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.

AUSTIN, TEXAS RECEIVED MAY 10, 1940

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

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

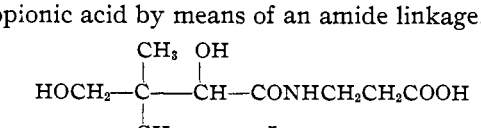
BY ERIC T. STILLER, STANTON A. HARRIS, JACOB FINKELSTEIN, JOHN C. KERESZTESY AND KARL FOLKERS

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.

(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).

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

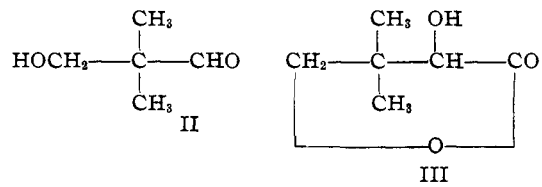
(3) Williams and Major, *Science*, **91**, 246 (1940).
(4) Stiller, Keresztesy and Finkelstein, THIS JOURNAL, **62**, 1779 (1940).

The method we have used to condense the two portions of the vitamin molecule is essentially that of Williams and his collaborators.[1]

The vitamin was first obtained by its resynthesis from the pure crystalline lactone of (+)α,γ-dihydroxy-β,β-dimethylbutyric acid, obtained from concentrates of pantothenic acid[4] by condensing it with β-alanine ethyl ester. The two substances were heated together at 70° and after the saponification of the resulting mixture of esters, a bacterial assay showed that the crude product contained 80% of pantothenic acid, based on the amount of lactone used. The vitamin was purified by the removal of the greater part of the β-alanine by precipitation from alcoholic solution by means of acetone in which pantothenic acid is soluble. The uncombined lactone was then removed by continuous ether extraction of the aqueous solution of the acid which had been previously neutralized with barium hydroxide or barium carbonate. The dry residue of free acids obtained on removal of the barium ions and the water was extracted with reagent acetone in order to remove the last traces of β-alanine. By this means pantothenic acid is obtained as a pale yellow viscous oil from which it is very difficult to remove the last traces of solvents. Pantothenic acid is dextro rotatory showing $[\alpha]^{25}D$ + 37.5°. It should be pointed out that this value may be a little low owing to the difficulty of freeing the larger samples from solvents. It forms a microcrystalline calcium salt; $[\alpha]^{26}D$ + 24.3°.

α-Hydroxy-β,β-dimethyl-γ-butyrolactone has been synthesized by Glaser[5] and by Köhn and Neustädter[6] by a cyanhydrin synthesis on α,α-dimethyl-β-hydroxypropionaldehyde, II. This aldol has been described by Wessely,[7] but on following his directions the aldol was not obtained by the condensation of isobutyraldehyde and formaldehyde. However, by modifying the procedure the aldol was obtained in good yield. The lactone was prepared from the aldol by a modification of the method of Köhn and Neustädter.[6] The aldol was converted into its bisulfite compound and this, in turn, into the corresponding cyanhydrin. After saponification, the lactone was isolated in good yield.

Levene and Haller[8] resolved γ-valerolactone as the cinchonidine salt of the corresponding hydroxy acid. The hydroxy acid was made by opening the lactone with barium hydroxide and then carefully removing the barium ion in the cold with sulfuric acid. Pope and Gibson[9] have completely resolved α-substituted acids by making use of the principle of equilibrium of salts. One mole of potassium hydroxide and one mole of alkaloid were added to two moles of the racemic acid. If the alkaloidal salt of one isomer is much less soluble than the salt of the other isomer, it will crystallize out in almost pure form, leaving the other isomer in solution as its potassium salt.



This principle has been applied successfully to the resolution of racemic α-hydroxy-β,β-dimethyl-γ-butyrolactone. The racemic lactone was converted into its sodium salt by heating with sodium hydroxide and the hot solution was treated with one-half molecular equivalent of quinine hydrochloride. The first crop of the crystalline quinine salt of the pure (+)α,γ-dihydroxy-β,β-dimethylbutyric acid was obtained in 86% yield. This quinine salt gave the (−)α-hydroxy-β,β-dimethyl-γ-butyrolactone when treated with hydrochloric acid and exhaustively extracted with ether. The quinine salt of the (−)acid is more soluble than quinine hydrochloride in cold water and hence cannot be obtained by this method. However, it was obtained from the barium salt and quinine sulfate. The two isomers were then separated readily by fractional crystallization. In this way, the (+)α-hydroxy-β,β-dimethyl-γ-butyrolactone was also obtained.

In order to increase the yield of the desired (−) lactone, the (+)isomer was successfully racemized by heating an aqueous solution of its sodium salt at 150° for eighteen hours. The rotation of the recovered lactone indicated 97–98% racemization and the quinine salt of the (+)acid was obtained in 84% yield from this racemate.

In another experiment, crude (+)lactone was refluxed with alcoholic sodium ethoxide when a fairly rapid change in rotation was obtained during the first sixteen hours followed by a much

(5) Glaser, *Monatsh.*, **25**, 46 (1904).

(6) Köhn and Neustädter, *ibid.*, **39**, 295 (1918).

(7) Wessely, *Monatsh.*, **21**, 231 (1900); *cf.* Fourneau, Benoit and Firmenich, *Bull. soc. chim.*, (4) **47**, 871 (1930); Sabetay and Bléger, *ibid.*, (4) **47**, 888 (1930).

(8) Levene and Haller, *J. Biol. Chem.*, **69**, 165 (1926).

(9) Pope and Gibson, *J. Chem. Soc.*, **101**, 939 (1912).

slower change, indicating that at least partial racemization was taking place.

The pure (−)lactone had the same melting point, mixed melting point and specific rotation, and gave the same *p*-nitrobenzoate as was obtained from the lactone derived from natural pantothenic acid, thus proving their chemical identity.

The synthetic (−)rotatory, (+)rotatory and racemic forms of the lactone were condensed with β-alanine ester and purified by the method described above and gave the synthetic (+)rotatory, (−)rotatory and racemic pantothenic acids, respectively. These acids, like the one derived from the natural lactone, were obtained as viscous oils. The free acids are difficult to free from the last traces of solvents and hence physical constants made on such viscous oils may vary. They form, however, microcrystalline calcium salts which do not have definite melting points but decompose with evolution of gas at temperatures between 150 and 160°. Bacterial assays of these products showed that the (+)pantothenic acid had the full activity of the natural vitamin, whereas the (−)form was inactive when prepared from a rigidly purified specimen of the (+)lactone. The racemic modification had half of the activity of natural (+)form. The bacterial assay of the corresponding calcium salts gave similar results as shown in Table I.

TABLE I

COMPARISON OF PANTOTHENIC ACID WITH ITS (−) AND *dl*-ISOMERS

Form		Assay, %[c] Free acid	Ca salt[a]	$[\alpha]_D$, Ca salt
(+)Form	Resynthesized	99–100	99–101	+24.27
	Synthetic	91–97[b]	99–100	+24.93
(−)Form		0	0	−23.80
d,l-Form		47–52	48–49[d]	0

[a] All the calcium salts were anisotropic. [b] The difficulty encountered in thoroughly drying these viscous oils probably accounts for the low assay value. [c] The bacterial assay values are correlated with a standard sample of a pantothenic acid concentrate, kindly supplied by Dr. R. J. Williams. [d] Equimolecular mixtures of (+) and (−)calcium pantothenate were assayed and they showed 48–50% activity.

We are indebted to Dr. Klaus Unna of the Merck Institute for Therapeutic Research for the preliminary determination of the biological activity of synthetic (+)pantothenic acid and its (−)isomer. The synthetic (+)pantothenic acid was tested in chicks according to the method proposed by Jukes and Lepkovsky.[10] It was found active in promoting growth and curing the dermatitis. The responses to 15 and 20 mg. per 100 g. of diet were not significantly different as to growth and curative effects. On the other hand, 10 mg. per 100 g. of diet produced weight increase approximately half of that obtained with the higher dose levels. A dose of 800 γ of the synthetic (+)pantothenic acid given as a single dose to pantothenic acid depleted rats produced a rapid and marked gain in weight whereas the same dose of the (−)form was practically without effect.

(10) Jukes and Lepkovsky, *J. Biol. Chem.*, **114**, 109 (1936).

Experimental Part

α,α-Dimethyl-β-hydroxypropionaldehyde, II.—A mixture of 200 g. of isobutyraldehyde and 224 g. of 40% formalin was stirred in an ice-bath and 160 g. of potassium carbonate was added in small portions at such a rate that the temperature of the reaction mixture did not exceed 20°. After all the potassium carbonate had been added, the stirring was continued for one hour. During this period, the reaction mixture reached room temperature. The viscous liquid was extracted with ether and dried over sodium sulfate. When the ether was removed, the residue solidified upon cooling. The aldol was purified by distillation. At 15 mm., the b. p. was 83–86° and the distillate immediately crystallized. After recrystallization from alcohol, the product was dried at 60° in vacuum, m. p. 96–97°.

Anal. Calcd. for $C_5H_{10}O_2$: C, 58.82; H, 9.80. Found: C, 59.09; H, 9.78.

Racemic α-Hydroxy-β,β-dimethyl-γ-butyrolactone, III.—A solution of 80 g. of sodium bisulfite was stirred and heated on the steam-bath with 72 g. of β,β-dimethyl-γ-hydroxypropionaldehyde until complete solution was obtained. The solution was then cooled to 10° and maintained between 5–10°, while a solution of 46 g. of potassium cyanide was added slowly. The stirring was continued for one hour before removing the ice-bath and permitting the reaction mixture to reach room temperature. The upper cyanhydrin layer was separated and combined with the subsequent ethereal extractions of the aqueous portion. The ethereal solution was then added slowly to 200 cc. of concentrated hydrochloric acid while maintaining the temperature between 10–15°. After the addition, the reaction mixture was kept at room temperature overnight. A sufficient amount of water was then added to dissolve the precipitated ammonium chloride and the solution was then heated. After the ether had been removed, the hydrolysis was completed by heating at 100° for three hours. The solution was then treated in the cold with a 30% solution of sodium hydroxide until almost neutral and finally treated with a saturated solution of sodium bicarbonate until the *p*H was 7.2. This solution was then continuously extracted with ether for sixteen hours. The ethereal extract was dried over sodium sulfate and then fractionated. The lactone boiled between 119–121° at 15 mm. and solidified to a glassy product. It was recrystallized by dissolving in cold ether and adding cold petroleum ether until just cloudy. Upon standing,

clusters of fine, colorless needles separated. After washing with petroleum ether, the product was dried over phosphorus pentoxide in vacuum, m. p. 56–58°; yield, 52 g.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.31, 55.55; H, 7.78, 7.81.

p-Nitrobenzoate of Racemic α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—This derivative was obtained by heating the lactone with one molecular equivalent of *p*-nitrobenzoyl chloride in pyridine in the usual manner. The product was recrystallized from alcohol in the form of long needles, m. p. 137–138°.

Anal. Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.69; N, 5.02. Found: C, 56.09; H, 4.45; N, 4.90.

The Quinine Salt of (+)α,γ-Dihydroxy-β,β-dimethylbutyric Acid.—A solution of 21 g. (0.1615 mole) of pure racemic lactone in 48.5 cc. of water was treated with 46 cc. of 3.872 *N* sodium hydroxide (10% in excess of theoretical) and heated to 80–90° when hydrolysis of the lactone was complete. After cooling, the excess alkali was neutralized with 2.5 *N* hydrochloric acid and the solution diluted to 400 cc. and again heated to 80–90° on a steam-bath. Thirty-two grams (0.0805 mole) of quinine hydrochloride was added to the hot solution with stirring. Separation of the crystalline quinine salt of (+)α,γ-dihydroxy-β,β-dimethylbutyric acid commenced after a small portion of the quinine hydrochloride had been added The solution and crystals were allowed to stand overnight in a refrigerator and then separated by filtration. After washing with water, the crystals were dried to constant weight in an oven at 60°. The yield of the first crop was 32.4 g. An additional 1.4 g. of pure material was obtained from the mother liquor on concentration. The total yield was 33.8 g. (86.5%); m. p. 189°; $[\alpha]^{25}D$ −130.5° in methanol: *C* = 1%.

Anal. Calcd. for $C_{26}H_{36}O_6N_2$: C, 66.08; H, 7.67; N, 5.93. Found: C, 66.00; H, 7.69; N, 5.94.

(−)α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—A solution of 20.1 g. (0.0425 mole) of the quinine salt $[\alpha]^{25}D$ −130°) in 50 cc. of 2.5 *N* hydrochloric acid (3 equivalents) was heated on the steam-bath for twenty minutes and then continuously extracted for eleven hours with ethyl ether. The ether solution was evaporated to dryness and the residue dried by distilling with alcohol and benzene. The crystalline residue was recrystallized from a little benzene and petroleum ether (b. p. 30–40°). The yield of (−)α-hydroxy-β,β-dimethyl-γ-butyrolactone was 3.95 g. (0.0304 mole) 71.5%); m. p. 89–90°; $[\alpha]^{25}D$ −50.7° in water, *C* = 2.05%. A once recrystallized sample had a melting point and mixed melting point of 90–91° with a sample of lactone (m. p. 91–92°) isolated from natural pantothenic acid.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.32; H, 7.80.

p-Nitrobenzoate of (−)α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—One gram of the synthetic (—)lactone was treated in pyridine with 1.57 g. of *p*-nitrobenzoyl chloride. After heating on the steam-bath, it was poured onto several volumes of cracked ice. The solid which separated was recrystallized three times from 95% alcohol. The yield of the pure *p*-nitrobenzoate was 0.7 g.; m. p. 112°. When mixed with the *p*-nitrobenzoate of the lactone from natural sources there was no depression in melting point.

Anal. Calcd. for $C_{13}H_{13}O_6N$: C, 55.91; H, 4.69; N, 5.02. Found: C, 56.10; H, 4.45; N, 4.94.

Quinine Salt of (−)α,γ-Dihydroxy-β,β-dimethylbutyric Acid.—The barium salt of α,γ-dihydroxy-β,β-dimethylbutyric acid was made by dissolving 65 g. (0.5 mole) of the *d,l*-lactone in 200 cc. of water and heating on the steam-bath with a 20% excess of barium hydroxide. On cooling crystallization took place, so the solution was made up to 500 cc., heated to 80° and neutralized with a stream of carbon dioxide. The solution was filtered from the barium carbonate and on standing a few long needle-like crystals separated.

An aliquot portion of this solution (0.1825 mole) was added to an equivalent amount (71.4 g.) of quinine sulfate which had been made to a paste with water. The quinine salt was brought into solution in 750 cc. of boiling water and quickly centrifuged from precipitated barium sulfate. Crystallization started almost immediately. These crystals proved to be the quinine salt of the dextrorotatory dihydroxy acid mixed with uncombined quinine sulfate. The aqueous filtrate contained the quinine salt of the levo rotatory dihydroxy acid. This was recovered by concentration to a small volume under reduced pressure when crystallization took place. The specific rotations of three successive crops were −143, −146, and −147°, respectively. The latter two crops were combined and recrystallized from 95% ethanol. It crystallized in fine needles in feather like designs which formed a much bulkier mass than did the quinine salt of the (+)acid; m. p. 176–178°; $[\alpha]^{25}D$ −146° in methanol; *C* = 1%. Mixed melting point with the isomer melting at 188–189° showed a depression of only four degrees.

Anal. Calcd. for $C_{26}H_{36}O_6N_2$: C, 66.08; H, 7.67; N, 5.93. Found: C, 66.23; H, 7.72; N, 5.80.

(+)α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—The quinine salt of the (−)acid (3.8 g.) ($[\alpha]^{25}D$ −146°) was treated with 10 cc. of 2.5 *N* hydrochloric acid on a steam-bath for one hour and then extracted ten times with ether. The (+)lactone was recovered and purified in the same manner as described for the (−)lactone. The crude yield was 0.7 g. (67%). After recrystallization from benzene, the m. p. was 91°; $[\alpha]^{25}D$ +50.1° in water; *C* = 2%.

Anal. Calcd. for $C_6H_{10}O_3$: C, 55.37; H, 7.75. Found: C, 55.19; H, 7.51.

p-Nitrobenzoate of (+)α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—The *p*-nitrobenzoate of the (+)lactone was made as described for the (−)lactone. After two recrystallizations the melting point was 114° and the mixed melting point with the *p*-nitrobenzoate of the (−)lactone was 135° which is about the melting point of the racemic form.

Racemization of (+)α-Hydroxy-β,β-dimethyl-γ-butyrolactone.—After the removal of the quinine salt of (+)α,γ-dihydroxy-β,β-dimethylbutyric acid in the previous experiment, the mother liquor was neutralized with sodium hydroxide and extracted with chloroform to remove the excess of quinine. The neutral solution was concentrated to 160 cc. and heated in a bomb tube for eleven hours at

150°. The observed rotation had changed from −0.37 to −0.04°. The solution was further concentrated to 50 cc. volume, acidified to *p*H 2 or lower with concentrated hydrochloric acid, heated twenty minutes on a steam-bath and extracted fifteen times with ether. The ether was concentrated to dryness and the residue dried by distilling with alcohol and benzene and sublimed at 90–110° (bath temperature) at 3 mm. pressure. The yield was 8.9 g. (42.6% based on original lactone); $[\alpha]^{25}D$ +5.84° in water; C = 1%. Since a 43.3% yield of quinine salt had already been obtained, this accounted for an 85.9% recovery of the original lactone.

From the rotation it was calculated that this lactone was about 88% in the racemic form. On this basis a resolution experiment was performed using one-tenth the quantities described in the previous experiment. The yield of quinine salt of the (+)acid was 84%; m. p. and mixed m. p. 188–190°; $[\alpha]^{25}D$ −130°. This definitely proved the racemization of the (−)acid to the *d,l*-form.

In a second experiment in which the heating period was eighteen hours instead of eleven the racemization was more nearly complete; $[\alpha]^{25}D$ +1.14. The *p*-nitrobenzoate of this racemized lactone gave a melting point of 128.5–130.5° which was raised to 133° by two recrystallizations. The mixed melting point with this ester of the racemic lactone was 135°.

In another racemization experiment, 2 g. of crude (+) lactone was refluxed under protection of a calcium chloride tube in 50 cc. of absolute alcohol containing one equivalent of sodium ethylate. The initial observed rotation was +0.47°; after sixteen hours it was +0.17°, and +0.12° at the end of forty-eight hours. This change in rotation indicated that partial racemization had taken place.

Resynthesis of (+)Pantothenic Acid.—A mixture of 0.9860 g. of α-hydroxy-β,β-dimethyl-γ-butyrolactone obtained from pantothenic acid concentrates and 2.2 g. of freshly distilled β-alanine ethyl ester were heated at 70° for two and one-half hours. After cooling the mixture, it was dissolved in 40 cc. of water and 40 cc. of 0.9 *N* barium hydroxide added and the mixture allowed to stand at room temperature for three hours. The barium ion was then removed quantitatively by means of 6 *N* sulfuric acid. The barium sulfate was removed by centrifuging and washed twice with small amounts of water. A bacterial assay on an aliquot of this solution indicated that 80% of the lactone had been converted into pantothenic acid. The solution was adjusted to *p*H 5–6 with pyridine and evaporated to dryness *in vacuo* at 25°. The resulting almost colorless sirup (3 g.) was finally dried in high vacuum over sulfuric acid. It was then dissolved in 5 cc. of methanol and 300 cc. of acetone was added slowly with vigorous stirring. The mixture was kept at 0° till the oil had separated and partially crystallized and the supernatant liquid was clear. The acetone insoluble material was twice reworked with methanol and acetone. The combined acetone–methanol liquors were evaporated to dryness *in vacuo* at 25°. The pale yellow viscous oil was dissolved in 10 cc. of water, neutralized to *p*H 7.5 with 0.9 *N* barium hydroxide and continuously extracted with ether for eighteen hours. By this means, a small amount of unchanged lactone was removed.

The barium ion was quantitatively removed with 6 *N* sulfuric acid, and the resulting barium sulfate was twice washed with water. The combined aqueous liquors were adjusted to between *p*H 5 and 6 by means of pyridine and evaporated to dryness at 25° *in vacuo*. The resulting pale yellow oil was dried in high vacuum at 40° and extracted twice with 100-cc. portions of reagent acetone, with vigorous shaking. The extracts were allowed to stand at 0° till the supernatant liquid had cleared. It was then filtered to remove a small amount of crystalline β-alanine. The acetone was removed *in vacuo* and the resulting pale yellow sirup was dried in high vacuum at 40°; yield 630 mg. (38%). Bacterial assays on this material showed it to have 99–100% activity.

Calcium (+)Pantothenate.—A solution of 600 mg. of (+)pantothenic acid in 5 cc. of water was neutralized with calcium carbonate. After removal of the excess calcium carbonate by filtration the solution was evaporated to dryness at 25° *in vacuo*. The resulting hard colorless glass on treatment with acetone gave a colorless micro-crystalline powder; yield 620 mg. It was purified by dissolving in the minimum amount of methanol and filtered from a trace of insoluble material. The methanol solution was then added slowly to a large volume of acetone with vigorous stirring. The colorless micro-crystalline powder was filtered off and dried at 78° in vacuum. It showed $[\alpha]^{25}D$ +24.27° (C = 1.566% in H_2O). A bacterial assay showed it to have 99–101% activity.

Anal. Calcd. for $Ca(C_9H_{16}O_5N)_2$: C, 45.35; H, 6.77; N, 5.88. Found: C, 45.52, 45.10; H, 6.83, 6.90; N, 5.59.

Racemic Pantothenic Acid.—A mixture of 4.16 g. of racemic α-hydroxy-β,β-dimethyl-γ-butyrolactone and 8 g. of freshly distilled β-alanine ethyl ester was heated at 70° for three hours. The resulting mixture was converted to the free acid by saponification with 300 cc. 0.45 *N* barium hydroxide. A bacterial assay of the resulting solution showed that 40% of the *d,l*-lactone had been converted into material having the physiological activity of pantothenic acid.

The isolation of the *d,l*-pantothenic acid was carried out as described above for the resynthesized pantothenic acid; yield 6.14 g. The *d,l* acid was a very pale yellow viscous oil which was extremely difficult to free from solvent. A bacterial assay showed it to have 47–52% activity.

Calcium Salt of Racemic Pantothenic Acid.—A solution of 5 g. of *d,l*-pantothenic acid dissolved in 25 cc. of water was neutralized with calcium carbonate and the calcium salt isolated as described above; yield 4.5 g. The salt was purified by solution in methanol and precipitation with acetone. A bacterial assay showed 48–49% activity. For analysis, the material was dried at 78° in vacuum. *Anal.* Calcd. for $Ca(C_9H_{16}O_5N)_2$: C, 45.35; H, 6.77; N, 5.88; Ca, 8.41. Found: C, 45.23; H, 6.63; N, 6.14; Ca, 8.41.

Benzylthiuronium Salt of Racemic Pantothenic Acid.—A solution of 0.1513 g. of *d,l*-pantothenic acid in 2 cc. of methanol was neutralized to *p*H 7.5 with 1 *N* sodium methoxide and 0.18 g. of benzylthiuronium chloride dissolved in 1.5 cc. methanol was added and the mixture allowed to stand at room temperature for an hour. The methanol was then evaporated at room temperature and the resulting sirup was extracted with hot acetone in order to free it from sodium chloride; on concentration of the lactone the ben-

zylthiuronium salt commenced to crystallize. After recrystallization from hot acetone, the salt was obtained as colorless needles, m. p. 135–136°; yield 0.23 g. *Anal.* Calcd. for $C_{17}H_{27}O_6N_3S$: C, 52.95; H, 7.07; N, 10.90. Found: C, 52.71; H, 6.99; N, 10.89.

Synthetic (+)Pantothenic Acid.—A mixture of 3.75 g. of synthetic (−)α-hydroxy-β,β-dimethyl-γ-butyrolactone and 7.5 g. β-alanine ethyl ester was heated at 70° for three hours. The resulting mixture of esters was saponified with 300 cc. 0.45 *N* barium hydroxide at room temperature for two and one-half hours. A bacterial assay showed that 66% of the lactone had been converted into pantothenic acid.

The isolation of the pure pantothenic acid was carried out as described above; yield 3.88 g. The product was a viscous almost colorless oil from which it was difficult to remove the last traces of solvents. Bacterial assays showed 91–97% activity.

Calcium Salt of Synthetic (+)Pantothenic Acid.—This salt was prepared and purified as described above from 3.5 g. of synthetic (+)pantothenic acid; yield 3.11 g. A bacterial assay showed 99–100% activity. When air dried it contained 5.82% H_2O. It showed $[\alpha]^{26}D$ +24.93° (*C* = 0.792%; H_2O). For analysis the salt was dried at 78° *in vacuo*. *Anal.* Calcd. for $Ca(C_9H_{16}O_5N)_2$: C, 45.35; H, 6.77; N, 5.88; Ca, 8.41. Found: C, 45.21; H, 6.78; N, 5.81; Ca, 8.30.

Synthetic (−)Pantothenic Acid.—A mixture of 2.88 g. of (+)α-hydroxy-β,β-dimethyl-γ-butyrolactone and 5.76 g. of freshly distilled β-alanine ethyl ester was heated at 70° for three hours. The resulting mixture of esters was saponified with 200 cc. 0.45 *N* barium hydroxide at room temperature for two and one-half hours. A bacterial assay showed no activity.

The isolation of the pure (−)pantothenic acid was carried out as described for the (+)acid. The product was a colorless viscous oil; yield 3.8 g. A bacterial assay of this material after it had been dried at 40° at 10^{-5} mm. for three hours showed no growth response.

Calcium Salt of (−)Pantothenic Acid.—The salt prepared from 3.5 g. of this material was obtained as a colorless microcrystalline powder by the method described above; yield 3.33 g. It showed $[\alpha]^{26}D$ −23.80° (*C* = 0.7976%; H_2O). Bacterial assays showed no activity. For analysis the calcium salt was dried at 78° C. *in vacuo*. *Anal.* Calcd. for $Ca(C_9H_{16}O_5N)_2$: C, 45.35; H, 6.77; N, 5.88; Ca, 8.41. Found: C, 45.46; H, 6.69; N, 5.72; Ca, 8.40.

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Summary

1. α-Hydroxy-β,β-dimethyl-γ-butyrolactone has been synthesized and resolved into its optical enantiomorphs.

2. The (−)form of the lactone has been shown to be identical with the lactone obtained from the hydrolysis of pantothenic acid.

3. (+)Pantothenic acid has been resynthesized from the lactone obtained from natural sources and isolated as its calcium salt.

4. (+)Pantothenic acid has been synthesized from synthetic (−)α-hydroxy-β,β-dimethyl-γ-butyrolactone and shown to have the same physiological activity as the resynthesized pantothenic acid.

5. Racemic and (−)pantothenic acids have been synthesized from the racemic and (+)α-hydroxy-β,β-dimethyl-γ-butyrolactones and isolated as their calcium salts. They were shown to have, respectively, 50% and 0% of the bacterial growth stimulation activity of (+)pantothenic acid.

6. The synthetic (+)pantothenic acid showed the expected biological activity when assayed on chicks and rats.

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