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80. Osamu Nagase: Investigations on Pantothenic Acid and Its Related Compounds. Ⅳ.*1 Chemical Studies. (3). Syntheses of D-Pantetheine 4′-Phosphate and N-D-Pantothenoyl-L-cysteine 4′-Phosphate.

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D-Pantetheine 4′-phosphate (Ⅵa) and D-pantothenoyl-L-cysteine 4′-phosphate (Ⅵb) was prepared by two different routes. The first involved reaction of D-pantothenonitrile 4′-dibenzyl phosphate (Ⅱ) with cysteamine or L-cysteine followed by acid hydrolysis and then reduction with sodium in liquid ammonia. In the second route, D-pantothenonitrile 4′-phosphate (Ⅶ) was treated with cysteamine or L-cysteine to form thiazoline (Ⅷ), which was then hydrolyzed.

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In the continuation of the preceding study, syntheses of D-pantetheine 4′-phosphate (Ⅵa) and N-D-pantothenoyl-L-cysteine 4′-phosphate (Ⅵb) were undertaken for the purpose of examining the scope of the thiazoline method[1] previously developed and supplying the synthetic substrates for biochemical studies. These two compounds were emphasized by Brown[2] to be the more possible intermediates than D-pantetheine and D-pantothenoyl-L-cysteine in the biosynthetic route from pantothenic acid to coenzyme A. D-Pantetheine 4′-phosphate was originally obtained by acidic or enzymatic decomposition of coenzyme A and identified with Acetobactor-stimulatory factor[3] (ASF). Its chemical synthesis was reported by Baddiley and Thain,[4] and by Moffatt and Khorana.[5] Khorana group used it as one of the starting materials in their total synthesis of coenzyme A. D-Pantothenoyl-L-cysteine 4′-phosphate has not yet been isolated from any natural source, but was chemically synthesized by Baddiley and Mathias.[6]

The starting material for the present syntheses was D-pantothenonitrile 4′-dibenzylphosphate (Ⅱ). Synthesis of Ⅱ was effected in 73.2% yield by direct phosphorylation of D-pantothenonitrile (Ⅰ) with dibenzylphosphorochloridate. Position of the phosphate bond in Ⅱ was confirmed by its conversion to D-pantetheine 4′-phosphate (Ⅵa) as will be described below. Therefore, the 2′-hydroxyl group in Ⅰ seems to be unaffected by this phosphorylating agent owing to steric hindrance of the two neighboring methyl groups as in the case of phosphorylation of D-pantetheine.[5]

Thiazoline ring closure was carried out by the fusion of Ⅱ with 2 equimolar cysteamine (Ⅲa) at 100° to give a pale yellow resinous product under evolution of ammonia. The structure of this substance, though not purified, was presumed to be Ⅳa by the following spectral evidences. It exhibited ultraviolet absorptions consistent with the thiazoline structure.[1] The diester form of phosphoric acid was evidenced by the infrared absorption bands at 1207 and 1047 cm^{-1} corresponding to those of barium dimethylphosphate ($(CH_3O)_2PO_2Ba_{1.5}$) at 1205 and 1053 cm^{-1}. According to Tsuboi,[7]

*1 Part Ⅲ. This Bulletin, **15**, 644 (1967).

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1) M. Shimizu, G. Ohta, O. Nagase, S. Okada, Y. Hosokawa: This Bulletin, **13**, 180 (1965).
2) G.M. Brown: J. Biol. Chem., **234**, 370 (1959).
3) *a*) G.D. Novelli, N.O. Kaplan, F. Lipmann: *Ibid.*, **177**, 97 (1949). *b*) G.D. Novelli, R.M. Flynn, F. Lipmann: *Ibid.*, **177**, 493 (1949).
4) J. Baddiley, E.M. Thain: J. Chem. Soc., **1953**, 1610.
5) J.G. Moffatt, H.G. Khorana: J. Am. Chem. Soc., **81**, 1265 (1959); **83**, 663 (1961).
6) J. Baddiley, A.P. Mathias: J. Chem. Soc., **1954**, 2803.
7) M. Tsuboi: J. Am. Chem. Soc., **79**, 1351 (1957).

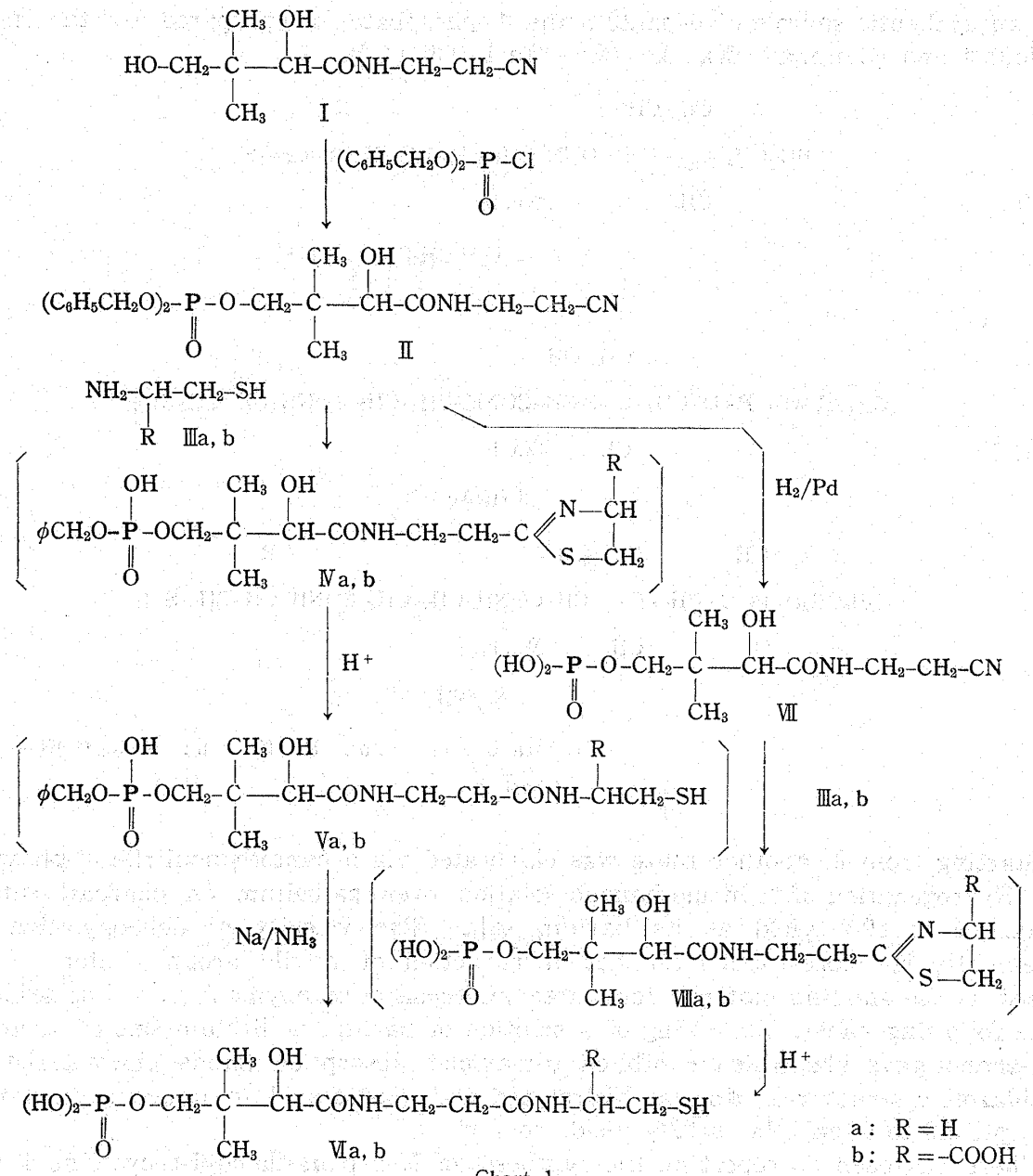

Chart 1.

those absorptions are ascribed to the vibration of PO_2^-. That the diester of the phosphoric acid was not 2,'4'-cyclic phosphate but monobenzyl 4'-phosphate was further evidenced as described below. Apparently, one of the benzyl groups in II was removed by cysteamine in the above thiazoline ring closure, and this fact corresponds to similar results described by Todd, *et al.*[8] Fission of the thiazoline ring was effected by heating IVa with acetic acid in 70% methanolic solution to give a viscous oily product. It did not exhibit ultraviolet absorptions characteristic of the thiazoline structure. The infrared spectrum of its barium salt showed the bands at 1048 and 1206 cm^{-1} assigned to the vibration of PO_2^- group and the bands at 697 and 736 cm^{-1} assigned to CH deformation of benzyl group. Therefore, this substance proved to be Va, though not purified. Debenylation of Va was effected with sodium and liquid ammonia. The product obtained as a barium salt in 73.2% yield from II was identified

8) *a*) V.M. Clark, A.R. Todd: J. Chem. Soc., **1950**, 2023. *b*) J. Baddiley, V.M. Clark, J.J. Michalski, A.R. Todd: *Ibid.*, **1949**, 815.

with an authentic sample of D-pantetheine 4′-phosphate (Ⅵa) prepared by the method of Moffatt and Khorana[5] (Ⅸa→Ⅹa→Ⅺa→Ⅵa in Chart 2).

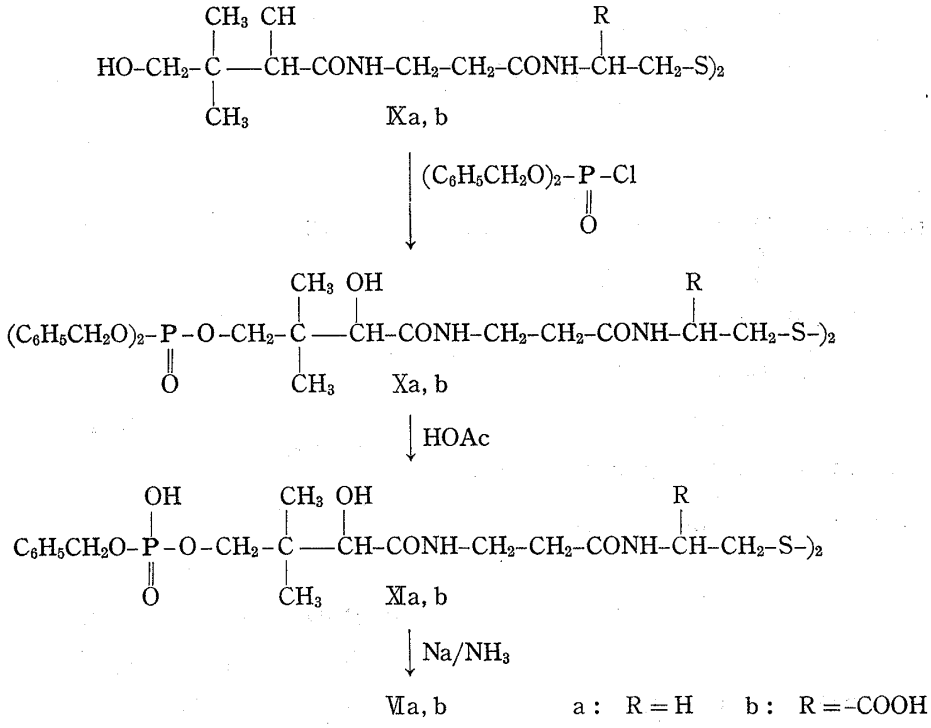

Chart 2.

Starting from Ⅱ, another route was elaborated via D-pantothenonitrile 4′-phosphate (Ⅶ). Hydrogenation of Ⅱ in methanolic solution over palladium on charcoal catalyst afforded Ⅶ in 69% yield as its barium salt. The velocity of debenzylation was conveniently far more rapid than that of reduction of nitrile group. Later, Ⅶ was utilized as the starting material for a total synthesis of coenzyme A as will be described in the following paper. Refluxing of a solution of barium or lithium salt of Ⅶ and Ⅲa in methanol gave Ⅷa, which exhibited ultraviolet absorption bands characteristic to the thiazoline structure. Ⅷa was hydrolyzed with oxalic acid in aqueous solution at about pH 5.0 to afford Ⅵa in 82% yield from Ⅶ.

There has been no report on the synthesis of N-D-pantothenoyl-L-cysteine 4′-phosphate (Ⅵb), except that of Baddiley and Mathias.[6] Starting from S-benzylpantothenoyl-L-cysteine, they prepared the desired compound by phosphorylation with dibenzylphosphorochloridate and debenzylation with sodium and liquid ammonia, but their paper did not give detailed description of physical properties. Prior to the application of the thiazoline method, the reaction sequence with modified phosphorylation of Moffatt and Khorana[5] (Ⅸb→Ⅹb→Ⅺb→Ⅵb in Chart 2) was employed for the purpose of obtaining a standard sample. In order to apply the thiazoline method, the present author used the same reaction as that for the synthesis of di-(D-pantothenoyl)-L-cystine (Ⅸb) in the preceding work.*1 Thiazoline ring closure was effected by refluxing ethanolic solution of Ⅱ with L-cysteine (Ⅲb) in the presence of one molar sodium ethoxide, and was confirmed by ultraviolet and infrared spectra. As in the case of synthesis of Ⅵa, removal of one of the benzyl groups was similarly observed. Ring fission of Ⅳb under acidic conditions followed by debenzylation with sodium and liquid ammonia afforded Ⅵb in 40% yield from Ⅱ. Another route starting from Ⅶ via Ⅷb gave Ⅵb in 63% yield. Three samples of Ⅵb produced by these different methods showed the same Rf value in paper partition chromatography. While the rotation

value of Ⅵb was ±0°, that of its disulfide form was −31°. The synthetic route of the standard sample started from di-(D-pantothenoyl)-L-cystine (Ⅸb) and involved the reduction with sodium and liquid ammonia in the last stage of synthesis. However, the possibility of racemization of cysteine seems to be excluded, because removal of the protecting group with sodium and liquid ammonia is generally considered not to cause racemization during the synthesis of peptides including L-cysteine.[9]

Experimental

Paper Chromatography——Paper chromatography was performed by the ascending technique using Toyo No. 50 paper. The solvent systems used were Solvent I, *n*-BuOH-HOAc-H_2O (5:2:3); Solvent Ⅱ, *n*-PrOH-conc. NH_4OH-H_2O (6:3:1). Phosphorus-containing compounds were located on chromatograms with the Hanes and Isherwood spray[10] followed by ultraviolet irradiation. Thiol compounds were located with the ammonia spray after the nitroprusside spray and disulfide compounds with the nitroprusside spray after the KCN spray. The Rf values of different compounds are shown in Table I.

Table I. Rf Values of Compounds

Compound	Rf	
	Solvent I	Solvent Ⅱ
Pantetheine 4′-phosphate (SH)	0.55	0.26
Pantetheine 4′-phosphate (SS)	0.34	0.25
Pantetheine 2′,4′-cyclic phosphate	0.56	0.63
Pantothenonitrile 4′-dibenzyl phosphate	0.91	
Pantothenonitrile 4′-phosphate	0.45	0.34
Pantetheine 4′-monobenzyl phosphate	0.75	0.73
N-Pantothenoylcysteine 4′-phosphate (SH)	0.46	0.10
N-Pantothenoylcysteine 4′-phosphate (SS)	0.42	0.10
N-Pantothenoylcysteine 4′-monobenzyl phosphate	0.68	0.55

D-Pantothenonitrile 4′-Dibenzyl Phosphate (Ⅱ)——D-Pantothenonitrile (I) (5.0 g., 0.025 mole) was dissolved in anhydrous pyridine (300 ml.) and the solution was cooled to −40° in a Dry Ice-acetone bath. A solution of dibenzyl phosphorochloridate[11] (from 13.1 g., 0.05 mole of dibenzyl phosphite[12] and 6.75 g., 0.05 mole of N-chlorosuccinimide) in benzene (80 ml.) was then added with shaking over a period of 20 min. The mixture was allowed to stand in the bath for 20 hr. during which the temperature arised slowly to −10°. Water (45 ml.) was added and after 15 min. at room temperature the solution was evaporated *in vacuo*. The residual pyridine was removed by several evaporations with MeOH, and the residue was dissolved in EtOAc (200 ml.) and washed three times each with 2*N* H_2SO_4, 10% sodium bicarbonate and saturated sodium sulfate (each 100 ml.). The organic layer was dried over sodium sulfate and evaporated *in vacuo* to give D-pantothenonitrile 4′-dibenzyl phosphate (Ⅱ) (8.6 g., 73.2%) as a thick syrup. For further purification, a 90% methanolic solution of the syrup (0.2 g.) was passed through a column of a mixture of "Amberlite IR 120 (H^+) and IRA 410 (OH^-) (1:1, 1 ml.) and the column was washed with 90% MeOH. The effluent was evaporated to dryness *in vacuo*, the residue was dissolved in ether (10 ml.), and petroleum ether (10 ml.) was added to precipitate an oil, which was collected and dried *in vacuo* over P_2O_5 to give an analytical sample. $[\alpha]_D^{23}$ +19.1° (c=1.1, EtOH). IR $\nu_{max}^{liq.film}$ cm^{-1}: 3340, 2250, 1665, 1523, 1256, 1035, 1015, 1000, 740, 700. UV λ_{max}^{EtOH} mμ (ε): 252 (340), 258 (440), 263.5 (390), 268.5 (260). *Anal.* Calcd. for $C_{23}H_{29}O_6N_2P \cdot 1/2H_2O$: C, 58.84; H, 6.44; N, 5.97. Found: C, 58.72; H, 6.41; N, 5.99.

D-Pantothenonitrile 4′-Phosphate (Ⅶ)——A solution of D-pantothenonitrile 4′-dibenzyl phosphate (Ⅱ) (4.7 g., 0.01 mole) in 90% MeOH (50 ml.) was hydrogenated at room temperature and atmospheric pressure over a 10% palladium-charcoal catalyst (1 g.). Hydrogen was absorbed quite rapidly in first 5 min. and then

9) *a*) J. P. Greenstein: J. Biol Chem., **128**, 241 (1939). *b*) C. W. Roberts, V. du Vigneaud: *Ibid.*, **204**, 871 (1953).
10) C. S. Hanes, F. A. Isherwood: Nature, **164**, 1107 (1949).
11) G. W. Kenner, A. R. Todd, F. J. Weymouth: J. Chem. Soc., **1952**, 3675.
12) O. M. Friedmann, D. L. Klass, A. M. Seligman: J. Am. Chem. Soc., **76**, 916 (1954).

very slowly. After absorption of 520 ml. of H_2 during 10 min., the catalyst was filtered off and the solvent was evaporated *in vacuo*. A solution of the residue in H_2O (50 ml.) was passed through a column of "Amberlite IR 120 (H^+)" (6 ml.) and the column was washed with H_2O. The combined eluate was adjusted to pH 7.3 with 0.1*N* $Ba(OH)_2$ and evaporated to dryness *in vacuo* after removal of a small amount of insoluble material. The residue was dissolved in MeOH (20 ml.), and the filtered solution was concentrated to a volume of approximately 15 ml. *in vacuo*. Ether (180 ml.) was added with stirring to give a precipitate which was dried over P_2O_5 *in vacuo* giving the barium salt of Ⅶ (3.11 g., 68.9%). $[\alpha]_D^{25}$ +11.4° (c=2.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3300, 2250 (C≡N), 1656, 1547, 1535, 1080, 978. *Anal.* Calcd. for $C_9H_{15}O_6N_2PBa \cdot 2H_2O$: C, 23.93; H, 4.24; N, 6.20; P, 6.87. Found: C, 24.14; H, 4.02; N, 6.35; P, 6.95. Lithium salt: $[\alpha]_D^{20}$ +18.2° (c=2.0, H_2O). *Anal.* Calcd. for $C_9H_{15}O_6N_2PLi_2 \cdot 1/2H_2O$: C, 35.90; H, 5.36; N, 9.30. Found: C, 35.95; H, 5.60; N, 9.17.

D-Pantetheine 4′-Phosphate (Ⅵa)——a) A mixture of D-pantothenonitrile 4′-dibenzyl phosphate (Ⅱ) (1.0 g., 2.13 mmoles) and cysteamine (0.36 g., 4.69 mmoles) was heated in N_2 at 100~105° for 30 min., during which evolution of NH_3 was observed, to give pale yellow glass. This is crude thiazoline Ⅳa. UV λ_{max}^{EtOH}: 250 mμ (inflexion). $\lambda_{max}^{1N\ HCl}$: 263 mμ. IR $\nu_{max}^{liq.film}$ cm^{-1}: 3350, 3200, 1652, 1640, 1630, 1207, 1075, 1047, 1020, 738, 697.

A solution of the above crude product in 70% MeOH (30 ml.) was adjusted to pH 4.9 with HOAc and heated in N_2 at 60° for 4 hr., during which a maximal ultraviolet absorption of the thiazoline ring disappeared and the pH of the solution changed to 4.6. After removal of the solvent, the residue was dissolved in 50% MeOH and passed through a column of "Amberlite IR 120 (H^+)" (6 ml.) and the column was washed with 50% MeOH (50 ml.). The eluate was concentrated *in vacuo* and the residue was dried *in vacuo* over P_2O_5 to give almost colorless viscous oil (Va) (850 mg.). IR ν_{max}^{KBr} cm^{-1}: 3290, 1655, 1548, 1530, 1240, 1210, 1032, 1006, 737, 697.

Barium salt of Va: A solution of the oil (0.38 g.) in 70% MeOH was adjusted to pH 7.0 with $Ba(OH)_2$ and concentrated *in vacuo* to a volume of 5 ml. After removal of a trace of insoluble material by filtration, the solution was evaporated to dryness *in vacuo* and the residue was dissolved in MeOH. Filtration and concentration *in vacuo* to a volume of 1 ml. and addition of dry, peroxide-free ether (30 ml.) gave a white precipitate which was reprecipitated to give the barium salt of Va (340 mg.). IR ν_{max}^{KBr} cm^{-1}: 3490, 3260, 3070, 3040, 1660, 1647, 1548, 1533, 1206, 1085, 1048, 1016, 736, 697.

Ⅵ (850 mg.) was dissolved in liquid ammonia (50 ml.) and sodium was added until a blue color persisted. A few drops of MeOH was added to destroy the blue color and ammonia was evaporated by gentle warming. The residue was dissolved in ice water (20 ml.) and "Amberlite IR 120 (H^+)" was added until the solution was acidic. The mixture was then poured into the top of a column of IR 120 (H^+) resin (8 ml.), which was then washed with H_2O. The effluent was brought to pH 7.4 with $Ba(OH)_2$, concentrated *in vacuo* to a volume of about 10 ml. and then filtered, and the filtrate was concentrated to dryness. The residue was dissolved in MeOH (10 ml.), separated from a trace of insoluble material and concentrated *in vacuo* to a small volume (2 ml.). Addition of dry, peroxide-free ether (30 ml.) gave a white precipitate which was collected and dried *in vacuo* over P_2O_5 to give the barium salt of D-pantetheine 4′-phosphate (Ⅵa) (770 mg., 73.2% yield based on Ⅱ). $[\alpha]_D^{23}$ +13.3° (c=2.25, H_2O) (reported +10.8°,[4] +11.7° to 12.8°,[5] +14.6°.[13]) IR ν_{max}^{KBr} cm^{-1}: 3290, 1653, 1565, 1542, 1078, 977. This was chromatographically and infrared spectrophotometrically identical with an authentic sample. Paper chromatography in solvent I showed that it was almost entirely in the thiol form (Rf 0.55), only a trace of the disulfide form (Rf 0.34) being present. *Anal.* Calcd. for $C_{11}H_{21}O_7N_2PSBa$: C, 26.76; H, 4.29; N, 5.68. Found: C, 27.01; H, 4.85; N, 5.20.

An authentic sample of Ⅵa was prepared by the procedure of Moffatt and Khorana,[5] $[\alpha]_D^{23}$ +13.6° (c=1.0, H_2O). *Anal.* Calcd. for $C_{11}H_{21}O_7N_2PSBa \cdot 2H_2O$: C, 24.94; H, 4.76; N, 5.29. Found: C, 25.01; H, 4.68; N, 4.54.

b) A solution of barium salt of Ⅶ (1.25 g., 2.77 mmoles) and cysteamine (0.255 g., 3.3 mmoles) in MeOH (5 ml.) was refluxed under N_2 for 6 hr. The reaction mixture was concentrated *in vacuo* to dryness to give a crude thiazoline (Ⅷa) as a white powder (1.3 g.). UV λ_{max}^{EtOH} mμ: 231.5, 248. $\lambda_{max}^{1N\ HCl}$: 265 mμ. A solution of the above crude product in H_2O (10 ml.) was adjusted to pH 5.0 with 1*N* oxalic acid and heated under N_2 at 60° for 2 hr. After removal of the white precipitate, the reaction mixture was passed through a column of "Amberlite IR 120 (H^+)" (8 ml.), which was then washed with H_2O. The combined effluent was adjusted to pH 7.2 with 0.1*M* $Ba(OH)_2$ and then evaporated *in vacuo* to dryness. The residue was dissolved in MeOH (10 ml.), separated from a trace of insoluble material and concentrated *in vacuo* to a volume of roughly 5 ml. The addition of dry, peroxide-free ether gave a white precipitate which was filtered and dried *in vacuo* over P_2O_5 giving the barium salt of D-pantetheine 4′-phosphate (1.20 g., 82.0% yield based on Ⅶ), $[\alpha]_D^{25}$ + 12.3° (c=2.3, H_2O). The IR spectrum and paper chromatograms were identical with that described above. *Anal.* Calcd. for $C_{11}H_{21}O_7N_2PSBa \cdot 2H_2O$: C, 24.94; H, 4.76; N, 5.29; P, 5.83. Found: C, 25.23; H, 4.51; N, 5.20; P, 5.63.

Disulfide of Ⅵa: To a solution of the barium salt of Ⅵa (247 mg.) in H_2O (10 ml.) was added dropwise aqueous 0.35% H_2O_2 solution until the solution gave no color with nitroprusside reagent. The reaction

13) J. M. Osbond: Brit. Pat., 749,715.

mixture was concentrated *in vacuo* to a volume of roughly 2 ml. Addition of EtOH (20 ml) gave a white precipitate which was collected and reprecipitated to give the barium salt of disulfide of Va (180 mg.), $[\alpha]_D^{20}$ +12.2° (c=1, H_2O). *Anal.* Calcd. for $C_{22}H_{40}O_{14}N_4S_2P_2Ba_2 \cdot 7H_2O$: C, 23.75; H, 4.89; N, 5.04; P, 5.57. Found: C, 23.45; H, 4.57; N, 4.71; P, 5.90.

N-D-Pantothenoyl-L-cysteine 4′-Phosphate (VIb)——a) An aqueous solution of the barium salt of N,N′-di-D-pantothenoyl-L-cystine (Xb, dihydrate, 2.0 g., 2.46 mmoles) was passed through a column of "Amberlite IR 120 (H^+)" (13 ml.) and the column was washed with water (150 ml.). The combined effluent was concentrated to dryness *in vacuo*. The residue was dried by three evaporations with anhydrous pyridine and dissolved in anhydrous pyridine (50 ml.). The solution was cooled to −36～−38° in a Dry Ice-acetone bath and a solution of dibenzyl phosphorochloridate (from 2.58 g., 9.84 mmoles of dibenzyl phosphite and 1.33 g. of N-chlorosuccinimide) in benzene (20 ml.) was added dropwise with shaking. It was allowed to stand in the bath for 1 hr. and then at room temperature for 13 hr. H_2O (8 ml.) was added and after 10 min. the solution was evaporated *in vacuo*. The residual pyridine was removed by several evaporations with MeOH. 2*N* H_2SO_4 (30 ml.) was added to the residue and the mixture was extracted with EtOAc (50, 20, 10 ml.). From the EtOAc-extract was obtained dibenzyl phosphate (1.96 g.). The aqueous layer containing an insoluble resinous oil was then extracted with methyl ethyl ketone (50, 20, 10 ml.). The organic layer was washed with saturated sodium sulfate, dried over sodium sulfate and evaporated *in vacuo* to give a pale yellow thick oil (0.84 g.). It was dissolved in HOAc (20 ml.), H_2O (20 ml.) added and the mixture heated on a boiling water-bath for 1 hr. The solvent was evaporated *in vacuo* and the residual HOAc was removed by several evaporations with MeOH. The residue (0.82 g.) was suspended in liquid ammonia (30 ml.) and sodium was added until a blue color persisted. MeOH was added to destroy the blue color and then ammonia was evaporated. The residual white powder was dissolved in ice water (30 ml.) and IR 120 (H^+) resin (12 ml.) was added until the solution was acidic. The mixture was applied to the top of a column of IR 120 (H^+) resin (8 ml.). The column was washed with H_2O (10 ml.). The eluate was adjusted to pH 7.0 with aqueous $Ba(OH)_2$ solution and filtered. The filtrate was concentrated to dryness *in vacuo* and the residue was redissolved in H_2O, filtered and concentrated to a volume of 1 ml. Addition of EtOH (10 ml.) gave white powder which was reprecipitated from H_2O with EtOH and then from H_2O with MeOH to give the barium salt of N-D-pantothenoyl-L-cysteine 4′-phosphate (0.32 g., 10.5% yield), $[\alpha]_D^{25}$ −7° (c=2, H_2O). It was reduced for 3 hr. with 50% aqueous 2-mercaptoethanol. Evaporation of the reaction mixture *in vacuo* and precipitation of the residue from H_2O with MeOH gave a pure sample of the barium salt of VIb, $[\alpha]_D^{15}$ +0° (c=2, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3370, 3250, 3090, 2920, 1643, 1585, 1545, 1395, 1120, 1080, 975. *Anal.* Calcd. for $C_{12}H_{20}O_9N_2PSBa_{1.5} \cdot H_2O$: C, 23.12; H, 3.56; N, 4.49. Found: C, 23.57; H, 3.87; N, 4.24.

b) To a solution of L-cysteine hydrochloride monohydrate (1.11 g., 6.3 mmoles) in EtOH (20 ml.) was added a solution of sodium ethoxide (from 0.29 g., 12.6 mmoles of Na) in EtOH (30 ml.). D-Pantothenonitrile 4′-dibenzyl phosphate (II) (1.41 g., 3 mmoles) in EtOH (10 ml.) was then added and the mixture was refluxed under N_2 for 9 hr. The insoluble material was filtered off and the filtrate was evaporated to dryness *in vacuo* to give crude IVb (2.11 g.), pale yellow amorphous powder. UV λ_{max}^{EtOH} mμ: 235, 248. $\lambda_{max}^{1N\ HCl}$: 264 mμ. IR ν_{max}^{KBr} cm^{-1}: 3320, 3070, 3040, 2940, 1660, 1645, 1630, 1610, 1530, 1398, 1225, 1095, 1080, 1043, 1020, 863, 735, 697.

A 70% aqueous methanolic solution of the crude IVb (2.1 g.; 60 ml.) was adjusted to pH 5.0 with HOAc and heated under N_2 at 60° for 2 hr. The reaction mixture was concentrated to dryness *in vacuo* and dissolved in 50% MeOH (60 ml.). The solution was passed through a column of IR 120 (H^+) resin (15 ml.) and the column was washed with 50% MeOH. The effluent was concentrated to dryness *in vacuo* to afford crude Vb (1.15 g.) as pale yellow powder. IR ν_{max}^{KBr} cm^{-1}: 3330, 3070, 3040, 2950, 1730, 1657, 1550, 1533, 1210, 1010, 738, 698.

The crude Vb was dissolved in liquid ammonia (40 ml.) and sodium was added until a blue color persisted. A few drops of MeOH was added to destroy the blue color and ammonia was evaporated. The residue was then worked up in the same way as described above to give the barium salt of VIb (0.75 g., 40.1% yield from II), $[\alpha]_D^{15}$ −5° (c=2, H_2O). This was reduced with 2-mercaptoethanol to yield a pure sample, $[\alpha]_D$ +0° (c=2, H_2O). It was identical with a sample described above in comparison of IR spectrum and paper chromatography. *Anal.* Calcd. for $C_{12}H_{20}O_9N_2SPBa_{1.5} \cdot H_2O$: C, 23,12; H, 3.56; N, 4.49. Found: C, 23.41; H, 4.09; N, 4.42.

c) To a solution of L-cysteine hydrochloride monohydrate (3.13 g., 17.8 mmoles) in MeOH (30 ml.) was added a solution of sodium methoxide prepared from Na (0.82 g., 35.6 mmoles) and MeOH (30 ml.). After removal of separated NaCl by filtration, barium salt of D-pantothenonitrile 4′-phosphate (VII) (5.0 g., 10.7 mmoles) in MeOH (70 ml.) was added. The mixture was refluxed under N_2 for 9 hr. Separated VIIIb (5.26 g.) was filtered. Concentration of the mother liquor gave an additional crop (2.58 g.). For purification, the first crop of VIIIb (300 mg.) was dissolved in H_2O (20 ml.) and passed through a column of IR 120 (Ba^{++}) (10 ml.). The eluate was evaporated to dryness *in vacuo* to yield the barium salt of VIIIb as white amorphous powder (320 mg.). $[\alpha]_D^{20}$ +0° (c=2, H_2O). UV $\lambda_{max}^{1N\ HCl}$: 265 mμ (ε 4130). $\lambda_{max}^{H_2O\ (pH\ 7)}$ mμ: 238, 250. IR ν_{max}^{KBr} cm^{-1}: 3360, 2960, 1640, 1588, 1535, 1400, 1080, 975. Paper chromatography in 70% EtOH showed

one spot (Rf 0.40). *Anal.* Calcd. for $C_{12}H_{18}O_8N_2PSBa_{1.5}\cdot 4H_2O$: C, 21.86; H, 3.97; N, 4.25. Found : C, 21.99; H, 4.00; N, 4.64.

A solution of the above crude product (Ⅷb, 7.4 g.) in H_2O (70 ml.) was adjusted to pH 5.0 with 1*M* oxalic acid and the separated barium oxalate was filtered off. The filtrate was heated under N_2 at 55° for 40 min., while a maximal ultraviolet absorption of the thiazoline ring disappeared and the pH of the solution changed to 4.2. The solution was filtered through a column of "Amberlite IR 120 (H^+)" (40 ml.) and the column was washed with water (300 ml.). The eluted solution was neutralized to pH 7.0 with 0.1*M* $Ba(OH)_2$ and concentrated *in vacuo* to a volume of 15 ml. Addition of MeOH (200 ml.) separated a white precipitate (5.3 g.) which was collected and reprecipitated from H_2O with CH_3OH to give barium salt of Ⅵb (4.4 g., 63.8% yield from Ⅶ), $[\alpha]_D^{20}$ +0° (c=2, H_2O). It was identical with the sample described above in all respect.

Disulfide of Ⅵb : The barium salt of Ⅵb was oxidized with H_2O_2 by usual way. The product was precipitated from H_2O with MeOH to give the barium salt of disulfide of Ⅵb, $[\alpha]_D^{20}$ −31° (c=2, H_2O). *Anal.* Calcd. for $C_{24}H_{38}O_{16}N_4P_2S_2Ba_3\cdot 2H_2O$: C, 23.16; H, 3.40; N, 4.50. Found : C, 23.12; H, 4.15; N, 4.66.

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