[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FLORIDA STATE UNIVERSITY]

# Unsaturated Amino Acids. IV. Synthesis of Halogen Substituted Allylglycines[1,2]

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A number of halogen-containing unsaturated amino acids was prepared as potential antagonists of the essential aliphatic amino acids. These were synthesized either by the addition of a halogen or halogen acid to propargylglycine or by condensation of the appropriate halide with diethyl formamidomalonate followed by dilute acid hydrolysis of the condensation product.

As part of an investigation of amino acid antagonists, a number of olefinic and acetylenic amino acids was previously prepared and tested for their ability to inhibit the growth of microörganisms.[4] Since 2-amino-4-bromo-4-pentenoic acid (γ-bromoallylglycine)[5] was more effective than 2-amino-4-pentenoic acid (allylglycine) or 2-amino-4-pentynoic acid (propargylglycine), as an inhibitor of the growth of *E. coli* (ATCC 9723), it was decided to prepare a series of these halogenated compounds as potential amino acid antagonists and to attempt to correlate changes in their structure with microbiological activity.

Using propargyglycine as a starting material, 2-amino-4-iodo-4-pentenoic acid (γ-iodo-allylglycine) was prepared by the addition of hydriodic acid in glacial acetic acid; 2-amino-4,5-dichloro-4-pentenoic acid (γ,ω-dichloroallylglycine) by chlorination in glacial acetic acid; and 2-amino-4,5-dibromo-4-pentenoic acid (γ,ω-dibromoallylglycine) by bromination in ethanol. The properties of these compounds are given in Table I.

The other amino acids in Table I were most conveniently prepared from the condensation product obtained from diethyl formamidomalonate (I) and the appropriate halide according to the method previously described.[6] The reaction sequence was

$$RCH_2X + I \xrightarrow[(\text{toluene})]{NaH} RCH_2C(COOC_2H_5)_2NHCHO \xrightarrow[HX]{10\text{–}15\%}$$

II

$$RCH_2CH(NH_2)COOH$$

III

R = $CH_2{=}CCl{-}$ (a); $CH_2{=}CBr{-}$ (b); *cis*- and *trans*-$CHCl{=}CH{-}$ (c); $CHBr{=}CH{-}$ (d); $CCl_2{=}CH{-}$ (e)

The intermediates IIa and IIb, obtained by this method, have been reported[6]; IIb and IId have been prepared using ethanolic sodium ethoxide.[7]

In Table II are listed the properties of new intermediates.

All of the condensation products obtained here were converted to the amino acids by hydrolysis with dilute acid. The mixed isomers of IIIc have been obtained[8] from the hydrolysis of the corresponding acetamidomalonate compound.

## Discussion

Although both hydrobromic and hydrochloric acids could be made to add to the acetylenic bond of propargylglycine by prolonged refluxing in aqueous solution, it was more convenient to prepare the amino acids from the appropriate condensation product. Similar attempts to add concentrated aqueous hydriodic acid were unsuccessful. However, a glacial acetic acid solution of hydrogen iodide was able to accomplish the desired conversion to γ-iodoallylglycine in 58% yield. γ,ω-Dichloroallylglycine was prepared in 48% yield in a similar manner using a solution of chlorine in glacial acetic acid. When the same reaction was attempted with bromine, extensive decomposition resulted. As a consequence, the reaction was performed with bromine in ethanol containing sufficient hydrobromic acid to effect solution of the amino acid to give a 67% yield of γ,ω-dibromoallylglycine. In these three cases, the halide required to prepare the condensation product was not readily available.

The preparation of 1,3-dibromopropene presented certain problems. The dehydration of dibromohydrin with phosphorus oxychloride[9] yielded a product too impure to permit the synthesis of an amino acid of unambiguous composition. A number of modifications of the reaction between acrolein and phosphorus pentabromide[10] were performed until it was found that the cautious dropwise addition of two moles of freshly distilled acrolein to a mixture of one mole of phosphorus tribromide and one-half mole of phosphorus pentabromide, while maintaining the temperature at 60°, gave a material which could be converted to 1,3-dibromopropene. Heating of the reaction product led to a copious evolution of hydrogen bromide and the spontaneous distillation of 1,3-dibromopropene. Refractionation gave a 30% yield of a *cis–trans* mixture.[11] Shortly after this method had been developed, Hatch and Harwell[12] reported a more straightfor-

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(3) National Science Foundation Fellow, 1953–1954.

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(5) The synthesis of this compound was reported by H. Gershon, Doctoral Dissertation, University of Colorado, 1950.

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(11) The authors wish to thank Dr. L. F. Hatch for examining our infrared spectrum and for providing unpublished data of the infrared spectra of the separated isomers.

(12) L. F. Hatch and K. E. Harwell, THIS JOURNAL, **75**, 6002 (1953).

TABLE I
AMINO ACIDS

| Substituted 2-amino-4-pentenoic acid | Yield, % | M.p., °C. (anal. sample) | Formula | Calculated, % C | H | N | X | Found, % C | H | N | X |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 4-Chloro | 50 | 226–226.5 | $C_5H_8NO_2Cl$ | 40.15 | 5.39 | 9.37 | 23.71 | 40.38 | 5.09 | .. | 23.55 |
| 4-Bromo | 70 | 215.5–217 | $C_5H_8NO_2Br$ | 30.95 | 4.16 | 7.22 | 41.19 | 30.98 | 4.04 | .. | ... |
| 4-Iodo | 58 | 213–214 | $C_5H_8NO_2I$ | 24.91 | 3.34 | 5.81 | 52.66 | 25.15 | 3.35 | 5.77 | 52.25 |
| *cis*-5-Chloro | 94 | 226–231 | $C_5H_8NO_2Cl$ | 40.15 | 5.39 | 9.37 | 23.71 | 40.01 | 5.49 | .. | ... |
| *trans*-5-Chloro | 91 | 230–234 | $C_5H_8NO_2Cl$ | 40.15 | 5.39 | 9.37 | 23.71 | 40.49 | 5.67 | .. | ... |
| 5-Bromo | 70 | 234–235.5 | $C_5H_8NO_2Br$ | 30.95 | 4.16 | 7.22 | 41.19 | 31.00 | 4.28 | .. | .. |
| 5,5-Dichloro | 76 | 201–202 | $C_5H_7NO_2Cl_2$ | 32.63 | 3.83 | 7.61 | 38.54 | 32.83 | 3.84 | 7.32 | 38.40 |
| 4,5-Dichloro | 48 | 209–210 | $C_5H_7NO_2Cl_2$ | 32.63 | 3.83 | 7.61 | 38.54 | 32.76 | 4.14 | 7.25 | 37.87 |
| 4,5-Dibromo | 67 | 222–224 | $C_5H_7NO_2Br_2$ | 22.00 | 2.59 | 5.13 | 58.56 | 22.48 | 2.55 | 4.86 | 58.53 |

TABLE II
CONDENSATION PRODUCTS

| Substituted diethyl formamidomalonate | Crude yield, % | M.p., °C. (Anal. sample) | Formula | Calculated, % C | H | Found, % C | H |
|---|---|---|---|---|---|---|---|
| *cis*-1-Chloroallyl | 84 | 79–80 | $C_{11}H_{16}NO_5Cl$ | 47.57 | 5.81 | 47.90 | 5.96 |
| *trans*-1-Chloroallyl | 66 | 84–85 | $C_{11}H_{16}NO_5Cl$ | 47.57 | 5.81 | 47.52 | 5.63 |
| 1-Bromoallyl | 95 | 69.5–70 | $C_{11}H_{16}NO_5Br$ | 41.01 | 5.01 | 41.29 | 5.23 |
| 1,1-Dichloroallyl | 83 | 86–87 | $C_{11}H_{15}NO_5Cl_2$ | 42.32 | 4.84 | 42.35 | 4.95 |

ward preparation and method for the separation of the isomers.

When the mixed isomers of 1,3-dibromopropene were condensed with I using sodium hydride in toluene or a dimethylformamide solution of the sodium salt,[6] usually a product with a much lower melting point than reported in Table I was obtained. This melting point was variable and apparently dependent upon the isomeric composition. In one fortuitous case, a pure *cis* isomer (m.p. 70°) was obtained. This condensation product could not be made to undergo further reaction with I under a variety of reaction conditions.[13]

All of the condensation products could be converted to the corresponding amino acid in good yield by relatively dilute acid hydrolysis. This is in contrast to the higher acid concentrations and longer reflux periods required with acetamidomalonate derivatives which, in the case of some amino acids, leads to lowered yields. In general, hydrolysis was accomplished by refluxing the condensation product in the appropriate 15% mineral acid for about four hours. The progress of the reaction could be followed by the evolution of carbon dioxide which usually ended in about three hours. The amino acid was recovered from the hydrolysis mixture either by use of an ion exchange resin or by precipitation from an alcohol solution of the hydrohalide salt with pyridine or ammonium hydroxide.

## Experimental[14]

**3-Bromo-1,1-dichloro-1-propene.**—A solution of 33.1 g. (0.30 mole) of freshly distilled 1,1-dichloropropene, b.p. 76.8–77.8°, in 250 ml. of dry carbon tetrachloride was brought to a slow reflux and to this was added about three-fourths of an intimate mixture of 62.4 g. (0.35 mole) of N-bromosuccinimide and 1.5 g. of freshly crystallized benzoyl peroxide. After refluxing for 30 minutes, the remainder of the solid was added and the refluxing was continued for an additional 3.5 hours. The reaction mixture was then cooled in an ice-bath and filtered. The resulting solution was fractionated through a 12-inch helix-packed column to give 46.3 g. (82% yield) of product boiling at 74.3–75.5° (40 mm.). This was refractionated to give pure 3-bromo-1,1-dichloro-1-propene as a pale yellow liquid, b.p. 74.8° (40 mm.), $n^{24.7}D$ 1.5351, $d^{14.5}_4$ 1.687.

**2-Amino-4-iodo-4-pentenoic Acid.**—A fuming solution of hydriodic acid in glacial acetic acid was prepared by mixing slowly with cooling stoichiometric amounts of acetic anhydride and freshly distilled constant boiling (57%) hydriodic acid. A mixture of 166 g. of this solution (0.2 mole of hydriodic acid) and 11.3 g. (0.1 mole) of 2-amino-4-pentynoic acid was heated to 60° for 30 minutes, heated to 100° briefly and then allowed to cool. The excess hydriodic acid was removed from the resulting black solution by evaporation to dryness *in vacuo*, and the very dark material was taken up in water. This was then added to an amount of Amberlite IR-4B resin in the basic form sufficient to neutralize the solution. Almost all of the color of the solution disappeared during this treatment. The resin was washed free of amino acid and the washings, after filtration, were concentrated to a volume of 100 ml. The solution was warmed to 60°, and an equal volume of ethanol was added. After standing at −15°, the product was filtered, washed with ethanol and then ether, and allowed to air dry. It gave an intense green Beilstein test for halogen and weighed 14.0 g. (58% yield). An analytical sample was obtained after two recrystallizations from 50% ethanol. A freshly prepared sample was pure white initially but slowly became yellow on exposure to light.

**2-Amino-4,5-dichloro-4-pentenoic Acid.**—A mixture of 100 g. of glacial acetic acid and 2.0 ml. of acetic anhydride was cooled in an ice-bath, and chlorine was bubbled through it until the weight had increased by 7.1 g. (0.1 mole). To the cool solution was added 11.3 g. of 2-amino-4-pentynoic acid, and the mixture was allowed to warm to room temperature with constant vigorous stirring. An exothermic reaction occurred which necessitated cooling to prevent the loss of chlorine. After two hours stirring at room temperature, the mixture was reduced to a small volume *in vacuo*, neutralized with a small amount of concentrated ammonium

(13) These data cannot be reconciled with the results obtained by Capkova-Jirku and co-workers.[7] They report that condensation of one mole of I with 1,3-dibromopropene in ethanolic sodium ethoxide gave IId (m.p. 50°) which would further react with I to give the disubstituted propene (m.p. 80°). It is unfortunate that these previous workers did not report their method of synthesis of 1,3-dibromopropene nor obtain a complete elemental analysis of their products. It is perhaps significant that they were unable to convert any of their condensation products to the corresponding amino acid. The condensation product reported here, the amino acid derived from it, and the benzoyl derivative of this amino acid all gave the analysis for carbon and hydrogen as calculated on the basis of a monoalkylated bromopropene, and infrared analysis of the high melting material and the same derivatives indicated that they were all almost pure *cis* isomers.

(14) All melting and boiling points are corrected. Melting points of amino acids were determined in sealed capillary tubes in a Hershberg apparatus with a rapid temperature rise to within 10° of the melting point. We wish to thank the General Aniline and Film Co. for generous samples of 3-bromopropyne and the Shell Development Co. for 2,3-dichloropropene and 1,3-dichloropropene.

TABLE III

BENZOYL DERIVATIVES OF AMINO ACIDS

| Substituted N-benzoyl-2-amino-4-pentenoic acid | M.p., °C. | Formula | Calculated, % C | Calculated, % H | Calculated, % X | Found, % C | Found, % H | Found, % X |
|---|---|---|---|---|---|---|---|---|
| 4-Chloro | 126.5–127.5 | $C_{12}H_{12}NO_3Cl$ | 56.81 | 4.77 | 13.98 | 57.15 | 4.56 | 14.00 |
| 4-Bromo | 141–142 | $C_{12}H_{12}NO_3Br$ | 48.34 | 4.06 | 26.81 | 48.64 | 4.21 | 27.16 |
| 4-Iodo | 144–146 | $C_{12}H_{12}NO_3I$ | 41.76 | 3.51 | 36.77 | 42.03 | 3.92 | 37.34 |
| *cis*-5-Chloro | 169–170 | $C_{12}H_{12}NO_3Cl$ | 56.81 | 4.77 | 13.98 | 56.92 | 4.85 | ... |
| *trans*-5-Chloro | 173.5–175 | $C_{12}H_{12}NO_3Cl$ | 56.81 | 4.77 | 13.98 | 56.96 | 4.82 | ... |
| 5-Bromo | 166–169 | $C_{12}H_{12}NO_3Br$ | 48.33 | 4.06 | 26.81 | 48.73 | 4.32 | ... |
| 4,5-Dibromo | 163–164 | $C_{12}H_{11}NO_3Br_2$ | 38.22 | 2.94 | 42.39 | 38.46 | 2.80 | 42.22 |
| 4,5-Dichloro | 159.5–160.5 | $C_{12}H_{11}NO_3Cl_2$ | 50.02 | 3.85 | 24.61 | 49.82 | 3.72 | ... |
| 5,5-Dichloro | 172.5–173.5 | $C_{12}H_{11}NO_3Cl_2$ | 50.02 | 3.85 | 24.61 | 50.17 | 3.81 | 24.79 |

hydroxide, and treated with an equal volume of ethanol. After standing at −15° overnight, the product was filtered to give 8.8 g. (48% yield) of light colorless flakes. A small sample was recrystallized three times from dilute ethanol to give the analytical sample.

**2-Amino-4,5-dibromo-4-pentenoic Acid.**—A solution of 20.0 g. of 2-amino-4-pentynoic acid in ethanol was prepared by warming a suspension of the amino acid in absolute ethanol and adding the minimum amount of concentrated hydrobromic acid. To this solution was added 16.0 g. of bromine, and then the mixture was warmed, with stirring, for two hours. After this period, the solution was almost colorless. It was then neutralized with concentrated ammonium hydroxide, at which time a considerable amount of darkening occurred. An equal volume of acetone was added and the solution was held at −15°. The precipitate which formed was filtered to give 12.2 g. of nearly white product. The mother liquors yielded 6.0 g. of slightly less pure product. These crops represent a 67% yield. An analytical sample was obtained after three recrystallizations from dilute ethanol.

**Preparation of Benzoyl Derivatives of the Amino Acids.**—A modification of the Schotten–Baumann reaction was used. One equivalent of the amino acid was dissolved in a small amount of water containing three equivalents of sodium hydroxide. This was cooled to 0°, and 1.5 equivalents of benzoyl chloride was added dropwise, with vigorous stirring. After stirring for an additional 10 minutes, the mixture was treated dropwise while still cold with concentrated hydrochloric acid until it was acidic to congo red test paper. The mixture was filtered and the solid dried. This solid was suspended in a small amount of hot benzene and filtered. Ether could not be used since the products were too soluble in this solvent. The residue was washed with small amounts of hot benzene, dried, and crystallized twice from ethanol–water to give the analytical samples. The melting points and analyses of these derivatives are shown in Table III.

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# The Enthalpy Change in the Hydrolysis of Creatine Phosphate

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The enthalpy change on hydrolysis of creatine phosphate has been measured calorimetrically. The hydrolysis was carried out at *p*H 8.0 and 25° by the combined action of the enzymes ATP[1]-creatine transphosphorylase (in the presence of ADP) and myosin and led to the result $\Delta H = -9.0 \pm 0.5$ kcal. per mole. This quantity was found to be essentially independent of ionic strength.

In view of the importance of creatine phosphate in the chemical events associated with muscle action, considerable interest attaches to the thermodynamics of its hydrolysis under physiological conditions. Previous determinations of the heat of hydrolysis of CrP involve considerable uncertainty, either because the reaction was carried out in complex muscle extracts of unknown composition[2] or because they depend on measurements at *p*H values far removed from neutrality.[3]

In the present work, the heat of hydrolysis of CrP is measured directly at *p*H 8.0 and 25°. The hydrolysis is carried out, in the presence of ADP, by combined use of the enzymes ATP-Cr transphosphorylase and myosin ATPase. The transphosphorylase catalyzes the reaction

$$CrP + ADP + H^+ \rightleftarrows Cr + ATP \qquad (1)$$

the equilibrium constant of which is close to unity at *p*H 8.0. The basis for writing reaction 1 with a hydrogen ion on the left side will be presented later. Myosin catalyzes the reaction

$$ATP + H_2O \rightleftarrows ADP + HPO_4^= + H^+ \qquad (2)$$

which proceeds essentially to completion at *p*H 8.0. Thus the over-all reaction

$$CrP + H_2O \rightleftarrows Cr + HPO_4^= \qquad (3)$$

is also carried essentially to completion. This fact, coupled with the fact that the reactions are sufficiently rapid so that practically complete hydrolysis could be achieved within a period suitable for direct calorimetry, simplified the analytical determination of the extent of reaction at the end of the calorimetric experiment.

## Experimental

Five-hour extracted myosin B was prepared by the method of Botts and Morales.[4] ATP-Cr transphosphorylase[5] and

(1) The following abbreviations are used: AMP, ADP, ATP, adenosine mono-, di- and triphosphate, respectively; Cr, creatine; CrP, creatine phosphate; tris, tris(hydroxymethyl)-aminomethane.

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