labelled acetate by the reaction sequence: $KC^{13}N \rightarrow C^{13}O_2 \rightarrow C^{13}H_3OH \rightarrow C^{13}H_3J \rightarrow C^{13}H_3COOH$ [3].

In view of the usefulness of methylene labelled glycine for studies of intermediary metabolism, an easy route to this compound from the sources of C^{13} and C^{14}, at present available, would be of some interest. The following method involves only three isolation stages from $KC^{13}N$ to $HOOC\text{-}C^{13}H_2NH_2$, gives an overall yield of 50 %, or more, and could be carried out in about 10 hours. An outline of the procedure is shown by the following scheme:



(I) (II) (III) (IV)

N · N′-diphenyl-thio-urea (I) is condensed with $KC^{13}N$ in the presence of basic lead carbonate in water-alcohol mixture to N · N′-diphenyl-cyano-formamidine (II). Since the reduction of cyano-groups to $—CH_2NH_2$ proceeds with extreme ease in ether solution with lithiumaluminum hydride [4] it was to be expected that the cyanoformamidine should be reduced in a similar way to the corresponding glycine-N · N′-diphenyl-amidine, provided that the amidine structure was not to be touched by the hydride. We found that this, actually, was the case, and that the glycine-N · N′-diphenyl-amidine was formed in a yield of 65—70 %. In view of the easy reducibility of amides [5]

and anilides [6] by lithium-aluminum hydride it is somewhat surprising that the amidine structure should be stable against this reducing agent. It is, however, to our knowledge not yet known, whether amidine groups in general are stable against hydrides, but it seems worth investigating if the N′ · N′-diphenyl-amidine structure, in its stability against reduction, as observed by us, could be used for protection of carboxyl groups when working with lithium-aluminum hydride and other agents of the same kind. The hydrolysis of glycine-N · N′-diphenyl-amidine is very slow with concentrated HCl at 100°, but proceeds with great ease and almost quantitative yield with barium hydroxide in water solution at boiling during one hour, yielding glycine and aniline.

For preparation of C^{13}-labelled glycine the commercial $KC^{13}N$ (Eastman Kodak) could be used directly; for C^{14}-labelled glycine high yield syntheses from $BaC^{14}O_3$ have been worked out for preparation of $KC^{14}N$ [7], the latter being at now commercially available.

EXPERIMENTAL

N · N′-Diphenyl-cyano-formamidine (II)

The following preparation is a small-scale procedure, worked out from the data for large-scale preparation given by Sandmeyer [8]. 7 g 95 % Potassium cyanide with about 5 % C^{13}-excess was dissolved in 20 ml of water. To this solution 30 g basic lead carbonate, 20 g N · N′-diphenyl-thio-urea (I) and 50 ml 95 % ethanol were added, and the mixture heated to 50—55° during one hour under efficient stirring. Gradually the mixture turns black (PbS), the reaction being ready when the filtrate of a small sample does not discolorize a small amount of lead carbonate at short boiling. The reaction being complete, 200 ml of cold water was added to the reaction mixture. After cooling the precipitate was filtered off, washed with cold water and treated with 200 ml of boiling ethanol, whereby the N · N′-diphenyl-cyano-formamidine was extracted from the lead sulphide and excess lead carbonate. The alcoholic extract was evaporated to about 50 ml and kept at 0°, whereby the main part of the amidine crystallized in light yellow plates, m. p. 139° (litt. 137°). Yield 14 g. An additional amount of 6 g of product could be isolated from the mother liquor. (This latter crude material does, however, contain at least 80 % of the amidine, so it was found to be quite satisfactory to evaporate, in later runs, the alcoholic extract to dryness and directly use this material (19 g, 90 % yield) for the subsequent reduction.)

Glycine-N · N′-diphenyl-amidine (III)

In a 2 litre three-necked flask, equipped with reflux condenser, separating funnel, high-speed mercury-seal stirrer and nitrogen inlet, 16 g of lithium-aluminum hydride was dissolved in 500 ml anhydrous ether. A solution of 19 g crude, but alcohol-free, N · N′-diphenyl-cyano-formamidine in 300 ml anhydrous ether was added at room temperature under a period of about 2 hours. The rate of addition was regulated to keep the ether in gentle reflux. A yellow precipitate was formed at the beginning of the reaction, gradually changing to white. After addition of the cyano-formamidine the

mixture was stirred for another 20 minutes, cooled to 0° and water (500 ml) added, drop by drop. During the reduction procedure dry nitrogen was slowly passed through the mixture. The yellow ether layer, after addition of the water, was separated and the cloudy water-phase 3 times extracted with 100 ml of ether. The combined extracts, dried for a short time over anhydrous sodium sulphate, were evaporated to dryness leaving an yellow oil, the main part of which solidifies on scratching. This reaction product (17.5 g) consists of glycine-N · N′-diphenylamidine and a small amount of aniline. For isolation of the pure product a small sample (1 g) was treated with 2 ml of methanol, sucked dry (m. p. 106°) and recrystallized from petrol ether, m. p. 112°.

Calculated for $C_{14}H_{15}N_3$:	18.65 % N
Estimated:	18.7, 18.8 % N

Glycine (IV)

16.5 g crude glycine-N · N′-diphenyl-amidine was heated at reflux with 80 ml 10 % solution of $Ba(OH)_2$ for one hour (oilbath at 130°). Aniline was shown to separate from the waterphase after 15 minutes. After the hydrolysis the alcaline mixture was extracted 3 times with 50 ml ether each, and barium ions removed by adding an exact amount of 10 % sulphuric acid. $BaSO_4$ was centrifuged off, boiled with 150 ml water, centrifuged and the combined Ba- and sulphate-free solutions evaporated to 10 ml. A small amount of Norite was added, the solution filtered and the glycine precipitated by addition of 5 volumes of ethanol and cooling to 0°. The colourless crystals were washed with some absolute ethanol and dried. Yield 3.8 g. Overall yield from KCN 48 % (Higher yields being obtained).

Calculated for glycine, $C_2H_5NO_2$:	18.7 % N
Estimated:	18.6 » »

M. P. of the ethyl ester hydrochloride 143° (litt. 144°).

A paper partition chromatogram in phenol-water of our glycine preparation showed the right R_F-value. As for control of the actual position of the labelled carbon atom in the glycine prepared a small sample was totally combusted in oxygen to CO_2, trapped as $BaCO_3$. Another sample was treated with ninhydrine liberating its carboxyl group as CO_2, trapped as $BaCO_3$. Mass-spectrometric analysis of the two carbonate preparations showed the following values:

Total glycine carbon:	2.17 % C^{13}-excess
Carboxyl carbon:	0.01 » C^{13}-excess

The isotope carbon was thus located to the methylene group, having a C^{13}-excess of 4.32 %.

SUMMARY

A new method is given for the synthesis of methylene labelled (C^{13}) glycine, the reaction steps being the preparation of N · N′-diphenyl-cyano-formamidine, glycine-N · N′-diphenyl-amidine and hydrolysis of the latter to glycine.

The overall yield from $KC^{13}N$ is round 50 % and the reaction steps work rapidly and satisfactory.

REFERENCES

1. Tolbert, B. M. *J. Biol. Chem.* **173** (1948) 205; Ostwald, R. *Ibid.* **173** (1948) 207.
2. Altman, K. J., Casarett, G. W., Masters, R. E., Noonan, T. E., and Salomon, K. *J. Biol. Chem.* **176** (1948) 319.
3. Ehrensvärd, G., and Hammarsten E. (1949). Unpublished.
4. Nystrom, R., and Brown, W. G. *J. Am. Chem. Soc.* **70** (1948) 3738.
5. Uffer, C., and Schlittler, B. *Helv. Chim. Acta* **31** (1948) 1397.
6. Ehrlich, T. *J. Am. Chem. Soc.* **70** (1948) 2286.
7. Loftfield, R. B. *U. S. Atomic Energy Commission, Oak Ridge, Tenn. Isotopes Branch Circular C—3.* June (1947).
8. Geigy and Co. D. R. P. 115169; Sandmeyer, J. In Ullmanns *Encyclopädie der technischen Chemie.* **6** (1930) 241.

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