adding ethyl acetate (45 ml.) until a permanent turbidity appeared. After the mixture had remained overnight at 4°, the crystals were collected, 0.19 g. A second crop, 0.13 g., was obtained from the mother liquor. The product was dried over phosphorus pentoxide *in vacuo* at 100° for analysis, m.p. 194.5–196° dec., $[\alpha]_D^{24}$ −62° (*c* 1.0, water).

Anal. Calcd. for $C_{19}H_{34}N_4O_7$.HCl: C, 48.87; H, 7.55; N, 12.00; Cl, 7.59. Found: C, 47.92; H, 7.71; N, 11.95; Cl, 7.59.

The expected amino acids were detected in approximately equal molar amounts upon paper chromatography of an acid hydrolysate of the tetrapeptide.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

A New Synthesis of DL-Glutamine

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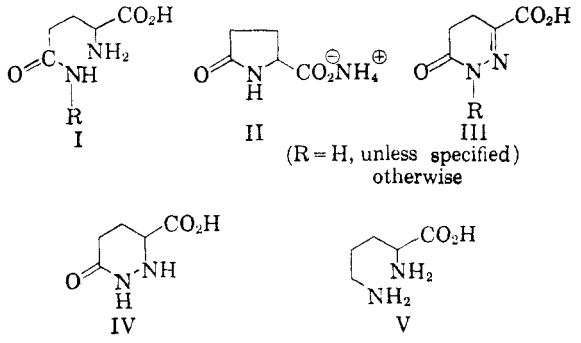
1,4,5,6-Tetrahydro-6-oxo-3-pyridazinecarboxylic acid has been converted to DL-glutamine by hydrogenation in water using palladium-on-carbon as the catalyst, and to DL-ornithine by hydrogenation in acetic acid using platinum oxide as the catalyst. A convenient preparation of benzylhydrazine is described.

Although glutamine (I. R = H),[1] the naturally occurring monoamide of glutamic acid, has shown promise as a therapeutic agent in several important medicinal areas,[2] extensive clinical investigation of this substance has been retarded by the prohibitive cost of both the natural L-isomer and the racemate. The tendency of glutamine to undergo cyclization to ammonium pyroglutamate (II) under rather mild conditions,[3] necessitates special precautions in the isolation of glutamine from natural sources and the use of blocking groups in synthetic procedures.[4] We wish to report here a simple synthetic sequence which obviates this inherent difficulty and makes DL-glutamine readily accessible.



The arrangement of atoms in 1,4,5,6-tetrahydro-6-oxo-3-pyridazinecarboxylic acid (III), readily prepared from α-ketoglutaric acid and hydrazine, made this compound appear particularly attractive as a potential intermediate for the desired synthesis. The conversion of III to glutamine requires only the saturation of the carbon-nitrogen double bond and the reductive cleavage of the nitrogen-nitrogen single bond. The possibility that both of these steps might be accomplished in one operation led us to undertake a study of the catalytic hydrogenation of III.[5,6]

Ethyl oxalosuccinate was prepared by the method of Friedman and Kosower[7] and hydrolyzed to α-ketoglutaric acid by the method of Blaise and

(1) For a review on the chemical characteristics and physiological roles of glutamine, see R. M. Archibald, *Chem. Rev.*, **37**, 161 (1945); for metabolism studies, see A. Meister, *Physiol. Rev.*, **36**, 103 (1956).

(2) D. B. Tower, *Neurology*, **5**, 113 (1955); L. L. Rogers and R. B. Pelton, *Texas Repts. Biol. Med.*, **15**, 84 (1957); J. M. Ravel, B. Felsing, E. M. Lansford, R. H. Trubey, and W. Shive, *J. Biol. Chem.*, **214**, 497 (1955); L. L. Rogers, R. B. Pelton, and R. J. Williams, *J. Biol. Chem.*, **214**, 503 (1955); **220**, 321 (1956); W. Shive and J. M. Ravel, *U.S. Pat.* **2,868,693**; E. A. Swinyard, L. Chin, F. R. Cole, and L. S. Goodman, *Proc. Soc. Exptl. Biol. Med.*, **94**, 12 (1957).

(3) The complete conversion of glutamine to ammonium pyroglutamate can be accomplished by heating the solid to its melting point or by holding an aqueous solution at 100° for one hour.

(4) (a) F. E. King and D. A. Kidd, *J. Chem. Soc.*, 3315 (1949). (b) M. Bergmann, L. Zervas, and L. Salzman, *Ber.*, **66B**, 1288 (1933); H. Nienberg, *Ber.*, **68B**, 2232 (1935); B. Vassel, *U.S. Pat.* **2,762,841**; R. M. Joyce and B. Vassel, *U.S. Pat.* **2,798,092**.

(5) Although there is ample precedent for the catalytic reduction of a carbon-nitrogen double bond to a carbon-nitrogen single bond, the catalytic reductive cleavage of a nitrogen-nitrogen single bond by hydrogen has seen only limited application. W. F. Whitmore and A. J. Revukas, *J. Am. Chem. Soc.*, **59**, 1500 (1937); **62**, 1687 (1940), reported the cleavage of azo compounds using Raney nickel catalyst and hydrogen. The presence of a small amount of isopropylamine as a by-product in the catalytic hydrogenation (platinum catalyst) of acetone semicarbazone to isopropylsemicarbazide was observed by D. W. Neighbors, A. L. Foster, S. M. Clark, J. E. Miller, and J. R. Bailey, *J. Am. Chem. Soc.*, **44**, 1557 (1922). The chemical reductive cleavage of substituted hydrazines to amines using sodium amalgam and acetic acid in alcohol has been reported by J. Tafel, *Ber.*, **19**, 1924 (1886).

(6) The cleavage of nitrogen-nitrogen bonds by the use of excess Raney nickel has received some application in recent years. In addition to work reported by C. Ainsworth, *J. Am. Chem. Soc.*, **76**, 5774 (1954); **78**, 1636 (1956); and R. L. Hinman, *J. Org. Chem.*, **22**, 148 (1957), there is the synthesis of L-glutamine from L-glutamic acid γ-hydrazide, S. Akabori and K. Narita, *Proc. Japan Acad.*, **29**, 264 (1953); S. Rath, U.S. Pat. **2,788,370**.

(7) L. Friedman and E. Kosower, *Org. Syntheses*, Coll. Vol. III, 510 (1955).

Gault.[8,9] By a slight modification of the procedure of Evans and Wiselogle,[10] III was prepared in 89% yield.

An exploratory hydrogenation of III was carried out on a semimicro scale using platinum oxide as the catalyst and glacial acetic acid as the solvent. Paper chromatographic analysis of the resulting mixture indicated the presence of glutamine, glutamic acid, *proline*, and *ornithine*, with the last named compound predominating. Similar results were obtained when dilute hydrochloric acid was employed as the solvent. On a preparative scale, using acetic acid as the solvent, DL-ornithine (V) was isolated as the monohydrochloride in 23% yield. We were unable to improve the yield by the use of other catalysts.

Our next attempt involved the use of Raney nickel as the catalyst. The sodium salt of III was dissolved in water and hydrogenated on a semimicro scale to give glutamine and glutamic acid only, as evidenced by paper chromatography. However, when the reaction was run on a preparative scale, we were unable to isolate any crystalline material.

Having thus failed to achieve the desired one-step transformation, we directed our efforts to the two-step approach. In an attempt to obtain hexahydro-6-oxo-3-pyridazinecarboxylic acid (IV), III was suspended in water and hydrogenated over 5% palladium-on-carbon. *DL-Glutamine was isolated in 27% yield.* Subsequent experiments using low-pressure hydrogenation equipment gave yields of 50–55% with a hydrogen absorption of 75% of the theoretical two moles. When the hydrogenation was conducted at 70 atmospheres a yield of 63% was obtained.

In this reaction hydrogenolysis of the nitrogen-nitrogen single bond may be preceded by saturation of the carbon-nitrogen double bond.[11] The fact that no hydrogen was taken up when benzhydrazide was subjected to identical hydrogenation conditions, however, would indicate that such is not the case and that, in fact, the reverse sequence might well pertain. Although the hydrogenation of IV would indeed shed some light on this aspect, numerous attempts to obtain this compound by both chemical and catalytic reduction of III have so far met with failure. An alternative route to IV, the reaction of α-bromo-γ-carbethoxybutyric acid with hydrazine, was also unsuccessful.

The use of monosubstituted hydrazines in this reaction sequence gave the corresponding substituted glutamines without incident. An alternative path to these same compounds involving alkylation of the ethyl ester of III was unsuccessful.

A convenient synthesis of benzylhydrazine is described.

EXPERIMENTAL

Melting points were determined in soft glass capillary tubes and are uncorrected.

1,4,5,6-Tetrahydro-6-oxo-3-pyridazinecarboxylic acid (III). To a well-stirred solution of 22 g. of α-ketoglutaric acid in 30 ml. of water was added 9 ml. of 85% hydrazine hydrate over a period of 3–4 min. The mixture became hot, and the product separated as a crystalline solid. The mixture was stirred at room temperature for 30 min. and then refrigerated for several hours. The solid was collected on a funnel, washed with 15 ml. of 2*N* hydrochloric acid, and allowed to dry in the air. The yield of acid, m.p. 196–197° dec., was 20 g. (88%). Crystallization from 2*N* hydrochloric acid gave a sample which analyzed for the hemihydrate, m.p. 194–195° dec. (lit.,[10] m.p. 195–196° dec.).

Anal. Calcd. for $C_5H_6N_2O_3 \cdot {}^1/_2H_2O$: C, 39.74; H, 4.67; N, 18.54. Found: C, 39.98; H, 4.84; N, 18.90.

Water of crystallization was removed by drying *in vacuo* at 100° for several hours.

Anal. Calcd. for $C_5H_6N_2O_3$: C, 42.25; H, 4.26; N, 19.71. Found: C, 42.55; H, 4.47; N, 19.51.

The ethyl ester was obtained as follows: A mixture of 20 g. of acid (hemihydrate), 300 ml. of ethanol, and 5 ml. of concd. sulfuric acid was allowed to reflux for 27 hr. After removal of a small amount of hydrazine sulfate by filtration, the filtrate was refrigerated and the resulting solid was collected on a filter. The yield of ester, m.p. 125–129°, was 13.7 g. (60.5%). An analytical sample, m.p. 133.5–135°, was obtained by recrystallization from ethanol.

Anal. Calcd. for $C_7H_{10}N_2O_3$: C, 49.40; H, 5.92; N, 16.46. Found: C, 49.83; H, 5.91; N, 16.85.

1-Benzyl-1,4,5,6-tetrahydro-6-oxo-3-pyridazinecarboxylic acid (III. R = $CH_2C_6H_5$). The procedure was the same as that used to prepare the parent compound. From 5.0 g. of α-ketoglutaric acid and 4.2 g. of benzylhydrazine there was obtained, after two recrystallizations from ethanol, 5.0 g. (63%) of the acid, m.p. 175–177.5°.

Anal. Calcd. for $C_{12}H_{12}N_2O_3$: C, 62.06; H, 5.21; N, 12.06. Found: C, 62.22; H, 5.09; N, 12.32.

1-Methyl-1,4,5,6-tetrahydro-6-oxo-3-pyridazinecarboxylic acid (III. R = CH_3). The procedure was similar to that used to prepare the parent compound. From 10.0 g. of α-ketoglutaric acid and 18.8 g. of methylhydrazine sulfate there was obtained, after recrystallization from 2*N* hydrochloric acid, 6.0 g. (56.5%) of the acid, m.p. 154–156.5°. A second crystallization from ethanol gave an analytically pure sample, m.p. 156–157°.

Anal. Calcd. for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.28; H, 5.27; N, 17.76.

1-Phenyl-1,4,5,6-tetrahydro-6-oxo-3-pyridazinecarboxylic acid (III. R = C_6H_5). The procedure was similar to that of Feofilaktov and Semenova.[12] A mixture of 5.0 g. of α-ketoglutaric acid, 6.5 ml. of phenylhydrazine, and 120 ml. of 3% hydrochloric acid was heated on the steam bath for 30 min., during which time a light tan crystalline solid separated. After recrystallization from ethanol, the yield of acid, m.p. 170–172°, was 4.8 g. (65%) (lit.,[12] m.p. 172°).

Anal. Calcd. for $C_{11}H_{10}N_2O_3$: C, 60.54; H, 4.62; N, 12.84. Found: C, 60.28; H, 4.47; N, 12.98.

(8) E. E. Blaise and H. Gault, *Bull. soc. chim. France*, **9**, 451 (1911).

(9) The hydrolysis conditions in the procedure of ref. (7) were found to be very unsatisfactory.

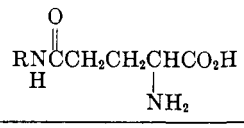
(10) R. C. Evans and F. Y. Wiselogle, *J. Am. Chem. Soc.*, **67**, 60 (1945).

(11) After completion of this work our attention was drawn to the report of M. K. Bach, *Biochimica et Biophysica Acta*, **26**, 104 (1957), who postulated a mechanism for the biological fixation of nitrogen which involves the identical sequence of reactions.

(12) V. V. Feofilaktov and N. K. Semenova, *Zhur. Obshchei Khim.*, **23**, 450 (1953); *Chem. Abstr.*, **48**, 444a (1954).

TABLE I

N-SUBSTITUTED GLUTARAMIC ACIDS



R	Crude Yield, %	M.P.	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Benzyl	46	209–210 dec.[a]	61.00	61.12	6.83	7.19	11.86	11.84
Phenyl	35	203–203.5[b, c]	59.45	59.34	6.35	6.36	12.60	12.63
Methyl	48	196–196.5[a]	44.99	45.18	7.55	7.64	17.49	17.42

[a] Recrystallized from ethanol and water. [b] One-half of the material was recovered from the catalyst. The material was purified by acidification of an alkaline solution. [c] Lit., [14] m.p. 208–209°.

DL-*Ornithine monohydrochloride* (V, HCl). 1,4,5,6-Tetrahydro-6-oxo-3-pyridazinecarboxylic acid (hemihydrate) (3.0 g.) was hydrogenated at low pressure and room temperature in 200 ml. of glacial acetic acid over platinum oxide. The catalyst was separated by filtration, the solvent was removed under reduced pressure, and the residue was taken up in 5 ml. of concd. hydrochloric acid and the solution was concentrated to dryness *in vacuo*. The residue was dissolved in 13 ml. of ethanol and partially neutralized with 2.8 ml. of concd. ammonium hydroxide. The resulting solid was separated by filtration, digested in 30 ml. of boiling ethanol for 10 min., collected on a filter, and washed with two 10-ml. portions of ethanol. After recrystallization from a mixture of water and ethanol, the yield of monohydrochloride, m.p. 220–223°, was 0.76 g. (23%). A second recrystallization raised the melting point to 226.5–227° (lit.,[13] m.p. 218°).

Anal. Calcd. for $C_5H_{12}N_2O_2 \cdot HCl$: C, 35.61; H, 7.77; N, 16.61. Found: C, 35.33; H, 8.02; N, 16.31.

DL-*Glutamine* (I. R = H). 1,4,5,6-Tetrahydro-6-oxo-3-pyridazinecarboxylic acid (hemihydrate), 6.7 g., was suspended in 50 ml. of water and hydrogenated over 2 g. of 5% palladium-on-carbon at room temperature and 70 atm. until hydrogen uptake ceased. (The hydrogenation may be carried out at 3 atm., but the yield is diminished somewhat.) The catalyst was separated by filtration and washed with 25 ml. of water. Acetone, 6–10 volumes, was added to the combined aqueous portions and the product separated as a white crystalline solid. The yield of glutamine, m.p. 168–169°, was 4.1 g. (63%). Recrystallization by the addition of acetone to an aqueous solution gave an analytical sample, m.p. 173–174.5° (lit.,[4a] m.p. 185–186°).

Anal. Calcd. for $C_5H_{10}N_2O_3$: C, 41.09; H, 6.90; N, 19.17. Found: C, 40.97; H, 6.93; N, 19.31.

The *N*-substituted glutaramic acids were prepared using a procedure identical with that for glutamine. The experimental results are summarized in Table I.

Benzylidenehydrazine.[15] Benzaldehyde (157.5 g.) was added dropwise to 164 g. of 100% hydrazine hydrate over a period of 20 min. while the temperature, which rose rapidly during the initial period, was maintained at 38–40° by intermittent ice bath cooling. Anhydrous magnesium sulfate was then added portionwise until no further rise in temperature occurred. Three volumes of ether were added and the ether solution was decanted and allowed to dry over solid potassium hydroxide. After removal of the ether under reduced pressure, the residue was distilled *in vacuo*. The yield of benzylidenehydrazine, b.p. 115–120° (8 mm.), n_D^{25} 1.6271, was 145.7 g. (82.6%) (lit.,[16] b.p. 140°, 14 mm.).

Anal. Calcd. for $C_7H_8N_2$: C, 69.97; H, 6.71; N, 23.32. Found: C, 69.32; H, 7.20; N, 22.79.

Benzylhydrazine. Benzylidenehydrazine (14.4 g.) was dissolved in 100 ml. of ethanol and hydrogenated at low pressure over 1.0 g. of 5% palladium-on-carbon. The theoretical amount of hydrogen was absorbed in 20 min., and after separation of the catalyst by filtration and removal of the ethanol under reduced pressure, the residue was distilled *in vacuo*. The yield of benzylhydrazine was 11.0 g. (75%), b.p. 105–110° (8 mm.), n_D^{25} 1.5568 (lit.,[17] b.p. 103°, 41 mm.).

Anal. Calcd. for $C_7H_{10}N_2$: C, 68.82; H, 8.25; N, 22.93. Found: C, 69.01; H, 8.16; N, 22.91.

The hydrochloride was crystallized from ethanol, m.p. 109–111° (lit.,[18] m.p. 111°).

Anal. Calcd. for $C_7H_{10}N_2 \cdot HCl$: C, 52.99; H, 6.99; N, 17.66. Found: C, 52.65; H, 7.08; N, 17.59.

Acknowledgment. The assistance of the following persons has been greatly appreciated: Mr. W. L. Brown and associates for the microanalyses, Dr. H. E. Boaz and associates for the spectral measurements, and Mr. H. L. Bird, Jr., for the paper chromatograms.

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