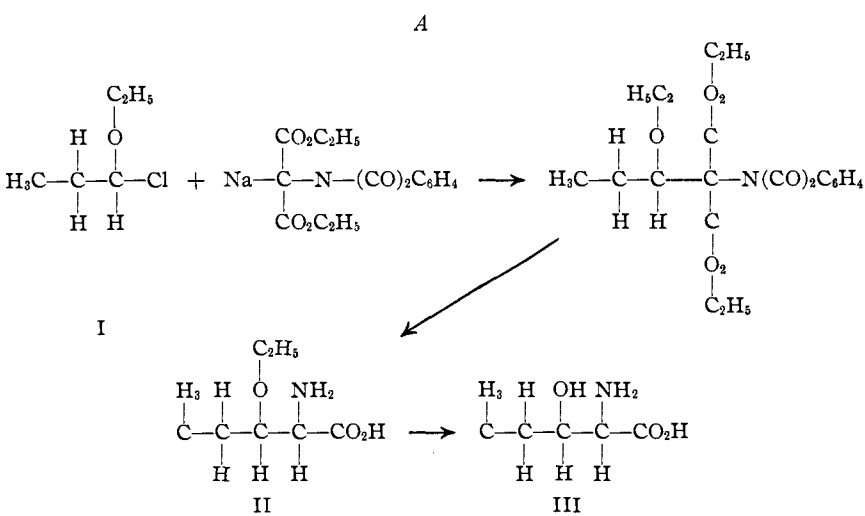
[CONTRIBUTION FROM THE SECTION ON BIOCHEMISTRY OF THE MAYO FOUNDATION]

THE SYNTHESIS OF TWO HYDROXYLAMINOVALERIC ACIDS, α-AMINO-β-HYDROXY- AND γ-AMINO-β-HYDROXYVALERIC ACIDS

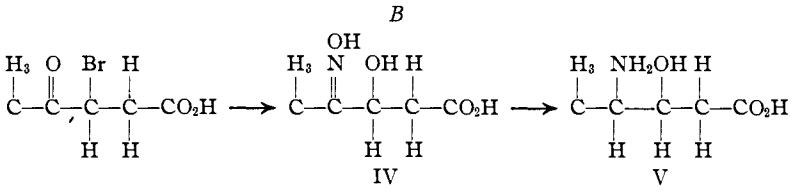
BY ARNOLD E. OSTERBERG

RECEIVED NOVEMBER 17, 1926 PUBLISHED FEBRUARY 5, 1927

The isolation of a crystalline substance by Eddy, Kerr and Williams[1] having the properties of Wilder's bios and which was believed to have the empirical formula $C_5H_{11}NO_3$, led me to determine whether or not certain hydroxylaminovaleric acids having this empirical formula would possess properties identical with those of the crystalline substance isolated from yeast. Of the hydroxylaminovaleric acids known, α-amino-δ-hydroxyvaleric acid has a melting point identical with that given by Eddy for bios, namely, 223°. Synthesis of this substance and its incorporation in yeast mediums did not affect yeast growth and gave negative results. In the search for other valeric acid derivatives having the same empirical formula, and which I thought might possibly have some catalytic effect on yeast growth, I have synthesized two new hydroxylaminovaleric acids. These are α-amino-β-hydroxy- and γ-amino-β-hydroxyvaleric acids. Neither of these agrees in physical and chemical properties with "bios" isolated from yeast and they are inert as regards their effect on yeast growth. These acids have been synthesized according to the following scheme.



[1] Eddy, Kerr and Williams, THIS JOURNAL, **46**, 2846 (1924).



Experimental Part

α-Chloropropyl-ethyl Ether, I.—This compound may be made in a manner similar to the general method used for the preparation of halogenated mixed ethers.[2]

To an ice-cold mixture of 100 cc. of propionaldehyde and 200 cc. of absolute ethyl alcohol is added dry hydrogen chloride gas until the solution is saturated. This saturation is completed as rapidly as possible, since the solution becomes very dark on standing any length of time. The reaction mixture separates into two layers. The top layer is removed and fractionated under diminished pressure; b. p., 57–60°, at 18 mm.; yield, 80%. This fraction is about 93% pure α-chloropropyl-ethyl ether and is satisfactory for further condensation reactions.

α-Amino-β-ethoxyvaleric Acid, II.—Forty-seven hundredths mole of α-chloropropyl-ethyl ether is added to a benzene suspension of 0.4 mole of sodium phthalimidomalonic ester immersed in ice water. This mixture is allowed to stand over a period of 12 hours with frequent shaking. At the end of this period the reaction is practically complete. The benzene solution is then washed with water several times to remove sodium chloride; it is then dried with calcium chloride and the benzene removed in a vacuum. The residue is dissolved in 500 cc. of 95% alcohol containing 160 cc. of 10 *N* sodium hydroxide. The saponification evolves considerable heat. To insure completion of hydrolysis the solution is boiled until most of the alcohol is off. Three hundred cc. of concd. hydrochloric acid is added. Considerable carbon dioxide is evolved at this point, indicating that decarboxylation of the malonic ester residue has taken place. The solution is then evaporated to dryness on a steam-bath. The residue is ground with concd. hydrochloric acid and filtered, the precipitate being washed thoroughly with concd. hydrochloric acid. The filtrate is concentrated to dryness and the residue is dissolved in water. To the aqueous solution is added 200 g. of lead monoxide and the mixture heated on a steam-bath for one hour. A precipitate consisting of an excess of lead oxide and lead chloride is then filtered off and the chlorides remaining in solution are removed by treatment with silver oxide. The silver and lead present in the filtrate are removed with hydrogen sulfide. The sulfide is filtered off and the filtrate concentrated to dryness. The residue, which consists of α-amino-β-ethoxyvaleric acid and some glycine, is dissolved in 200 cc. of hot, 80% ethyl alcohol, cooled and allowed to stand several hours. The crystals which separate are then filtered off. This product still contains a small amount of glycine. The pure α-amino-β-ethoxyvaleric acid is obtained by evaporating the filtrate to dryness and taking up the residue in absolute ethyl alcohol; yield, 13.5 g.; m. p., 227°.

Anal. Calcd. for $C_7H_{15}NO_3$: C, 52.13; H, 9.37; N, 8.69. Found: C, 51.65; H, 9.20; N, 8.70.

α-Amino-β-hydroxyvaleric Acid, III.—This may be obtained from the ethoxy derivative by refluxing with concd. hydrobromic acid.

[2] Litterscheid and Thimme, *Ann.*, **334**, 49 (1904). Wedekind, *Ber.*, **36**, 1383 (1903).

Twenty g. of α-amino-β-ethoxyvaleric acid is refluxed for one hour with 200 cc. of concd. hydrobromic acid. The excess of hydrobromic acid is removed by concentrating in a vacuum and the residue is dissolved in water. The remaining halogen is removed by treatment with silver oxide and the silver removed by hydrogen sulfide. The free amino acid is obtained by concentrating the filtrate and dissolving the residue in warm, 80% ethyl alcohol. On cooling, a small amount of glycine separates. The filtrate is concentrated and the residue treated with hot, 95% alcohol; yield of α-amino-β-hydroxyvaleric acid, 7.5 g.; m. p., 220°.

Anal. Calcd. for $C_5H_{11}NO_3$: C, 45.07; H, 8.33; N, 10.52. Found: C, 44.66; H, 8.10; N, 10.40.

β-Hydroxylevulinic Acid Oxime, IV.—This is prepared with only slight modification according to the method of Wolff.[3]

An aqueous solution containing 0.32 mole of β-bromolevulinic acid is treated at 65° with 0.32 mole of an aqueous solution of sodium carbonate until the bromides are quantitatively removed. To this solution is then added 0.32 mole of hydroxylamine hydrochloride which has been neutralized with sodium carbonate. This mixture is allowed to stand for three days. It is then concentrated to dryness, 32 cc. of 10 *N* sulfuric acid is added and the product extracted with ether. The crystals, which separate after removal of the ether, are filtered off and washed with ether; yield of oxime, 10 g.; m. p., 145°.

β-Hydroxy-γ-aminovaleric Acid, V.—Eight and five-tenths g. of β-hydroxylevulinic acid oxime is reduced in 200 cc. of 50% alcohol with an excess of sodium amalgam. The temperature is maintained below 20° by immersion in cold water and vigorous stirring. Twenty-three g. of sodium (five times the calculated amount) made into a 2.5% amalgam is used. The solution is maintained acid by frequent additions of glacial acetic acid; in all, 55 cc. is used. When the reduction is complete, 100 cc. of 10 *N* sulfuric acid is added and the resulting sodium sulfate is filtered off. The filtrate is then concentrated to dryness. Absolute ethyl alcohol is added to the residue and the product allowed to stand several hours. The remaining sodium sulfate which separates is then filtered off. The filtrate is again concentrated to dryness in a vacuum and the residue dissolved in 25 cc. of 95% alcohol. The crystalline amino acid is obtained from this solution by adding approximately 150 cc. of absolute alcohol, in which it is insoluble; yield, 2.5 g.; m. p., 188°.

Anal. Calcd. for $C_5H_{11}NO_3$: C, 45.07; H, 8.33; N, 10.52. Found: C, 44.89; H, 8.18; N, 10.36.

Summary

α-Amino-β-hydroxy- and γ-amino-β-hydroxyvaleric acids have been prepared. These acids, as well as the other known hydroxylaminovaleric acids, do not possess the properties of "bios."

ROCHESTER, MINNESOTA

[3] Wolff, *Ann.*, **264**, 229 (1891).