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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY¹]

The Formation of 4-Carboxy-2-azetidinone from Asparagine in Phosphate Buffer

BY EUGENE A. TALLEY, THOMAS J. FITZPATRICK² AND WILLIAM L. PORTER

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The β -lactam, 4-carboxy-2-azetidinone, was synthesized from L- and DL-asparagine by cyclization in phosphate buffer of pH 6.7 at 100°. In addition to this compound, four compounds were formed which were ninhydrin positive.

While investigating analytical methods for use with the amides of potatoes, we had occasion to heat asparagine in phosphate buffer, as in the glutamine method described by Vickery and Pucher^{3a} and Hamilton.^{3b}

Although these investigators had found asparagine not to interfere to an appreciable extent in the

(1) A laboratory of the Eastern Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture. Article copyrighted; reprint rights reserved.

(2) Candidate for a Ph.D. degree at the University of Massachusetts, Amherst, Massachusetts.

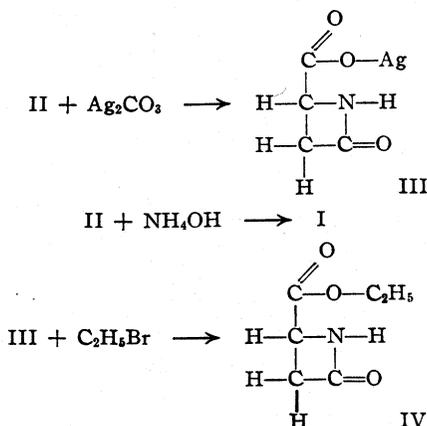
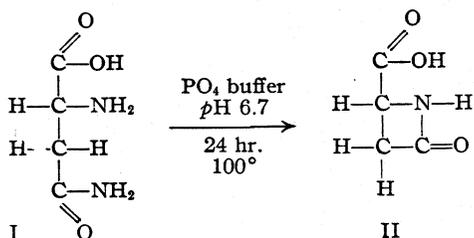
(3) (a) G. W. Pucher and H. B. Vickery, *Ind. Eng. Chem., Anal. Ed.* **12**, 27 (1940); (b) P. B. Hamilton, *J. Biol. Chem.*, **158**, 375 (1945).

glutamine determination, we found, by means of ion-exchange techniques, that definite reaction did occur in their time limit. From reaction mixtures resulting from longer periods of heating, we isolated a compound which was ninhydrin negative and Rydon-Smith⁴ positive, indicating a secondary amide. This compound was similar in some properties to pyroglutamic acid produced from glutamine under the same conditions.

Efforts to identify the compound led to the conclusion that it was 4-carboxy-2-azetidinone (pyro-

(4) H. N. Rydon and P. W. G. Smith, *Nature*, **169**, 922 (1952).

aspartic acid). Elemental analyses, molecular weight determinations on the parent compound and its ethyl ester, neutralization equivalent and some of its reactions are conclusive evidence of the β -lactam structure.



Considerable difficulty was encountered in synthesizing IV by the ordinary esterification methods. This suggested that compound II is highly hydrogen bonded, an indication further supported by preliminary infrared analyses.

Compound II was a minor component of the reaction of I in phosphate buffer. The major component was aspartic acid. In addition to these, the reaction mixture contained four other ninhydrin-positive compounds in appreciable quantities as shown by resolution on the Moore-Stein ion-exchange columns.⁵ One of these compounds produced a brown color with ninhydrin. The identification of these materials is now under investigation. Use of the L- or the DL-form of asparagine produces the β -lactam.

The basic β -lactam structure is present in the penicillin molecule. Gilman and Speeter synthesized 1,4-phenyl-2-azetidinone by the reaction of ethyl bromoacetate with benzalaniline.⁶ Sheehan and Izzo⁷ synthesized 1-phenyl-2-azetidinone from phenyl isocyanate and diazomethane. No references to the unsubstituted carboxyazetidinone described here have been found. However, Sheehan and Bose⁸ were able to synthesize 1-phenyl-4,4-dicarboxy-2-azetidinone from which they synthesized 1-phenyl-4-carboxy-2-azetidinone. Attempts to synthesize the unsubstituted carboxy- β -lactam were unsuccessful.⁹

The reduced form of II, azetidine-2-carboxylic acid, has been isolated from *Convallaria majalis* L.

(Lily-of-the-Valley) by Fowden¹⁰ who was also successful in synthesizing this compound by bromination of γ -aminobutyric acid followed by refluxing with barium hydroxide to remove hydrogen bromide and effect ring closure. Attempts to reduce the ring carbonyl of II to produce azetidine-2-carboxylic acid have, so far, been unsuccessful.

Compound II was stable to hydrolysis in 6 N hydrochloric acid at the boiling temperature for 24 hours. It also resisted hydrolysis at room temperature with 0.4 N barium hydroxide but papergrams indicated the production of trace quantities of two ninhydrin-positive compounds with R_f values of 0.31 and 0.49 in phenol.

Experimental

4-Carboxy-2-azetidinone (II).—To L-asparagine (7 g.) was added 585 ml. of pH 6.7 buffer ($\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 24.4 g., KH_2PO_4 , 16.28 g. per 100 ml., diluted 1-25 with water just before use). The flask was closed loosely with a small beaker, placed in a covered boiling water-bath and heated for 24 hours. The contents were cooled and run through a column (dia. 94 mm., height 50 mm.) of Dowex-50 cation resin, 200-400 mesh, 12% cross-linked, in the [H⁺] ion form. The aspartic acid formed in the reaction and any unreacted asparagine were adsorbed. The eluate was passed through a column (dia. 94 mm., height 20 mm.) of Dowex-2 anion resin, 200-400 mesh, 10% cross-linked, in the [OH⁻] ion form. The β -lactam and the phosphate ion were adsorbed. After washing with water, the β -lactam was preferentially eluted with 800 ml. of 1 N formic acid. The eluate was evaporated to dryness in a rotating still at temperatures below 45° to remove all traces of formic acid. The dried sample was dissolved in a minimum of water at 50-60°, filtered, cooled slowly and placed in a refrigerator overnight. The needle-like crystals were filtered off and recrystallized from water. Repeated runs gave yields varying from 0.30 to 0.35 g. corresponding to 4.3 to 5.0% after two to three recrystallizations.

Anal. Calcd. for $\text{C}_4\text{H}_5\text{O}_3\text{N}$: C, 41.74; H, 4.38; N, 12.18; mol. wt., 115.09. Found: C, 41.45; H, 4.24; N, 12.04; mol. wt. (freezing point depression using glacial acetic acid), 103.2; neut. equiv., 109.3; sublimation range, 191-193°.

Hydrolysis of 4-Carboxy-2-azetidinone (II).—Concentrated ammonium hydroxide at room temperature gave yields of 3.1% of asparagine after 6 hours at room temperature, 11.3% after 24 hours, 20.6% after 48 hours, 39.2% after 96 hours, 86.7% after 14 days and 98% after 26 days, with the production of approximately 2.5-3.0% of aspartic acid. This is a straight-line relationship up to 96 hours. These values were obtained by the Moore-Stein technique.

Ethyl Ester of 4-Carboxy-2-azetidinone (IV).—A sample of 4-carboxy-2-azetidinone (1.0 g.) was dissolved in water. To this was added silver carbonate (1.2 g.) and the suspension was boiled for 5 minutes. The hot suspension was filtered to remove excess silver carbonate, the filtrate was cooled slowly, and the solution was placed in the refrigerator. The crystals were removed by filtration and the mother liquor concentrated to obtain a second crop. The yield was 1.89 g. of the silver salt. This silver salt was suspended in benzene and, after adding 50 ml. of ethyl bromide, the suspension was refluxed at 30° for 50 hours in the dark. The silver bromide formed was removed by filtration. The benzene and excess ethyl bromide were removed by heating on the steam-bath. More benzene was added and evaporated to incipient precipitation. The solution was cooled slowly and allowed to crystallize. Plates, resembling fish scales, were removed by filtration. After recrystallization from benzene, the yield was 0.15 g. corresponding to 15% based upon the weight of β -lactam used.

Anal. Calcd. as $\text{C}_6\text{H}_9\text{O}_3\text{N}$: C, 50.34; H, 6.34; N, 9.79; mol. wt., 143.14. Found: C, 50.62; H, 6.51; N, 9.54; mol. wt. (elevation of boiling point using benzene), 160, (freezing point depression using glacial acetic acid), 128; m.p. (microscopic stage, corrected), 96°.

(10) L. Fowden, *Nature*, **176**, 347 (1955).

PHILADELPHIA 18, PA.

(5) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

(6) H. Gilman and M. Speeter, *THIS JOURNAL*, **65**, 2255 (1943).

(7) J. C. Sheehan and P. T. Izzo, *ibid.*, **70**, 1985 (1948).

(8) J. C. Sheehan and A. K. Bose, *ibid.*, **72**, 5158 (1950).

(9) J. C. Sheehan, private communication.