phenyl). Further support for the pyrimidine structure was furnished by running the reaction with acetonitrile in place of benzonitrile to give a known pyrimidine (see next section).

5-Cyano-4,6-diamino-2-methylpyrimidine.[18] A heavy-walled polymer tube (25 mm. × 21 cm.) was charged with 2.0 g. (18.5 mmoles) of 1,1-diamino-2,2-dicyanoethylene, 5.0 g. (120 mmoles) of acetonitrile, and 0.29 g. (3.7 mmoles) of piperidine. The mixture was frozen, the air was replaced by nitrogen, and the tube was sealed. It was heated in a Carius furnace at 180–190° for 1 hr. The tube was then cooled and opened and its contents removed with the aid of a few ml. of absolute alcohol. The pyrimidine, obtained as pale yellow crystals, was separated by filtration and air-dried; wt. 1.65 g. (60%); m.p. 397–398° on a block.[19] Recrystallization from acetic acid gave a colorless product of unchanged melting point. The infrared spectrum is similar to that of the 2-phenyl analog.

Anal. Calcd. for $C_6H_7N_5$: C, 48.3; H, 4.7; N, 47.0 Found: C, 48.4; H, 4.8; N, 45.7.

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[18] Dr. J. R. Roland did this experiment.

[19] Z. Budesinsky and J. Kopecky [*Collection Czechoslov. Communs.*, 20, 52 (1955)], who prepared this pyrimidine by a 3-step synthesis, report m.p. > 360°.

[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, UNIVERSITY OF FLORIDA COLLEGE OF MEDICINE, AND THE DEPARTMENT OF CHEMISTRY, OHIO STATE UNIVERSITY]

Synthesis and Properties of Isoserine. A Novel Bromhydrination Method

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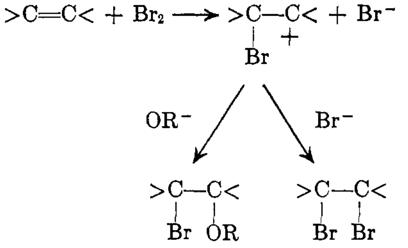
The bromination of methyl acrylate was carried out in the presence of silver nitrate, which prevented formation of methyl dibromopropionate as a byproduct. Isoserine was synthesized by hydrolysis of the product of amination of methyl bromohydroxypropionate. The copper-catalyzed oxidative deamination of isoserine yielded tartronic acid semialdehyde; under these conditions, hydroxypyruvic acid was produced from serine. Other chemical properties of isoserine are described.

Isoserine inhibits several enzymes of serine metabolism in mammals[1] and interferes with pantothenic acid synthesis from β-alanine in yeast.[2] The compound was first synthesized by Melikoff[3] by treatment of α-chloro-β-hydroxypropionic acid with aqueous ammonia. The apparent rearrangement of the hydroxyl group was explained by postulating that glycidic acid is the intermediate, as amination of glycidic and α-hydroxy-β-chloropropionic acids also led to the same product. Mattocks and Hartung,[4] however, obtained the methyl ester of *N*-benzylserine by treatment of methyl α-bromo-β-hydroxypropionate with anhydrous benzylamine. Since the base strengths of benzylamine and ammonia are similar,[5] it was of interest to study the reaction of the same ester with anhydrous ammonia.

When the preparation of methyl α-bromo-β-hydroxypropionate by treatment of methyl acrylate with alkaline bromine water[4] was attempted, a large proportion of the product proved to be methyl α,β-dibromopropionate. Bartlett and Tarbell[6] concluded from kinetic studies that the reaction of olefins with bromine in alcohols occurs with the following mechanism:



Although the predominant product early in the reaction is the bromoalkoxy adduct, more of the dibromo derivative is formed as the first reaction proceeds and bromide ion concentration is increased. Bromide ion also reacts with the halogen molecule to form the tribromide ion, thus diminishing the bromine concentration and slowing the first reaction.

If the reaction of olefins with halogens in water is similar to that in alcohols, the addition of silver nitrate to precipitate the bromide ions as formed should greatly increase the proportion of bromhydrin among the products. It was found that when methyl acrylate was treated with bromine in an aqueous silver nitrate solution in the cold, a yield of over 80% of crude bromhydrin was afforded. Distillation of the product at reduced pressure caused great losses by decomposition, but a small amount of methyl bromohydroxypropionate was obtained.

Treatment of the bromohydroxy ester with aqueous ammonium hydroxide (analogous to Meli-

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[1] (a) S. K. Fellner and K. C. Leibman, *Federation Proc.*, 19, 9 (1960); (b) K. C. Leibman, *Federation Proc.*, 20, 235 (1961).

[2] N. Nielson and G. Johansen, *Naturwiss.*, 31, 235 (1943).

[3] P. Melikoff, *Ber.*, 13, 1265 (1880).

[4] A. M. Mattocks and W. H. Hartung, *J. Biol. Chem.*, 165, 501 (1946).

[5] pK_b in water at 25°: ammonia, 4.74; benzylamine, 4.70. Calculated from data in *Internat. Critical Tables*, 6, 281 (1929); 7, 240 (1930).

[6] P. D. Bartlett and D. S. Tarbell, *J. Am. Chem. Soc.*, 58, 466 (1936).

koff's procedure[3]), with anhydrous ammonia in dry ether (analogous to the Mattocks and Hartung synthesis[4]), or with liquid anhydrous ammonia, produced the ester of isoserine in all cases. After hydrolysis, isoserine was identified by elemental analysis, melting points, diphenylurea derivative, and copper contents of the copper salts (Fischer and Leuchs[7] showed that while the copper salt of serine contains one atom of copper per two serinate residues, that of isoserine contains one organic residue per copper atom, as the copper forms a salt with both the carboxyl and α-hydroxyl groups). Almost one molar equivalent of formaldehyde was produced from isoserine on oxidation with periodate. Isoserine gave the same color as did serine in the colorimetric ninhydrin procedure of Troll and Cannan.[8]

Traube and Schönewald[9] demonstrated the copper-catalyzed oxidative deamination of alkylamines and glycine. Evidence is presented here that under these conditions isoserine is oxidized to tartronic acid semialdehyde and serine to hydroxypyruvic acid. These products were identified by the formation of derivatives and by specific enzymic and colorimetric reactions.

The alum-catalyzed reaction of serine with pyridoxal yields pyruvic acid.[10] Alanine, but not β-alanine, also transaminates with pyridoxal.[11] Under similar conditions, isoserine did not react with pyridoxal to form an oxo acid, nor did it interfere with the formation of pyruvic acid from serine.

EXPERIMENTAL

Reaction of methyl acrylate with alkaline bromine water. The method of Mattocks and Hartung[4] was followed, starting with 43.5 g. (0.5 mole) of methyl acrylate. After neutralization, the reaction mixture was allowed to stand for 36 hr. at room temperature, after which time it had separated into two layers. The upper aqueous layer was extracted with one liter of ether in four portions. The collected ether extracts were dried over anhydrous calcium sulfate, filtered, and evaporated. A yellow-brown liquid remained, identified as crude methyl bromohydroxypropionate, weighing 27.4 g. (30%).

Anal. Calcd. for $C_4H_7O_3Br$: Br, 43.7. Found: Br, 38.7.

The water-insoluble layer was dissolved in ether, dried over anhydrous calcium sulfate, and the solvent was removed under reduced pressure. The residue (46 g., 37%) was distilled at 57–60°/2.5 mm., and again at 196–201° at atmospheric pressure. The recorded boiling point of methyl α,β-dibromopropionate is 203–206°.[12]

Anal. Calcd. for $C_4H_6O_2Br_2$: Br, 65.2. Found: Br, 65.8.

Reaction of methyl acrylate with aqueous bromine in the presence of silver nitrate. The vapors of 25.7 ml. (80.0 g., 0.5 mole) of bromine, diluted with air, were passed through a mixture of 85.0 g. (0.5 mole) of silver nitrate, 43.5 g. (0.5 mole) of freshly-distilled methyl acrylate, and one liter of distilled water with stirring. The temperature was maintained between −2° and 0°, and the rate of flow of vapors was adjusted so that the reaction mixture was only very slightly yellow. After all the bromine was consumed, the entire reaction mixture, including the yellow precipitate, was shaken with ether and filtered. The precipitate was washed twice more with ether, and the filtrate extracted several times. The precipitate was dried to constant weight, and 91.8 g. (0.49 mole) of silver bromide was recovered. The combined ether extracts were dried over anhydrous calcium sulfate, filtered, and the ether removed under reduced pressure. A clear amber liquid remained, weighing 74.4 g. (81.3%).

Anal. Calcd. for $C_4H_7O_3Br$: Br, 43.7. Found: Br, 39.8.

A portion of the crude product was shaken with aqueous sodium bisulfite, extracted with ether, dried over anhydrous calcium sulfate, filtered, and the solvent removed under reduced pressure. The residue was distilled; a small amount of pale yellow distillate, which darkened upon standing, was collected at 88–90°/4.5 mm., while a large proportion of tar remained in the stillpot.

Anal. Calcd. for $C_4H_7O_3Br$: C, 26.2; H, 3.83; Br, 43.7; saponification equivalent, 91.5. Found: C, 25.4; H, 3.85; Br, 43.0; saponification equivalent, 90.7.

Isoserine. A. Amination with ammonium hydroxide. In a sealed pressure bottle, 74 g. (0.4 mole) of the crude bromohydrin ester was heated with 400 ml. of ammonium hydroxide (sp. gr. 0.90) at 80° for 12 hr. The mixture was then evaporated to a thick syrup under reduced pressure. To this was added 42 g. (1.0 mole) of lithium hydroxide monohydrate and enough water to make the final concentration of LiOH about 3 *M*. The mixture was refluxed for 48 hr., neutralized with hydrochloric acid, filtered, treated with decolorizing charcoal, and filtered hot. Absolute ethanol was then added until a dense turbidity developed, after which the mixture was allowed to stand overnight and then filtered. Lithium chloride is quite soluble in alcohol. The precipitated isoserine was purified by dissolving in a minimum quantity of water, reprecipitating with ethanol, filtering, washing with ethanol and ether, and drying in air. The yield, enlarged by recovering more isoserine from the mother liquors after concentration, was 16.5 g. (39%) m.p., 247.5° with decomposition after three recrystallizations (lit., 248°[7]).

The copper salt was prepared by adding an excess of freshly-precipitated, well-washed cupric hydroxide to a solution of isoserine, boiling for 10 min., filtering, concentrating to a small volume, and allowing to stand for several days. The deep blue needles were filtered and dried at 110° for 2 days.

Anal. Calcd. for isoserine salt: Cu, 38.2; for serine salt: 23.4. Found: Cu, 39.1.

Diphenylurea derivative: m.p., 181.5–182.5° (lit., 181–184°[7,13]).

B. Amination with anhydrous liquid ammonia. A mixture of 36.6 g. (0.2 mole) of the crude bromhydrin ester and approximately 50 ml. (2 moles) of anhydrous liquid ammonia were heated in a Carius tube at 70° for 66 hr. After slow evaporation of ammonia, hydrolysis and precipitation were carried out as before. The yield was 6.4 g. (30%); m.p. 247° dec. after two recrystallizations; mixture with product of *A*, m.p. 246–247° dec.; mixture with DL-serine, m.p. 219° dec.

Anal. Calcd. for $C_3H_7O_3N$: C, 34.3; H, 6.65; N, 13.3. Found: C, 34.1; H, 6.29; N, 13.1.

C. Amination with anhydrous ammonia in ether. Anhydrous ammonia was bubbled slowly through a solution of 36.6 g. (0.2 mole) of the crude bromohydrin ester in 1 liter of dry ether for 9 hr. at 0° with stirring in a vessel protected from

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(7) E. Fischer and H. Leuchs, *Ber.*, **35**, 3787 (1902).

(8) W. Troll and R. K. Cannan, *J. Biol. Chem.*, **200**, 803 (1953).

(9) W. Traube and A. Schönewald, *Ber.*, **39**, 178 (1906).

(10) D. E. Metzler and E. E. Snell, *J. Biol. Chem.*, **198**, 353 (1952).

(11) E. E. Snell, *J. Am. Chem. Soc.*, **67**, 194 (1945).

(12) (a) G. Münder and B. Tollens, *Ann.*, **167**, 222 (1873); (b) F. Weger, *Ann.*, **221**, 61 (1883); (c) P. E. Verkade, *Rec. trav. chim.*, **41**, 208 (1922); (d) A. V. Ipatov, *J. Gen. Chem. (U. S. S. R.)*, **15**, 187 (1945); *Chem. Abstr.*, **40**, 1783 (1946).

(13) C. Neuberg and P. Mayer, *Biochem. Z.*, **3**, 116 (1907).

moisture by a drying tube filled with anhydrous calcium sulfate. The reaction mixture was stirred further for 20 hr., and then allowed to stand overnight. After removing solvent under reduced pressure, hydrolysis and precipitation were performed as above. A small amount of white crystals was obtained which melted at 246.5–247.0° dec.; mixture with product from *B*, m.p. 247.0–247.5° dec.

Periodate oxidations. Oxidations of isoserine and of serine were performed as described by Frisell *et al.*[14] A solution of isoserine containing 525 μg. (5.0 μmoles) produced 4.6 μmoles of formaldehyde (92%). Under the same conditions, a 96% yield of formaldehyde was obtained from DL-serine (Mann Research Labs., chromatographically pure).

Ninhydrin reaction. When measured as described by Troll and Cannan,[8] isoserine showed a molar extinction of 2.45×10^4, whereas in the case of serine, the molar extinction was 2.41×10^4, both at 570 mμ.

Oxidation of isoserine to tartronic acid semialdehyde. To a mixture of 5.25 g. (50 mmoles) of isoserine, 2.0 g. (50 mmoles) of sodium hydroxide, and 100 ml. of distilled water was added 10 g. of electrolytic copper powder in a 500-ml. Erlenmeyer flask equipped with two flexible tubes. The flask was flushed with oxygen, after which the outlet was closed and the inlet tube connected to a vessel filled with oxygen over a hydraulic trough. The flask was shaken gently for 24 hr. The reaction mixture was filtered, and the deep blue supernatant liquid (pH = 11) was shaken with batches of about 100 g. of Dowex-50W-X8 resin (H form) and filtered. This was repeated until the solution was free of copper (ferrocyanide test), at which point the pH was 1.7. The solution was then titrated with a saturated solution of barium hydroxide in freshly-boiled distilled water to the first persistent pink color of phenolphthalein. The precipitate was allowed to settle, and the supernatant fluid was carefully drawn off and treated with absolute ethanol. The combined precipitates were filtered with suction, and washed successively on the filter with absolute ethanol and ether. After drying in a stream of air, the cream-colored powder weighed 2 g. (18%).

Anal. Calcd. for $C_3H_2O_4Ba$: Ba, 57.4. Found: Ba, 57.2.

In a similar preparation, the reaction mixture, after treatment with Dowex-50W, was titrated to pH 6 with 4*M* lithium hydroxide, and the resulting solution was frozen, lyophilized, and the dry powder stored over calcium chloride at −15°.

Tartronic acid semialdehyde phenylosazone, prepared as described by Fischer *et al.*,[15] melted at 215° (lit., 209°,[15] 218°[16]). Mesoxalic acid semialdehyde bisphenylhydrazone, which is the same compound, was prepared from mesoxalic acid semialdehyde, which had been synthesized as reported by Fenton[17]; m.p., 214°; mixture with tartronic acid semialdehyde phenylosazone, m.p. 214°.

Glyoxal bisphenylhydrazone was prepared from tartronic acid semialdehyde by the method of Fischer *et al.*[15]; m.p., 166°. Mixture with bisphenylhydrazone prepared from glyoxal, m.p. 166°. Under the same conditions, mesoxalic acid semialdehyde was not decarboxylated, and the derivative of m.p. 214° was obtained.

Other reactions of tartronic acid semialdehyde. The compound gave positive reactions in Tollens' and Pinoff's tests, as reported by Fischer *ct al.*[15] It did not react in the naphtharesorcinol colorimetric method for hydroxypyruvate,[18a] nor did it oxidize reduced diphosphopyridine nucleotide in the presence of hydroxypyruvate reductase.[18] In the colorimetric method for keto acids of Friedemann and Haugen,[19] tartronic acid semialdehyde showed a weak reaction (E_{520} for 1 μmole of barium salt was 0.1).

Oxidation of serine to hydroxypyruvic acid. In a manner similar to that described for isoserine, 5.15 g. of DL-serine in 100 ml. of water containing 196 mg. of sodium hydroxide was shaken in oxygen overnight with 15 g. of copper powder. The reaction mixture, after filtration, was freed of copper by treatment with Dowex-50W resin, titrated with 1*M* lithium hydroxide to pH 7.8, treated with decolorizing charcoal in the cold, filtered, frozen, and lyophilized. An off-white powder (1.6 g.) was obtained. Assay of the product by the enzymic method of Dickens and Williamson[18a] showed the lithium hydroxypyruvate content to be 24%. The naphtharesorcinol test[18a] was positive.

Transamination reactions. A mixture of 20 μmoles of amino acid, 5 μmoles of ammonium aluminum sulfate, and 20 μmoles of pyridoxal hydrochloride in 5 ml. of 0.1*M* sodium acetate buffer solution, pH 4.7, were heated in a boiling water bath for 3 hr. Pyruvic acid was determined as described by Metzler and Snell.[20] The yield of pyruvic acid from serine was 13.0 μmoles; no keto acid, as measured in this test, was produced from isoserine. When a mixture of 20 μmoles each of serine and isoserine was treated in the same manner, 12.8 μmoles of pyruvic acid was obtained.

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