ference in the case of lithium amounts to only 16 kcal., thereby indicating a relatively greater interaction energy of the lithium ion with ammonia than with water.

Acknowledgment.—We wish to express our appreciation to the Research Corporation for a Frederick Cottrell grant in aid of research which made this investigation possible. We are also indebted to the Sigma Xi for an earlier grant supporting exploratory work. We acknowledge the assistance of Mr. Sumner P. Wolsky who performed some of the calibration experiments.

Summary

The heats of reaction of lithium, sodium, potassium and cesium metals with dilute solutions of ammonium bromide and ammonium chloride have been determined in a liquid ammonia calorimeter at −33°. Combination of these heats of reaction with the known heats of solution of the metals in pure liquid ammonia has given the heat of reaction of each dilute metal solution with ammonium ion. Exothermic heats of reaction varying from 39.7 to 41.6 kcal. (mean 40.4 ± 1 kcal.) indicate within experimental error an identical reaction in each case and, therefore, a close similarity in the nature of the dilute solutions of the alkali metals in liquid ammonia. A redetermination of the heat of solution of lithium in liquid ammonia gave −9.6 kcal. for ΔH instead of the reported value −8.0 kcal. From the measured heats of reaction and corresponding free energy changes relative partial molal ionic entropies and entropies of solvation of the alkali metal ions in liquid ammonia have been calculated.

BOSTON, MASS. RECEIVED JANUARY 5, 1949

[CONTRIBUTION FROM THE LABORATORY FOR THE STUDY OF HEREDITARY AND METABOLIC DISORDERS AND THE DEPARTMENTS OF BIOLOGICAL CHEMISTRY AND MEDICINE, UNIVERSITY OF UTAH COLLEGE OF MEDICINE]

The Relationship between Homoserine and its Lactone[1]

BY MARVIN D. ARMSTRONG

The preparation of the optical isomers of homoserine (α-amino-γ-hydroxybutyric acid) was described in a previous publication[2] and a brief review was made of its earlier literature. Since the first synthesis of homoserine by Fischer and Blumenthal in 1907[3] little appeared to have been added to our knowledge of the physical and chemical properties of the compound and its derivatives. The purpose of the present investigation was to examine some of these properties, particularly the relationship of homoserine to its γ-lactone and its diketopiperazine.

The early studies of homoserine by Fischer and Blumenthal indicated that in acid solutions it possibly existed only in the lactone form; the conversion to a lactone is in analogy with the behavior of homocysteine which forms the corresponding thiolactone.[4] Fischer and Blumenthal also prepared free α-aminobutyrolactone from its hydrochloride and found that it reacts with itself to form a diketopiperazine. The formation of a diketopiperazine is likewise similar to the reaction of homocysteine thiolactone which reacts with itself in neutral solution to form homocysteine diketopiperazine.[5] It thus was of interest to find whether homoserine could exist in the open form in acid solutions and whether the lactone of homoserine reacted in aqueous solution to form the corresponding diketopiperazine as well as opening to form homoserine.

The nitrous acid amino nitrogen determination showed both homoserine and its lactone to contain the calculated amount of amino nitrogen; homoserine diketopiperazine showed no amino nitrogen under the conditions of the determination. Solutions, ranging from 1 N in sodium hydroxide to 6 N in hydrochloric acid, of the free acid after standing for several days suffered no loss in their content of amino nitrogen; this indicated that no measurable amount of diketopiperazine was formed from homoserine itself. Solutions of the lactone behaved somewhat differently; in the presence of even a slight amount of base the lactone ring opened to form homoserine, and its solutions showed no loss of amino nitrogen. Dilute (1%) neutral or slightly acidic (less than 1 mole of acid/mole of lactone) solutions of the lactone did not show the formation of any significant amount of diketopiperazine; more concentrated neutral or slightly acidic solutions of the lactone, however, showed the formation of a mixture of homoserine and its diketopiperazine. The formation of homocysteine diketopiperazine in good yield from homocysteine thiolactone may be understood to occur as a result of the much greater insolubility of this diketopiperazine in water; this would speed the formation of diketopiperazine at the expense of the reaction forming homocysteine.

The availability of optically active homoserine and its lactone hydrobromide made it appear likely that the relationship of the free amino acid to its lactone could be studied polarimetrically,

(1) This research was supported by a grant from the United States Public Health Service. Presented in part before the Division of Biological Chemistry at the 112th meeting of the American Chemical Society, New York, September 16, 1947.

(2) M. D. Armstrong, THIS JOURNAL, **70**, 1756 (1948).

(3) E. Fischer and H. Blumenthal, *Ber.*, **40**, 106 (1907).

(4) B. Riegel and V. du Vigneaud, *J. Biol. Chem.*, **112**, 149 (1935–1936).

(5) V. du Vigneaud, W. I. Patterson and M. Hunt, *J. Biol. Chem.*, **126**, 217 (1938).

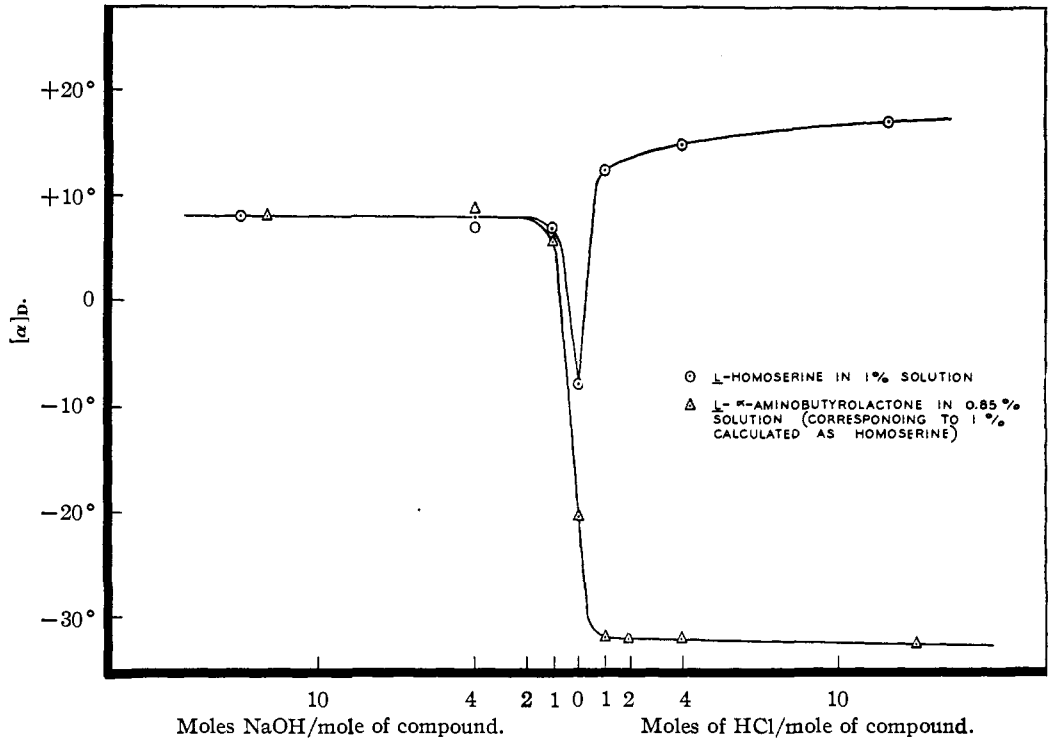
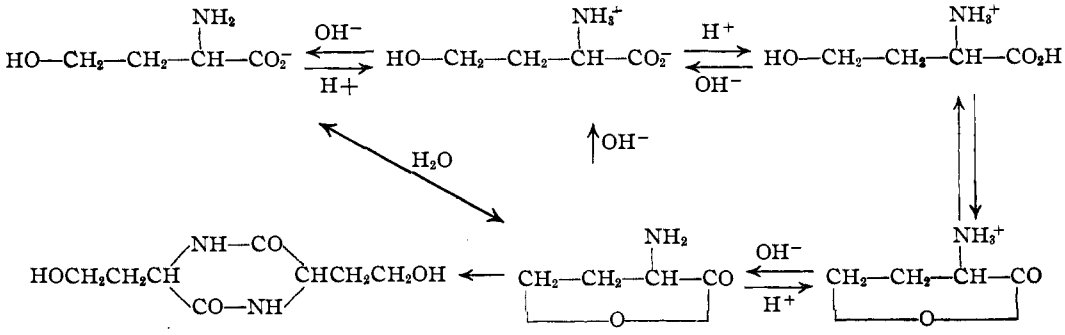


Fig. 1.—The effect of acid and alkali on the rotation of L-homoserine and its lactone.

provided the initial rotations of homoserine and its lactone were widely enough separated. Accordingly, measurements were made of the rotations of L-homoserine and of L-α-aminobutyrolactone in solutions containing varying amounts of acid and alkali. For convenience a concentration of the lactone hydrobromide equivalent in molarity to a 1% solution of homoserine was used and the specific rotations are reported on this basis. The results (Fig. 1) show that the specific rotation of L-homoserine is affected by acid and alkali in the same manner as that of the other natural amino acids. L-α-Aminobutyrolactone, on the other hand, shows a quite different behavior. In the presence of 1 mole of base the lactone ring is immediately opened to yield a salt of homoserine; 1 mole of acid causes it to exhibit a negative maximum of rotation which is not increased significantly at higher acid concentrations.

Mutarotation was found to occur in acidic solutions of the amino acid or its lactone. The change in rotation with respect to time is shown in Fig. 2 for three concentrations of acid; the speed with which equilibrium was reached varied with the strength of acid. With the use of the observed initial rotations of homoserine and of its lactone (extrapolated to zero time) it became possible to calculate the percentage of each present in an equilibrium mixture. In Fig. 3 is shown the effect of acid concentration on the percentage of homoserine present in such a solution.

The above data indicate that in solutions of homoserine the following changes may occur.



Experimental

L- and DL-homoserine, L- and DL-α-aminobutyrolactone hydrobromide, and inactive homoserine diketopiperazine were prepared in the manner previously described.[1]

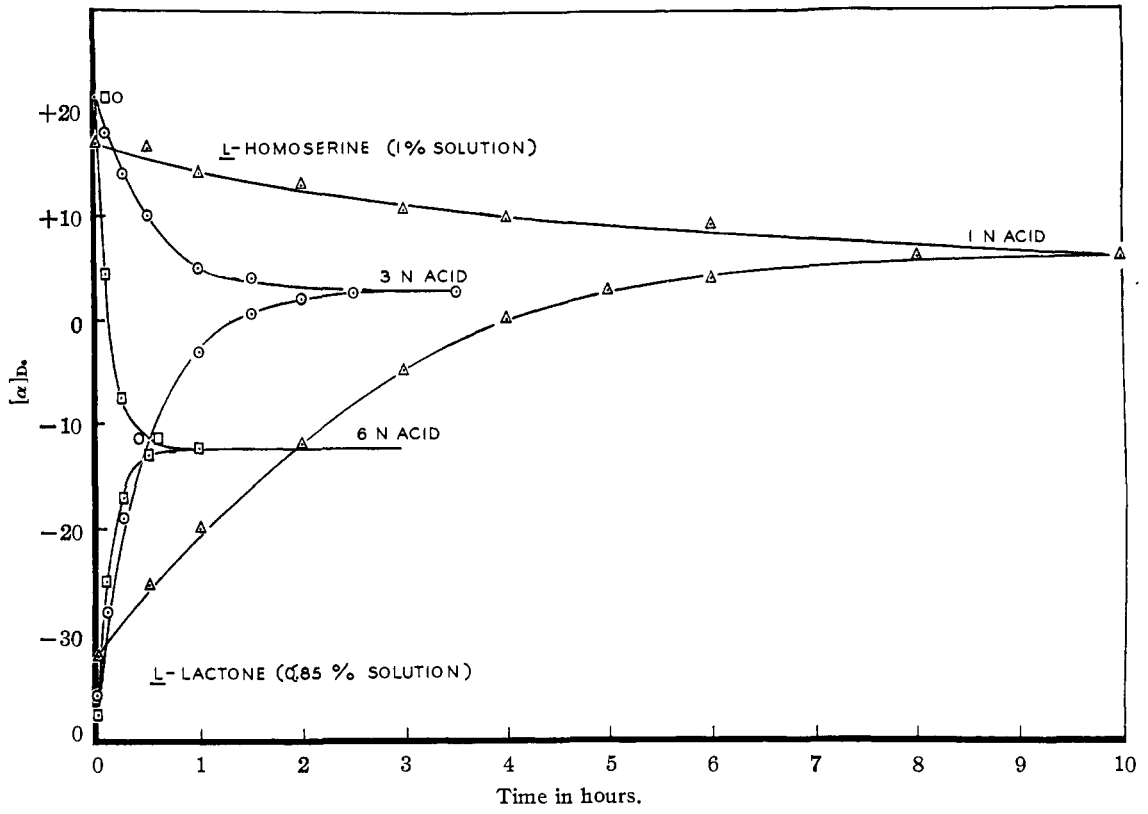


Fig. 2.—The effect of acid concentration on the equilibration of homoserine and its lactone.

The Solubility of L- and DL-Homoserine.—Because of the high solubility of these compounds in water and the unavailability of a considerable amount of the optically active compound for experimentation, a roughly quantitative estimate of their solubility was made as follows: 500.0 mg. of the compound was placed in a tared flask and distilled water was added dropwise to the flask until most of the compound had dissolved. The flask was shaken for a period of two hours between the addition of each drop of water. The amount of water necessary just to dissolve the compound was determined by reweighing the flask. DL-Homoserine: temperature, 30°; 504.4 mg. required 400 mg. of water; solubility, 125 g. of DL-homoserine in 100 g. of water. L-Homoserine: temperature, 30°; 404.4 mg. required 367 mg. of water for complete solution; solubility, 110 g. of L-homoserine in 100 g. of water. It will be noted that this approximation gives a minimum value for the solubility of the compounds.

Test for Diketopiperazine Formation from Homoserine and α-Aminobutyrolactone.—Analytical data were determined for homoserine and its derivatives:

TABLE I

	N Calcd., %	N Found, % Kjeldahl	Amino	Ninhydrin
Homoserine	11.76	11.93	11.77	11.78
α-Aminobutyrolactone hydrobromide	7.69	7.75	8.15[6]	0
Homoserine diketopiperazine	13.86	13.81	0	0

One per cent. solutions of homoserine and of α-aminobutyrolactone[7] were allowed to stand for several days with varying amounts of acid or alkali; at the end of this time the solutions showed no change in amino nitrogen content.

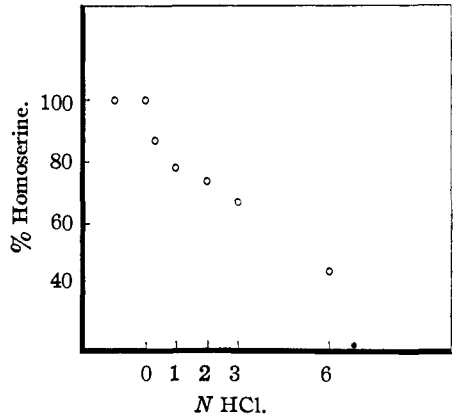


Fig. 3.—The effect of acid concentration on the composition of an equilibrium mixture of homoserine and its lactone. One per cent. solutions were used.

Opening of the Lactone Ring of α-Aminobutyrolactone in Aqueous Solution.—An attempt was made to follow the experimental conditions which gave a 95.5% yield of homocysteine diketopiperazine from homocysteine thiolactone hydrochloride.[5] To a solution of 10.0 g. of DL-α-

(6) After standing under conditions which open the lactone ring, a value of 7.72% amino N is found.

(7) Solutions of α-aminobutyrolactone were obtained by adding the calculated amounts of standard acid or alkali to a solution of its hydrobromide.

aminobutyrolactone hydrobromide in 100 ml. of water was added 4.62 g. of sodium bicarbonate. The resulting solution was allowed to stand overnight at room temperature. No precipitate had formed at this time so the solution was evaporated on a steam-bath to yield a thick semi-crystalline sirup. This sirup was dissolved in 18 ml. of water and the resulting solution was diluted with 100 ml. of absolute ethanol and let stand overnight in a refrigerator. The crystalline product was collected on a filter, washed with absolute alcohol, and was air dried; yield, 5.0 g.; m. p., 160–163° dec. An additional 0.6 g. of product having the same melting point was obtained by reworking the mother liquors.

The combined fractions were recrystallized by dissolving them in 20 ml. of hot water, adding 40 ml. of absolute ethanol, and allowing the solution to stand for several days in a refrigerator; 0.7 g. of flat parallelograms, m. p. 197–200°, was obtained. This compound gave a negative ninhydrin test and a mixed melting point with *meso*-homoserine diketopiperazine (m. p. 200–202°) showed no depression.

Anal. Calcd. for $C_8H_{14}O_4N_2$: N, 13.86. Found: N, 13.61.

The filtrate from this compound was diluted with 20 ml. of absolute ethanol and was allowed to stand in a refrigerator several more days: 1.4 g. of well formed flat needles was obtained, m. p. 183–185° dec. This compound gave a strongly positive ninhydrin test and a mixed melting point with DL-homoserine (m. p. 186–187°) showed no depression.

Anal. Calcd. for $C_4H_9O_3N$: N, 11.76. Found: N, 11.75.

A solution of homoserine in water was evaporated to dryness on a steam-bath in the same manner as in the above experiment. After it was redissolved in water an amino nitrogen determination showed that no loss in amino nitrogen had occurred, hence no diketopiperazine had formed under the conditions used.

The Optical Rotation of L-Homoserine and L-α-Aminobutyrolactone.—Rotation was measured using a 2-dm. tube and monochromatic light from a sodium vapor lamp. A 1% solution of homoserine was used in aqueous solutions containing varying amounts of acid and alkali; a 1.53% solution of α-aminobutyrolactone hydrobromide (equimolar with the 1% solution of homoserine) was used in a corresponding manner. The proper ratios of acid and alkali to the compounds were obtained by previously preparing standardized solutions containing the correct amounts of acid or alkali. The finely powdered compounds were dissolved in the solvent, placed in a polarimeter tube and the rotations of the solutions were measured as quickly as possible and thereafter at intervals. In cases where rapid mutarotation occurred the rotations at zero time were estimated by extrapolation. All rotations were measured at 25 ± 3°; there is no measurable error over this amount of variation in temperature due to a temperature coefficient for these compounds.

The results of the experiments are shown graphically in Figs. 1, 2 and 3.

Summary

The relationships between homoserine, its lactone and its diketopiperazine have been studied.

In basic and in neutral aqueous solution homoserine itself is stable and does not transform into either its lactone or diketopiperazine. In acidic solution homoserine is in equilibrium with its lactone; increasing amounts of the lactone are present in more strongly acid solutions.

In basic solution the lactone ring of α-aminobutyrolactone is opened to form homoserine; in solutions containing 1 mole or more of acid per mole of lactone the lactone is in equilibrium with homoserine. In dilute neutral solution the lactone ring opens to form homoserine and in dilute solutions containing less than 1 mole of acid per mole of lactone the lactone is in equilibrium with homoserine; in more concentrated neutral and slightly acidic solutions a mixture of homoserine and its diketopiperazine is formed.

SALT LAKE CITY, UTAH RECEIVED APRIL 18, 1949

[CONTRIBUTION FROM THE DIVISION OF CHEMISTRY OF THE NATIONAL RESEARCH COUNCIL]

The Synthesis of Pseudoconhydrine[1]

BY LÉO MARION AND WILLIAM F. COCKBURN

Pseudoconhydrine, one of the Hemlock group of alkaloids, occurs in the common hemlock, *Conium maculatum* L., along with coniine, N-methylconiine, γ-coniceine and conhydrine. It was discovered in the residues from the isolation of coniine and found to be an isomer of conhydrine, with the empirical formula $C_8H_{17}ON$.[2] Further investigation showed the base, like conhydrine itself, to be an hydroxyconiine,[3] while a study of the exhaustive methylation demonstrated that the hydroxyl group occupies position 5 of the piperidine nucleus.[4] Pseudoconhydrine is thus 5 - hydroxy - 2 - *n* - propylpiperidine[4] (VI), a structure now confirmed by the total synthesis of the alkaloid, the resolution of the synthetic base into its optical isomers and the preparation of derivatives.

Attempts to sulfonate conyrine (2-propylpyridine) in the 5-position with oleum and a mercury catalyst were largely unsuccessful, apparently owing to oxidation of the propyl side-chain, with the formation of tarry by-products. A small amount of impure material was isolated, but the method was not considered sufficiently profitable and was abandoned. The following scheme, however, proved successful.

2-Methylpyridine-5-sulfonic acid (I) was obtained by sulfonation of α-picoline[5] and converted to 5-hydroxy-2-methylpyridine (II) by potash fusion.[6] Addition of ethereal diazomethane to an aqueous–methanolic solution of II yielded the

(1) Published as National Research Council Bull. No. 2002.

(2) A. Ladenburg and G. Adam, *Ber.*, **24**, 1671 (1891).

(3) K. Löffler, *ibid.*, **42**, 116, 960 (1909).

(4) E. Späth, F. Kuffner and L. Ensfellner, *ibid.*, **66**, 591 (1933).

(5) S. M. McElvain and M. A. Goese, THIS JOURNAL, **65**, 2233 (1943).

(6) O. Wulff, U. S. Patent 1,880,645; *C. A.*, **27**, 513 (1933).