Acknowledgment.—We are indebted to Mr. B. A. Krukoff for the task of obtaining the plant materials, for the determinations of plants and for his suggestions and advice on botanical matters. We wish to express our appreciation to Dr. Ralph E. Heal for the bioassays. The coöperation of Messrs. Douglass Hayman, R. N. Boos and associates on microanalyses was very helpful.

Summary

An insecticidal alkaloid, ryanodine, tentatively formulated as $C_{25}H_{35}NO_9$ or $C_{26}H_{31}NO_9$, has been isolated from root and stem material of *Ryania speciosa* Vahl.

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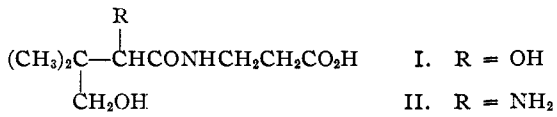
[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF MERCK & CO., INC.]

An Amino Analog of Pantothenic Acid

BY FREDERICK W. HOLLY, RODERICK A. BARNES,[1a] FRANK R. KONIUSZY AND KARL FOLKERS

An amino analog of pantothenic acid, as an ethyl ester, has been synthesized. This analog, ethyl DL-β'-(α-amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate, showed no vitamin activity when assayed for pantothenic acid activity for *Lactobacillus arabinosus* or when tested in pantothenic acid deficient rats. In the presence of pantothenic acid, however, the analog had vitamin activity for *Lactobacillus arabinosus*.

Of the many analogs of pantothenic acid (I) which have been prepared,[1] the amino analog, β'-(α-amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionic acid (II), has not been included.

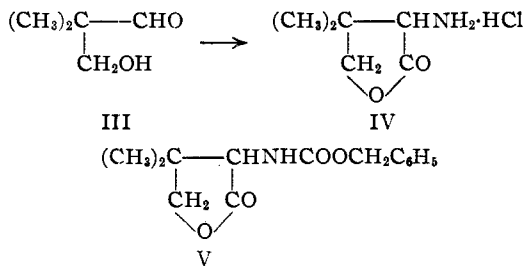


I. R = OH

II. R = NH_2

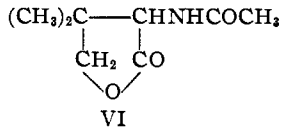
It was of interest to synthesize and test biologically this amino analog, because the compound possesses structural relationship to the naturally occurring α-amino acids.

α,α-Dimethyl-β-hydroxypropionaldehyde[2] (III) was converted into DL-α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride[2a] (IV) by a modified Strecker reaction.[3]



III IV

V



VI

The N-carbobenzoxy derivative of the amino lactone (V) was prepared by reaction of the amino lactone hydrochloride with carbobenzoxy chloride in diethylaniline–benzene solution. The N-acetyl derivative of the amino lactone (VI) was obtained by reaction of the amino lactone hydrochloride with acetic anhydride in pyridine solution, acetyl chloride in benzene solution, or with acetyl chloride in benzene–dioxane in the presence of barium carbonate.

Condensation of α-carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone with β-alanine ethyl ester yielded ethyl DL-β'-(α-carbobenzoxyamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate (VII), which was obtained as an oil. The carbobenzoxy group of the β-alanine derivative (VII) was readily removed by hydrogenolysis of the compound over a palladium–charcoal catalyst[4] in ethanol–hydrochloric acid solution. This reaction yielded the hydrochloride of the amino analog of pantothenic acid as an ethyl ester (VIII). Since this ethyl ester (VIII) was an oil, it was characterized further by reaction with acetic anhydride in pyridine to give a crystalline diacetyl derivative (IX). This diacetyl derivative (IX) had the same melting point and infrared absorption spectrum as another sample of the diacetyl derivative which had been synthesized by alternative reactions.

The alternative synthesis of the diacetyl derivative (IX) started with DL-α-acetylamino-β,β-dimethyl-γ-butyrolactone (VI). Reaction of this acetylamino lactone with β-alanine ethyl ester gave ethyl DL-β'-(α-acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate (X) and then acetylation of this compound gave ethyl DL-β'-(α-acetylamino-β,β-dimethyl-γ-acetoxybutyrylamino)-propionate (IX).

Dr. H. B. Woodruff of the Microbiological Department has kindly assayed the amino analog

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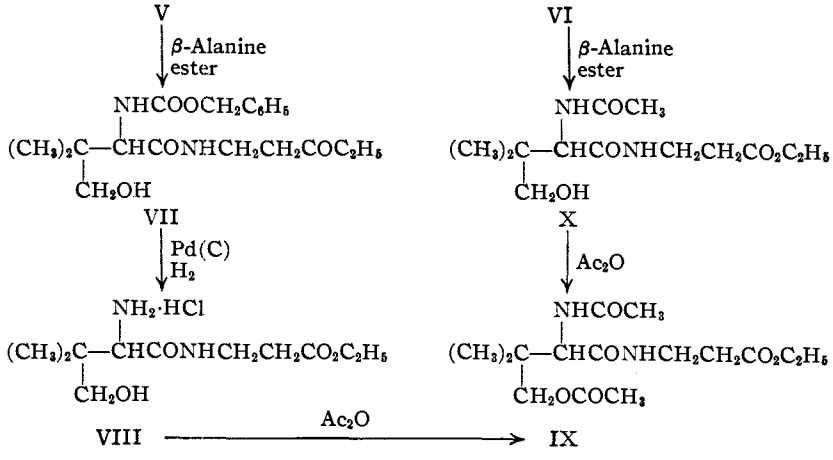
(1) Many references to the literature in this field have been cited by Cheldelin and Schink, THIS JOURNAL, **69**, 2625 (1947). See also McIlwain and Hughes, *Biochem. J.*, **39**, 133 (1945).

(2) Stiller, Harris, Finkelstein, Keresztesy and Folkers, THIS JOURNAL, **62**, 1785 (1940).

(2a) The acid corresponding to this lactone has now been reported by Ackermann and Kirby (*J. Biol. Chem.*, **175**, 483 (1948)) as possibly being a naturally occurring amino acid (note added in proof).

(3) Clarke and Bean, "Organic Syntheses," Coll. Vol. II, John Wiley & Sons, Inc., New York, N. Y., 1943, p. 29.

(4) Mozingo, "Organic Syntheses," Vol. XXVI, John Wiley & Sons, Inc., New York, N. Y., 1946, p. 78, procedure B.



(VIII) for pantothenic acid activity for *Lactobacillus arabinosus* and found that when an amount of pantothenic acid sufficient to yield one-half maximum growth was mixed with an equal quantity of the analog (0.02 microgram of each compound/10 ml. of broth), maximum growth resulted. The analog had less than 0.66% of the vitamin activity of an equal quantity of pantothenic acid when used as a substitute for it, and had no antagonistic action to the growth effect of pantothenic acid when added at ten times the weight of pantothenic acid which yields maximum cell growth.

We are indebted to Dr. Gladys Emerson and Miss Elizabeth Wurtz of the Merck Institute for Therapeutic Research for testing the ethyl ester of the amino analog (VIII) and the N-acetyl derivative (X) of this ester in pantothenic acid deficient rats. Both compounds showed no pantothenic acid activity when tested by the prophylactic method at a level equivalent to 1 mg. of calcium pantothenate per day, which is tenfold the optimal dose of pantothenic acid. Since ethyl pantothenate and its monoacetyl derivative in which the α-hydroxyl is acetylated were as active on a molar basis as pantothenic acid when tested in rats and chicks,[5] it is to be expected that the ethyl esters would be active in rats if the amino analog (II) and its N-acetyl derivative were active.

Experimental

DL-α-**Amino-β,β-dimethyl-γ-butyrolactone Hydrochloride.**—One hundred and seventy-five grams of α,α-dimethyl-β-hydroxypropionaldehyde[2] in 500 ml. of methanol was added with stirring during a period of one hour to a mixture of 120 g. of ammonium chloride, 105 g. of sodium cyanide, and 250 ml. of methanol. The mixture was refluxed for five hours and left at room temperature overnight.

One liter of absolute ether was added to the mixture and a precipitate was removed by filtration and washed with ether. To the filtrate, 750 ml. of concentrated hydrochloric acid and 400 ml. of water were added. The solution was distilled until the temperature of the vapors reached 105°. Two hundred milliliters of concentrated hydrochloric acid was added and the mixture was refluxed for six hours. The solution was then concentrated under reduced pressure to a small volume, and a precipitate was removed by filtration. Concentration of the filtrate gave a mixture of ammonium chloride and α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride. The mixture was extracted with absolute ethanol, and absolute ether was added to the alcoholic extract until it became turbid. The solution was allowed to stand overnight at room temperature, and the ammonium chloride was removed by filtration. After the filtrate was concentrated to about 400 ml., the amine hydrochloride was precipitated by the addition of 2 l. of ether. An oil separated which crystallized slowly to give 75 g. of tan product, m. p. 192–196°. A 2-g. sample was recrystallized three times from a mixture of alcohol and ether at room temperature to give 0.8 g. of α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride, m. p. 224–225°.

Anal. Calcd. for $C_6H_{12}NO_2Cl$: C, 43.51; H, 7.31; N, 8.46. Found: C, 43.71; H, 7.15; N, 8.67.

DL-α-**Carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone.**—To a suspension of 17 g. of α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride in 160 ml. of benzene, were added 29 g. of carbobenzoxy chloride (90%) and 50 ml. of diethylaniline. The mixture was heated on a steam-bath for thirty minutes, cooled to 30°, extracted with 200 ml. of 2.5 *N* hydrochloric acid and then with 100 ml. of water. After the benzene solution was dried over sodium sulfate for fifteen minutes, the mixture was filtered and 200 ml. of petroleum ether was added to the filtrate. A white crystalline product separated and the mixture was cooled in ice; the DL-α-carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone was collected on a filter, washed with petroleum ether, and dried *in vacuo*; yield, 15.3 g., m. p. 116–116.5° (micro-block).

Anal. Calcd. for $C_{14}H_{17}NO_4$: C, 63.86; H, 6.51; N, 5.32. Found: C, 64.27; H, 6.38; N, 5.49.

DL-α-**Acetylamino-β,β-dimethyl-γ-butyrolactone.**—To 1.65 g. of α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride in 10 ml. of pyridine was added 2 g. of acetic anhydride. The mixture became warm and was left without cooling for five minutes, and was then concentrated under reduced pressure. The residual mixture of oil and crystals was dissolved in 10 ml. of absolute alcohol at 70°. The solution was cooled and the crystalline product was collected on a funnel, washed with absolute alcohol, and dried under reduced pressure to give 1.13 g. of α-acetylamino-β,β-dimethyl-γ-butyrolactone, m. p. 164–165°. After a sample was recrystallized from absolute alcohol, it melted at 163.5–164.5°.

Anal. Calcd. for $C_8H_{13}NO_3$: C, 56.12; H, 7.65; N, 8.18. Found: C, 56.38; H, 7.73; N, 7.92.

Two alternative methods for preparing the acetylamino lactone were by treatment of α-amino-β,β-dimethyl-γ-butyrolactone hydrochloride with a solution of acetyl chloride in benzene, or with acetyl chloride and barium carbonate in a mixture of benzene and dioxane.

Ethyl DL-β′-(α-**Acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate.**—A mixture of 3.4 g. of α-acetylamino-β,β-dimethyl-γ-butyrolactone and 2.6 g. of ethyl β-aminopropionate was heated on a steam-bath for two and one-half hours. The crystalline solid which formed was dissolved in chloroform and washed with 1 *N* hydrochloric acid and then with water. Removal of the chloroform under reduced pressure gave a white crystalline residue. Recrystallization of the product from benzene–petroleum ether yielded 2.8 g. of ethyl DL-

(5) Unna and Mushett, *Am. J. Physiol.*, **135**, 267 (1942); Harris, Boyack and Folkers, THIS JOURNAL, **63**, 2662 (1941).

β′-(α-acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate, m. p. 106–112° (micro-block). A 1.6-g. sample was recrystallized twice from benzene; m. p. 113–114° (micro-block), yield 1 g.

Anal. Calcd. for $C_{13}H_{24}N_2O_5$: C, 54.15; H, 8.39; N, 9.72. Found: C, 54.49; H, 8.06; N, 9.63.

Ethyl DL-β′-(α-Acetylamino-β,β-dimethyl-γ-acetoxybutyrylamino)-propionate.—Two grams of ethyl DL-β′-(α-acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate was added to a solution of 15 ml. of acetic anhydride and 12 ml. of pyridine, and the mixture was heated on a steam-bath for five minutes. The solution was concentrated *in vacuo* to a crystalline residue which was recrystallized four times from a mixture of chloroform and ether to give 1 g. of ethyl DL-β′-(α-acetylamino-β,β-dimethyl-γ-acetoxybutyrylamino)-propionate, m. p. 127–128° (micro-block).

Anal. Calcd. for $C_{15}H_{26}N_2O_6$: C, 54.53; H, 7.93; N, 8.48; mol. wt., 330.38. Found: C, 54.52; H, 7.79; N, 8.91; mol. wt., 334 (ebullioscopic in benzene).

Ethyl DL-β′-(α-Carbobenzoxyamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate.—A mixture of 2.5 g. of ethyl β-aminopropionate and 3.8 g. of α-carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone was heated on a steam-bath for two and one-half hours. The oil which formed was dissolved in 25 ml. of benzene and the solution was extracted with two 20-ml. portions of 1 *N* hydrochloric acid, and then with two 20-ml. portions of water. The benzene solution was dried over potassium carbonate, filtered, and concentrated under reduced pressure. A sample of the oily product was heated at 70–90° under a pressure of 0.01 mm. for five minutes.

Anal. Calcd. for $C_{19}H_{28}N_2O_6$: N, 7.37. Found: N, 7.49.

Ethyl DL-β′-(α-Amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate Hydrochloride.—A solution of 7.5 g. (0.02 mole) of ethyl DL-β′-(α-carbobenzoxyamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate in 75 ml. of ethanol containing 2 ml. of concentrated hydrochloric acid was hydrogenated over 8 g. of a palladium–Darco catalyst[4] (5% palladium) for one and six-tenths hours, during which time 0.016 mole of hydrogen was absorbed. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to an oil. A 1.5-g. sample of the oil was heated for five minutes at 90° in a mixture of acetic anhydride and pyridine and concentrated under reduced pressure. The oily residue was dissolved in 50 ml. of chloroform and washed twice with 25 ml. of water. Evaporation of the chloroform yielded a crystalline product that was recrystallized from chloroform–ether; 0.9 g. of ethyl DL-β′-(α-acetylamino-β,β-dimethyl-γ-acetoxybutyrylamino)-propionate, m. p. 128–129° (micro-block). A mixture with the diacetyl derivative prepared from the N-acetyl derivative as described above, melted at 128–129° (micro-block). The region of the absorption spectrum between 9 μ and 13 μ was observed on the crystalline diacetyl derivatives mulled in petrolatum. In column A of Table I are the absorption bands for the diacetyl derivative obtained from the amine; in column B are the bands for that obtained from the N-acetyl derivative. Only strong bands are recorded.

TABLE I

INFRARED ABSORPTION DATA

A. μ	B. μ
12.89	12.88
12.31	12.29
11.66	11.67
11.31	11.31
10.78	10.77
9.65	9.61

Acknowledgment.—We are indebted to Mr. D. W. Hayman and Mr. R. N. Boos and their associates for the microanalyses, to Dr. J. B. Conn for the molecular weight determination, and to Dr. N. R. Trenner for the infrared absorption data.

Summary

DL-α-Amino-β,β-dimethyl-γ-butyrolactone hydrochloride, DL-α-acetylamino-β,β-dimethyl-γ-butyrolactone and DL-α-carbobenzoxyamino-β,β-dimethyl-γ-butyrolactone have been prepared. Condensation of the substituted lactones with β-alanine ethyl ester has yielded ethyl DL-β′-(α-acetylamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate and ethyl DL-β′-(α-carbobenzoxyamino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate. Ethyl DL-β′-(α-amino-β,β-dimethyl-γ-hydroxybutyrylamino)-propionate hydrochloride, an amino analog of ethyl pantothenate, has been prepared by hydrogenolysis of the N-carbobenzoxy derivative and characterized as the N,O-diacetyl derivative. The amino analog of pantothenic acid showed no pantothenic acid activity when assayed in rats, or when assayed for *Lactobacillus arabinosus*. In the presence of pantothenic acid, the analog has vitamin activity for *Lactobacillus arabinosus*.

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