sis) from 10,500 to 11,500. Calculated as calcium salt this corresponds to a potency of about 12,500.

For the physiological tests the derivative was hydrolyzed to pantothenic acid by allowing to stand for one hour with 1 *N* alcoholic potassium hydroxide at room temperature.

Analyses of this material seemed to show definitely the presence of *one* rather than *two* acetyl groups. Acetic acid found: 23, 26, 26%. This was interpreted to mean that if two hydroxyl groups were present as indicated above, one of them must be unreactive under the acetylation conditions used.

Low yields, limited supplies of suitable material and unexpected decomposition during distillation as well as lack of time prevented extending this study to a satisfactory conclusion.

Discussion

Evidence has been presented for the formation of an α-hydroxylactone as a cleavage product of pantothenic acid. It was presumed to be a γ- or δ-lactone, and the former was indicated by the previously reported[11] condensation with acetone, acetaldehyde and benzaldehyde which would necessitate the formation of seven-membered rings in case the second hydroxyl was in the δ-position.

By the time the work reported in this paper had been completed the vitamin properties of pantothenic acid had been recognized,[21,22] and an increased interest in the compound was manifest. A co-operative arrangement was entered into whereby all of the material in this paper and other unpublished results were turned over to the Merck Research Laboratories, where the work was continued and the exact structure of the lactone determined.

We wish to express our keen appreciation to Professor J. W. E. Glattfeld of the University of Chicago for supplying us with generous samples of a number of hydroxy acids and lactones, to the Rockefeller Foundation for support of the research and to our collaborators in the Merck Research Laboratories, who have carried the work ahead to a successful identification of the lactone.

Summary

1. The presence of an α-hydroxyl group in the non-β-alanine portion of pantothenic acid has been shown.

2. This cleavage product is capable of spontaneous lactonization under acid conditions and is indicated to be an α-hydroxy-γ-lactone.

3. There are no adjacent hydroxyl groups in the pantothenic acid molecule and the absence of a β-hydroxy group in the lactone portion is indicated.

4. The groups CH_3CO— and CH_3CHOH— are not present in pantothenic acid.

5 The synthetic β-alanine derivatives of several α-hydroxy-γ-lactones: *i.e.*, α-hydroxy-γ-*n*-valero-lactone, α-hydroxy-β-methyl-γ-butyrolactone, and α-hydroxy-α-methyl-γ-butyrolactone, show definite but slight physiological activity.

6. A rapid method for the preparation of a concentrate containing about 20% pantothenic acid is described.

RECEIVED MAY 10, 1940

(21) Jukes, THIS JOURNAL, **61**, 975 (1939).

(22) Woolley, Waisman and Elvehjem, *ibid.*, **61**, 977 (1939).

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

Pantothenic Acid. VI. The Isolation and Structure of the Lactone Moiety

BY ERIC T. STILLER, JOHN C. KERESZTESY AND JACOB FINKELSTEIN

It has been reported by Williams and his coworkers[1,2,3] that β-alanine is an essential constituent of the molecule of pantothenic acid. They further showed that this amino acid was combined by means of an amide linkage with a dihydroxy acid which was capable of ready lactonization.

These findings were confirmed for the chick anti-dermatitis factor by Woolley, Waisman and Elvehjem,[4] thus identifying pantothenic acid with a member of the vitamin B complex. This latter fact was also established by Jukes.[5]

Pantothenic acid from natural sources has been found to be extremely difficult to purify and so far the natural vitamin has not been isolated in a state of purity. This lack of success is probably due to its hydrophilic nature and also to the lack of suitable precipitating reagents.

(1) Williams, Weinstock, Jr., and Mitchell, Abstracts, Division of Organic Chemistry, Amer. Chem. Soc., Milwaukee, Wis., 1938.

(2) Weinstock, Jr., Mitchell, Pratt and Williams, THIS JOURNAL, **61**, 1421 (1939).

(3) Mitchell, Weinstock, Jr., Snell, Stanbery and Williams, *ibid.*, **62**, 1776 (1940).

(4) Woolley, Waisman and Elvehjem, *J. Biol. Chem.*, **129**, 673 (1939); THIS JOURNAL, **61**, 977 (1939).

(5) Jukes, *ibid.*, **61**, 975 (1939).

In view of the fundamental findings of Williams and his co-workers[1,2,3] in regard to the two constituents of the pantothenic acid molecule it was unnecessary to isolate pure pantothenic acid in order to determine its structure, since when the structure of the hydroxy acid fragment of the vitamin molecule was elucidated the complete structure of pantothenic acid was concurrently determined.

After preliminary work in our laboratories on the isolation of the chick anti-dermatitis factor we were fortunate in the spring of 1939 in obtaining the collaboration of Dr. R. J. Williams, and details from him of his unpublished findings.

In a preliminary announcement[6] it was recorded that the non-nitrogenous portion from the pantothenic acid molecule had been isolated in crystalline form, from concentrates of pantothenic acid, and had been characterized as α-hydroxy-β,β-dimethyl-γ-butyrolactone. The present paper deals with the details of the isolation and characterization of this lactone.

Since a characteristic property of pantothenic acid is, that it yields on hydrolysis a hydroxy acid which readily lactonizes,[3,4] the initial object of our research was to obtain concentrates of pantothenic acid as free as possible from other hydroxy acids capable of lactonization.

In the past, in order to concentrate pantothenic acid or the chick anti-dermatitis factor, use has been made of adsorption on charcoal, extraction from acid solution with immiscible solvents, *e. g.*, ether and amyl alcohol, fractionation of alkaline earth and alkaloidal salts. By such means Williams, *et al.*,[7] were able to effect a very considerable concentration of this factor and obtained a product which contained approximately 90% of calcium pantothenate.

Saponification of such concentrates which Dr. R. J. Williams very kindly turned over to us, and similar ones prepared by similar means in our own laboratory, invariably gave a lactone fraction which would not crystallize. More recently Woolley[8] has described the isolation of a crystalline derivative of the non-nitrogenous portion of the vitamin molecule prepared from such concentrates.

A method of preparing concentrates from alcoholic liver extract was devised which would be free of interfering hydroxy acids capable of lactonization after saponification. Use was made of part of the procedure described in Part V[3], using various adsorptions and elutions from charcoal. These steps were successfully applied to large quantities of alcoholic liver extract. In some cases, however, alcoholic liver extracts were obtained which showed very great losses of pantothenic acid during the first alkaline Norit treatment. The concentrate of free acids obtained by these adsorptions and elutions was further fractionated with solvents (alcohol, acetone and ether) and finally precipitated as a mixture of barium salts.

By this means concentrates were obtained containing from 3–40% of pantothenic acid[9] which were uniformly satisfactory for the isolation of the lactone moiety from the vitamin molecule.

The concentrates were hydrolyzed with normal alkali and then acidified and heated in order to lactonize the hydroxy acids. After careful neutralization, the hydrolyzates were subjected to continuous ether extraction. On removal of the solvent, a crystalline solid was obtained in 55–60% of the theoretical yield.

The product was readily purified by molecular sublimation and crystallization from an ether–petroleum ether mixture. It melted at 91–92°, and showed $[\alpha]^{26}D$ −49.8°. Analysis and molecular weight determination by the freezing point method, using water as the solvent, indicated a formula $C_6H_{10}O_3$. The molecular weight determinations, using benzene as the solvent, gave increasing values, with increasing concentration of the solute. Anomalous molecular weights were also obtained by the Rast method.

Direct titration showed the absence of a free carboxyl group, but on heating one equivalent of alkali was consumed which indicated the presence of a lactone group. The stability of the lactone and the rate of lactonization of the free hydroxy acid suggested a γ- rather than a δ-lactone.

The substance possessed one active hydrogen atom and one hydroxyl group and the latter was shown by the formation of a monoacetate. The *p*-nitrobenzoate and 3,5-dinitrobenzoate were also prepared.

(6) Williams and Major, *Science*, **91**, 246 (1940).

(7) Williams, Truesdail, Weinstock, Jr., Rohrman, Lyman and McBurney, THIS JOURNAL, **60**, 2719 (1938).

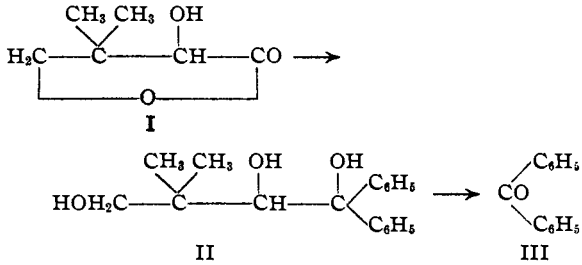
(8) Woolley, *Science*, **91**, 245 (1940).

(9) In developing this method of concentration and at all stages of the isolation of the lactone, invaluable use was made of the bioassay technique developed by Williams and his collaborators (private communication from Dr. R. J. Williams) using the growth stimulation of the microörganisms: *Streptococcus lactis*, *Saccharomyces cerevisiae Gebrüder Mayer*, and *Lactobacillus casei* (Snell, Strong and Peterson, *J. Bact.*, **38**, 293 (1939)). The assays were correlated with a standard sample of a pantothenic acid concentrate kindly supplied by Dr. Williams. In the early part of the work described in this paper the assays were carried out by Mr. E. F. Pratt, and later by Mr. M. Kasha, to whom we wish to express our thanks for their invaluable assistance.

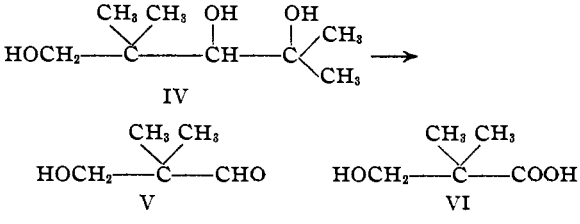
A Kuhn–Roth determination gave a value corresponding to 26% of one C-methyl group, and such a result would be expected from the *gem*-dimethyl grouping as shown in I.

Oxidation by means of cold alkaline barium permanganate was fairly rapid until the equivalent of 5 oxygen atoms had been used, thereafter, an additional oxygen atom was more slowly consumed. The only product which was obtained from the oxidation mixture was a small yield of acetone which was isolated as the *p*-nitrophenylhydrazone. This result is also in conformity with the *gem*-dimethyl grouping as shown in I.

The hydroxy lactone I was converted into the crystalline hydroxydiphenylcarbinol, II, by treatment with phenylmagnesium bromide. On oxidation with lead tetraacetate, benzophenone, III, was formed, which was characterized as the 2,4-dinitrophenylhydrazone. The above hydroxydiphenylcarbinol, therefore, must be a 1,2-glycol, and the lactone, in turn, must possess a hydroxyl group in the position α- to the carbonyl group of the lactone bridge. These facts are demonstrated in the formulas I, II and III.



The complete carbon skeleton of the lactone, I, was determined by its conversion into the hydroxydimethylcarbinol, IV, with methylmagnesium iodide. On oxidation with lead tetraacetate, the carbinol yielded the aldehyde, V, which on oxidation with alkaline silver hydroxide gave α,α-dimethyl-β-hydroxypropionic acid, VI, m. p. 124–125°. The melting point showed no depression when it was mixed with an authentic specimen of α,α-dimethyl-β-hydroxypropionic acid prepared from α,α-dimethyl-β-hydroxypropionaldehyde by

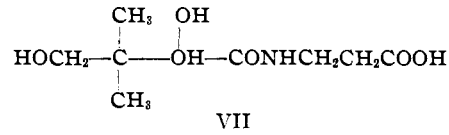


a Cannizzaro reaction.[10] Thus the lactone must have the structure as represented in I.

An attempt to replace the hydroxyl groups of the hydroxy lactone by refluxing with 48% hydrobromic acid failed and the lactone itself was recovered unchanged. This failure to brominate the lactone is thus analogous to the finding of Glaser,[11] who was unable to reduce α-hydroxy-β,β-dimethyl-γ-butyrolactone by means of fuming hydriodic acid. The neopentyl alcohol structure of the hydroxylactone probably accounts for the resistance to bromination. *dl*-α-Hydroxy-β,β-dimethyl-γ-butyrolactone has been synthesized and resolved into its optical components.[12] The levo-rotatory form of the synthetic lactone was chemically and physically identical with the lactone obtained from natural sources.

The identity of the natural and synthetic lactones was confirmed by the assay method of Williams, *et al.*,[3] following their condensation with β-alanine ester and subsequent saponification of the resulting esters. The acids so obtained, in both cases, showed the same degree of stimulation of bacterial growth.[9]

Thus, pantothenic acid is α,γ-dihydroxy-β,β-dimethylbutyryl-β'-alanide, as represented by VII.



Experimental Part

Preparation of Concentrates.—Sixty-four liters of a concentrate of the alcohol soluble fraction of liver extract was diluted with 192 liters of water and sufficient concentrated ammonia water added to bring the *p*H of the solution to above 9.5. To the solution, 6400 g. of Norit A was then added and the mixture vigorously stirred for thirty minutes. It was then filtered in a large suction filter pot and the Norit well washed by resuspending in water and refiltering. The Norit was then discarded.

The filtrate and washings were acidified to *p*H 3 with 50% sulfuric acid and volume was brought to 380 liters with water. This solution was then stirred with 10,400 g. of Norit A for one hour. The mixture was then filtered and the Norit washed with 50 liters of water. The filtrate and washings were discarded. The Norit was immediately resuspended in 64 liters of water containing 640 cc. of concd. ammonia water. After thirty minutes of vigorous stirring, the mixture was filtered and the Norit re-extracted twice more using the same quantity of water and ammonia water. The charcoal was then discarded.

(10) Wessely, *Monatsh.*, **22**, 66 (1901).

(11) Glaser, *ibid.*, **25**, 46 (1904).

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

The combined eluates were adjusted to *p*H 3 with 50% sulfuric acid and then stirred for one hour with 3200 g. of Norit A. After filtration, the charcoal was well washed with water and the washings and filtrate discarded. The charcoal was immediately eluted by stirring with 16 liters of ethanol containing 80 cc. of pyridine. The elution was repeated three more times using the same quantities of alcohol and pyridine. The charcoal was then discarded.

The combined eluates were concentrated *in vacuo* to a thick sirup. (All concentrations in the entire procedure were run at a water-bath temperature not exceeding 40°.) Absolute alcohol was slowly added in 20-cc. portions, grinding and thoroughly mixing each portion with the gum until it became quite thin. Then five liters of absolute alcohol was added slowly with stirring, producing a gummy precipitate. This was allowed to stand in the refrigerator overnight.

The next day the alcoholic solution was separated from the gum which was reworked by dissolving in 50–75 cc. of 95% ethanol, and then adding, with stirring, five liters of absolute alcohol and allowing to stand in the refrigerator overnight. The resulting solution, after separating from the insoluble material, was combined with the main solution and concentrated to a thick sirup.

This thick sirup was dissolved in approximately 200 cc. of absolute alcohol, and five liters of acetone was added slowly with vigorous stirring. After standing in the refrigerator overnight, the supernatant solution was decanted from the gummy precipitate which was immediately reworked by dissolving in 100 cc. of absolute alcohol and reprecipitating with five liters of acetone.

The combined acetone solutions were concentrated to a gum and treated with 150 cc. of absolute alcohol, followed by five liters of absolute ethyl ether. After chilling, the solution was decanted and the gummy residue reworked with alcohol and ether in the same manner as the original precipitation.

The combined alcohol–ether filtrates were concentrated until free from alcohol and ether, picked up in one liter of water and extracted three times with 150-cc. portions of ethyl ether. The combined ether extracts were in turn washed with 100 cc. of ether.

The aqueous fractions were combined and brought to *p*H 6.8–7.2 with barium hydroxide and the precipitate which formed was filtered off and discarded. The filtrate was concentrated to 250 cc., refiltered and extracted with three 100-cc. portions of ethyl ether. The aqueous solution was then evaporated to a thick gum. This gum was then thoroughly triturated with 250-cc. portions of acetone. The insoluble material was filtered, washed with three 20-cc. portions of anhydrous ethyl ether and dried in a desiccator *in vacuo*. The product was an amorphous powder, which weighed 100 g. and contained, by yeast test, approximately 10% of barium pantothenate.

Isolation of the Lactone $C_6H_{10}O_3$.—The concentrate of the barium salts was hydrolyzed in 20-g. portions with 370 cc. of *N* sodium hydroxide on a steam-bath at 100° for one hour. After cooling, the solution was adjusted to *p*H 2.3 with 6 *N* sulfuric acid. The barium sulfate was removed and washed twice with 50-cc. portions of water. The combined filtrate and washings were heated at 100° on the steam-bath for thirty minutes. After cooling, the solution was adjusted to *p*H 7.5 with solid sodium bicarbonate and then transferred to a continuous extraction apparatus, and extracted with ether for twenty-four hours. The ether extract was dried thoroughly with anhydrous sodium sulfate and evaporated to dryness. The crystalline material (55–60%) was sublimed in high vacuum (25° at 10^{-4} mm.), when it was obtained as a colorless microcrystalline film on the condenser. The lactone is readily recrystallized from ether–petroleum ether or isoamyl ether and is obtained in clusters of colorless prismatic needles, m. p. 92–93°.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75; mol. wt., 130. Found: C, 55.40, 55.44, 55.33; H, 7.71, 7.77, 7.64; neut. equiv., 134, 133, 130; mol. wt. (freezing-point, water), 138; C-methyl determinations gave 3.0% C-CH_3. Calcd. for $C_6H_{10}O_3$: (1C-CH_3), 11.53%.

The Zerewitinoff determination data are summarized in Table I.

TABLE I

DETERMINATION OF ACTIVE HYDROGEN ATOMS

Substance, mg.	Methane, cc. at 26.5°	Methane, cc. at 100°	Active hydrogen atoms	
3.841	0.62	0.65	0.94	0.99
3.734	.67	.70	1.04	1.10

The lactone has no free carboxyl group but on heating with *N* sodium hydroxide it consumed one equivalent, due to the saponification of a lactone group.

The lactone possesses a hircine odor and is readily soluble in water, alcohol, ether, acetone, sparingly soluble in isoamyl ether and very sparingly soluble in petroleum ether. When pure, the lactone is quite stable in air but in the crude form, when first isolated, it contains small amounts of impurities which rapidly turn brown in air. It is levorotatory showing $[\alpha]^{27}D$ −49.8° (*C* = 2%; H_2O). An aqueous solution is not acid to litmus even on standing for forty-eight hours or on boiling for a short time.

Acetate of the Lactone.—A solution of 64.2 mg. of the lactone in 1 cc. of dry pyridine was treated with 0.5 cc. of freshly distilled acetic anhydride and allowed to stand at room temperature overnight. The solvents were then evaporated under reduced pressure and the almost colorless viscous oil was transferred to a sublimation apparatus. The major portion of the material sublimed as a colorless crystalline film at 40° (10^{-5} mm.). It had m. p. 41–42°; the yield was 71 mg.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.81; H, 7.03. Found: C, 55.82; H, 6.93.

3,5-Dinitrobenzoate of the Lactone.—A solution of 33.7 mg. of the lactone in 2 cc. of freshly distilled dry pyridine was treated with 118.1 mg. of freshly prepared 3,5-dinitrobenzoyl chloride and the mixture heated at 100° for one and one-half hours. After cooling in ice, the mixture was adjusted to *p*H 2 with 6 *N* sulfuric acid and the crystalline precipitate extracted with chloroform. The combined chloroform extracts were washed with dilute sodium bicarbonate solution and finally with water. On evaporation of the chloroform, after drying over anhydrous sodium sulfate, 64.4 mg. of slightly colored needles was obtained, m. p. 153–154°. After recrystallization from alcohol, in which it is sparingly soluble, the dinitrobenzoate

was obtained as very faintly yellow needles, m. p. 156–157°.

Anal. Calcd. for $C_{18}H_{12}O_8N_2$: C, 48.16; H, 3.73; N, 8.64. Found: C, 48.38, 48.26; H, 3.74, 3.66; N, 8.57, 8.66.

p-Nitrobenzoate of the Lactone.—The *p*-nitrobenzoate was prepared from 25.8 mg. of the lactone and 60 mg. of freshly prepared *p*-nitrobenzoyl chloride, dissolved in 1 cc. dry pyridine as above; yield was 66.3 mg. After two recrystallizations from alcohol, it was obtained as fine colorless needles, m. p. 112°.

Anal. Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.69; N, 5.02. Found: C, 56.07, 56.01; H, 4.57, 4.58; N, 5.02, 5.04.

Saponification and Relactonization of the Lactone.—A solution of 0.1311 g. of the lactone in 2 cc. of *N* alcoholic sodium hydroxide was heated at 100° in a sealed tube for one hour. The solution after cooling was made up to 10 cc. with 50% aqueous ethyl alcohol. The specific rotation of the sodium salt was $[\alpha]^{26.5}D$ +22.19°, 6 *N* hydrochloric acid (0.5 cc.) was added and after thirty-one hours, the mixture reached equilibrium and showed $[\alpha]^{26}D$ −50.4°.

Oxidation of the Lactone.—To a solution of 104.4 mg. of the lactone in 4 cc. of water at 50°, barium hydroxide solution was added until the solution remained alkaline to phenolphthalein on standing at 50° for thirty minutes. The *p*H of the cooled solution during the oxidation was maintained between 8 and 8.5 by the addition of aqueous barium hydroxide. One-tenth molar barium permanganate solution was added in 1-cc. portions. At the beginning, the oxidizing solution was used up fairly rapidly till about 12 cc. had been added when the rate of oxidation slowed down. After the equivalent of 6 atoms of oxygen (16.0 cc.) had been added, the oxidation was interrupted, and the manganese dioxide and barium carbonate were centrifuged off, and thoroughly washed with water. The combined alkaline liquor and washings (*ca.* 50 cc.) were transferred to a distillation flask and about 12 cc. was distilled off into a receiver cooled in an ice–salt mixture. A solution of 250 mg. of freshly recrystallized *p*-nitrophenylhydrazine dissolved in 5 cc. of glacial acetic acid was added to the distillate. Crystallization took place immediately and, after standing for some time, the fine orange-red needles were filtered off; yield was 8.3 mg. The product was dissolved in a small amount of dry benzene and petroleum ether was added to incipient turbidity. On standing, acetone *p*-nitrophenylhydrazone crystallized as clusters of orange-red needles, m. p. 146–147°; mixed m. p. with an authentic sample, 147–148°.

The Diphenyl Carbinol from the Lactone and Phenylmagnesium Bromide.—To a Grignard reagent prepared from 5.8 g. of freshly distilled bromobenzene and 1 g. of magnesium ribbon in 50 cc. of dry ether, a solution of 400 mg. of the lactone in 15 cc. of ether was added slowly. The solution was then refluxed for two hours and allowed to stand overnight at room temperature. The solution was poured onto ice and acidified with dilute hydrochloric acid. The aqueous solution, after separation of the ethereal layer, was extracted five times with ether. The combined ether extracts were dried over anhydrous sodium sulfate and the ether removed by evaporation. The crystalline residue was recrystallized from a mixture of ethyl acetate and petroleum ether and obtained as fine colorless needles, m. p. 154–155°; yield 600 mg. The mother liquor gave a further 270 mg. For analysis, the material was recrystallized from benzene.

Anal. Calcd. for $C_{18}H_{22}O_3$: C, 75.50; H, 7.74. Found: C, 75.55, 75.48; H, 7.43, 7.76.

Oxidation of the Carbinol.—To a solution of 277.3 mg. of the carbinol dissolved in 15 cc. of benzene, 432.5 mg. (1 mole) of lead tetraacetate was added and the mixture was thoroughly stirred at 48° for seventy minutes. After cooling, the solution was concentrated to a third of its volume and 4 volumes of ether was added. The lead salts were filtered off and washed with ether. The filtrate and washings were evaporated to dryness and the residue (265.2 mg.) was taken up in 5 cc. ether and shaken with 2 cc. of a saturated solution of sodium bisulfite. After the ethereal layer had been separated, the bisulfite layer was twice extracted with ether. The combined ethereal extracts were washed with dilute hydrochloric acid and then with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The residual oil (146.6 mg.) was dissolved in 3 cc. of absolute alcohol and filtered from a trace of flocculent material; 3 cc. of a solution of 2,4-dinitrophenylhydrazine (prepared from 1 g. of the hydrazine, dissolved in 2 cc. of concentrated sulfuric acid and 15 cc. absolute alcohol) was added to the alcoholic solution. Immediate crystallization of tiny brick-red plates occurred. After standing for some hours, the crystalline material was collected, yield 238.6 mg., m. p. 234–235°; mixed m. p. with benzophenone 2,4-dinitrophenylhydrazone, 234–236°. From the alcoholic mother liquors, on addition of another 1 cc. of the hydrazine solution, a further 60.7 mg. of the hydrazone was obtained. For analysis, the product was recrystallized from chloroform.

Anal. Calcd. for $C_{19}H_{14}O_4N_4$: C, 62.95; H, 3.89; N, 15.46. Found: C, 62.51, 62.62; H, 3.96, 3.83; N, 15.49, 15.40.

Degradation of the Lactone $C_6H_{10}O_3$ to the Acid $C_3H_{10}O_3$.—To a Grignard solution prepared from 1 g. of magnesium and 3 cc. of methyl iodide in 50 cc. of ether, a solution of 684.6 mg. of the lactone in 30 cc. of dry ether was added. When the addition was complete, the solution was gently refluxed for two hours and then allowed to stand at room temperature overnight. The mixture was decomposed with ice and dilute sulfuric acid and the ether layer separated; the aqueous phase was then extracted ten times with ether. The combined ether extracts were dried over sodium sulfate and on evaporation gave 370 mg. of an almost colorless viscous oil.

The aqueous mother liquor was saturated with ammonium chloride and continuously extracted with ether for eighteen hours. By this means, a further 480 mg. of the product was obtained.

The combined yields of the carbinol, IV (850 mg.), were dissolved in 75 cc. dry benzene and the solution maintained at 50°. 2.33 g. (1 mol.) of freshly recrystallized lead tetraacetate was added in small portions during half an hour with vigorous stirring. After the last addition, the temperature and stirring were maintained a further two and one-half hours, when the solution no longer gave a positive starch–iodide test. Fifty cc. of benzene was re-

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.

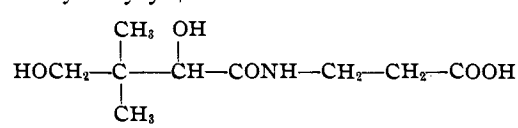
Acknowledgments.—The authors wish to express their great appreciation to Dr. R. J. Williams for making available to them much unpublished work and for much helpful advice. They further wish to acknowledge their appreciation for the help given by Messrs. E. F. Pratt and H. K. Mitchell during the summer 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



HOCH$_2$—C(CH$_3$)(CH$_3$)—CH(OH)—CONH—CH$_2$—CH$_2$—COOH

RAHWAY, N. J. RECEIVED MAY 10, 1940

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

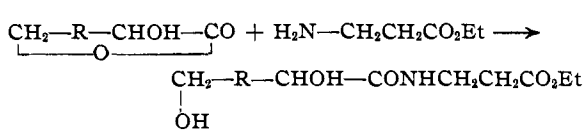
Pantothenic Acid. VII. Partial and Total Synthesis Studies

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



CH$_2$—R—CHOH—CO + H$_2$N—CH$_2$CH$_2$CO$_2$Et ⟶ CH$_2$—R—CHOH—CONHCH$_2$CH$_2$CO$_2$Et

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).