moved by distillation at 25° under reduced pressure, and then 75 cc. of dry ether was added, the lead salts filtered off and washed thoroughly with anhydrous ether. The combined filtrate and washings were evaporated to dryness in vacuum at 25°. The residue (504 mg.) was an almost colorless oil which gave a strong positive test for an aldehyde with Schiff reagent.

The aldehyde (504 mg.) was dissolved in 15 cc. of absolute alcohol, and a solution of 2 g. silver nitrate (calcd. 1.78 g.) in 10 cc. of water added. During a half-hour a 0.5 *N* aqueous solution of 0.71 g. sodium hydroxide (calcd. 0.706 mg.) was added dropwise, with vigorous stirring. There was an immediate formation of a black precipitate of silver and after the final addition of alkali, the stirring was continued for three hours. The silver was removed by filtration and thoroughly washed with water. The filtrate, which was slightly alkaline and gave a negative Schiff test, was evaporated at 30° under reduced pressure to 10 cc. It was acidified to *p*H 2.3 with dilute sulfuric acid and continuously extracted with ether for sixteen hours. The ether extract was dried thoroughly with sodium sulfate and then evaporated to dryness. The residue was a pale yellow viscous oil which crystallized on standing, yield 350 mg. After recrystallization from ether–petroleum ether, it was obtained as colorless needles, m. p. 124–125°; mixed m. p. with an authentic specimen of hydroxypivalic acid, 124–125.5°.

Anal. Calcd. for $C_5H_{10}O_3$: C, 50.85; H, 8.5. Found: C, 50.78; H, 8.59.

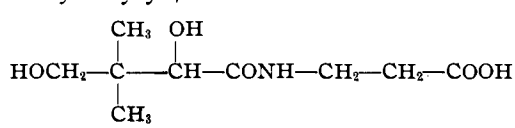
Acknowledgments.—The authors wish to express their great appreciation to Dr. R. J. Williams for making available to them much unpublished work and for much helpful advice. They further wish to acknowledge their appreciation for the help given by Messrs. E. F. Pratt and H. K. Mitchell during the summer of 1939. They also wish to express their indebtedness to Drs. R. T. Major and K. Folkers for their interest and advice, to Messrs. D. F. Hayman and W. Reiss, and H. S. Clark for carrying out the microanalyses and to Messrs. M. Kasha, H. Koones, E. Rickes and W. B. Wright for their assistance throughout the investigation.

Summary

1. Concentrates of pantothenic acid have been prepared, and from the hydrolyzates of these concentrates a crystalline lactone has been isolated.

2. The lactone has been characterized as the lactone of α-γ-dihydroxy-β,β-dimethylbutyric acid by degradative methods.

3. Pantothenic acid is (+) α,γ-dihydroxy-β,β-dimethylbutyryl-β′-alanide



RAHWAY, N. J. RECEIVED MAY 10, 1940

[CONTRIBUTION FROM THE CHEMICAL LABORATORIES OF OREGON STATE COLLEGE AND THE UNIVERSITY OF TEXAS]

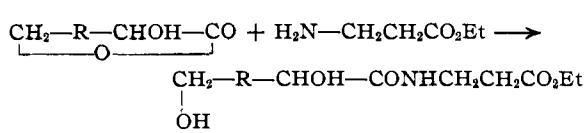
Pantothenic Acid. VII. Partial and Total Synthesis Studies

BY ROGER J. WILLIAMS, HERSCHEL K. MITCHELL, HARRY H. WEINSTOCK, JR., AND ESMOND E. SNELL

Partial Synthesis Experiments

The fact that a partial synthesis of pantothenic acid was first accomplished in June, 1938, has been alluded to.[1] Since subsequent to the identification of the lactone moiety[2] the method used in this partial synthesis served as a basis for a practical synthesis it is desirable to record the details of this earlier work.

Our previous finding that the non-nitrogenous cleavage product of pantothenic acid was a lactone[3] led us to attempt to produce the ester of pantothenic acid by the "ammonolysis" of this lactone (the exact structure of which was not known) as follows



Methods for hydrolyzing the ester of pantothenic acid without cleavage of the "peptide" linkage have previously been reported.[4] Such experiments were found to be successful and yields up to approximately 50% of theoretical were obtained, calculated on the basis of original pantothenic acid from which the impure lactone for the reaction was derived. An example is cited below.

Calcium pantothenate, 1 mg. of potency 6200, was heated at 100° for one-half hour with 1.1 ml. of 0.5 *N* hydrochloric acid. A 0.15-ml. portion equivalent to 850 milligram units of the original was removed and evaporated

(1) Williams, *Science*, **89**, 486 (1939).
(2) Stiller, *et al.*, THIS JOURNAL, **62**, 1785 (1940).
(3) Mitchell, *et al.*, *ibid.*, **62**, 1776 (1940).
(4) Williams, *et al.*, *ibid.* 454 (1939).

to dryness in a small centrifuge cone. Approximately 10 mg. of β-alanine methyl ester was added and the mixture was heated to 65–75° for thirty minutes. After hydrolyzing the ester linkage with 0.3 *N* sodium carbonate solution at 30° for ninety minutes, physiological tests using *Streptococcus lactis*[3] were performed. The results indicated a recovery of 370–420 units of activity or 43–49% resynthesis based upon the activity of the original material which was destroyed by cleavage.

It was also found at this time that yields up to about 15% resynthesis could be obtained by heating hydrolyzed pantothenic acid in alcoholic solution in a sealed tube with the sodium salt of β-alanine.

Total Synthesis Experiments

Subsequent to the identification of the lactone cleavage product of pantothenic acid[2] and the first complete synthesis of pantothenic acid using the lactone–β-alanine ester condensation[5] we have found an exceedingly simple and effective method for condensing the two portions of the molecule. This avoids both the use of the β-alanine esters which polymerize to solids on standing,[6] and the hydrolysis of the ester group after condensation. It yields directly the salt of pantothenic acid. A typical experiment is cited below:

The sodium salt of β-alanine was prepared by adding one equivalent of alkali to the free amino acid, evaporating to dryness, powdering in a mortar and drying over phosphorus pentoxide. α-Hydroxy-β,β-dimethyl-γ-butyrolactone, 3.27 g. dried over phosphorus pentoxide, was melted in a wide-mouthed test-tube and kept at 95–100° while 2.75 g. of the powdered β-alanine salt was added. The material was stirred intermittently and the heating continued for one hour after the two ingredients were well mixed. As heating proceeded the material became more and more viscous and on cooling set to a hard brittle mass of pure white material. Physiological tests with *Lactobacillus casei* ε showed the production of 37,670 gram units of pantothenic acid activity. The potency of the preparation (wt. 6.076 g.) is therefore 6200 compared with 12,100 for the synthetic dextrorotatory calcium pantothenate (Merck) assayed at the same time. Even allowing for possible slight activity of the levo antipode,[5] the material obtained in this manner appears to be essentially pure racemic sodium pantothenate. Being made from carefully dried reagents, it is probably drier than can be made by evaporating water from a moist salt of pantothenic acid.

(5) Stiller, *et al.*, THIS JOURNAL, **62**, 1785 (1940).
(6) Abderhalden, *Z. biol. Chem.*, **85**, 118 (1913).

Summary

1. Before the exact structure of the lactone portion was known, partial synthesis of pantothenic acid was accomplished by treating the impure lactone with β-alanine ester and subsequently hydrolyzing the ester group. The yields calculated on the basis of the original active compound were up to approximately 50%.

2. Subsequent to the identification of the lactone portion, a simple and effective method of synthesis of pantothenic acid was discovered. By heating the dry lactone with dry sodium salt of β-alanine, approximately a theoretical yield of sodium pantothenate was obtained directly.

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

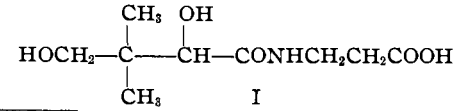
Pantothenic Acid. VIII. The Total Synthesis of Pure Pantothenic Acid

BY ERIC T. STILLER, STANTON A. HARRIS, JACOB FINKELSTEIN, JOHN C. KERESZTESY AND KARL FOLKERS

The resynthesis of pantothenic acid from the lactone fraction of the hydrolysates of pantothenic acid concentrates has previously been described by Williams and his collaborators[1] and also by Woolley, Waisman and Elvehjem.[2] The crude lactone fraction was recombined with the second moiety of the vitamin molecule, *i. e.*, β-alanine to give material which possessed the physiological activity of pantothenic acid.

It has been announced previously[3] that pure pantothenic has been synthesized. The present paper describes the details of this synthetic work.

In the preceding paper[4] it has been shown that pantothenic acid, I, consists of (+)α,γ-dihydroxy-β,β-dimethylbutyric acid joined with β-aminopropionic acid by means of an amide linkage.



I

(1) Mitchell, Weinstock, Snell and Williams, THIS JOURNAL, **62**, 1784 (1940). Williams, *Science*, **89**, 486 (1939). See also Snell, Strong and Peterson, *J. Bact.*, **38**, 293 (1939).
(2) Woolley, Waisman and Elvehjem, THIS JOURNAL, **61**, 977 (1939); *J. Biol. Chem.*, **129**, 673 (1939).
(3) Williams and Major, *Science*, **91**, 246 (1940).
(4) Stiller, Keresztesy and Finkelstein, THIS JOURNAL, **62**, 1779 (1940).