m.p. 110–112°, yield 48%), was dissolved in chloroform and crystallization was induced by adding petroleum ether; yield, after purification, 18.3 g., m.p. 111–112°. This material was readily soluble in dioxane and chloroform, moderately soluble in alcohol and hot water and gave a color with ferric chloride solution.

Anal. Calcd. for $C_{12}H_{13}N_3O_3$: C, 58.30; H, 5.30; N, 16.98. Found: C, 58.62; H, 5.22; N, 17.03.

1-Benzyl-4-carboxamido-5-hydroxyl-1,2,3-triazole.—The ammonolysis of the 4-carbethoxy-5-hydroxy derivative under the conditions employed for 1-benzyl-4-carbethoxy-5-amino-1,2,3-triazole required 72 hours for completion. From 24.72 g. (0.1 mole) of starting material in 400 ml. of ammonia saturated ethylene glycol, 21.0 g. of white crystals melting at 136–147° were obtained after acidification of the aqueous mixture. Recrystallization from 95% alcohol gave 16.8 g. of product (77%) melting at 165–170°. Several additional recrystallizations brought the melting point to 174–175° (with decomposition).

Anal. Calcd. for $C_{10}H_{10}N_4O_2$: C, 55.04; H, 4.62; N, 25.68. Found: C, 55.05; H, 4.61; N, 55.52.

The product from debenzylation of the above material with sodium and liquid ammonia proved difficult to purify. It was found to be more convenient to prepare the 4-carboxamido-5-hydroxy-1,2,3-triazole by the procedure of Dimroth[7] from malonamide and phenyl azide which gave the compound in a 51 per cent. yield, m.p. 196°. This reaction could not be accomplished using benzyl azide in place of the phenyl azide.

1-Benzyl-4-carbethoxy-5-chloro-1,2,3-triazole.—After heating 49.44 g. (0.2 mole) of 1-benzyl-4-carbethoxy-5-hydroxy-1,2,3-triazole, 41.66 g. of phosphorus pentachloride and 45 ml. of phosphorus oxychloride on the steam-bath for one hour, the phosphorus oxychloride was removed at 90° *in vacuo* (water aspirator). The oily residue was placed over potassium hydroxide in a vacuum desiccator overnight, then taken up in a little warm absolute alcohol, seeded and cooled in a freezer. Large white crystals (23.6 g., 44%), melting at 67–68°, were obtained after washing with cold alcohol, then with ether and drying. Further recrystallization from 95% alcohol did not alter the melting point.

Anal. Calcd. for $C_{12}H_{12}N_3O_2Cl$: C, 54.24; H, 4.55; N, 15.81; Cl, 13.35. Found: C, 54.28; H, 4.63; N, 15.66; Cl, 13.40.

1-Benzyl-4-carbethoxy-5-mercapto-1,2,3-triazole Sodium Salt.—Five-hundred ml. of absolute alcohol containing 5.40 g. (0.1 mole) of sodium methoxide was saturated with hydrogen sulfide. After adding 26.56 g. of 1-benzyl-4-carbethoxy-5-chloro-1,2,3-triazole, the solution was heated under reflux for 24 hours. The mixture was concentrated *in vacuo* to a volume of 200 ml. and the inorganic precipitate was removed. Concentration was continued to a final volume of 100 ml. and six volumes of ether were added giving 8.61 g. of white crystals, m.p. 241–242° (with decomposition). The filtrate was evaporated to dryness *in vacuo* and the residue was stirred with 400 ml. of ether. An additional 0.61 g. of product with the same melting point was removed; total yield 9.22 g. (32%). Recrystallization from isopropyl alcohol gave a product melting at 248–249° (with decomposition).

Anal. Calcd. for $C_{12}H_{12}N_3O_2SNa$: C, 50.51; H, 4.24; N, 14.73. Found: C, 50.74; H, 4.65; N, 14.69.

The ether filtrate was evaporated to dryness and the resulting sirup was dissolved in a small amount of warm 95% alcohol. The solution was cooled giving 12.1 g. (46%) of starting material, m.p. 62–64°. The above reaction, when run for four hours at 100° in a sealed tube, resulted in only about 25% conversion of the starting material. A reaction run at 100° for 12 hours gave only a 10% yield of the desired product along with a water and dilute alkali-insoluble material melting at 116–118°.

1-Benzyl-4-carboxy-5-mercapto-1,2,3-triazole.—The ester was converted in a nearly quantitative yield to the acid, m.p. 123° (with bubbling), by brief warming with dilute alkali followed by acidification.

Anal. Calcd. for $C_{10}H_9N_3O_2S$: C, 51.04; H, 3.85; N, 17.86. Found: C, 50.95; H, 3.68; N, 17.87.

1-Benzyl-4-carboxamido-5-mercapto-1,2,3-triazole.—The ammonolysis of 11.40 g. (0.04 mole) of 1-benzyl-4-carbethoxy-5-mercapto-1,2,3-triazole sodium salt was accomplished in nearly quantitative yield by heating at 100° for 15 hours in 50 ml. of ethylene glycol saturated with ammonia at 0°, yield 9.27 g. (99%), m.p. 205–206° (with bubbling, sintering at 199°). Recrystallization from approximately 1.5 liters of 95% alcohol gave 7.41 g. of cream colored crystals with the same melting point.

Anal. Calcd. for $C_{10}H_{10}N_4OS$: C, 51.26; H, 4.30; N, 23.92. Found: C, 51.33; H, 4.37; N, 23.98.

4(5)-Carboxamido-5(4)-mercapto-1,2,3-triazole.—The debenzylation of 7.03 g. (0.03 mole) of the 1-benzyl-5-mercapto derivative resulted in 2.81 g. (65%) of tan crystals melting at 194–195° (with decomposition). The decomposition point was somewhat dependent on the rate of heating. Recrystallization from hot water gave a white product with the same decomposition point.

Anal. Calcd. for $C_3H_4N_4OS$: C, 24.99; H, 2.80; N, 38.87. Found: C, 25.15; H, 2.78; N, 38.81.

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[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

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

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The β-lactam, 4-carboxy-2-azetidinone, was synthesized from L- and DL-asparagine by cyclization in phosphate buffer of *p*H 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 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–Smith[4] 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-

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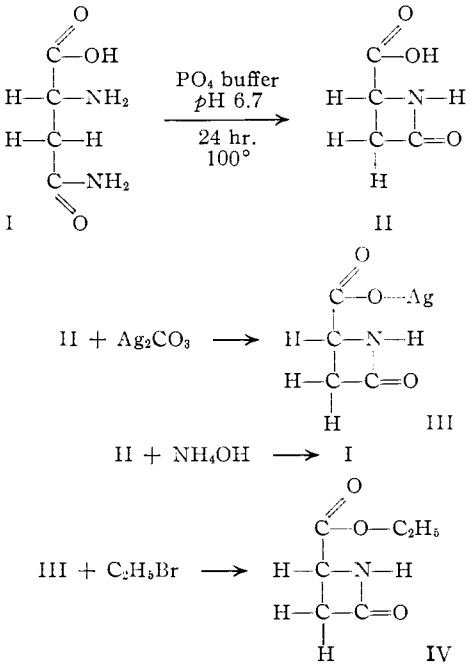
(3) (a) G. W. Pucher and H. B. Vickery, *Ind. Eng. Chem., Anal. Ed.* **12**, 27 (1940); (b) P. B. Hamilton, *J. Biol. Chem.*, **158**, 375 (1945).

(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 Izzo[7] synthesized 1-phenyl-2-azetidinone from phenyl isocyanate and diazomethane. No references to the unsubstituted carboxyazetidinone described here have been found. However, Sheehan and Bose[8] 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 Fowden[10] 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 *p*H 6.7 buffer ($Na_2HPO_4 \cdot 7H_2O$, 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 $C_4H_5O_3N$: 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 $C_6H_9O_3N$: 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°.

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