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

# Synthesis of Pantetheine and S-Acetylpantetheine

BY EDWARD WALTON, ANDREW N. WILSON, FREDERICK W. HOLLY AND KARL FOLKERS

RECEIVED OCTOBER 23, 1953

Pantetheine has been synthesized by the condensation of (−)-pantolactone with β-alanyl-S-benzyl-β-aminoethanethiol followed by the reductive removal of the benzyl group. Preparation of β-alanyl-S-benzyl-β-aminoethanethiol by both the carbobenzyloxy and phthaloyl methods for peptide synthesis is described. Pantetheine was converted into its S-acetyl derivative by means of thiolacetic acid in aqueous solution.

The preparation of pantetheine (*Lactobacillus bulgaricus* factor) by condensation of methyl pantothenate with β-aminoethanethiol has been described.[1] The present paper describes an alternative synthesis of pantetheine and its conversion into S-acetylpantetheine.[2-4] During the course of this work, additional syntheses of pantetheine have been described.[5-8]

In our method, S-benzyl-β-aminoethanethiol (II) was prepared from commercial β-aminoethanethiol (I) and benzyl chloride. N-(N-Carbobenzyloxy-β-alanyl)-S-benzyl-β-aminoethanethiol (V) was prepared by condensing S-benzyl-β-aminoethanethiol and N-carbobenzyloxy-β-alanyl chloride (III). The conversion of N-(N-carbobenzyloxy-β-alanyl)-S-benzyl-β-aminoethanethiol into N-(β-alanyl)-S-benzyl-β-aminoethanethiol (VII) was accomplished by reduction with sodium in liquid ammonia, followed by rebenzylation of the sodium mercaptide.

N-(β-Alanyl)-S-benzyl-β-aminoethanethiol has been synthesized by another method. N-Phthaloyl-β-alanyl chloride (IV) was allowed to react with S-benzyl-β-aminoethanethiol to produce N-(N-phthaloyl-β-alanyl)-S-benzyl-β-aminoethanethiol (VI), which was converted into N-(β-alanyl)-S-benzyl-β-aminoethanethiol by treatment with hydrazine hydrate. (−)-Pantolactone was condensed with N-(β-alanyl)-S-benzyl-β-aminoethanethiol to produce S-benzylpantetheine (VIII), which in turn was converted into pantetheine (IX) using sodium and liquid ammonia to remove the benzyl group.

A sample of pantetheine, which was fully active as indicated by enzymatic assay,[9] had a molecular weight (ebullioscopic) of 272 ± 10 as compared with a theoretical value of 278. Polarographic analysis gave the following result: $E_{1/2}$ −0.479 volt *vs.* S.C.E., $I_{d/c}$ = 7.28 μamp./mg./cc. (anodic) (in 0.2 *M* ethylenediaminetetraacetate, 0.01% gelatin, *p*H 9.0).

Treatment of pantetheine with thiolacetic acid in aqueous solution[10] yielded S-acetylpantetheine (X). Acetyl determination indicated the presence of one acetyl group. Polarographic analysis did not show the half-wave potential characteristic of a thiol function. The infrared absorption spectrum did not show absorption in the 5.75 μ region, characteristic of the stretching frequency of the ester-carbonyl group. Iodometric titration further confirmed the absence of a thiol group. Treatment of the S-acetyl derivative with zinc and dilute hydrochloric acid, and subsequent iodine titration showed the absence of a disulfide linkage. Hydrolysis of the S-acetyl derivative with dilute alkali at room temperature regenerated pantetheine as evidenced by iodometric titration.

**Acknowledgments.**—We are indebted to Dr. J. B. Conn for polarographic data and molecular weight determinations, to Mr. R. N. Boos and associates for microanalyses, and to Mr. R. W. Walker for infrared spectral data. We also wish to express our appreciation to Dr. G. E. Boxer for his helpful interest during the course of this work and for the enzymatic assays.

## Experimental

**S-Benzyl-β-aminoethanethiol (II).**—A solution of 127 g. (1 mole, 115 ml.) of benzyl chloride in 300 ml. of methanol was added to a stirred solution of 77 g. (1 mole) of β-aminoethanethiol and 54 g. (1 mole) of sodium methoxide in 700 ml. of methanol. The mixture warmed spontaneously during the addition and afterwards the solution was refluxed an additional 30 minutes. The reaction mixture was cooled and the precipitate of sodium chloride was removed by filtration. The filtrate was concentrated to remove almost all of the solvent and the residue was added to water. The strongly basic solution was extracted three times with 200-ml. portions of ether. The ether extracts were combined, washed twice with 100-ml. portions of water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The oily residue (139 g.) was distilled through a short column under reduced pressure and 87.3 g. (52%) of S-benzyl-β-aminoethanethiol boiling at 95–99° (0.6 mm.) was collected as product. A 5-g. portion of the product was dissolved in 50 ml. of ethanol and excess dry hydrogen chloride was added. The solution was diluted with 200 ml. of ether which caused the separation of 5.8 g. of S-benzyl-β-aminoethanethiol hydrochloride, m.p. 106–131°. Repeated recrystallizations of the hydrochloride from ethanol–ether, methanol–ether, and ethanol yielded an analytical sample, m.p. 120–136°.[11]

*Anal.* Calcd. for $C_9H_{14}NSCl$: C, 53.06; H, 6.93; N, 6.88; Cl, 17.40. Found: C, 53.00; H, 6.75; N, 7.18; Cl, 17.37.

Another 5-g. portion of free amine in 10 ml. of methanol was treated with an excess of anhydrous hydrogen bromide. The solution was diluted with 100 ml. of anhydrous ether, and the precipitated solid was removed and washed with several portions of ether. The product was recrystallized from 15 ml. of methanol by the addition of 200 ml. of ether

(1) E. E. Snell, G. M. Brown, V. J. Peters, J. A. Craig, E. L. Wittle, J. A. Moore, J. M. McGlohon and O. D. Bird, THIS JOURNAL, **72**, 5349 (1950).

(2) R. Schwyzer, *Helv. Chim. Acta*, **35**, 1903 (1952).

(3) T. E. King, C. J. Stewart and V. H. Cheldelin, *Science*, **117**, 439 (1953).

(4) J. Baddiley and F. M. Thain, *ibid.*, **117**, 439 (1953).

(5) J. Baddiley and F. M. Thain, *J. Chem. Soc.*, 800 (1952).

(6) T. E. King, C. J. Stewart and V. H. Cheldelin, THIS JOURNAL, **75**, 1290 (1953).

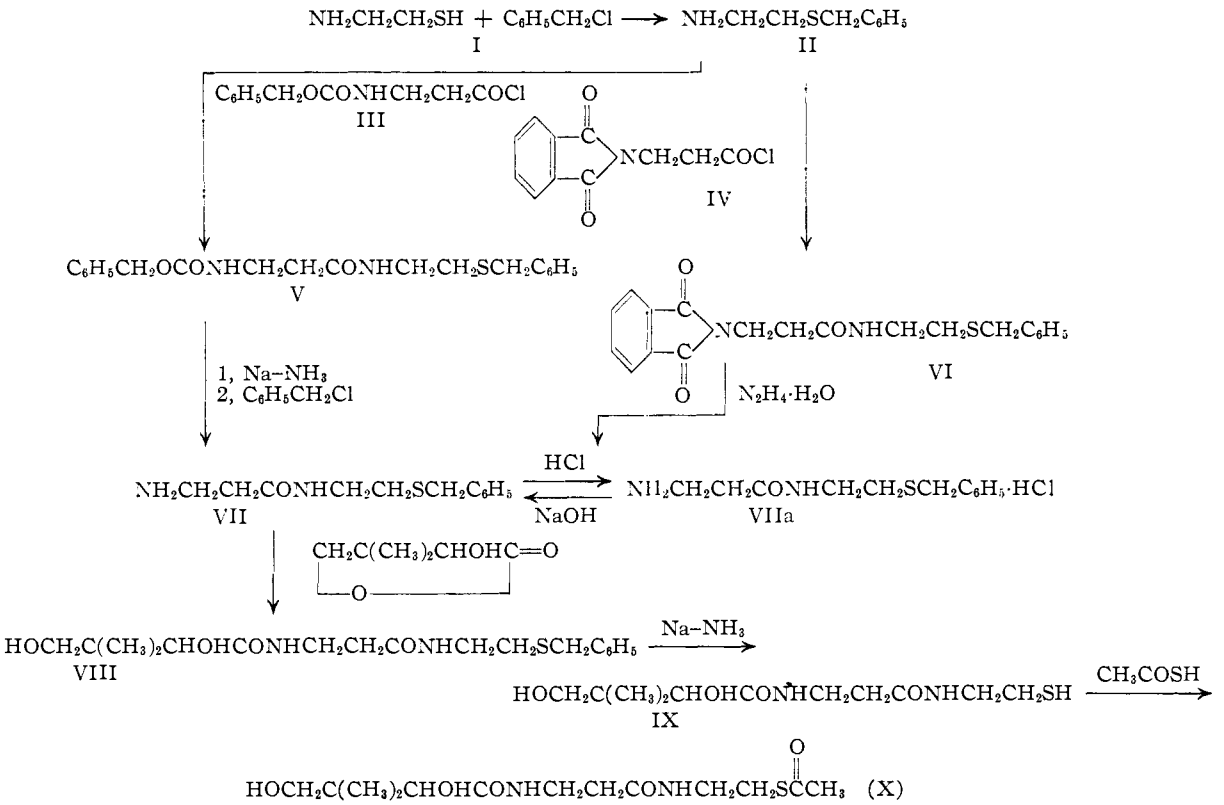
(7) E. L. Wittle, J. A. Moore, R. W. Stipek, F. E. Peterson, V. M. McGlohon, O. D. Bird, G. M. Brown and E. E. Snell, *ibid.*, **75**, 1694 (1953).

(8) M. Viscontini, K. Adank, M. Merkling, K. Ehrhardt and P. Karrer, *Helv. Chim. Acta*, **36**, 835 (1953).

(9) F. Lynen, E. Reichert and L. Rueff, *Ann.*, **574**, 1 (1951).

(10) I. B. Wilson, THIS JOURNAL, **74**, 3205 (1952).

(11) The melting point of this compound as reported by Baddiley and Thain (ref. 5) is 119–120°.




to yield 6.9 g. of S-benzyl-β-aminoethanethiol hydrobromide, m.p. 126–129°. A portion of the product was recrystallized several times to give an analytical sample, m.p. 129–130°.

*Anal.* Calcd. for $C_9H_{14}NSBr$: C, 43.55; H, 5.69; N, 5.64; S, 12.92; Br, 32.20. Found: C, 43.77; H, 5.44; N, 5.85; S, 13.09; Br, 32.67.

**N-(N-Carbobenzyloxy-β-alanyl)-S-benzyl-β-aminoethanethiol (V).**—A suspension of 22.3 g. (0.1 mole) of N-carbobenzyloxy-β-alanine[12] in 400 ml. of dry ether was cooled to 0°. The cold mixture was treated with 22.9 g. (0.11 mole) of phosphorus pentachloride and the mixture was stirred an additional 45 minutes. The cold solution was filtered and concentrated under reduced pressure at a temperature of 10°. The residue, redissolved in 60 ml. of dry ether, was added to a mixture of 16.7 g. (0.1 mole) of S-benzyl-β-aminoethanethiol, 40 ml. of 2.5 *N* sodium hydroxide solution, 60 ml. of water and 200 ml. of ether at a temperature of 10° over a 16-minute period. In order to adjust the mixture to about *p*H 9, 25 ml. of 2.5 *N* sodium hydroxide solution was added. The stirring was continued an additional 15 minutes, and the product was filtered and washed twice with 100-ml. portions of water. The dried product (32 g., 85%), m.p. 118–120°, was recrystallized by dissolving the material in 100 ml. of methanol and adding 200 ml. of isopropyl ether to the solution. There was obtained 28.2 g. of N-(N-carbobenzyloxy-β-alanyl)-S-benzyl-β-aminoethanethiol,[5] m.p. 119–120°.

*Anal.* Calcd. for $C_{20}H_{24}N_2O_3S$: C, 64.49; H, 6.49; N, 7.52. Found: C, 64.96; H, 6.20; N, 7.54.

**N-(β-Alanyl)-S-benzyl-β-aminoethanethiol Hydrochloride (VIIa).**—About 600 ml. of liquid anhydrous ammonia was added to 28.2 g. (0.075 mole) of N-(N-carbobenzyloxy-β-alanyl)-S-benzyl-β-aminoethanethiol. Sodium (7.4 g., 0.322 mole) was added to the stirred mixture until a permanent blue color was obtained. The reaction mixture was cooled in a Dry Ice–alcohol bath and 10.1 g. (0.0875 mole) of benzyl chloride was added slowly. The ammonia was allowed to evaporate at room temperature and near the end of the evaporation 21.2 g. (0.322 equivalent) of ammonium sulfate was added. The last traces of ammonia were removed under reduced pressure. Ice was added to the residue and then enough hydrochloric acid to adjust the resulting solution at *p*H level 2. The solution was extracted twice with 100-ml. portions of ether. The aqueous phase was made strongly alkaline with 30% sodium hydroxide solution and the liberated amine was extracted three times with 200-ml. portions of chloroform. The chloroform extracts were combined, washed three times with 100-ml. portions of ice-water, and dried over anhydrous sodium sulfate. After removal of the drying agent, the solution was concentrated under reduced pressure. The residual oil was dissolved in 100 ml. of methanol, cooled and treated with excess dry hydrogen chloride. The product was crystallized by the addition of 500 ml. of ether; 18 g. (88%) of N-(β-alanyl)-S-benzyl-β-aminoethanethiol hydrochloride, m.p. 163–165°, was obtained. A sample recrystallized for analysis melted at 165–167°.

*Anal.* Calcd. for $C_{12}H_{19}N_2OSCl$: C, 52.44; H, 6.97; N, 10.20; S, 11.67; Cl, 12.90. Found: C, 52.67; H, 6.72; N, 10.30; S, 11.96; Cl, 12.56.

**N-Phthaloyl-β-alanyl Chloride (IV).**—A small amount of the solvent was distilled from a suspension of 21.9 g. (0.1 mole) of N-phthaloyl-β-alanine[13] in 250 ml. of benzene in order to remove water. A reflux condenser was added to the system and a total of 20.8 g. (0.1 mole) of phosphorus pentachloride was added to the warm mixture in five equal portions. A vigorous reaction occurred after each addition. Heating and stirring were continued an additional 0.5 hour. The practically clear solution was filtered and the filtrate was concentrated to a small volume. The addition of petroleum ether caused the crystallization of 22 g. (93%) of the acid chloride, m.p. 102–104°.

**N-(N-Phthaloyl-β-alanyl)-S-benzyl-β-aminoethanethiol (VI).**—A solution of 22 g. (0.093 mole) of N-phthaloyl-β-alanyl chloride in 60 ml. of dry dioxane was added dropwise to a suspension of 16.3 g. (0.097 mole) of S-benzyl-β-aminoethanethiol in 260 ml. of ice-water and 40 ml. of 2.5 *N* sodium hydroxide solution. The addition required 15 minutes and the reaction mixture was stirred an additional 2 hours. The precipitated solid was removed and dried at 60° under reduced pressure. The product (22.7 g.), m.p. 105–120°, was recrystallized from 180 ml. of ethyl acetate to yield 18.5 g. of the phthaloyl derivative, m.p. 113–123°. The solid

(12) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(13) R. A. Turner, THIS JOURNAL, **75**, 2388 (1953).

was dissolved in 300 ml. of chloroform and the solution was washed successively with a saturated sodium bicarbonate solution, water, 1 *N* hydrochloric acid, and finally 3 times with water. The chloroform solution was concentrated to dryness and the residue was recrystallized from 100 ml. of ethyl acetate. The recovery was 16.8 g., m.p. 113–123°, of N-(N-phthaloyl-β-alanyl)-S-benzyl-β-aminoethanethiol.

*Anal.* Calcd. for $C_{20}H_{20}N_2O_3S$: C, 65.19; H, 5.47; N, 7.61. Found: C, 65.14; H, 5.72; N, 7.78.

**N-(β-Alanyl)-S-benzyl-β-aminoethanethiol Hydrochloride (VIIa).**—A mixture of 7.37 g. (0.02 mole) of N-(N-phthaloyl-β-alanyl)-S-benzyl-β-aminoethanethiol and 1.18 g. (0.02 mole, 1.15 ml.) of 85% hydrazine hydrate in 50 ml. of ethanol was heated at the reflux temperature. Complete solution was obtained rapidly and after about 15 minutes a precipitate began to separate. Heating was continued a total of 1 hour. The reaction mixture was concentrated to dryness and 150 ml. of water and 50 ml. of 1 *N* hydrochloric acid were added. To ensure complete dissolution of the acid soluble components, the mixture was warmed to about 50° and then cooled to room temperature. The undissolved solid was removed and the filtrate was concentrated under reduced pressure. The residue (5.8 g.), m.p. 130–150°, was recrystallized 3 times from alcohol–ether to yield 4.0 g. of N-(β-alanyl)-S-benzyl-β-aminoethanethiol hydrochloride, m.p. 164.5–166.5°.

**S-Benzylpantetheine (VIII).**—A solution of 30% sodium hydroxide was added to a solution of 8.8 g. (0.032 mole) of N-(β-alanyl)-S-benzyl-β-aminoethanethiol hydrochloride in 80 ml. of ice and water until the free amine separated. The oil was extracted into several 50-ml. portions of chloroform. The combined chloroform extracts were washed with two portions of water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The residual oil was dissolved in 30 ml. of methanol and 4.2 g. (0.032 mole) of (−)-pantolactone was added. The solution was refluxed for 1 hour and the solvent was removed under reduced pressure. The viscous oil remaining was dissolved in about 200 ml. of chloroform and extracted with 25 ml. of 0.5 *N* hydrochloric acid followed by three 25-ml. portions of water. The chloroform solution was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure; 11 g. of S-benzylpantetheine was obtained, $[\alpha]^{24}D$ +20° (*c* 1.7 in methanol).

*Anal.* Calcd. for $C_{18}H_{28}N_2O_4S$: C, 58.67; H, 7.64; N, 7.60. Found: C, 58.71; H, 7.52; N, 7.89.

**Pantetheine (IX).**—To 5.4 g. (0.0147 mole) of S-benzylpantetheine in a flask cooled in a mixture of Dry Ice and alcohol was added 150 ml. of liquid anhydrous ammonia. The cooling bath was removed and 1.37 g. (0.0596 mole) of sodium was added in small portions with stirring. At the end of the addition a permanent blue color was obtained. About 10 g. of ammonium sulfate was added and stirring was continued while the ammonia evaporated. The residue was dissolved in ice-water and the mixture was extracted with three 70-ml. portions of 1-butanol. The combined 1-butanol extracts were washed with three 50-ml. portions of water and dried over anhydrous magnesium sulfate. The solvent was removed at reduced pressure to yield 4.2 g. of pantetheine. Enzymatic assay indicated that the product was 80 to 90% pure.

**S-Acetylpantetheine (X).**—Pantetheine (10 g., 0.036 mole) was dissolved in 60 ml. of water and to this solution was added 20 ml. (0.28 mole) of freshly distilled thiolacetic acid. The mixture was allowed to stir overnight at room temperature. The reaction mixture, which had become homogeneous, was concentrated under reduced pressure. The yield of S-acetylpantetheine, a pale yellow viscous oil, was quantitative.

*Anal.* Calcd. for $C_{13}H_{24}N_2O_5S$: C, 48.73; H, 7.55; N, 8.75; acetyl, 13.43. Found: C, 48.39; H, 7.40; N, 8.18; acetyl, 13.59.

RAHWAY, N. J.

---

[CONTRIBUTION FROM WARNER–CHILCOTT RESEARCH LABORATORIES]

# The Preparation of Some Nitrogenous Derivatives of Conidendrin

BY JOHN SWIDINSKY, FREEMAN H. MCMILLAN AND JOHN A. KING

RECEIVED OCTOBER 20, 1953

Ten amides have been prepared by the reaction of methylated *l*-conidendrin with a variety of amines and three of these amides have been reduced to the corresponding amines by lithium aluminum hydride. It has been observed that the lactone linkage in the dehydrogenated methylated conidendrin is very stable and non-reactive.

As a member of the group of lignans[1] which occur in woody tissue, *l*-conidendrin (I) has been known for many years. It was first isolated from sulfite waste liquor by Lindsey and Tollens[2] in 1892, and the proof of its skeletal and functional group structure was summarized by Haworth in the 1942 Tilden Lecture[3]; the stereochemistry of the hydroaromatic ring has not been established although it has been deduced (Haworth, ref. 3) that the molecule has the *trans* (1:2), *trans* (2:3) structure (using Haworth's numbering system, in which the aryl group is at position 1). Its isolation and identification a few years ago by Brauns[4] and Pearl[5] as a constituent to the extent of 0.6% of the weight of the wood charged of sulfite waste liquor of western hemlock have stimulated interest in the use of it[6] or its demethylated derivative[7,8] as antioxidants in edible oils and have led to the development of commerical processes for its isolation,[9,10] isomerization[11,12] and demethylation.[11,13]

The probable close structural relationship of conidendrin to podophyllotoxin and to α- and β-peltatin[14,15] and the recent observations concerning the high necrotizing activity for mouse sarcoma 37 of these latter substances[16,17] make a study of chemical

(1) The term lignan was proposed by Haworth some years ago to include the group of natural products characterized by the 2,3-dibenzylbutane skeleton (as such or cyclized). *Cf. Ann. Repts. on Progress of Chem. (Chem. Soc.*, **33**, 270 (1936)).

(2) J. B. Lindsey and B. Tollens, *Ann.*, **267**, 341 (1892).

(3) R. D. Haworth, *J. Chem. Soc.*, 448 (1942).

(4) F. E. Brauns, *J. Org. Chem.*, **10**, 216 (1945).

(5) I. A. Pearl, *ibid.*, **10**, 219 (1945).

(6) P. Swartling, *Proc. 12th Intern. Dairy Congr. (Stockholm)*, **2**, 375 (1949); *C. A.*, **44**, 2664 (1950).

(7) G. S. Fisher, L. Kyame and W. G. Bickford, *J. Am. Oil Chemists' Soc.*, **24**, 340 (1947).

(8) R. N. Moore and W. G. Bickford, *ibid.*, **29**, 1 (1952).

(9) H. B. Lackey, W. W. Moyer and W. M. Hearon, *Tappi*, **32**, 469 (1949).

(10) H. B. Lackey (to Crown Zellerbach Corporation), U. S. Patent 2,577,470, December 4, 1951.

(11) W. M. Hearon, H. B. Lackey and W. W. Moyer, THIS JOURNAL, **73**, 4005 (1951).

(12) W. M. Hearon and V. V. Jones (to Crown Zellerbach Corporation), U. S. Patent 2,610,970, September 16, 1952.

(13) W. M. Hearon (to Crown Zellerbach Corporation), U. S. Patent 2,612,508, September 30, 1952.

(14) J. L. Hartwell and W. E. Detty, THIS JOURNAL, **72**, 246 (1950).

(15) J. L. Hartwell and A. W. Schrecker, Paper No. 5 presented before the Division of Medicinal Chemistry at the 117th Meeting of the American Chemical Society, Philadelphia, Pa., April 10, 1950.

(16) J. L. Hartwell, THIS JOURNAL, **69**, 2918 (1947).

(17) J. L. Hartwell and W. E. Detty, *ibid.*, **70**, 28 (1948).