[Chem. Pharm. Bull. 15(5) 713~715 (1967)]

UDC 577.16 : 547.995.07

Seizaburo Okada, Osamu Nagase, and Masao Shimizu : Investigations on Pantothenic Acid and Its Related Compounds. Ⅶ. Chemical Studies. (5).*[1] Synthesis of D-Pantothenic Acid 4′-Phosphate.

(*Central Research Laboratory, Daiichi Seiyaku Co., Ltd.**[2])

(Received June 3, 1966)

D-Pantothenic acid 4′-phoshate (Ⅱ) was shown by Brown¹⁾ to be the first metabolite in the biosynthesis of coenzyme A from D-pantothenic acid. There have so far been three independent reports²⁾~⁴⁾ on its chemical synthesis, each of which lacks the detailed description on its properties. The requirement of synthetic substrate for the biochemical studies in this series prompted chemical synthesis of Ⅱ, the results of which are reported here.

Atherton⁴⁾ claimed the process for the manufacture of Ⅱ by phosphorylation of D-pantothenonitrile with phenyl phosphorodichloridate followed by hydrolysis with barium hydroxide. In the present study, synthesis of Ⅱ was effected by hydrolysis of D-pantothenonitrile 4′-phosphate (I) with barium hydroxide in 81% yield. As described in the previous paper,⁵⁾ I was synthesized by phosphorylation of D-pantothenonitrile with dibenzyl phosphorochloridate followed by catalytic hydrogenation and confirmed by its conversion to D-pantetheine 4′-phosphate. The process of alkaline hydrolysis seems to involve the possibility of racemization of pantoic acid moiety.⁶⁾ But the result of microbiological assay with *Lactobacillus arabinosus* 17-5 after digestion with calf intestinal alkaline phosphatase showed the growth response corresponded with theoretical recovery of D-pantothenic acid, excluding the occurence of racemization. In contrast to pantothenic acid, Ⅱ is stable under alkaline conditions as shown by the high yield of Ⅱ from I and the microbiological activity of Ⅱ described above. The fact is in agreement with that described by King and Strong.³⁾

Baddiley and Thain²⁾ synthesized racemic pantothenic acid 4′-phosphate by phosphorylation of racemic pantothenic acid 2′-benzyl ether with diphenyl phosphorochloridate followed by catalytic hydrogenation, while King and Strong³⁾ independently used free pantothenic acid as the starting material, which was phosphorylated with the same agent and hydrolyzed with sodium hydroxide. The follow-up of the latter method showed the concomitant formation of 2′,4′-cyclic phosphate (Ⅲ) besides Ⅱ. Ⅲ could be hydrolyzed to Ⅱ with barium hydroxide. As another phosporylating agent, cyanoethyl phosphate also was tried, with which methyl D-pantothenate (Ⅳ) was phosphorylated in the presence of dicyclohexylcarbodiimide (DCC) and hydrolyzed with sodium hydroxide to afford Ⅱ in 27% yield.

During the biochemical study, Ⅱ was found to be partially active for the growth of *Lactobacillus arabinosus* 17-5 for which pantothenic acid 4′-phoshate had ben reported

---

*[1] Part 4. This Bulletin, **15**, 655 (1967).

*[2] Minamifunabori-cho, Edogawa-ku, Tokyo (岡田清三郎, 長瀬 脩, 清水正夫).

1) G.M. Brown : J. Biol. Chem., **234**, 370 (1959).

2) J. Baddiley, E.M. Thain : J. Chem. Soc., **1951**, 246.

3) T.E. King, F.M. Strong : J. Biol. Chem., **191**, 515 (1951).

4) F.R. Atherton : U.S. Pat., 2,870,188 (1959).

5) O. Nagase : This Bulletin, **15**, 648 (1967).

6) W.S. Pierpoint, D.E. Hughes, J. Baddiley, A.P. Mathias : Biochem. J., **61**, 368 (1955).

to be inactive.3,6) The cause for this discrepancy will be explained in the following paper.7)

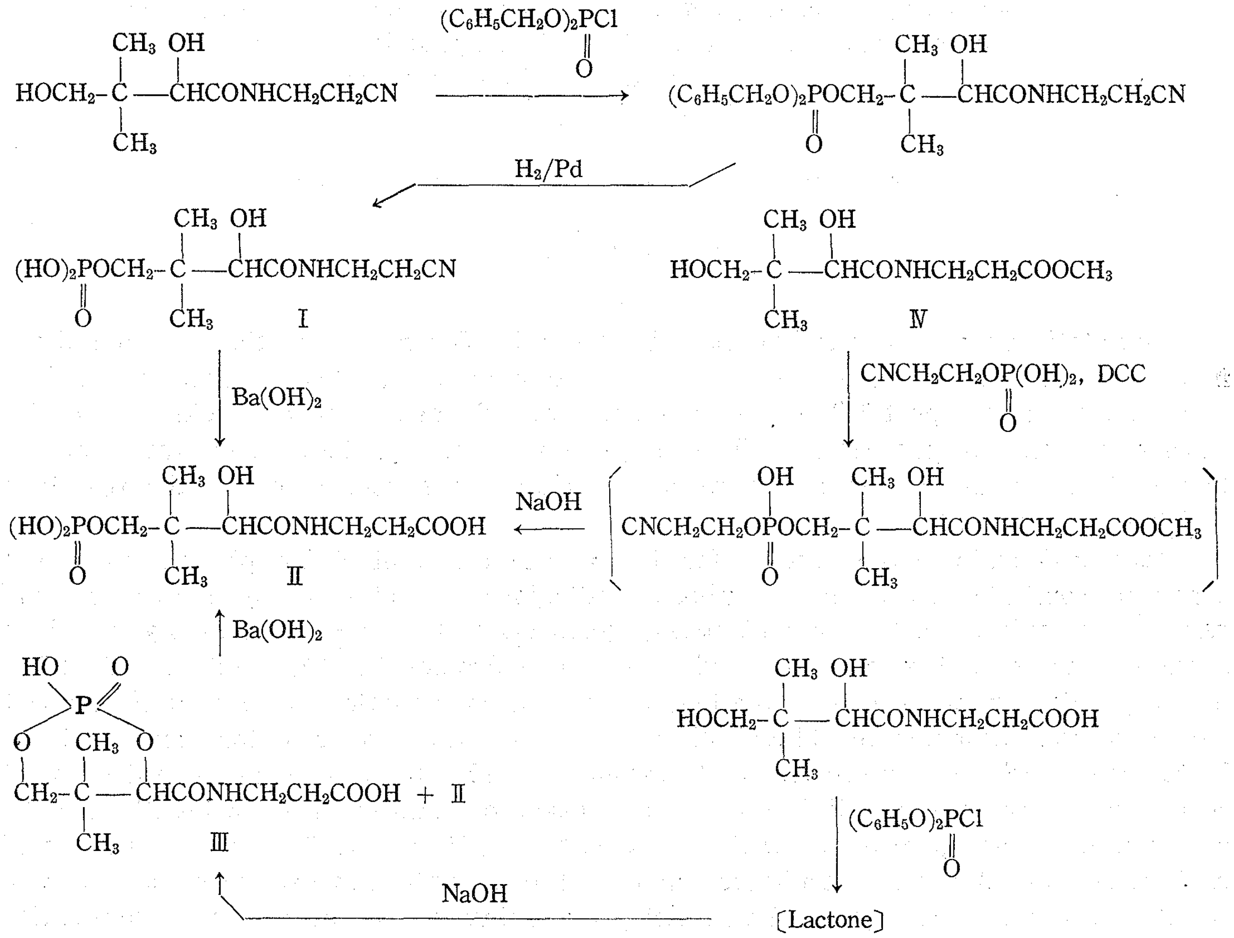

Chart 1.

Experimental

Paper chromatography was carried out using an ascending technique on Toyo Roshi No. 50. Solvent systems : solvent I, PrOH-NH_4OH-H_2O (6:3:1); solvent II, BuOH-AcOH-H_2O (5:2:3); solvent III, isobutyric acid-0.5*N* NH_4OH (10:6). Detection : the Hanes and Isherwood spray8) followed by ultraviolet irradiation.

Nuclear magnetic resonance (NMR) spectrum of free acid was measured in D_2O at 25°, 60 Mc. Band positions are given as upfield displacement in p.p.m. from DHO.

D-Pantothenic Acid 4′-Phosphate (II)——a) A solution of the barium salt of D-pantothenonitrile 4′-phosphate (I)5) (226 mg.) in 0.3*N* $Ba(OH)_2$ (25 ml.) was refluxed for 3.5 hr. and then passed through a column of Amberlite IR 120 (H^+). The eluate and washings were neutralized to pH 7.2 with $Ba(OH)_2$ and evaporated to dryness *in vacuo*. Addition of EtOH (10 ml.) to an aqueous solution of the residue (1 ml.) gave a white precipitate which was dried *in vacuo* over P_2O_5 giving the barium salt of D-pantothenic acid 4′-phosphate (225 mg. 81% yield). $[\alpha]_D^{24}$ +9.0°(c=3.3, H_2O). IR ν_{max}^{KBr} cm^{-1} : 3295, 2945, 2875, 1642, 1565, 1407, 1112~1072, 975, 800. NMR : 1.05 (1H, singlet), 1.23 (2H, triplet), 1.53 (2H, triplet), 2.45 (2H, triplet), 4.10 (3H, singlet), 4.16 (3H, singlet). Paper chromatography : Rf 0.34 in solvent I, Rf 0.47 in solvent II, Rf 0.53 in solvet III. Pantothenic acid content*3 : 95.5~102.1% of theoretical amount of D-pantothenic acid was recovered after phosphatase treatment. *Anal.* Calcd. for $C_9H_{15}O_8NPBa_{1.5}\cdot 3H_2O$: C, 19.43; H, 3.80; N, 2.52; P, 5.57; Ba, 37.03. Found : C, 19.20; H, 4.07; N, 2.28; P, 5.68; Ba, 37.60.

A solution of I in 1*N* NaOH was refluxed for 7.5 hr. and working-up in the same manner as described above gave the barium salt of II in a yield of 37.2%, $[\alpha]_D^{21}$ +9.1°(c=1.5, H_2O).

---

*3 This determination was performed by Y. Abiko. Experimental details are described in the following papaer.7)

7) Y. Abiko, M. Shimizu : This Bulletin, in press.

8) C. S. Hanes, F. A. Isherwood : Nature, **164**, 1107 (1949).

b) A solution of methyl D-pantothenate (116 mg., 0.5 mmole) and 2-cyanoethyl phosphate[9] (from the barium salt, 161 mg., 0.5 mmole) in pyridine was evaporated to dryness *in vacuo* three times with anhydrous pyridine. The residue was dissolved in anhydrous pyridine (0.5 ml.) and dicyclohexylcarbodiimide (103 mg., 0.5 mmole) was added. The solution was left overnight at room temperature, and water (1 ml.) was added. After 30 min. the mixture was filtered to remove dicyclohexylurea. The filtrate was concentrated to dryness *in vacuo* and the residue was dissolved in water (6.5 ml.) and 2*N* NaOH (3.5 ml.) was added. The solution was heated in a boiling water bath for 40 min., and passed through a column of Amberlite IR 120 (H^+). The column was washed with water. The combined effluent was neutralized with $Ba(OH)_2$ and then worked up as described above to give the barium salt of II (72.8 mg.). Paper chromatography, infrared (IR) spectrum and microbiological activity were identical with those of the sample described above.

c) Phosphorylation of D-pantothenic acid (2.19 g.) with diphenyl phosphorochloridate (7.45 g.) was carried out by the method of King and Strong[3] to give lactone of pantothenic acid 4′-diphenyl phosphate as amorphous powder (3 g., 69.2%). Its infrared spectral data supported the structure proposed by King, *et al.*[3] and then by Hunahashi, *et al.*[10] IR ν_{max}^{KBr} cm^{-1}: 3310, 3060, 2950, 1745, 1670, 1590, 1488, 1323, 1283, 1213, 1188, 1160, 1023, 945, 770~753, 687. *Anal.* Calcd. for $C_{21}H_{24}O_7NP$: C, 58.13; H, 5.57; N, 3.23. Found: C, 57.65; H, 5.30; N, 3.38.

A suspension of the lactone (140 mg.) in 0.5*N* NaOH (4 ml.) was heated under reflux for 1 hr. and the reaction mixture was passed through a column of Amberlite IR 120 (H^+). The effluent was washed with ether, neutralized with $Ba(OH)_2$, concentrated to a small volume, and EtOH was added to precipitate a barium salt (71 mg.). Paper chromatography in solvent I showed that it was a mixture of pantothenic acid 2′,4′-cyclic phosphate (III) (Rf 0.50), II (Rf 0.31) and an unidentified compound (Rf 0.42). IR spectrum of the mixture in KBr exhibited the strong absorption of PO_2^- group (1228, 1083 cm^{-1}) and NMR spectrum showed the signals for the P-O-C-H proton (0.57, 1H, doublet, J=2.5 c.p.s). Refluxing of the lactone in 1*N* NaOH for 3 hr. gave almost the same result.

Hydrolysis of 2′,4′-cyclic phosphate to 4′-phosphate was performed by using $Ba(OH)_2$.[4,11] A solution of the barium salt (60 mg.) in 0.3*N* $Ba(OH)_2$ (13 ml.) was refluxed for 4.5 hr. and then worked up in the same manner as described in a) to give the barium salt of II (31.9 mg.).

The authors wish to express their gratitude to Dr. J. Shinoda, Chairman of the Board of Directors, and Dr. T. Ishiguro, President of this Company for their kind encouragement. The authors are indebted to Mr. B. Kurihara and Miss E. Kosaka for elemental analyses and to Mr. I. Ito for measurement of NMR spectra.

---

9) G.M. Tener: J. Am. Chem. Soc., **83**, 159 (1961).
10) S. Hunahashi, M. Miyano, I. Uritani: Vitamins (Kyoto), **7**, 323 (1954).
11) J. Baddiley, E.M. Thain: J. Chem. Soc., **1951**, 3421.