

Studies on Heavy-Nitrogen Labeled Compounds. III.¹⁾
Preparation of ¹⁵N-Labeled α -Hydroximinopropionic Acid

By Yoshishige SATO, Teruhiko MESHI and Norio SUGIMOTO

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There are a number of methods of preparing ¹⁵N-labeled compounds by using (¹⁵NH₄)₂SO₄ as a starting substance but there are not many methods which resort to K¹⁵NO₃. However, the use of K¹⁵NO₃ as a starting substance will be required, when reactions including diazotization are performed. In 1950, Clusius et al.²⁾ succeeded in preparing Na¹⁵NO₂ by the reaction of aqueous solution of sodium hydroxide with a mixture of oxygen and nitric oxide obtained by decomposition of K¹⁵NO₃. Using Na¹⁵NO₂ obtained, they prepared potassium azide-¹⁵N³⁾, phenyl hydrazine ¹⁵N²⁾ and diphenyl hydrazine-¹⁵N⁴⁾. Bothner-By and Friedman⁵⁾, in 1951, synthesized 3,5-dinitrobenzoylazide-3-¹⁵N according to the same method as described above.

In this paper synthesis of α -hydroximinopropionic acid-¹⁵N was performed by using K¹⁵NO₃ as a starting substance. For prelimi-

nary experiment, K¹⁴NO₃, K¹⁴NO₂ and ¹⁴NH₂OH·HCl were employed for preparing K¹⁴NO₂, ¹⁴NH₂OH·(1/2)H₂SO₄ and CH₃C(¹⁴NOH)COOH, respectively. Then, using K¹⁵NO₃, CH₃C(¹⁵NOH)·COOH was prepared without separating the intermediate products: K¹⁵NO₂ and ¹⁵NH₂OH·(1/2)H₂SO₄.

Experimental and Results

Preliminary Experimental by Use of ¹⁴N-Compound. — *Preparation of K¹⁴NO₂ from K¹⁴NO₃.* — In order to reduce KNO₃ to KNO₂, lead, copper, sodium bisulfite, sodium formate and zinc were used and potassium hydroxide was sometimes employed as an accelerating agent for the reduction in a fused state. The amount of potassium nitrite was measured by the titration with N/10-KMnO₄ solution. The data are shown in Table I.

When a mixture of potassium hydroxide with copper, sodium formate or zinc is employed as a reducing agent, a high yield can be obtained as seen from this table.

On the other hand, in the case of the reduction of the aqueous solution, it was found that potassium nitrite was obtained with a greater yield than in the case of the fusion method. The procedure is as follows. Ten milliliter of aqueous solution containing 4 g. of potassium nitrate was

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2) K. Clusius and M. Hoch, *Helv. Chim. Acta.*, 33, 2122 (1950).

3) K. Clusius and H. Hurzeler, *ibid.*, 36, 1326 (1953).

4) K. Clusius and M. Vecchi, *ibid.*, 36, 933 (1953).

5) A. A. Bothner-By and L. Friedman, *J. Am. Chem. Soc.*, 73, 5391 (1951).

TABLE I. YIELD OF KNO_2 , THE REDUCING AGENTS AND THE REDUCTION TIME

Reducing agents	Yield of KNO_2 %	Amounts of reduction agents for 2 g. of KNO_3 g.	Fusion time, hr.
Pb	64*	2.4	2 ⁶⁾
Pb	80.2*	2	5 ⁷⁾
Pb	65~70*	4	7 ⁷⁾
Pb	91~93*	2.4	8 ⁷⁾
Pb	62	6	0.5
Pb+KOH	50	Pb 4, KOH 1.2	2
NaHSO_3	59	2	
$\text{HCOONa} + \text{KOH}$	80	HCOONa 1.4, KOH 1	0.5
Cu	82	1.37	1
Cu+KOH	83	Cu 1.37, KOH 1.2	1
Zn+KOH	79	Zn 1.3, KOH 1.2	1

* From literature.

mixed with 30 ml. of 30% aqueous ammonia solution and the mixture was kept below 10°C . Then, 2.6 g. of zinc dust was added in small portions to the mixture. The zinc-oxide was filtered off and the filtrate was concentrated to 3 ml. under vacuo. The yield of potassium nitrite was found to be 85%, when measured by titration with potassium permanganate solution.

Synthesis of Hydroxylamine Hydrochloride.—Using potassium nitrite as a starting substance, hydroxylamine hydrochloride was synthesized according to the method of organic syntheses⁹⁾. The aqueous solution of potassium carbonate saturated with sulfur dioxide gas was added to the aqueous solution containing 1.7 g. of potassium nitrite in a vessel, the temperature of which was kept at 0°C . The mixed solution was kept at pH 3 with 7% aqueous solution of sulfurous acid. Two milliliter of acetone was added to it. The acetoxime was hydrolyzed with concentrated hydrochloride. The yield of hydroxylamine hydrochloride was about 70%.

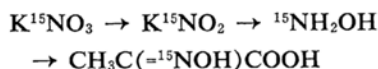
Synthesis of α -Hydroximinopropionic Acid.—In 4 ml. of aqueous solution containing 0.9 g. of pyruvic acid, 0.732 g. of hydroxylamine hydrochloride was added and allowed to stand for one

hour. Hydrochloric acid was neutralized by addition of 0.7 g. potassium carbonate powder. After the aqueous solution was neutralized, the solution was concentrated to dryness in vacuo. The solid residue was extracted with 100 ml. of ether, and α -hydroximinopropionic acid was obtained by removing ether. The yield is 85% on the basis of hydroxylamine hydrochloride and about 50% on the basis of potassium nitrate.

Synthesis of α -Hydroximinopropionic Acid- ^{15}N .— α -Hydroximinopropionic acid- ^{15}N was synthesized by the method described above. The procedure is as follows. Two and a half grams of K^{15}NO_3 (^{15}N atom % : 88%) was dissolved in an aqueous ammonia solution and kept below 10°C . Zinc powder (1.6 g.) was added for the reduction of K^{15}NO_3 to K^{15}NO_2 . After the deposited zinc oxide was removed by filtration, the solution was concentrated to 10 ml., kept at 0°C and made pH 3 with 7% sulfurous acid. Further 0.4 ml. of concentrated sulfuric acid was added to the solution in order to hydrolyze the reaction product and refluxed for five hours. Into the hydrolyzed solution, 50% aqueous solution containing 1.5 g. pyruvic acid was added and allowed to stand for one hour. Using the powder of potassium carbonate, the liberated sulfuric acid was neutralized and its solution was concentrated to dryness in vacuo. The residue was extracted with 100 ml. of ether. Water in the extracted ether solution was removed by anhydrous sodium sulfate and ether was removed in vacuo to give α -hydroximinopropionic acid- ^{15}N . The yield is 1.55 g. and 61.5% on the basis of K^{15}NO_3 .

Summary

By use of K^{15}NO_3 as a starting material, α -hydroximinopropionic acid- ^{15}N was synthesized in accordance with the following reactions:



The above reactions were carried out without separating any intermediate products. The yield of α -hydroximinopropionic acid- ^{15}N was about 61% on the basis of K^{15}NO_3 .

Osaka Research Laboratory
Tanabe Seiyaku Co., Ltd.
Kashimacho, Higashiyodogawa-ku
Osaka

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