equation and with a second order rate equation of simultaneous opposing reactions.

The "concentration" equilibrium constants, $K_{H/Na}$, were found to be constant to better than 20% over a concentration range of the water phase varying from 0.001 to about 1 N, the average values of $K_{H/Na}$ being 1.25 on AMBERLITE IR-100AG, 1.20 on IONAC C-200, 1.01 on DOWEX 30, and 0.66 on ZEOKARB. The assumptions underlying the mass action equation were discussed and $K_{H/Na}$ was shown to be proportional to an equilibrium constant expressed in terms of activities, the proportionality factor being the constant ratio of the activity coefficients of the hydrogen and sodium ions in the exchanger phase.

Confirming previously published views, the exchange velocities were shown to be reasonably well represented by a second order rate equation. The velocity constants k and k' of the forward and reverse reactions were found to depend somewhat upon the ionic strength of the water phase, a less than twofold increase of k and k' being observed as the concentration decreased from 0.02 to 0.001 N. This variation was probably due to interionic effects since the ratios, k/k', of the velocity constants, determined independently using solutions of approximately the same ionic strengths, were of the same order as the equilibrium constants, $K_{H/Na}$.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF COLORADO]

Unsaturated Amino Acids. II. Allylglycine, β-Methallylglycine and Crotylglycine[1,2]

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Existing knowledge of the relationship between chemical structure and biological antagonism indicates that an interchange of a vinylene radical and a sulfur atom in biologically important compounds may form metabolite antagonists.[4] From this it seemed possible that a metabolite–antagonist relationship may exist between the two simple sulfur-containing, naturally occurring amino acids, cysteine and methionine, and their vinylene analogs. Allylglycine (2-amino-4-pentenoic acid, IVa) is so related to cysteine; it and two of its methyl homologs are the subject of this paper. These homologs, β-methallylglycine (2-amino-4-methyl-4-pentenoic acid, IVb) and crotylglycine (2-amino-4-hexenoic acid, VIII), were studied because of the interesting biological properties of allylglycine. These amino acids have also been found to be active biologically and have been studied in considerable detail.[5,6] Of these amino acids, allylglycine has been synthesized by Sörenson,[7] and it and β-methallylglycine have recently been synthesized by Albertson[8] by the alkylation of ethyl acetamidomalonate or ethyl acetamidocyanoacetate with allyl and β-methallyl chloride and subsequent hydrolysis of the alkylation products to the amino acids. We have used Albertson's method for these compounds and a similar method for crotylglycine.

The unsaturated alkylated intermediates give poor yields of amino acids when subjected to acid hydrolysis. In agreement with Fillman and Albertson,[9] we have found that these low yields are due to partial or complete lactonization across the γ,δ-double bond[10] with the formation of α-amino-γ-lactones. Our results are summarized in the flow sheet.

In contrast to Albertson's report, we have found that the hydrolysis of the allyl intermediate Ia with concentrated hydrochloric acid results in partial lactonization to IIIa,[11] as well as partial formation of allylglycine hydrochloride IIa. The difference between his results and ours may be explained by the fact that only in concentrated hydrochloric acid will lactonization of allylglycine occur rapidly. We have found that treatment of allylglycine with concentrated hydrochloric acid at reflux results in the lactonization of about 30% of the amino acid in six hours, whereas refluxing with 6 N acid for twenty hours results in lactonization of only 11%. Treatment of lactone hydrochloride IIIa with concentrated hydrochloric acid does not yield any of the unsaturated amino acid hydrochloride IIa, indicating that an equilibrium between the unsaturated amino acid and the lactone was not obtained in our hydrolysis.

Treatment of allylglycine hydrochloride IIa

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(2) This paper was presented before the Division of Organic Chemistry at the Chicago meeting of the American Chemical Society, April 19–23, 1948.

(3) American Cyanamid Company Fellow.

(4) Complete references to this literature have been given in another paper.[5]

(5) Dittmer, Goering, Goodman and Cristol, THIS JOURNAL, **70**, 2499 (1948).

(6) Our synthesis of the third methyl homolog, α-methallylglycine (2-amino-3-methyl-4-pentenoic acid), led to a mixture of diastereoisomers, which has not yet been separated. The analog of methionine has also been prepared and will be reported in a separate communication.

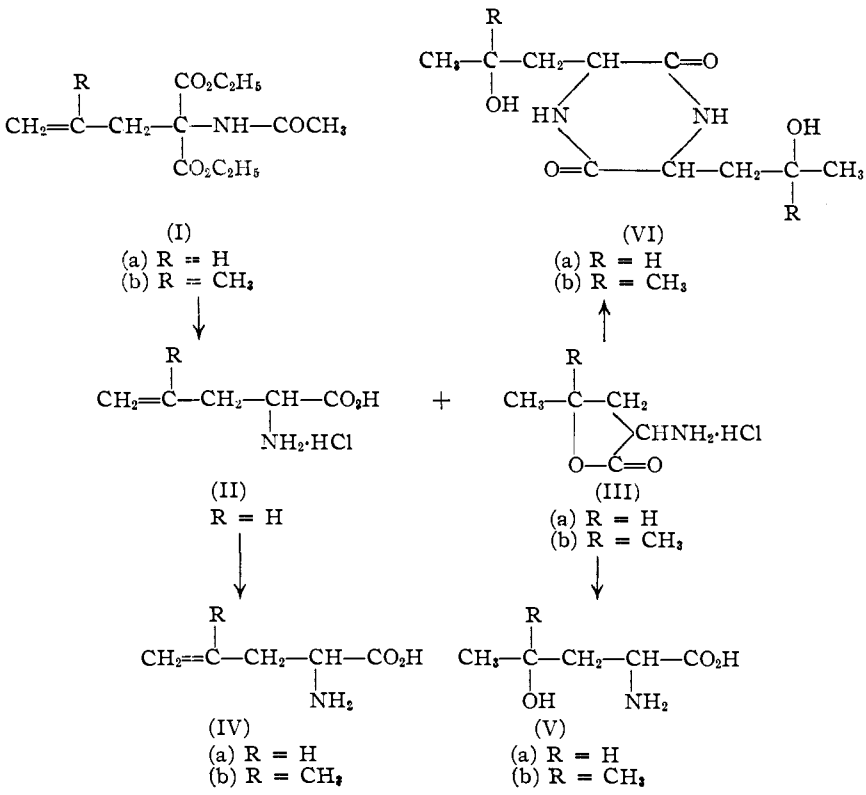
(7) Sörenson, *Ber.*, **41**, 3389 (1908).

(8) (a) Albertson and Archer, THIS JOURNAL, **67**, 308 (1945); (b) Albertson, *ibid.*, **68**, 450 (1946).

(9) Fillman and Albertson, *ibid.*, **70**, 171 (1948).

(10) Linstead and Ryden, *J. Chem. Soc.*, 580 (1933).

(11) Fischer and Leuchs, *Ber.*, **35**, 3787 (1902).

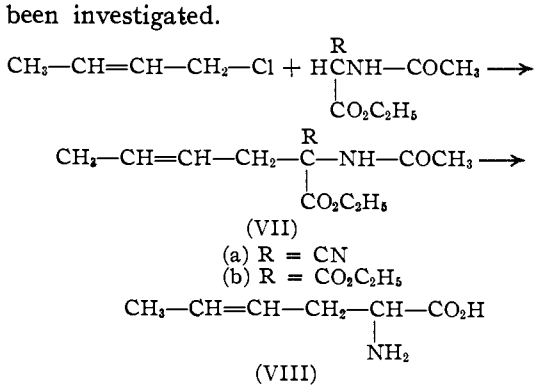


with pyridine gave the free amino acid. Treatment of the lactone IIIa with dilute base gave α-amino-γ-hydroxyvaleric acid Va.[9,11] Characterization of these compounds is described in the experimental section.

When ethyl methallylacetamidomalonate (Ib) was hydrolyzed in concentrated hydrochloric acid only the lactone IIIb was obtained, in agreement with Fillman and Albertson.[9] Attempts to prepare methallylglycine hydrochloride from the free amino acid[8b] by adding one equivalent of dilute hydrochloric acid and removing the water by evaporation in the cold resulted only in the lactonized compound. The greater ease of lactonization of methallylglycine compared with allylglycine is predictable assuming a carbonium ion intermediate. In the case of methallylglycine a comparatively readily formed tertiary carbonium ion is involved, whereas in the lactonization of allylglycine a less readily formed secondary carbonium ion is the intermediate. The formation of the γ-lactones instead of the δ-lactones is also predictable by analogous reasoning. Linstead[10] has shown that allylacetic acid is lactonized in acid to γ-valerolactone free of the δ isomer.

Treatment of the lactone IIIb with base gave γ-hydroxyleucine.[12] Characterizations of the intermediates involved in these reactions are given in the experimental section.

Crotylglycine (VIII) was prepared by alkylating both ethyl acetamidomalonate and ethyl acetamidocyanoacetate with crotyl chloride,[13] and hydrolyzing the resulting intermediates with base. Ethyl crotylacetamidomalonate (VIIb) was identified by its reduction to a compound identical with that obtained by the condensation of *n*-butyl bromide and ethyl acetamidomalonate. Albertson[8] reported that he was not successful in crystallizing the *n*-butyl derivative, but our preparation, prepared by reduction, crystallized without difficulty and melted at 41–42°. When the product prepared by direct alkylation with *n*-butyl bromide was seeded with the other preparation, it also crystallized and the preparations proved to be identical. The acid hydrolysis of ethyl crotylacetamidomalonate gave very low yields of amino acid presumably due to lactonization. The extent of this lactonization has not been investigated.



The structure of crotylglycine VIII was established by reduction of the intermediate to the saturated compound and by ozonolysis of VIII as described below. Karrer and Itschner[14] have described the preparation of crotylglycine by another method, but the melting point of their benzoyl derivative (157°) was different from that obtained by us (139°). It is possible that these workers had a different geometric or other type of isomer.

As these three unsaturated amino acids are of considerable biological significance in the development of theories of metabolite antagonism, it was

(12) Dakin, *J. Biol. Chem.*, **154**, 549 (1944).

(13) Crotyl chloride was obtained by fractional distillation of a butenyl chloride mixture; see Roberts, Young and Winstein, THIS JOURNAL, **64**, 2163 (1942).

(14) Karrer and Itschner, *Helv. Chim. Acta*, **18**, 782 (1935).

of importance that the structures of the products be proved. Hydrogenation of the amino acids or of the intermediates to the known saturated compounds constitutes proof of the carbon skeleton. This was done in each case, but this does not establish the position of the double bonds. Since these amino acids were obtained by hydrolysis using either acid or basic catalysis, and as double-bond migrations have been observed under similar conditions, it seemed essential to prove the position of the double bonds, which was done by ozonolysis. Allylglycine IVa upon ozonolysis followed by oxidation of the decomposed ozonide gave formic acid and aspartic acid. β-Methallylglycine IVb gave formaldehyde upon ozonolysis, and crotylglycine VIII gave acetaldehyde.

Experimental

α-Amino-γ-valerolactone Hydrochloride (IIIa) and Allylglycine (IVa).—Ethyl allylacetamidomalonate (Ia)[8b] (12.5 g.) was refluxed for eight hours with concentrated hydrochloric acid, and the product was concentrated under reduced pressure to a colorless viscous sirup. The sirup was crystallized from an absolute ethanol–ether mixture with separation of the crystalline lactone hydrochloride (IIIa). Excess ether caused the isomeric allylglycine hydrochloride (II) to oil out and had to be avoided. Recrystallization from absolute ethanol gave 1.9 g. (26%) of IIIa which melted at 194–196°. An analytical sample melted at 197–198°.[15a]

Anal. Calcd. for $C_5H_{10}O_2NCl$: N, 9.24. Found: N, 9.00.

The neutral **α-benzamido-γ-valerolactone** was prepared from the above lactone hydrochloride in the usual manner[16] and, after recrystallization from aqueous acetone and aqueous ethanol, melted at 141°.[15b]

Anal. Calcd. for $C_{12}H_{13}O_3N$: N, 6.39. Found: N, 6.20.

The neutral **α-(ω-phenylureido)-γ-valerolactone** was prepared from the lactone hydrochloride in the usual manner and melted at 165–165.5°.[15c] This checks with the properties of the same derivative previously prepared[11] from α-amino-γ-hydroxyvaleric acid (Va).

Anal. Calcd. for $C_{12}H_{14}O_3N_2$: N, 11.96. Found: N, 11.58.

Compound IIIa was converted to the free lactone, b. p. 125° (15 mm.), and to 3,6-diketo-2,5-bis-(2-hydroxypropyl)-piperazine (VIa), m. p. 173–174°, as described by Fischer.[11] Fischer reported a m. p. of 223–225° for this compound.

Anal. Calcd. for $C_{10}H_{18}O_4N_2$: N, 12.17. Found: N, 12.06.

Treatment of the mother liquors from the recrystallization and isolation of the lactone hydrochloride with pyridine gave 1.90 g. (34%) of allylglycine (IVa). An analytical sample melted at 255–258° (dec.) when heated rapidly in a m. p. tube,[15d] after recrystallization from water.

Anal. Calcd. for $C_5H_9O_2N$: N, 12.17. Found: N, 11.95.

The benzoyl derivative of IVa melted at 108°.[15e]

Conversion of Allylglycine (IVa) to α-Amino-γ-valerolactone Hydrochloride (IIIa).—Treatment of allylglycine with refluxing concentrated hydrochloric acid for five and one-half hours yielded a mixture from which 28% of the lactone hydrochloride IIIa and 32% of the unconverted allylglycine were recovered.

Treatment of allylglycine at reflux with 6 *N* hydrochloric acid for twenty hours gave 11% of the lactone hydrochloride and 70% of the original allylglycine.

Allylglycine Hydrochloride (II).—A solution of allylglycine in an equivalent of 0.1 *N* hydrochloric acid was evaporated at room temperature in an air stream. The residue was recrystallized from ether–absolute ethanol and melted at 164–168° (dec.).

Anal. Calcd. for $C_5H_{10}O_2NCl$: N, 9.24. Found: N, 9.30.

α-Amino-γ-hydroxyvaleric Acid (Va).—When IIIa was refluxed with 2 *N* sodium hydroxide for fifteen minutes, and the solution worked up in the usual fashion, 44% of α-amino-γ-hydroxyvaleric acid (Va) was obtained melting at 212° (uncor.)[15f] when heated rapidly in a capillary.

Anal. Calcd. for $C_5H_{11}O_3N$: N, 10.52. Found: N, 10.42.

Reduction of Allylglycine to α-Aminovaleric Acid.—To a solution of 200 mg. of allylglycine in 15 ml. of water was added 10 mg. of $PtO_2 \cdot H_2O$, and the mixture subjected to hydrogenation; 1.02 equivalents of hydrogen were taken up. The product was pure α-aminovaleric acid. A benzoyl derivative melted at 151–152°[15g] and when mixed with known α-benzamidovaleric acid, m. p. 151–152°, the m. p. was not depressed.

α-Amino-γ-methyl-γ-valerolactone Hydrochloride (IIIb).—When ethyl methallylacetamidomalonate[8] (8.45 g.) was refluxed for eight hours with concentrated hydrochloric acid and worked up as described for IIIa, 4.8 g. (93%) of IIIb was obtained. After recrystallization from ether–ethanol IIIb melted at 210–211°.[15h]

Anal. Calcd. for $C_6H_{12}O_2NCl$: C, 43.51; H, 7.31; N, 8.46; Cl, 21.41. Found: C, 43.60; H, 7.25; N, 8.41; Cl, 21.33.

The neutral **α-benzamido-γ-methyl-γ-valerolactone** was prepared from IIIb and after recrystallizations from aqueous ethanol melted at 178°.[15i]

Anal. Calcd. for $C_{13}H_{15}O_3N$: N, 6.01. Found: N, 5.97.

The neutral **α-(ω-phenylureido)-γ-methyl-γ-valerolactone** which had previously been prepared from γ-hydroxyleucine (Vb) was prepared from the above lactone hydrochloride and melted at 191–192°.[15j]

Anal. Calcd. for $C_{13}H_{16}O_3N_2$: C, 62.88; H, 6.50. Found: C, 62.72; H, 6.14.

The lactone hydrochloride was converted to **3,6-diketo-2,5-bis-(2-hydroxy-2-methylpropyl)-piperazine (VIb)** by converting the hydrochloride to the free lactone which changed to the diketopiperazine on standing. After recrystallizations from absolute ethanol the compound melted at 243–243.5°.

Anal. Calcd. for $C_{12}H_{22}O_4N_2$: N, 10.85. Found: N, 10.72.

Conversion of β-Methallylglycine (IVb) to α-Amino-γ-methyl-γ-valerolactone Hydrochloride (IIIb).—When β-methallylglycine[8b] (IVb) was dissolved in one equivalent of 1 *N* hydrochloric acid and the solution evaporated to dryness in the cold by an air stream, a crude preparation of IIIb was obtained which after one recrystallization melted at 202–204°. The melting point of a mixture with pure IIIb (m. p. 210–211°) was raised to 204–207°.

γ-Hydroxyleucine (Vb).—IIIb was converted to γ-hydroxyleucine in 81% yield by hydrolysis with 2 *N* sodium hydroxide. An analytical sample melted at 211–212° (dec.) with rapid heating in a capillary and at 230° on a Dennis m. p. bar.[15k]

Anal. Calcd. for $C_6H_{13}O_3N$: N, 9.52. Found: N, 9.28.

The phenylureido derivative of Vb was prepared in the usual manner but had been converted to the lactone form.[12] After recrystallizations from aqueous ethanol the com-

(15) All melting points are corrected: (a) ref. 11 gives m. p. 198–200°; (b) ref. 9 gives 140–141°; (c) ref. 11 gives 165–166°; (d) ref. 9 gives 212–215°; (e) ref. 8b gives 108°, ref. 9 gives 105–106°; (f) ref. 11 gives 212°; (g) ref. 8b gives 152°; (h) ref. 9 gives 208–209°; (i) ref. 9 gives 176°; (j) ref. 9 gives 200–201°, ref. 12 gives 188–189°; (k) ref. 9 gives 230–232°; ref. 12 gives 226–228°.

(16) All benzoyl derivatives of amino acids were prepared according to the method of Steiger, *J. Org. Chem.*, **9**, 396 (1944).

pound melted at 191–192°. When this derivative of Vb was mixed with the phenylureido derivative of IIIb, m. p. 191–192°, the m. p. was not depressed.

Ethyl crotylacetamidomalonate (VIIb) was prepared from crotyl chloride and ethyl acetamidomalonate according to the general procedure developed by Albertson[8b] in 80% yield. The product was crystallized from an ether-petroleum ether solution in a Dry Ice–acetone bath and after recrystallization melted at 47–48°.

Anal. Calcd. for $C_{13}H_{21}O_5N$: N, 5.16. Found: N, 5.05.

Reduction of Ethyl Crotylacetamidomalonate.—Hydrogenation of a solution of VIIb in ethanol over Raney nickel catalyst at room temperature and 30 pounds pressure was conducted and the product after recrystallization from ether–petroleum ether mixture, melted at 41–42°. This compound was identical with the compound obtained by the condensation of *n*-butyl bromide and ethyl acetamidomalonate which had previously been reported as an oil.[8b] Acid hydrolysis of this compound gave *dl*-norleucine which was identified by its benzoyl derivative.

Ethyl crotylacetamidocyanoacetate (VIIa) was prepared by alkylating ethyl acetamidocyanoacetate in the usual manner[8b] with crotyl chloride. The yield was 76%. An analytical sample was prepared by recrystallization from aqueous acetone and aqueous alcohol and melted at 56.5°.

Anal. Calcd. for $C_{11}H_{16}O_3N_2$: N, 12.49. Found: N, 12.31.

Crotylglycine.—By using Albertson's method[8b] for basic hydrolysis of substituted ethyl acetamidocyanoacetates VIIa was converted to crotylglycine (VIII) in 50% yield. Snyder's[17] method for basic hydrolysis of substituted ethyl acetamidomalonates gave a 30% conversion of VIIb to VIII. Crotylglycine was purified by recrystallization from aqueous ethanol and an analytical sample decomposed at *ca.* 260° when heated rapidly in a capillary.

Anal. Calcd. for $C_6H_{11}O_2N$: N, 10.84. Found: N, 10.58.

Crotylglycine was benzoylated in 89% yield. The compounds obtained by the basic hydrolysis of VIIa and VIIb were shown to give identical benzoyl derivatives. **2-Benzamido-4-hexenoic acid** was purified by recrystallization from aqueous acetone and aqueous ethanol and melted at 139°.

Anal. Calcd. for $C_{13}H_{15}O_3N$: N, 6.01. Found: N, 5.93.

Ozonolysis of Unsaturated Amino Acids. (A) Allylglycine.—A solution of 1.15 g. (0.01 mole) of allylglycine (IVa) in 10 ml. of water was ozonized at 0°. The products of ozonization were identified by the oxidation of the aldehydes to the corresponding acids by use of silver oxide as described by Young.[18] The oxidized solution was acidified, distilled, and Dyer constants[19] of the distillate showed that the only volatile acid was formic acid. This establishes a terminal double bond in allylglycine.

A solution of 1 g. of the benzoyl derivative of allylglycine in 4 ml. of glacial acetic acid was treated with an equivalent amount of ozone at room temperature. The ozonide was decomposed with water and oxidized with alkaline permanganate according to the procedure of Klosterman and Painter.[20] The resulting α-benzamidosuccinic acid melted at 163–165°, and the m. p. was not depressed when the compound was mixed with an authentic sample.[21]

(B) Crotylglycine.—A solution of 129 mg. of crotylglycine (VIII) in one ml. of water was ozonized at 0°. The aqueous solution was made basic with 1 *N* sodium hydroxide and steam distilled, trapping the volatile aldehyde in 0.5 ml. of ethanol at −15°. The methone derivative of the aldehyde was prepared according to the method of Horning and Horning[22] and after recrystallization melted at 139–140.5°. When the sample was mixed with an authentic sample prepared from acetaldehyde, its melting point was not depressed.

(C) β-Methallylglycine (IVb).—The same procedure as for crotylglycine was employed and a methone derivative obtained with a m. p. of 186–187°. When this sample was mixed with an authentic sample prepared from formaldehyde, its melting point was not depressed.

Acknowledgment.—The authors wish to acknowledge the assistance of the Shell Development Company and of the Shell Chemical Company in furnishing the allylic chlorides used in this research and of Mrs. Philippa Szerwo for some of the analyses.

Summary

The reactions involved in syntheses of allylglycine and methallylglycine from allyl chloride and methallyl chloride and ethyl acetamidomalonate have been investigated. By similar methods crotylglycine was synthesized.

Acid hydrolysis of ethyl methallylacetamidomalonate resulted in complete lactonization yielding α-amino-γ-methyl-γ-valerolactone hydrochloride. This was converted by base to γ-hydroxyleucine.

Acid hydrolysis of ethyl allylacetamidomalonate resulted in the formation of α-amino-γ-valerolactone hydrochloride in 26–33% yield and allylglycine in 30–35% yield. The α-amino-γ-valerolactone hydrochloride was converted to α-amino-γ-hydroxyvaleric acid.

Crotylglycine was prepared by basic hydrolysis of ethyl crotylacetamidomalonate and ethyl crotylacetamidocyanoacetate in 30 and 50% yields, respectively.

The structures of these three amino acids have been established by reduction to known compounds and by ozonolysis.

BOULDER, COLORADO RECEIVED MARCH 29, 1948

(17) Snyder and Smith, THIS JOURNAL, **66**, 350 (1944).

(18) Young, McKinnis, Webb and Roberts, *ibid.*, **68**, 293 (1946).

(19) Dyer, *J. Biol. Chem.*, **28**, 445 (1917).

(20) Klosterman and Painter, THIS JOURNAL, **69**, 1674 (1947).

(21) Fischer, *Ber.*, **32**, 2461 (1899).

(22) Horning and Horning, *J. Org. Chem.*, **11**, 95 (1946).