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Investigations on Pantothenic Acid and Its Related Compounds. XIII.[1] Chemical Studies. (6).[2] Syntheses of Homopantethine and Some Other Pantethine Analogs[3]

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Four pantethine analogs (homopantethine (VII), α-methyl-(XIIIb) and β-methyl-pantethine (XIIIc), and oxypantetheine (XVI)) were prepared. Synthesis of VII from D-pantothenonitrile (I) and homocysteamine by using the thiazine derivative (III) as an intermediate was established. XIIIb and XIIIc were synthesized by the thiazoline method described previously, and XVI by the general method.

Cysteamine moiety of coenzyme A (CoA) contains the functional group for enzymatic transacylation reactions. Prior to the attempted structural modification on this moiety in CoA, we undertook to synthesize some corresponding pantetheine or patethine analogs and to examine their microbiological activity. There have so far been reported several papers about such compounds: *e.g.* homopantethine (VII),[5] oxypantetheine (XVI),[6] selenopantethine[7] and so on.[6]

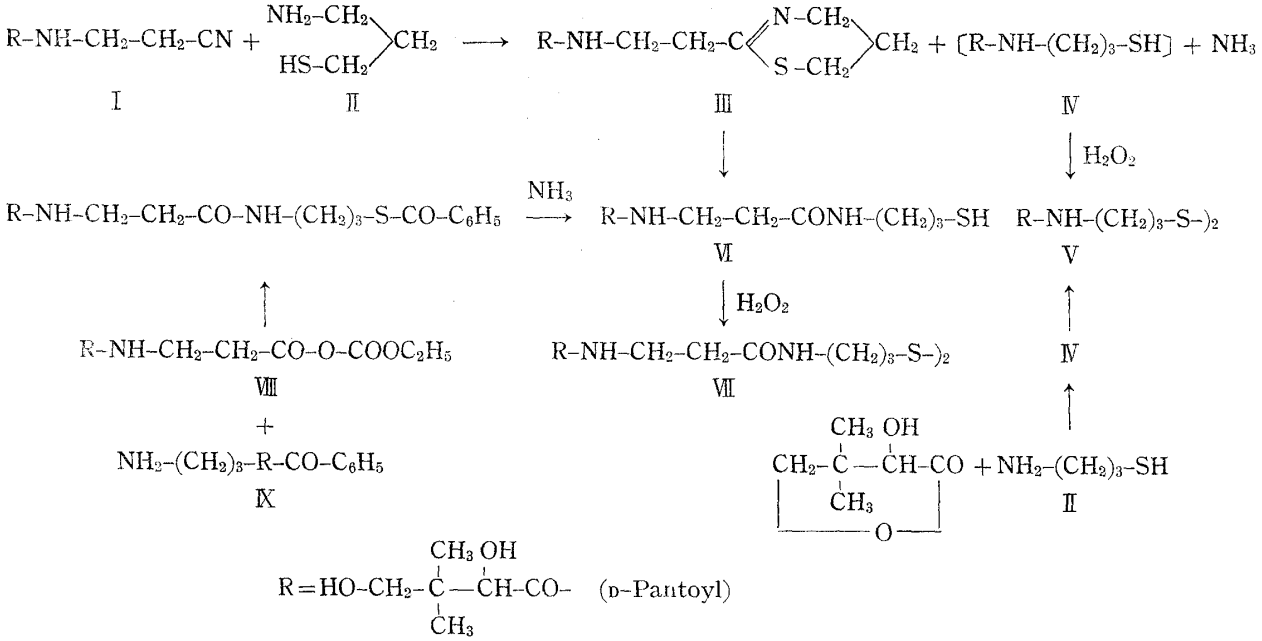

Chart 1. Synthesis of Homopantethine

1) Part XII: T. Suzuki, Y. Abiko, and M. Shimizu, *J. Biochem.* (Tokyo), **62**, 642 (1967).
2) Part (5): S. Okada, O. Nagase, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **15**, 711 (1967).
3) A part of this work was presented at the General Meeting of Pharmaceutical Society of Japan, April, 1967, Kyoto.
4) Location: *Minamifunabori-cho, Edogawa-ku, Tokyo.*
5) E. Felder, L. Fumagalli, and D. Pitré, *Helv. Chim. Acta*, **46**, 752 (1963).
6) *a*) C.J. Stewart, V.H. Cheldelin, and T.E. King, *J. Biol. Chem.*, **215**, 319 (1955). *b*) J. Baddiley and A.P. Mathias, *J. Chem. Soc.*, **1954**, 2803. *c*) J.A. Moore and E.L. Wittle, U.S. Patent 2807644 (1957).
7) W.H.H. Günther and H.G. Mautner, *J. Am. Chem. Soc.*, **82**, 2762 (1960).

In Part I of this series,[8] a novel synthesis of pantethine from pantothenonitrile was established through the intermediate formation of thiazoline derivative followed by hydrolysis thereof (hereafter generally referred to as the thiazoline method). (I→XIa→XIIa→XIIIa in Chart 2). It interested us to extend this method from five membered intermediate to six membered one in expectation of elaborating more convenient synthesis of homopantethine. As to the published synthetic method of homopantethine (VII), there has been only one by Felder, *et al.*[5] which is based on the reaction of ethyl pantothenyl carbonate (VIII) with S-acylhomocysteamine (IX) as shown in Chart 1. In accordance with the case of thiazoline closure, refluxing a solution of D-pantothenonitrile (I) and homocysteamine (3-mercaptopropylamine) (II) in ethanol afforded two different substances after partition chromatography over Celite. The faster eluting substance gave color characteristic of SH group on paper chromatogram, and its oxidized product with alkaline hydrogen peroxide was identified with N,N'-di-D-pantoylhomocystamine (V) by comparison with the authentic sample. The SH compound(D-pantoylhomocysteamine (IV)) was thought to have been yielded by transacylation between I and II during the reaction time. The slower eluting substance gave no instantaneous color with nitroprusside-KCN on paper chromatogram. The viscous oily substance purified from this portion was confirmed to be 2-(2-D-pantamidoethyl)-5,6-dihydro-4*H*-1,3-thiazine (III) by its ultraviolet (λ_{max}^{MeOH}: 235 mμ ($\varepsilon=5.0\times10^3$), $\lambda_{max}^{MeOH\text{-}HCl}$: 251 mμ ($\varepsilon=7.6\times10^3$))[9] and infrared spectra (ν_{max}^{KBr}: 1633 cm^{-1} (C=N)).[10] Ratio of III to IV in the yield was about 2:1. Formation of 2-alkyl-5,6-dihydro-4*H*-1,3-thizaine from alkylnitrile and homocysteamine has not been published, though not a few other methods known.[11]

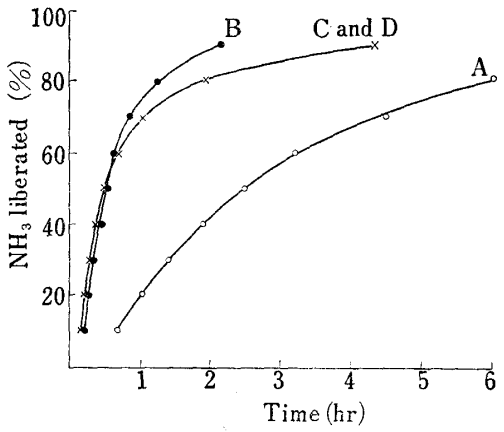

Fig. 1. Rate of Thiazine or Thiazoline Ring Closure

—○— A: I+$NH_2CH_2CH_2CH_2SH$ (1.2 equivalents)
—●— B: I+$NH_2CH_2CH_2CH_2SH$ (1.2 equivalents)+HCl (0.1 equivalent)
—×— C: I+$NH_2CH_2CH_2SH$ (1.2 equivalents)
—×— D: I+$NH_2CH_2CH_2SH$ (1.2 equivalents)+HCl (0.1 equivalent)

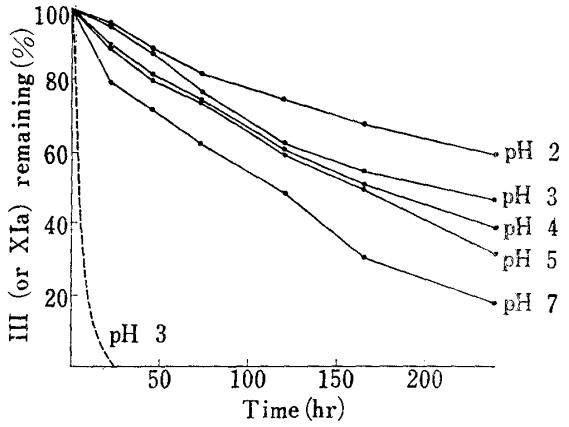

Fig. 2. Stability of III in Aqueous Solutions at 25°

———— : thiazine compound (III)
- - - - - - : thiazoline compound (XIa)[8]

The corresponding secondary product by transacylation had not been obtained in the case of thiazoline ring closure.[8] In order to examine this difference and to improve the yield of VII, the experimental conditions were studied in various ways. As the result, addition of a minute amount of hydrochloric acid in the reaction mixture was found very effective in repressing formation of IV. Therefore, the rates of five and six membered ring closure

8) M. Shimizu, G. Ohta, O. Nagase, S. Okada, and Y. Hosokawa, *Chem. Pharm. Bull.* (Tokyo), **13**, 180 (1965).
9) R.B. Martin and A. Parcell, *J. Am. Chem. Soc.*, **83**, 4830 (1961).
10) A.I. Meyers, *J. Org. Chem.*, **26**, 218 (1961).
11) R.C. Elderfield and E.E. Harris, "Heterocyclic Compounds," Vol. 6, Interscience Publishers, Inc., New York, N.Y., 1957, p. 604.

were investigated by measuring the amount of ammonia liberated in the presence or absence of hydrochloric acid as shown in Fig. 1. Addition of hydrochloric acid did not affect the rate of thiazoline ring closure, whereas that of dihydrothiazine ring closure was conspicuously accelerated. In the absence of hydrochloric acid, the latter was far slower than the former. Formation of IV by transacylation was presumably caused due to slowness of the desired main reaction.

As in the case of thiazoline, preliminary test for stability of III at various acidities was performed by measuring the decrease of ultraviolet absorption as shown in Fig. 2. Hydrolysis of III proceeded most rapidly at pH 7.0. But the rate of hydrolysis at this pH value was shown to be about 10 times slower than that obtained for the corresponding thiazoline derivative (XIa) at pH 3. This results agreed in principle with the experiment of Martin, *et al.*[9] For the purpose of preparing homopantetheine (VI), an aqueous solution of III was adjusted to pH 7.0 with oxalic acid and heated at 60° under nitrogen for 17 hours until the disappearence of ultraviolet absorption. At the end point of the reaction time, pH value of the reaction mixture was lowered to 3.65 due to the disappearence of basic structure (III). As far as this experimental condition was employed, the formation of S-acyl compound was not observed. Homopantetheine (VI) was oxidized to homopantethine (VII) with alkaline hydrogen peroxide. Purification with ion exchange resin afforded colorless glassy substance, properties of which were in good agreement with those reported by Felder, *et al.*[5] To sum up the above result, the standardized synthetic method of homopantethine is as follows. Condensation of I with II is carried out in the presence of 0.1 equimolar hydrochloric acid. The product, without isolation, is hydrolyzed and oxidized to give homopantethine in 57% yield based on I.

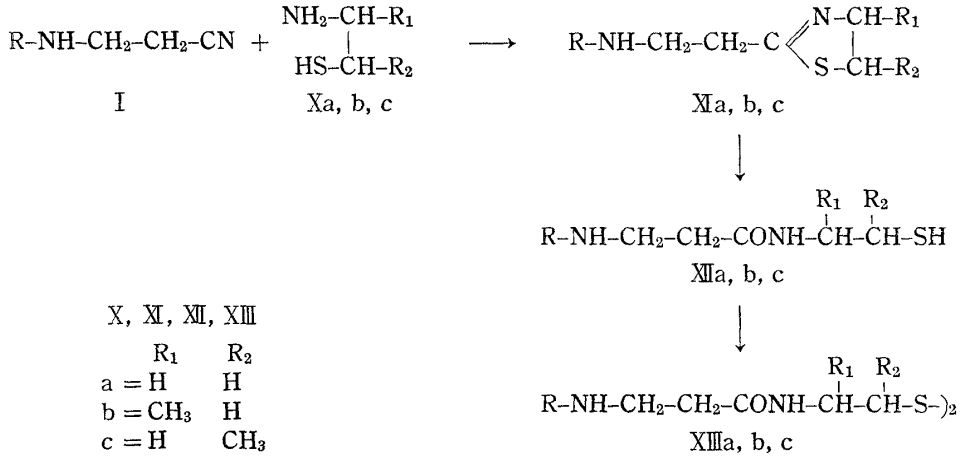

Chart 2. Syntheses of α- and β-Methylpantethine

Pantethine derivatives having methyl group as side chain on cystamine moiety were prepared by application of the thiazoline method as shown in Chart 2. In place of cysteamine (Xa), α-methylcysteamine (*dl*-2-amino-1-propanethiol) (Xb) and β-methylcysteamine (*dl*-1-amino-2-propanethiol) (Xc) were condensed with D-pantothenonitrile (I) followed by hydrolysis and oxidation to yield diastereoisomeric α-methyl- (XIIIb) and β-methylpantethine (XIIIc), respectively, both of which gave one spot on paper chromatogram and satisfactory analytical values.

Condensation of I with 2-aminoethanol (XIV) to obtain oxypantetheine (XVI) through oxazoline intermediate (XV), in accordance with the thiazoline method, was attempted but in vain. The only product obtained was N-D-pantoyl-2-aminoethanol (XVII) yielded possibly by transacylation. Therefore, XVI was synthesized by the general method based on peptide synthesis as shown in Chart 3. This compound had been already synthesized by Stewart, *et al.*[6a] and described to have only 85% purity owing to the contamination of D-pantolactone.

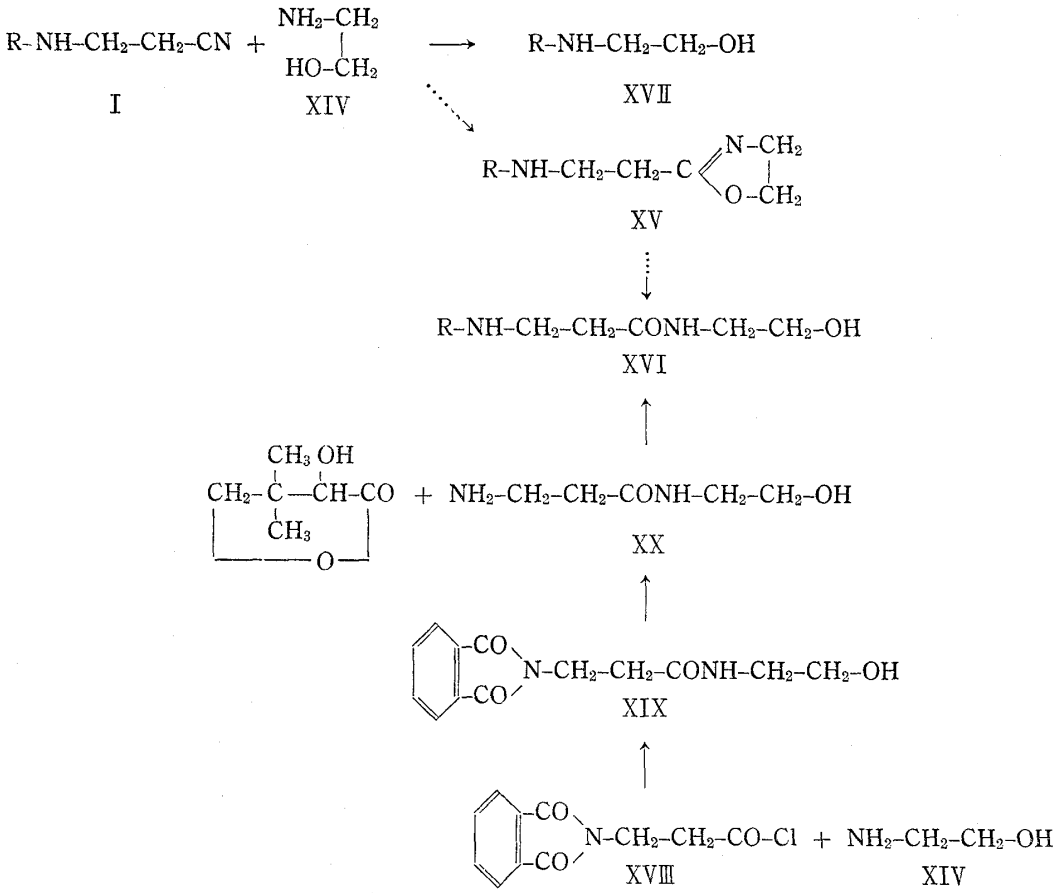

Chart 3. Synthesis of Oxypantetheine

Our sample ($[\alpha]_D^{24}$ +19.9°) proved paper chromatographically and infrared spectrographically homogeneous after purification with ion exchange resin.

Four pantethine or pantetheine analogs described above were examined on their microbiological properties using *Lactobacillus bulgaricus* B1, details of which will be reported in the following paper.

Experimental[12]

Paper Chromatography——Ascending paper chromatography (PPC) on Toyo Roshi No. 50 paper was carried out in the following solvent systems: solvent I, BuOH saturated with water; solvent II, methyl ethyl ketone saturated with water. Thiol compounds were detected on chromatograms by the ammonia spray after the nitroprusside spray, and disulfide compounds by the nitroprusside spray after the KCN spray. Compounds containing amino or amide functions were detected by the ninhydrin spray followed by heating. The *Rf* values of all compounds described are shown in Table I.

3-Amino-1-propanethiol (Homocysteamine) (II)——The free base of II was prepared from 3-chloropropylamine and sodium hydrogensulfide by the procedure of Turk, *et al.*[13] Freshly distilled product was used in all experiments reported here, bp 154—156°, mp 98—105° (reported[13] bp 156°, mp 97—105°).

2-(2-D-Pantamidoethyl)-5,6-dihydro-4*H*-1,3-thiazine (III)——A solution of D-pantothenonitrile (I) (2.0 g) and II (1.09 g) in EtOH (12 ml) was refluxed under N_2 for 8 hr, during which an evolution of ammonia was observed. Concentration of the reaction mixture afforded an oily substance (3.06 g). The product (2 g) was mixed with Celite (535) (5 g), and the mixture was applied to the top of a column prepared from Celite (300 g) and H_2O (300 ml) saturated with methyl ethyl ketone (MEK)–methyl isobutyl ketone (MBK) (1:1), and eluted with MEK–MBK (1:1) saturated with H_2O. Fractions of each 10 ml were collected. Eluate of fraction Nos. 75—115 was evaporated *in vaccuo* and the residue was dissolved in MeOH. The solution was

12) All melting points are uncorrected. Elemental analyses were performed by Mr. B. Kurihara, Miss E. Kosaka and Miss K. Takahashi in this Laboratory.

13) S.D. Turk, R.P. Louthon, R.L. Cobb, and C.R. Bresson, *J. Org. Chem.*, **27**, 2846 (1962).

Table I. *Rf* Values of Compounds

Compound	*Rf*	
	Solvent I	Solvent II
Homocysteamine	0.43	0.78
Homocystamine	0.38	0.43
2-(2-Pantamidoethyl)-5,6-dihydro-4*H*-1,3-thiazine	0.79	0.58
Homopantetheine	0.76	0.58
Homopantethine	0.72	0.26
N-Pantoylhomocysteamine		0.83
N,N'-Dipantoylhomocystamine	0.83	0.82
2-(2-Pantamidoethyl)-4-methyl-2-thiazoline	0.86	0.91
α-Methylpantetheine	0.82	0.82
α-Methylpantethine	0.81	0.48
2-(2-Pantamidoethyl)-5-methyl-2-thiazoline	0.82	0.92
β-Methylpantetheine	0.75	0.76
β-Methylpantethine	0.73	0.48
Oxypantetheine	0.54	0.26
N-Pantoyl-2-aminoethanol	0.67	0.49

filtered and evaporated to dryness to give III (632 mg) as viscous oil. $[\alpha]_D^{27}$ +28.2° (c=1.0, MeOH). UV λ_{max}^{MeOH}: 235 mμ (ε 5000), $\lambda_{max}^{1N\,HCl}$: 251 mμ (ε 7600). IR ν_{max}^{KBr} cm^{-1}: 1650 (amide I), 1633 (C=N), 1527 (amide II), 1074, 1044 (C-O). *Anal.* Calcd. for $C_{12}H_{22}O_3N_2S\cdot\frac{1}{2}CH_3OH$: C, 51.70; H, 8.33; N, 9.65. Found: C, 51.39; H, 8.13; N, 9.37.

The residue (370 mg) obtained from fraction Nos. 35—56 was dissolved in H_2O, and the solution was passed through a column of Amberlite IR 120 (H^+). Evaporation of the effluent gave a thiol compound (IV) (250 mg.), pale yellow oil. An aqueous solution of IV was adjusted to pH 8.8 with 10% NH_4OH, and 3% H_2O_2 was added until the solution was free of thiol (nitroprusside test). The reaction mixture was passed through a column of a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-) and the column was washed with H_2O. The combined effluent was evaporated *in vacuo* and the residue was dissolved in a small volume of MeOH. Addition of ether gave an oily precipitate, which was collected and dried *in vacuo* to give N,N'-di-D-pantoylhomocystamine (V), colorless glass. The IR spectrum and PPC were identical with those of an authentic sample described below. *Anal.* Calcd. for $C_{18}H_{36}O_6N_2S_2$: N, 6.36. Found: N, 6.56.

An authentic sample of V was prepared by fusion of D-pantolactone (1.3 g) with II (0.91 g) at 90° for 2.5 hr, and by oxidation of the product with 3% H_2O_2 in aqueous solution. The oxidized product was purified as described above. *Anal.* Calcd. for $C_{18}H_{36}O_6N_2S_2$: C, 49.06; H, 8.24; N, 6.36. Found: C, 48.50; H, 8.51; N, 6.34.

Rate of Thiazine or Thiazoline Ring Closure——Pantothenonitile (I) (2.00 g, 10 mmoles) and homocysteamine (II) (1.09 g, 12 mmoles) or cysteamine (Xa) (0.96 g, 12 mmoles) and EtOH (10 ml) were placed in a 30 ml round-bottomed flask, fitted with a reflux condenser and an inlet bute extending nearly to the surface of the contents. To the upper end of the condenser was attached a tube leading to a flask containing H_2O(40 ml), 1 N HCl(factor=1.088; 0.92 ml, 1 mmole), and one drop of methyl orange reagent for the absorption of ammonia. A current of N_2 was passsed into the reaction flask, which was set in an oil bath maintained at 100°. The reaction mixture was stirred and refluxed gently. Liberated ammonia was led into the absorption flask, and absorbed in HCl solution with stirring. When the contents of the absorption flask turns a yellow color, a further 1 N HCl (1 mmole) was added. Thus, the amount of ammonia liberated was plotted against time. Experiment in the presence of hydrochloric acid was performed as described above except that 35% HCl (0.1 ml) was added to the reaction system. Results are shown in Fig. 1.

Stability of III in Aqueous Solution——The rate of disappearance of III was followed by measuring the decrease of ultraviolet absorption maximum. The procedure was carried out at room temperature (25°) and at various pHs such as pH 2,3,4,5, and 7 in different periods ranging to 240 hr. Wave lengths of maximal absorption are 251 mμ at pH 2—4, 247 mμ at pH 5, and 238 mμ at pH 7. Fig. 2 shows the time courses of decrease of III in various acidities.

D-Homopantetheine (VI)——The following procedure was carried out in the stream of N_2. An aqueous solution of III (264 mg) was neutralized to pH 7.0 with 2 N oxalic acid and heated at 60° for 17 hr, during which pH of the solution changed to 3.65. The reaction mixture was passed through a column of a mixture of Amberlite IR 4B (OH^-) and IRC 50 (H^+) resin (each 1 ml) and the column was washed with H_2O. The combined effluent was evaporated *in vacuo* giving a pale yellow oil (206 mg), which was further purified by precipitation from methanolic solution with ether. The separated oil was dried *in vacuo* over P_2O_5 to yield homopantetheine (VI) (190 mg). $[\alpha]_D^{22}$ +26.3° (c=1.0, MeOH). IR ν_{max}^{KBr} cm^{-1}: 2550 (SH), 1653 (amide I),

1535 (amide II), 1078, 1042 (C–O). *Anal.* Calcd. for $C_{12}H_{24}O_4N_2S$: C, 49.29; H, 8.27; N, 9.58. Found: C, 50.02; H, 8.51; N, 8.99.

D-Homopantethine (VII)——a) A solution of VI (120 mg) in H_2O (3.5 ml) was adjusted to pH 8.6 with 10% NH_4OH, and 3% H_2O_2 was added under ice-cooling until the reaction mixture gave no further color with sodium nitroprusside reagent. The reaction mixture was passed through a column of a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-) (each 1.5 ml). The eluted solution was evaporated *in vacuo* to dryness, the residue (89 mg) was precipitated from MeOH solution with AcOEt. The separated oil was dried *in vacuo* over P_2O_5 to give homopantethine (VII) (76 mg), colorless galss. $[\alpha]_D^{22}$ +27.9° (c=1.0, MeOH) (reported[5] $[\alpha]_D^{23}$ +24.4°). *Anal.* Calcd. for $C_{24}H_{46}O_8N_4S_2 \cdot \frac{1}{2}H_2O$: C, 48.71; H, 8.01; N, 9.47. Found: C, 48.88; H, 7.84; N, 9.01.

b) To a solution of I (7.0 g) and II (3.85 g) in iso-PrOH (30 ml) was added 35% HCl (0.35 ml) and the mixture was refluxed under N_2 for 6 hr. Evaporation of the solvent *in vacuo* gave the crude thiazine (III) (11.0 g). A solution of this crude thiazine in H_2O (40 ml) was adjusted to pH 7.0 with 1 N oxalic acid, and heated at 60° for 15 hr. The solution was cooled with ice-water, adjusted to pH 8.2 with 10% NH_4OH, and oxidized with 3% H_2O_2 until it no longer colored sodium nitroprusside reagent. The reaction mixture was passed through a column of a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-) (each 30ml). The eluate and washings were evaporated *in vacuo* to give 5.8 g (56.8%) of VII. The IR spectrum was identical with that described above. PPC showed that it contained a trace of the mixed disulfide of homopantetheine and pantoylhomocysteamine.

***dl*-2-Amino-1-propanethiol (*α*-Methylcysteamine) (Xb)**——Preparation of 4-methyl-2-thiazolidinethione from 2-aminopropanol and carbon disulfide followed the method of Bluestone[14] for the synthesis of 5-methyl-2-thiazolidinethione. It had mp 96—98° (reported[15] mp 98.5—99°). A mixture of 4-methyl-2-thiazolidinethione (0.5 g) and 35% HCl (3 ml) was heated in a sealed tube at 165° for 13 hr, and then evaporated *in vacuo*. Recrystallization of the residue from EtOH gave the hydrochloride of Xb, needles, mp 94—97° (reported[16] mp 90—94°). *Anal.* Calcd. for $C_3H_{10}NSCl$: C, 28.23; H, 7.90; N, 10.98. Found: C, 28.27; H, 7.96; N, 10.88.

***dl*-1-Amino-2-propanethiol (*β*-Methylcysteamine) (Xc)**——The hydrochloride of Xc was prepared from 5-methyl-2-thiazolidinethione[14] (5 g) and 35% HCl (30 ml) by the procedure of Gabriel, *et al.*[17] Recrystallization of the product from EtOH gave a pure sample, needles, mp 84—88° (reported mp 87—88°,[17] mp 91.5—92.5°[13]).

***α*-Methylpantethine (XIIIb)**——To a solution of sodium ethoxide prepared from Na (69 mg) and EtOH (15 ml) was added hydrochloride of Xb (382 mg), and after stirring I (500 mg) was added. The mixture was heated at reflux temperature under N_2 for 6 hr. Separated NaCl was filtered off and the filtrate was concentrated *in vacuo*. The residue was dissolved in acetone (15 ml), insoluble material was filtered, and the filtrate was evaporated to dryness to afford the crude thiazoline (XIb) (620 mg). UV λ_{max}^{EtOH} mμ: 228.5, 247. $\lambda_{max}^{1N\,HCl}$: 265 mμ. IR $\nu_{max}^{liq.\,film}$ cm^{-1}: 1650 (amide I), 1630 (C=N), 1528 (amide II), 1075, 1043 (C–O), 1016 (thiazoline ring).

A solution of the crude thiazoline (620 mg) in H_2O (5 ml) was adjusted to pH 5.2 with 1 N oxalic acid, and heated under N_2 at 60° for 4 hr to yield *α*-methylpantetheine (XII). The resultant solution was adjusted to pH 8.0 with 10% NH_4OH, and oxidized with 3% H_2O_2 until it no longer colored sodium nitroprusside reagent. The reaction mixture was filtered through a column of a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-). Evaporation of the eluted solution *in vacuo* and drying of the residue over P_2O_5 afforded *α*-methylpantethine (XIIIb) (480 mg). For further purification, the product was dissolved in EtOH, AcOEt was added and the precipitated oil was dried *in vacuo* over P_2O_5 to give a pure sample, colorless amorphous powder. $[\alpha]_D$ +16.5° (c=1.9, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1650 (amide I), 1545, 1525 (amide II), 1077, 1037 (C–O). *Anal.* Calcd. for $C_{24}H_{46}O_8N_4S_2$: C, 49.46; H, 7.96; N, 9.61. Found: C, 49.34; H, 8.10; N, 9.84.

***β*-Methylpantethine (XIIIc)**——A solution of I (2.0 g) and Xc (prepared from the hydrochloride (1.53 g) and Na (0.276 g)) in EtOH (15 ml) was refluxed under N_2 for 6 hr. Treatment of the reaction mixture as described for XIb gave the crude thiazoline (XIc) (2.81 g). UV λ_{max}^{EtOH} mμ: 230, 246—247 (inflexion). $\lambda_{max}^{1N\,HCl}$: 263.5 mμ. IR $\nu_{max}^{liq.\,film}$ cm^{-1}: 1625 (C=N), 965 (thiazoline ring).

A solution of the crude thiazoline (1.97 g) in H_2O (10 ml) was adjusted to pH 5.1 with 1 N oxalic acid, and heated at 60° for 3 hr. The reaction mixture was filtered through a column of Amberlite CG 4B (OH^-). and CG 50 (H^+), and the eluted solution was concentrated *in vacuo* to yield *β*-methylpantetheine (XIIc) (2.0 g). Purification of the product by precipitation from EtOH solution with peroxide-free ether gave an analytical sample, colorless viscous oil. $[\alpha]_D^{25}$ +18.9° (c=2.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 2550 (SH). *Anal.* Calcd. for $C_{12}H_{24}O_4N_2S$: C, 49.27; H, 8.27; N, 9.58. Found: C, 49.06; H, 8.10; N, 9.57.

14) H. Bluestone, U.S. Patent 2860962 (1958).
15) S. Gabriel and H. Ohle, *Ber.*, **50**, 814 (1917).
16) M. Böse, *Ber.*, **53**, 2001 (1920).
17) S. Gabriel and E. Leupold, *Ber.*, **31**, 2838 (1898).

Oxidation of XIIc with H_2O_2 and treatment of the reaction mixture as described for α-methyl analog gave β-methylpantethine (XIIIc) (1.23 g). Purification by precipitation from EtOH solution with AcOEt gave an analytical sample, colorless amorphous powder. $[\alpha]_D^{25}$ +15.5° (c=2.0, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1650 (amide I), 1550, 1527 (amide II), 1075, 1038 (C–O). *Anal.* Calcd. for $C_{24}H_{40}O_8N_4S_2$: C, 49.46; H, 7.96; N, 9.61. Found: C, 49.36; H, 8.05; N, 9.95.

Reaction of D-Pantothenonitrile (I) with 2-Aminoethanol (XIV)——a) A solution of I (2.0 g) and XIV (0.92 g) in EtOH (10 ml) was refluxed for 36 hr, and then concentrated *in vacuo* to give an oil (2.4 g). A part of the oil (0.6 g) was dissolved in H_2O (10 ml), adjusted to pH 4.7 with 0.1 M oxalic acid and allowed to stand at room temperature for 2 days, during which pH of the solution was maintained at 4.7—7.0 with addition of oxalic acid and $Ba(OH)_2$. The solution was passed through a column of a mixture of Amberlite IR 120 (H^+) and Amberlite IRA 410 (OH^-) (each 5 ml). The effluent was evaporated *in vacuo* to give a galss (400 mg). Its thin-layer chromatography (TLC) using silica gel (Silica Rider, Daiichi Pure Chemicals Co., Ltd.) showed 2 spots indicating that the product was N-pantoyl-2-aminoethanol (XVII) (*Rf* 0.60 in MEK saturated with H_2O, *Rf* 0.34 in PrOH–NH_3–H_2O (6:3:1)) contaminated with unreacted I (*Rf* 0.78 in MEK saturated with H_2O, *Rf* 0.55 in PrOH–NH_3–H_2O (6:3:1)). IR spectrum also showed a presence of a small amount of I (C≡N band at 2250 cm^{-1}). In one experiment the reaction went to completion and I was not contained in the product, $[\alpha]_D^{22}$ +56.1° (c=1.2, MeOH).

An authentic sample of XVII was prepared by fusion of D-pantolactone (1.3 g) with 2-aminoethanol (0.61 g) at 100° for 2 hr. The product was purified by treatment with a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-) to yield a pure sample as an oil (1.3 g). $[\alpha]_D^{24}$ +55.1° (c=1.8, MeOH) (reported[18] $[\alpha]_D^{20}$ +31.5° (H_2O). *Anal.* Calcd. for $C_8H_{17}O_4N \cdot \frac{1}{2}H_2O$: C, 47.99; H, 9.06; N, 7.00. Found: C, 48.40; H, 9.39; N, 6.80.

b) A reaction of I (2.0 g) with XIV (0.92 g) was carried out by fusion at 100° for 9.5 hr, and then the product was hydrolyzed and purified as described above to yield racemic N-pantoyl-2-aminoethanol (83.8%). Recrystallization of the product from acetone gave a pure sample, needles, mp 88—90°. $[\alpha]_D^{24}$ 0° (c=1.15, MeOH). *Anal.* Calcd. for $C_8H_{17}O_4N$: C, 50.24; H, 8.96; N, 7.33. Found: C, 50.33; H, 8.54; N, 7.41.

c) To a solution of I (2.0 g) and XIV (0.67 g) in EtOH (10 ml) was added 35% HCl (0.1 ml) and the mixture was refluxed for 22.5 hr. Hydrolysis and purification of the product as described above afforded a colorless glass (yield 80.3% calculated as XVII). TLC in MEK saturated with H_2O showed that it was a mixture of XVII, I and a trace of oxypantetheine (*Rf* 0.17).

N-(Phthalyl-β-alanyl)-2-aminoethanol (XIX)——To an aqueous solution of 2-aminoethanol (XIV) (1.22 g) and $NaHCO_3$ (2.02 g) was added dropwise a solution of phthalyl-β-alanylchloride (XVIII)[19] (4.75 g) in dioxane (100 ml) at a temperature of 0—4° over 90 min. After stirring for 1.5 hr at 0°, the reaction mixture was adjusted to pH 7.2 with 1N HCl, concentrated *in vacuo*, and the separated solid was filtered. Recrystallization of the crude product (3.2 g) from EtOH afforded white needles of XIX (2.8 g), mp 155—158°. *Anal.* Calcd. for $C_{13}H_{14}O_4N_2$: C, 59.53; H, 5.38; N, 10.68. Found: C, 59.61; H, 5.08; N, 10.88.

N-β-Alanyl-2-aminoethanol Oxalate (XX)——The phthalyl compound (XIX) (2.1 g, 8 mmoles) was suspended in EtOH (20 ml), and 80% hydrazine hydrate (0.55 ml, 8.8 mmoles) was added. The mixture was refluxed for 3 hr, and then concentrated *in vacuo*. The residue was dissolved in H_2O (20 ml) and an aqueous solution of oxalic acid (1.5 g; 15 ml, 12 mmoles) was added. The separated phthalylhydrazine was filtered off. To the filtrate was added 0.2 M $Ba(OH)_2$ (13 ml, 2.6 mmoles), and the precipitated barium oxalate was removed by filtration. The filtered solution was concentrated *in vacuo* to dryness. Recrystallization of the residue from EtOH gave N-β-alanyl-2-aminoethanol oxalate (XX) (1.39 g), mp 116—118° (reported[6a] mp 122—123°). *Anal.* Calcd. for $C_5H_{12}O_2N_2 \cdot (COOH)_2$: C, 37.84; H, 6.35; N, 12.61. Found: C, 37.89; H, 6.41; N, 12.66.

D-Oxypantetheine (XVI)——To a solution of XX (667 mg) in MeOH (15 ml) was added a solution of sodium methoxide prepared from Na (138 mg) and MeOH (5 ml), and the separated sodium oxalate was filtered off. The filtrate was concentrated *in vacuo* to dryness to yield a hygroscopic white powder, which was redissolved in EtOH (10 ml). To the solution was added D-pantolactone (390 mg) and the mixture was refluxed for 9 hr. The solvent was evaporated *in vacuo*, and the residue was dissolved in H_2O. The solution was filtered through a column of a mixture of Amberlite IR 120 (H^+) and IRA 410 (OH^-) (each 6 ml), and the column was washed with H_2O. The eluted solution was concentrated *in vacuo* and the residue was dried *in vacuo* over P_2O_5 to give 517 mg of XVI as colorless glass. $[\alpha]_D^{24}$ +19.9° (c=1.1, H_2O). IR ν_{max}^{KBr} cm^{-1}: 1653 (amide I), 1540 (amide II), 1070, 1040 (C–O). *Anal.* Calcd. for $C_{11}H_{22}O_5N_2$: C, 50.37; H, 8.45; N, 10.68. Found: C, 50.01; H, 8.58; N, 10.73.

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