[CONTRIBUTION FROM THE CHEMICAL INSTITUTE, FACULTY OF SCIENCE, UNIVERSITY OF ZAGREB]

CONTRIBUTION TO THE KNOWLEDGE OF γ-AMINOCROTONIC ACID. VINYLOGS OF α-AMINO ACIDS. I

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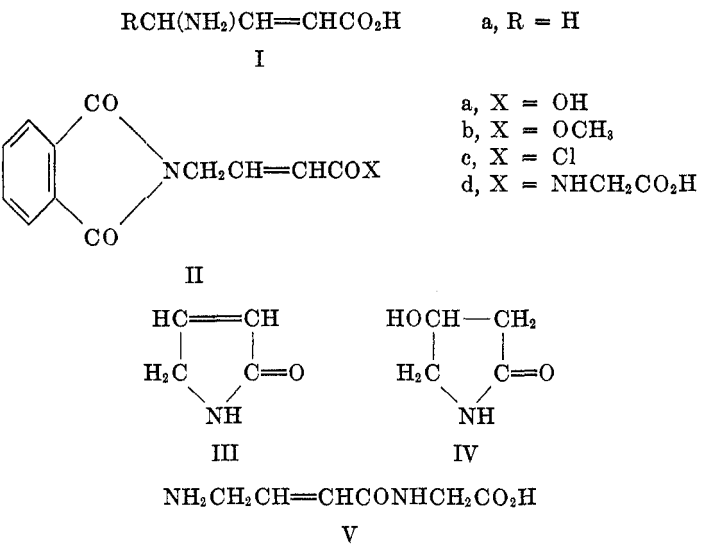
It is possible that the vinylogs of α-amino acids of the formula I, according to the principle of vinylogy (1), could prove to be interesting compounds, *inter alia*, as substrates for certain enzymes.



We have therefore attempted the synthesis of γ-aminocrotonic acid (Ia), the vinylog of glycine. A similar attempt also was made recently by Langenbeck and Boser (2) with the object of comparing certain catalytic reactions of γ-aminocrotonic acid with those of glycine. Langenbeck and Boser, however, found it impossible to repeat Rambaud's procedure (3) for the preparation of the picrate of γ-aminocrotonic acid. They therefore carried out the condensation of γ-bromocrotonic acid methyl ester with potassium phthalimide, obtaining γ-phthalimidocrotonic acid methyl ester (IIb), from which compound the phthaloyl group was split off with hydrazine hydrate. From the reaction mixture only the lactam of γ-aminocrotonic acid (III) could be isolated.

Our preparation of γ-aminocrotonic acid was carried out *via* the condensation of phthalimidoacetaldehyde with malonic acid in pyridine following Doebner's procedure (4), and γ-phthalimidocrotonic acid (IIa) was thus obtained. It could be separated by fractional crystallization into two isomers, with the melting points 179–180° and 218° respectively. Both these isomers were converted to γ-aminobutyric acid by catalytic hydrogenation. Fission of the phthaloyl group with hydrochloric acid gave no γ-aminocrotonic acid, but in repeated preparations, the only product obtained was γ-amino-β-hydroxybutyric acid, earlier de-

scribed by Bergmann, Brand, and Weinmann (5) and by Tomita (6). The behavior of γ-phthalimidocrotonic acid on hydrolysis with mineral acids is evidently analogous to the behavior of crotonic acid; under similar conditions this compound gave β-hydroxybutyric acid (7). Hydrazinolysis of γ-phthalimidocrotonic acid in boiling ethanol yielded only oily products. Closer examination of the conditions of hydrazinolysis showed that the procedure could be also successfully carried out at room temperature by shaking a suspension of γ-phthalimidocrotonic acid in ethanol with an equimolar quantity of hydrazine hydrate during 8 to 10 days. From the hydrolyzate obtained in this manner, white prisms of γ-aminocrotonic acid, m.p. 164° (decomp.) were isolated in a poor yield. This compound gave a yellow color with ninhydrin and the paper chromatogram showed a yellow spot, $R_f = 0.64$.

Heating of γ-aminocrotonic acid with a ninhydrin solution does not result in the evolution of carbon dioxide, under the conditions developed by Van Slyke and co-workers (8) for the determination of free α-amino acids by the ninhydrin-carbon dioxide reaction. Hydrogenation of γ-aminocrotonic acid over platinum oxide catalyst required one mole of hydrogen and yielded γ-aminobutyric acid.

From the chloride of γ-phthalimidocrotonic acid (IIc) and glycine, we prepared γ-phthalimidocrotonylglycine (IId). Hydrazinolysis of this compound according to Sheehan's method (9) gave the crystalline γ-aminocrotonylglycine (V), the vinylog of glycylglycine. Hydrogenation of this dipeptide over platinum oxide catalyst gave γ-aminobutyrylglycine.

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EXPERIMENTAL

All melting points are uncorrected unless otherwise stated.

Amberlite IR-4B (20–50 mesh) was used after treating with an aqueous 5% sodium carbonate solution and washing with redistilled water.

γ-Phthalimidocrotonic acid (IIa). A mixture of phthalimidoacetaldehyde, prepared according to Radde (12) (19 g., 0.1 mole), malonic acid (10.4 g., 0.1 mole), and pyridine (10 ml.) was heated under reflux for three hours at 90–100°. After cooling, 10% sulfuric acid was added (50 ml.), and the mixture was left at room temperature overnight. The crystals which had separated were filtered off, washed with diluted sulfuric acid and water, and dried. The crude γ-phthalimidocrotonic acid (23 g., 100% showed m.p. 130–135°. Repeated recrystallization from ethanol yielded 7 g. (30%) of white needles, m.p. 179–180°. γ-Phthalimidocrotonic acid could be sublimed at 150–160°/0.03 mm.

Anal. Calc'd for $C_{12}H_9NO_4$ (231.20): C, 62.34; H, 3.92.

Found: C, 62.10; H, 3.93.

The ethanolic mother liquors left over from the purification of γ-phthalimidocrotonic acid were evaporated to dryness and the residue was recrystallized from glacial acetic acid. White crystals of the same empirical formula as γ-phthalimidocrotonic acid, but of m.p. 218° were obtained. The same substance could also be obtained if γ-phthalimidocrotonic acid of m.p. 175° was repeatedly recrystallized from glacial acetic acid.

Anal. Found: C, 62.71; H, 3.83.

γ-Phthalimidobutyric acid. Hydrogenation over Adams' PtO_2 catalyst (30 mg.) was carried out with a solution of γ-phthalimidocrotonic acid (m.p. 179°, 0.23 g., 1 millimole) in ethanol (40 ml.) at atmospheric pressure and 20°. In three hours 0.9 millimole of hydrogen

was absorbed. The catalyst was filtered off, and from the filtrate white platelets of γ-phthalimidobutyric acid (0.22 g.) were obtained, which after repeated recrystallization from benzene had m.p. 117.5°. [Gabriel and Colman (11) reported m.p. 117–118°.]

Anal. Calc'd for $C_{12}H_{11}NO_4$ (233.22): C, 61.80; H, 4.75.

Found: C, 61.98; H, 4.83.

Hydrogenation of γ-phthalimidocrotonicutyric acid of m.p. 218° gave, under the same conditions, also γ-phthalimidobutyric acid, with m.p. 117.5°.

Anal. Found: C, 62.24; H, 4.96.

γ-Amino-β-hydroxybutyric acid. Hydrolysis of γ-phthalimidocrotonic acid with hydrochloric acid. γ-Phthalimidocrotonic acid (m.p. 179°, 2.95 g., 0.0125 mole) was refluxed for 10 hours with concentrated hydrochloric acid (40 ml.). After cooling, crystals of phthalic acid separated (1.7 g., 79%). The filtrate was extracted with ether, the solvent evaporated under reduced pressure, and γ-amino-β-hydroxybutyric acid hydrochloride (1.80 g., 89%) remained. This residue was dissolved in 1100 ml. of distilled water and passed through a column containing Amberlite IR-4B (15 g.) at a flow rate of 750–800 ml./hr. The free acid thus obtained was repeatedly recrystallized from water-ethanol (1:10) and white platelets of pure γ-amino-β-hydroxybutyric acid (0.6 g., 39%) were obtained, m.p. 213° (corr.). [Tomita (6) reported m.p. 214°.]

Anal. Calc'd for $C_4H_9NO_3$ (119.12): C, 40.33; H, 7.62.

Found: C, 40.62; H, 7.67.

This acid shows a marked ninhydrin reaction (10). The R_f value of γ-amino-β-hydroxybutyric acid is 0.54, with phenol-water as mobile phase, and at 18° (mean of six determinations). Whatman No. 1 paper was used.

γ-Benzamido-β-hydroxybutyric acid was prepared for identification purposes from γ-amino-β-hydroxybutyric acid. γ-Benzamido-β-hydroxybutyric acid had m.p. 176°. [Tomita and Sendju (10) reported m.p. 176°.]

Anal. Calc'd for $C_{11}H_{13}NO_4$ (223.22): C, 59.18; H, 5.87.

Found: C, 59.14; H, 5.81.

The lactam of γ-amino-β-hydroxybutyric acid was also prepared for identification purposes. Distillation of γ-amino-β-hydroxybutyric acid at 130°/0.03 mm. gave the lactam which, when recrystallized from ethanol, had m.p. 122–123°. [Tomita (6) reported m.p. 118°.]

Anal. Calc'd for $C_4H_7NO_2$ (101.10): C, 47.52; H, 6.98.

Found: C, 47.62; H, 6.89.

γ-Aminocrotonic acid (Ia). A mixture of γ-phthalimidocrotonic acid (m.p. 179°, 13.87 g., 0.06 mole) and of an equimolar quantity of a molar solution of hydrazine hydrate in ethanol (64 ml.) was kept at room temperature for a week, with stirring. The ethanol then was removed *in vacuo*, the dry residue suspended in water (175 ml.), the suspension brought to *p*H 5.5 with glacial acetic acid, and the mixture shaken for three hours at room temperature. The precipitated phthalyl hydrazide (7.5 g., 77% of the theoretical) was filtered off and the aqueous filtrate was evaporated to dryness under reduced pressure. An oily residue remained, (8.0 g.) which was dissolved in water (18 ml.), and after addition of ethanol (50 ml.), 2.2 g. of oily precipitate separated. The mother liquor was treated with ethanol (100 ml.) and a further 1.4 g. of oily precipitate was obtained. A repeated precipitation from the mother liquor from ethanol (250 ml.) yielded 0.44 g. (7.3%) of γ-aminocrotonic acid in the form of colorless prisms with m.p. 164° (decomp.). In a series of preparations the best yields were not above 10%. All of these precipitations and crystallizations were carried out at 0°. For analysis the substance was recrystallized from water-ethanol (1:5), m.p. unchanged.

Anal. Calc'd for $C_4H_7NO_2$ (101.10): C, 47.52; H, 6.98; N, 13.85.

Found: C, 47.52; H, 6.92; N, 14.01.

An aqueous solution of γ-aminocrotonic acid gave a yellow color with an aqueous solution of ninhydrin. On heating, the color became a deeper yellow, then purple, and finally violet.

Chromatography of γ-aminocrotonic acid on Whatman No. 1 paper, at 19°, using phenol-

water as mobile phase, after developing with a ninhydrin solution gave a spot of an intense yellow color, which became violet after standing; $R_f = 0.64$ (mean of four determinations). A violet spot, $R_f = 0.89$ was also present on the chromatogram of the crude hydrolyzate, and belongs to a hitherto unidentified compound.

Hydrogenation of γ-aminocrotonic acid (50 mg.) was carried out over Adams' PtO_2 catalyst (40 mg.) and 0.9 mole of hydrogen was absorbed. Paper chromatography of the hydrogenated product under the same conditions as described above gave only the spot of γ-aminobutyric acid, $R_f = 0.83$.

With 50 mg. of γ-aminocrotonic acid, the Van Slyke determination of free α-amino acid by the ninhydrin-carbon dioxide reaction (8) was carried out. However, the same CO_2-content was found as in the blank analysis.

If the hydrazinolysis of γ-phthalimidocrotonic acid was carried out in boiling ethanol an oily reaction product was obtained, the chromatogram of which showed two spots: a weak yellow one, $R_f = 0.64$ (corresponding to γ-aminocrotonic acid) and a violet one, $R_f = 0.89$ which belongs to the above mentioned unidentified compound.

γ-Phthalimidocrotonyl chloride. γ-Phthalimidocrotonic acid (m.p. 179°, 2.31 g., 0.01 mole) was dissolved in thionyl chloride (40 ml.) at 40–50°, and left at room temperature for six hours. The excess thionyl chloride was removed under reduced pressure, and the crude crystalline γ-phthalimidocrotonyl chloride, m.p. 112–115°, was recrystallized from benzene-petroleum ether to the constant m.p. 122–123°. The colorless needles (1.5 g., 60%) were sublimed at 110°/0.02 mm.

Anal. Calc'd for $C_{12}H_8ClNO_3$ (249.65): C, 57.73; H, 3.23.
Found: C, 58.04; H, 3.26.

γ-Phthalimidocrotonylglycine was prepared according to the procedure described by Sheehan and Frank (9) for the preparation of peptides. To a suspension of glycine (0.45 g., 0.006 mole) and magnesium oxide (0.36 g.) in water (20 ml.), a solution of γ-phthalimidocrotonyl chloride (m.p. 115–117°, 1.5 g., 0.006 mole) in dioxane (8 ml.) was added dropwise and with stirring during a half hour, at 5°. Stirring was continued for ten minutes at room temperature, and the reaction mixture adjusted to *p*H 5 with concentrated hydrochloric acid. After a few minutes, separation of the dipeptide began. The mixture was left at room temperature overnight and the white precipitate of γ-phthalimidocrotonylglycine was filtered off and recrystallized repeatedly from ethanol. White needles of the pure compound were obtained, (0.79 g., 46%), m.p. 220–221°.

Anal. Calc'd for $C_{14}H_{12}N_2O_5$ (288.25): C, 58.33; H, 4.20.
Found: C, 58.37; H, 4.21.

γ-Aminocrotonylglycine (V). To a solution of γ-phthalimidocrotonylglycine (0.58 g., 0.002 mole) in ethanol (20 ml.) an equimolar quantity of a molar ethanolic hydrazine hydrate solution was added, and the mixture was refluxed for two hours. After evaporating the reaction mixture to dryness under reduced pressure, 2 *N* hydrochloric acid (5 ml.) was added. After standing for a few hours, phthalyl hydrazide separated (0.275 g., 84.4%), was filtered off, washed with water, and discarded. The combined filtrate and washings were evaporated to dryness *in vacuo*, and the crystalline residue was dissolved in distilled water (160 ml.) and passed through a column containing Amberlite IR-4B (4 g.) at a flow rate of 200 ml./hr. After the column was washed with water (100 ml.), the combined filtrate and washings were evaporated to dryness under reduced pressure, in an atmosphere of nitrogen. The crude γ-aminocrotonylglycine remained (0.29 g., 93%), m.p. 198–199°. Repeated recrystallization from water-ethanol (1:3) yielded 0.13 g. (40%) of colorless prisms, m.p. 218° (decomp.). Paper chromatography, using phenol-water as mobile phase, on Whatman paper No. 1, and at 19°, after developing with ninhydrin, showed that γ-aminocrotonylglycine has $R_f = 0.66$, the spot being at first yellow, then violet. An aqueous solution of γ-aminocrotonylglycine gives a strong reaction with ninhydrin. The color was first yellow, then brown and on further heating, deep purple.

Anal. Calc'd for $C_6H_{10}N_2O_3$ (158.16): C, 45.56; H, 6.37; N, 17.72.
Found: C, 45.44; H, 6.29; N, 17.47.

γ-Phthalimidobutyrylglycine. Hydrogenation over Adams' PtO_2 catalyst (90 mg.) was carried out with a suspension of γ-phthalimidocrotonylglycine (0.6 g., 0.002 mole) in ethanol (30 ml.) at atmospheric pressure and 20°. After absorption of an equimolar quantity of hydrogen, the catalyst was filtered off, and the filtrate was evaporated to dryness under reduced pressure. γ-Phthalimidobutyrylglycine remained (0.56 g., 95%). Repeated recrystallization from ethanol gave colorless needles, m.p. 178°.

Anal. Calc'd for $C_{14}H_{14}N_2O_5$ (290.27): C, 57.93; H, 4.86.

Found: C, 58.08; H, 4.89.

γ-Aminobutyrylglycine. A solution of γ-aminocrotonylglycine (m.p. 217.5°, 0.12 g., 0.75 millimole) in water (8 ml.) was hydrogenated over Adams' PtO_2 catalyst (30 mg.). After an equimolar quantity of hydrogen was absorbed, the catalyst was filtered off, and the filtrate was evaporated to dryness under reduced pressure. The crystalline crude γ-aminobutyrylglycine (100 mg., 83.3%) remained, m.p. 209-211°, which after recrystallization from water-ethanol (1:5) had m.p. 214° (colorless prisms), yield 50 mg., 41%. An aqueous solution of γ-aminobutyrylglycine gave a pale violet color with ninhydrin, which deepened on heating.

Anal. Calc'd for $C_6H_{12}N_2O_3$ (160.17): C, 44.99; H, 7.55.

Found: C, 44.99; H, 7.48.

The paper chromatogram of γ-aminobutyrylglycine on Whatman paper No. 1, at 19°, using phenol-water as mobile phase, showed a violet spot, $R_f = 0.84$.

SUMMARY

1. The preparation of pure γ-aminocrotonic acid, which was obtained in poor yields by hydrazinolysis of γ-phthalimidocrotonic acid at room temperature, is described.

2. Hydrolysis of γ-phthalimidocrotonic acid with concentrated hydrochloric acid, in the usual manner, yielded γ-amino-β-hydroxybutyric acid.

3. Using standard methods, γ-aminocrotonylglycine was prepared, the vinylog of glycylglycine.

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