Title

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Abstract

Aspartic-3-C^{14} acid hydrochloride has been prepared by a method that is also suitable for the preparation of aspartic-4-C^{14} acid hydrochloride. A procedure has been developed by which the aspartic acid may be purified without the use of ion exchange columns.

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PREPARATION OF C^{14} LABELED DL-ASPARTIC ACID

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Aspartic acid has been prepared by means of the following sequence of reactions, suitable for synthesizing aspartic acid labeled in either the 3 or 4 position.

$$C^{14}H_3\text{-}COONa \xrightarrow{HCl} C^{14}H_3\text{-}COOH \xrightarrow{Cl_2} C^{14}H_2Cl\text{-}COOH \xrightarrow{KOH} C^{14}H_2Cl\text{-}COOK$$

$$\xrightarrow{(C_2H_5)_2SO_4} C^{14}H_2Cl\text{-}COOC_2H_5$$

$$C_2H_5OOC\text{-}C^{14}H_2Cl + NaC(COOC_2H_5)_2\text{-}NH\text{-}CO\text{-}CH_3 \longrightarrow C_2H_5OOC\text{-}C^{14}H_2\text{-}C(COOC_2H_5)_2\text{-}NH\text{-}CO\text{-}CH_3$$

$$\xrightarrow{HCl} HOOC\text{-}C^{14}H_2\text{-}CH(NH_2)\text{-}COOH \cdot HCl$$

EXPERIMENTAL

Ethylchloroacetate-2-C^{14}

The chloroacetic acid was prepared by a procedure similar to that of Hughes and Tolbert.[1] Anhydrous acetic acid was prepared from dry sodium acetate-2-C^{14} (1.85 g., 22.5 millimole, 13.3 μC/mg.) by a method described elsewhere for the preparation of propionic acid.[2] The acetic acid was distilled *in vacuo* into the chlorination apparatus (Figure 1) which contained 30 mg. red phosphorous, 15 mg. iodine, and 0.2 ml. (2.8 millimole) acetyl chloride. The reaction mixture was heated to approximately 40° to allow any water present to react with the acetyl chloride. Suitable precautions must be observed to accomodate any excess pressure which may develop. The bottom of the reaction tube was cooled in liquid nitrogen until all gaseous products were collected. The liquid nitrogen was then replaced by a dry ice-isopropyl alcohol bath and the excess hydrogen chloride removed *in vacuo*.

The apparatus and its frozen contents were then transferred to a steam bath where the condenser was filled with dry ice-isopropyl alcohol, and opened to the atmosphere (through a drying tube). Chlorine gas, passed through a phosphorous pentoxide tower, was bubbled through the reaction mixture at 100° for 2 hours. Upon cooling, the mixture solidified. The dry ice-isopropyl alcohol mixture was then poured out, most of the liquid chlorine allowed to escape by evaporation, and the condenser stopcock closed. The reaction tube was immediately immersed in liquid nitrogen to prevent pressure from developing. The chloroacetic acid was distilled into the bottom of the reaction tube by filling the cold-finger condenser with boiling water and by gently heating other exposed parts of the apparatus with a small flame. Both the condenser and liquid nitrogen bath were removed, and 1 ml. of water was added to the frozen mixture. This mixture was warmed, mixed (the chlorine allowed to escape), and then totally transferred with about 5 ml. more water to a 75 ml. pear shaped flask. After addition of phenolphthalein, the solution was immersed in an ice bath and neutralized with about 20-25 ml. of approximately 2N potassium hydroxide. The end point is indicated by the appearance of a blue color. The solution was frozen in a dry ice-isopropyl alcohol bath and then broken into small lumps with a spatula. The flask and frozen contents were then placed in a vacuum dessicator (Figure 2) and freeze-dried (36-50 hr.). Ethyl sulfate (15 ml.) was added to the dry solid, a reflux-distillation head (Figure 3) put in place, and the reaction vessel immersed in an oil bath. The oil bath was heated to 135° and the mixture allowed to reflux at that temperature for 1/2 hour. The temperature of the oil bath was then raised to 170° and the distillate collected for a period of about 2 hours. The reaction was judged to be complete when the black residue had acquired a granular appearance or when a blackish material began condensing in the reflux column. At this point the condenser was turned to the reflux position and about 1-1.5 ml. of n-decane were introduced into the flask through the condenser. The condenser was then returned to the distillation position and the n-decane distilled, carrying with it

any residual ethylchloroacetate. When the distillation was completed, the receiving vessel was removed and stoppered to prevent any access of moisture. If the reaction mixture is heated for too long beyond the stopping point described, it may suddenly decompose into a frothy carbonacious mass.

In addition to the desired product, ethylchloroacetate, and the n-decane, the distillate contains some ether, ethyl alcohol, and iodine. These impurities have been found not to interfere with any of the subsequent steps. Analysis of the distillate (by hydrolysis on inactive runs and by radioactive assay on active runs) indicates a yield of between 70% and 80% based on sodium acetate.

Aspartic-3-C^{14} acid

The ethanol used as a solvent for the following condensation was dried by distilling from metallic sodium and ethyl phthalate. Diethylacetamidomalonate (7.32 g., 33.8 millimole, a 100% molar excess) was dissolved in 40 ml. of dried ethanol in a 100 ml. round bottom flask fitted with a reflux condenser and protected from the air with a drying tube. To this solution was added .51 g. metallic sodium (22.2 millimole, a 25% molar excess). When the sodium was dissolved, the ethylchloroacetate was rinsed into the flask with about 5-10 ml. of dried ethanol. The solution was then allowed to reflux on the steam bath. Sodium chloride started to precipitate as soon as the solution began to boil. After 4 hours, the reflux condenser was removed and the ethanol distilled off. The last traces being removed at a pressure of about 30 cm. Hg. Concentrated hydrochloric acid (50 ml.) was then added to the residue and the mixture heated under reflux for 72 hours. The hydrolysate was then evaporated to dryness on a steam bath and final traces of moisture and hydrogen chloride removed in vacuo.

Purification of Aspartic Acid

It has been found that most amino acids prepared by condensing organic halides with diethylacetamidomalonate followed by hydrolysis of the product are contaminated with other radioactive compounds. The best way to obtain a pure product is to

fractionate the mixture by means of an ion-exchange column. In seeking to avoid the difficulties involved in the use of an ion-exchange column, it was found that aspartic acid prepared as shown could be purified through the preparation of its copper salt.

To this end, the crude hydrolysate was dissolved in a small quantity of water and transferred to a beaker. Copper chloride (7.67 g. $CuCl_2 \cdot 2H_2O$, a 33% molar excess over theoretical total amino acid yield) was then added to the hydrolysate solution and the total volume brought to 150 ml. This solution was then titrated (with stirring) to pH 5, using about 97 ml. of 1 N sodium hydroxide. The solution was then placed in the refrigerator for at least 2 days.

Approximately 4 ml. of Johns Manvile "Celite Analytical Filter Aid" was then added to the cold mixture. The precipitate was filtered onto a .25 cm. bed of "Celite" and washed with ice water until the filtrate was chloride free. The precipitate was then re-dispersed in about 150 ml. water, about .5 ml. concentrated hydrochloric acid added, and hydrogen sulfide gas bubbled through the solution with stirring for 2-1/2 hours. The solution was heated on a steam bath for 15 minutes and then allowed to cool to room temperature with continuous stirring. It was filtered through a .5 cm. bed of "Celite" which was then rinsed with slightly acid water (1 ml. concentrated hydrochloric acid in 200 ml. water) until most of the activity was removed.

Concentrated hydrochloric acid (25 ml.) was added to the filtrate which was then evaporated to dryness on a steam bath. The aspartic acid hydrochloride was dissolved in a mixture of 25 ml. concentrated hydrochloric acid and 25 ml. water and again dried. It was then redissolved in a small quantity of water, treated with activated charcoal, and filtered into a tared sample container.

Two dimensional paper chromatography[3] showed that there were no amino acids other than aspartic acid, and that there was less than 1% of a radioactive impurity which may have been a chromatographic anomaly.

The yield of aspartic-3-C^{14} acid hydrochloride was 2190 g., 5.7 μC/mg., 66% based on starting activity. The theoretical specific activity was 5.6 μC/mg.

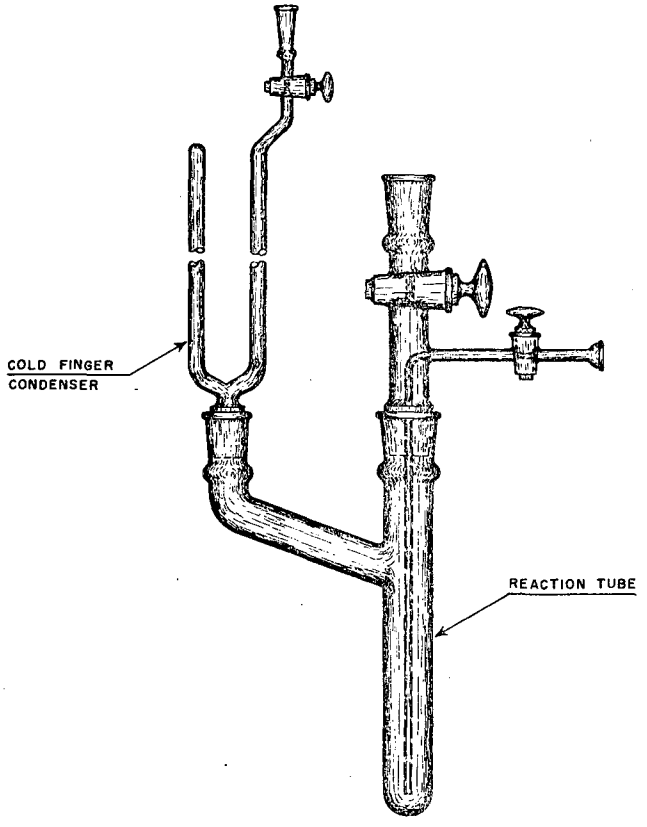
Aspartic-4-C^{14} acid hydrochloride has been prepared in similar yields by starting with carboxyl labeled sodium acetate.

REFERENCES

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

(2) Ostwald, R., Adams, P.T., and Tolbert, B.M., J.A.C.S. 74, 2425 (1952).

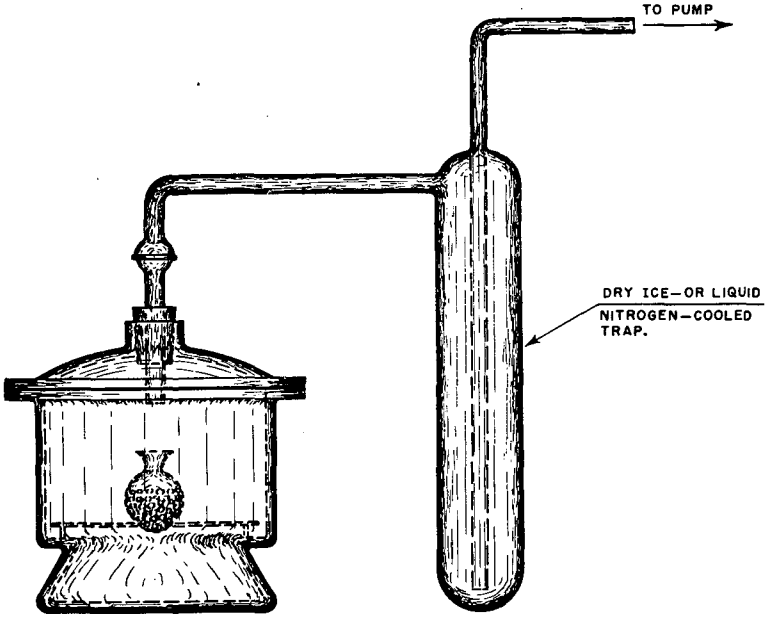
(3) Benson, A.A., et.al., J.A.C.S., 72, 1710 (1950).



CHLORINATION APPARATUS.

MU-4763

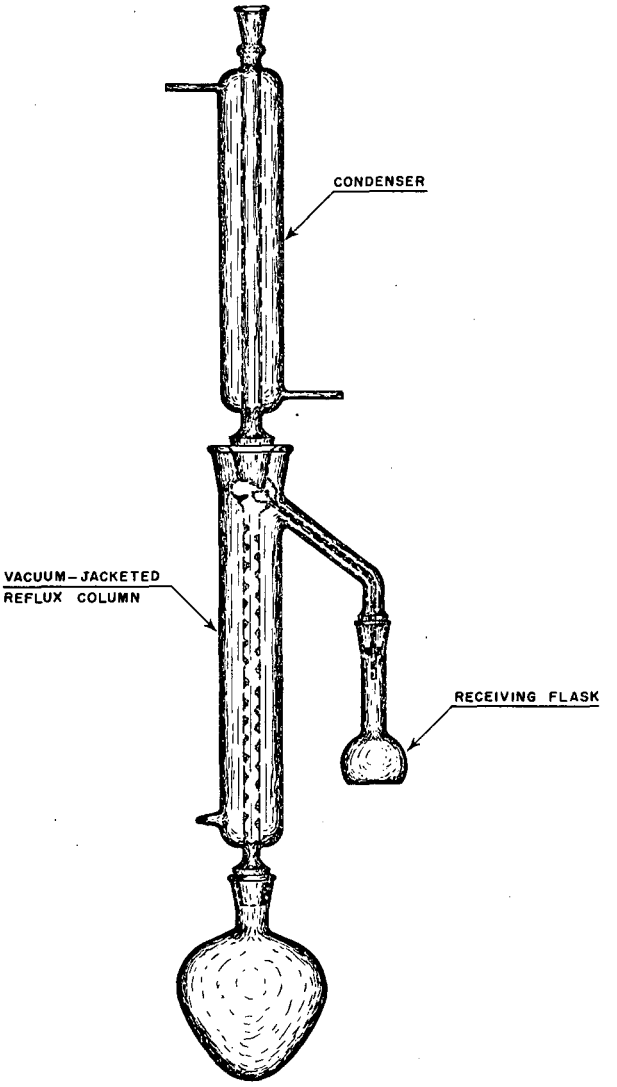
Fig. 1



VACUUM DESSICATOR.

MU-4762

Fig. 2



REFLUX-DISTILLATION HEAD.

MU-4761

Fig. 3