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25. Masao Shimizu, Genkichi Ohta, Osamu Nagase, Seizaburo Okada, and Yasuhiro Hosokawa : Investigations on Pantothenic Acid and its Related Compounds. I. Chemical Studies. (1). A Novel Synthesis of Pantethine.

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Pantothenic acid (PaA) in most living cells is present as coenzyme A (CoA). As shown in Chart 1, the biosynthesis of CoA from PaA has been studied by two groups of workers in recent years.[1,2] Among the intermediates thereof, all chemically synthesized, pantetheine (N-[3-(2-mercaptoethylamino)-3-oxopropyl]-2,4-dihydroxy-3,3-dimethylbutyramide) or the corresponding disulfide, pantethine, was originally discovered as growth factor for *Lactobacillus bulgaricus* (LBF)[3] and later demonstrated to be a part of CoA-structure.[4] The present paper deals with a novel synthesis of pantethine (Ⅷ).

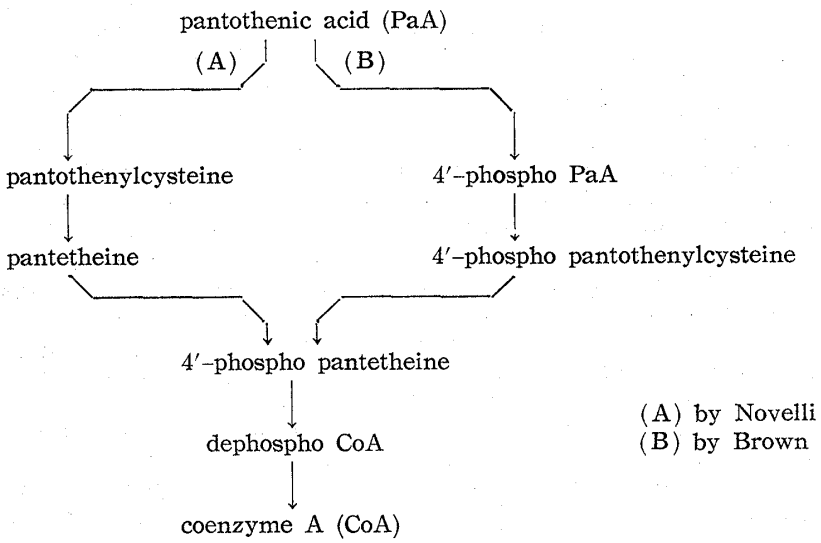

Chart 1. The Proposed Routes for the Biosynthesis of CoA

R.E. Basford, *et al.*[5] examined a commercially available sample of CoA using chromatographic and colorimetric techniques to detect the presence of four substances biologically active as CoA, one of which was reported to include a thiazoline moiety.[5] In view of the recent studies on the natural products such as bacitracin-A[6] or firefly luciferin,[7] synthesis of an equivalent to pantetheine possessing a thiazoline ring, *i.e.* 2-(2-D-pantamidoethyl)-2-thiazoline (Ⅳ) was attempted. Through the utilization of this compound, the new method was established as shown in Chart 2.

The initial step of the synthesis was the condensation of D-pantolactone (X) with 3-aminopropionitrile (Ia). The reaction was carried out at 50° to avoid decomposition

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1) L. Levintow, G.D. Novelli : J. Biol. Chem., **207**, 761 (1954).
2) G.M. Brown : J. Biol. Chem., **234**, 370 (1959); M.B. Hoagland, G.D. Novelli : *Ibid.*, **207**, 767 (1954).
3) W.L. Williams, E. Hoff-Gφrgensen, E.E. Snell : J. Biol. Chem., **177**, 933 (1949).
4) J. Baddiley, E.M. Thain : J. Chem. Soc., **1951**, 2253.
5) R.E. Basford, F.M. Huennekens : J. Am. Chem. Soc., **77**, 3878 (1955).
6) W. Stoffel, L.C. Craig : *Ibid.*, **83**, 145 (1961).
7) E.H. White, F. McCapra, G.E. Field : *Ibid.*, **85**, 337 (1963).

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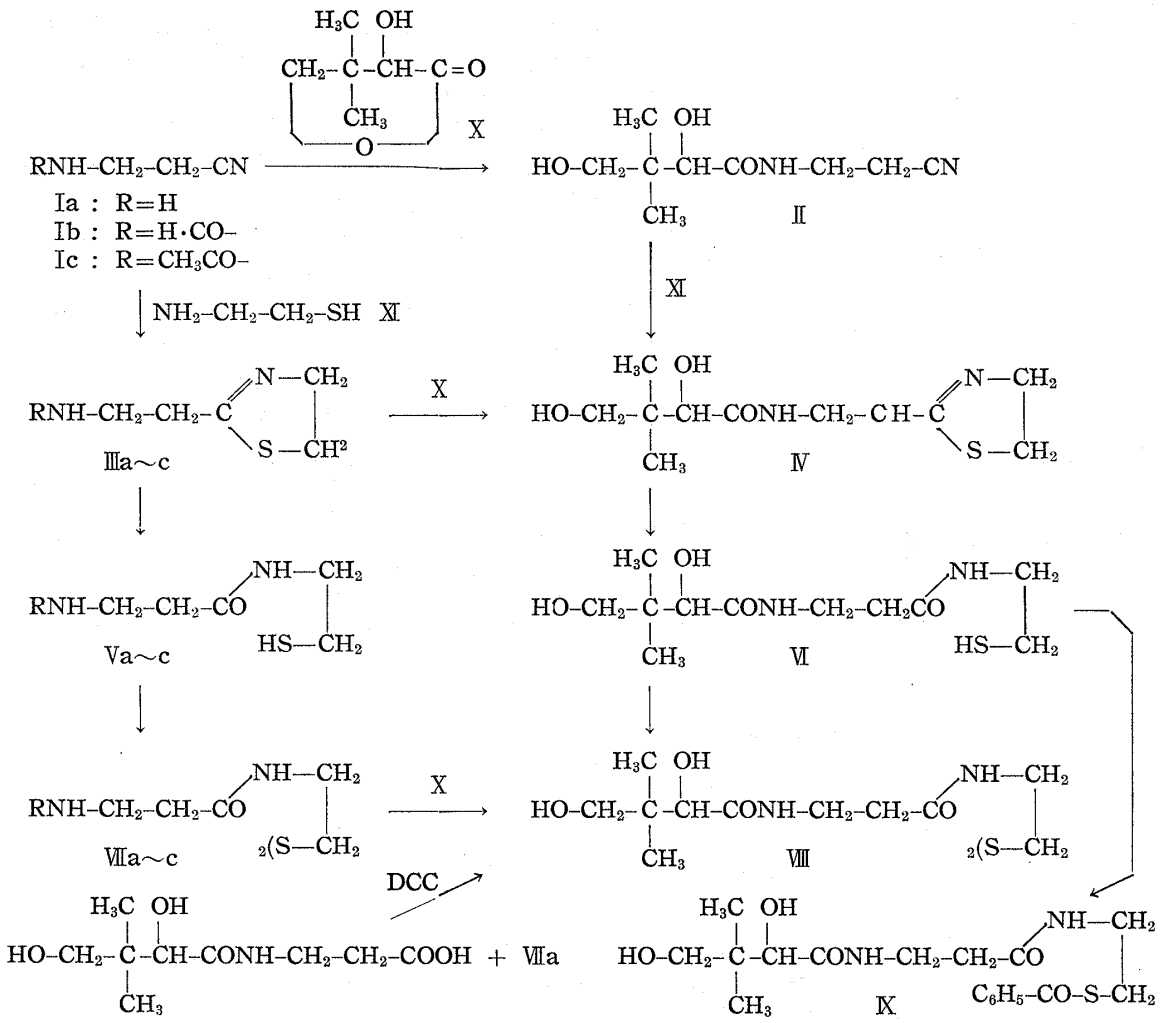

Chart 2. A Novel Synthesis of Pantethine

of Ia, and crystalline D-pantothenonitrile (II) was obtained in a yield of 92%. This compound has been prepared only in an oily state in previously published experiments.[8] Next, the thiazoline ring was formed by an application of the method of Kuhn and Drawert.[9] Treatment of the nitrile (II) in alcoholic solution with cysteamine (XI) at reflux temperature gave the desired compound (IV) in almost quantitative yield. An analytically pure sample as viscous oil was afforded by partition chromatography of the crude product, followed by molecular distillation. The structure of IV was confirmed by its ultraviolet[6,9,10] and infrared spectra.[11] This compound is hygroscopic and gradually hydrolyzed in open air. Despite its feeble basicity no solid derivatives were obtained.

Previous studies on the hydrolysis of 2-alkyl-2-thiazolines have shown the following results.[10,12] The products of hydrolysis are N-acyl- and S-acyl-2-mercaptoethylamines, and 2-thiazolidinol derivatives have been postulated as the intermediates.

8) *a*) M. B. Moore: U. S. Pat., 2,369,839 (1954). *b*) F. R. Atherton: *Ibid.*, 2,870,188 (1959). *c*) cf. W. Shive, E. E. Snell: J. Biol. Chem., **160**, 287 (1945).
9) R. Kuhn, F. Drawert: Ann., **590**, 55 (1954).
10) R. B. Martin, S. Lowey, E. L. Elson, J. T. Edsall: J. Am. Chem. Soc., **81**, 5089 (1959).
11) *a*) W. Otting, F. Drawert: Chem. Ber., 88, 1469 (1955). *b*) J. Roggero, J. Metzger: Compt. rend., **249**, 2529 (1959).
12) *a*) J. C. Crawhall, D. F. Elliott: J. Chem. Soc., **1951**, 2071; **1952**, 3094. *b*) H. Behringer, K. Kuchinka: Ann., **650**, 171 (1961). *c*) H. A. Smith, G. Gorin: J. Org. Chem., **26**, 820 (1961).

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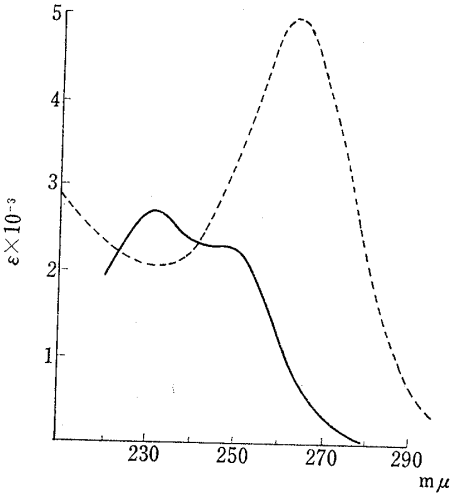

Fig. 1. Ultraviolet Absorption Spectra of Ⅳ

——— in C_2H_5OH
– – – – – in *N* HCl

Hydrolysis with boiling water or acetic acid is effective for the preparation of the N-acyl derivative. When the rate of hydrolysis of 2-methyl-2-thiazoline was plotted against pH, a bell-shaped curve with a maximum at about pH 3 was obtained.

While 2-methyl-2-thiazoline was stable in strongly acidic solutions it was slowly hydrolyzed near pH 7. Predominant formation of N-acetyl derivative was observed especially at higher pH values, whereas the S-acetyl derivative was also formed but only at higher acidities. A generally accepted sequence is shown in Chart 3.

For the preliminary test, hydrolysis of Ⅳ at various acidities was followed by the decrease of ultraviolet absorption maximum. As shown in Fig. 2, hydrolysis was slow at pH 7 or in *N* hydrochloric acid and most rapid at pH 3. At pH 2, formation of S-acyl compound was indicated by the

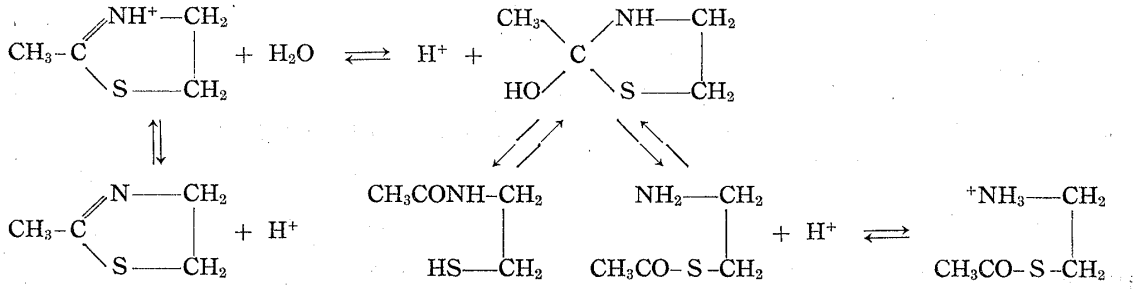

Chart 3. Scheme for the Interrelationship between 2-Methyl-2-thiazoline and N-Acetyl- and S-Acetyl-2-mercaptoethylamine

appearance of a new band at 230 mμ characteristic of the -S-CO group. This result was in accordance with that of 2-methyl-2-thiazoline and the reaction scheme was assumed the same as shown above. In practice, hydrolysis with 0.1*N* acetic acid under nitrogen at room temperature for 24 hours furnished pantetheine (Ⅵ) of high quality, which was purified by means of ion-exchange resin and further identified by the crystalline S-benzoate (Ⅸ). Instead of acetic acid, weakly acidic ion-exchange resin could similarly be used; hydrolysis in boiling water for six hours was also effective. In the proceeding of hydrolysis, the acidity of the reaction mixture increases due to the disappearance of basic thiazoline moiety. When hydrolyzed with hydrochloric acid at 60°, the rate of increase was rapid and the concomitant cleavage of the amide bond in resultant pantetheine was observed. In model experiments with pantethine (Ⅷ), the amide linkage was confirmed

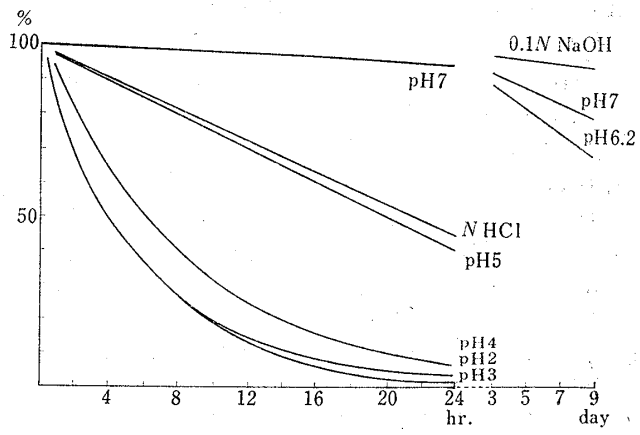

Fig. 2. Preliminary Test for Stability of Ⅳ at Various PHs (Decrease of Ⅳ)

PH 3: at 265 mμ PH 4: at 260 mμ
PH 5: at 250 mμ PH 6: at 247~248 mμ

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to be stable under conditions of 60° for one hour in a solution of pH 3.75 but not at pH 2.1. The reaction course was examined by detection of an infrared absorption band at 1780 cm^{-1} of pantolactone in the spectrum of treated pantethine. For the preparation of pantethine, it was found most suitable to carry out the hydrolysis at 60° between an initial pH of 5.30 and final pH of 3.5 by the use of acetic acid or preferably oxalic acid.

Pantetheine (Ⅵ) was readily oxidized with hydrogen peroxide under weakly alkaline conditions to afford pantethine (Ⅷ). The purification of Ⅷ was performed by percolating the reaction mixture through a column of acidic and basic ion-exchange resin (1:1) in mono-bed system, although a minute amount of Ⅷ was decomposed during the process. This method of purification, being more convenient than reported procedures,[13b] could separate Ⅷ from various impurities such as amines, acids, inorganic salts and also pantolactone. The presence of D-pantothenonitrile (Ⅱ), which is a conceivable impurity, could be excluded when slight excess of cysteamine (Ⅺ) was used in the conversion of Ⅱ into Ⅳ. It is not necessary, therefore, to isolate the intermediates, Ⅳ and Ⅵ, for the preparation of pantethine. Thus, the present method gave pantethine in a yield of 76~82% from the nitrile (Ⅱ).

As shown in Chart 2, 3-aminopropionitrile (Ia) and its acyl derivatives (Ib, c) were similarly converted into thiazoline derivatives (Ⅲa, b, c). The freshly distilled compound (Ⅲa), which is unstable in open air giving a resin-like polymerized mass after some days, was allowed to react with pantolactone to yield Ⅳ. Alethine (Ⅶa) and its acyl derivatives (Ⅶb, c) were synthesized through Ⅴa, b, c from Ⅲa, b, c. In order to synthesize alethine as the material for the preparation of pantethine, it is most convenient to select 2-formylaminopropionitrile (Ib) as starting material, because Ⅶb is readily deacylated by methanolic hydrochloric acid at room temperature.

The published methods of synthesis of pantethine and its derivatives[13] were based on the general procedures of peptide synthesis. These involved (1) condensation of pantolactone with alethine derivatives, (2) reaction between cysteamine derivatives and functional derivatives of pantothenic acid such as ester, azide and mixed anhydride, or (3) reaction of ethyl pantothenyl carbonate with ethyleneimine to form pantothenoyl ethyleneimine, followed by ring opening with thioacid to give S-acyl pantetheine.

The first type of synthesis has been recommended in spite of the multiple steps required for the preparation of alethine. A feature of the present method is to use 3-aminopropionitrile as starting material instead of β-alanine used in other published procedures. Pantethine obtained by the present method showed a single spot in paper chromatograms, having neither prominent ultraviolet absorption, nor bands corresponding to pantolactone or pantothenonitrile in the infrared spectrum. Its properties were in agreement with those reported and its biological activities were almost identical with authentic sample.

Pantethine is viscous oil or glassy mass and does not crystallize. For this reason, elemental analysis has been shown to be unreliable. Its content has been estimated only by microbiological assay. However two methods have proved to be practical means of quantitative analysis. First, by the method of Siggia, *et al.*[14] the disulfide linkage of pantethine can be effectively titrated with potassium bromide-potassium trioxybromide solution at 40~50° : the sample was shown to have purity of 98~100%. Second, the colorimetric method of pantothenate was applied.[15] Pantethine was hydrolyzed by acid and resultant pantolactone was converted to the hydroxamic acid, which in the

13) *a*) E. E. Snell, G. M. Brown : Advances in Enzymology, **14**, 49 (1953). *b*) E. E. Snell, E. L. Wittle : Methods in Enzymology, Ⅲ, 918 (1957). *c*) J. Baddiley : Advances in Enzymology, **16**, 1 (1955).
14) S. Siggia, R. L. Edsberg : Anal. Chem., **20**, 938 (1948).
15) E. G. Wollish, M. Schmall : *Ibid.*, **22**, 1033 (1950).

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presence of ferric chloride exhibited a color with a maximal absorption at 500 mμ. When tested by this colorimetric method, the observed content of pantethine was 100～103%.

In experiments related to those so far reported, a few observations on S-benzoylpantetheine (Ⅸ) deserve mention here. This derivative of pantetheine is crystalline (m.p. 116～117°), and has been suggested to be available as a standard substance for microbiological assay.[16] As described above, S-benzoate was prepared for characterization of pantetheine by reaction with one equivalent of benzoyl chloride at room temperature under alkaline conditions. Since pantetheine is unstable in alkaline solution and more so the S-benzoate, the reaction was conducted between pH 8 and 9. Buffer solutions were used to maintain the above pH range. It was observed that the S-benzoate is readily soluble in borate buffer whereas it is sparingly soluble in other buffer solutions or in water. The S-benzoate was recovered on acidification from its borate buffer solution. A crystalline substance was obtained from the same solution by salting out with sodium chloride. This crystalline substance was presumed to be a complex salt formed by sodium borate with the S-benzoate at the 1:3-diol grouping of the pantoyl moiety, although the purity and elemental analysis were unsatisfactory.

In addition to the novel synthesis described above, another one-step synthesis of pantethine was elaborated. The condensation of pantothenic acid (Ⅻ) with cystamine was slowly effected in the presence of N,N′-dicyclohexylcarbodiimide at room temperature in an unsatisfactory yield of 33.7%: Nevertheless this method is promising for providing a simple route to isotopically labelled pantethine.

Experimental

Melting points are uncorrected. UV spectra were measured on a Hitachi EPU-2 spectrophotometer, and IR spectra were measured on a Hitachi EPI-2 spectrophotometer.

D-Pantothenonitrile (II)[8a,b]——A mixture of D-pantolactone (X) (400 g.) and 3-aminopropionitrile (Ia) (215 g.) was heated at 50° for 4 hr. The reaction mixture was crystallized from $CH_3COOC_2H_5$ (150 ml.) to separate Ⅱ as prisms, m.p. 82～84°(410 g.). From the mother liquor, the second crop (137 g.) and the third crop (22 g.) with the same melting point were obtained. The total yield was 569 g. (92.4%). Recrystallization from the same solvent afforded an analytical sample, m.p. 82～84°, $[\alpha]_D^{20}$ +31.5°(c=1.75, H_2O). IR: ν_{max}^{KBr} 2250 cm^{-1} (C≡N). *Anal.* Calcd. for $C_9H_{16}O_3N_2$: C, 54.00; H, 8.04; N, 13.99. Found: C, 54.03; H, 8.05; N, 14.34.

2-(2-D-Pantamidoethyl)-2-thiazoline (IV)——a) To a solution of sodium ethoxide prepared from Na (0.63 g.) and C_2H_5OH (15 ml.) was added a solution of cysteamine hydrochloride (3.12 g.) in C_2H_5OH (5 ml.), and the separated NaCl was filtered off. To the filtrate was added the nitrile (Ⅱ) (5.0 g.) and the mixture was refluxed under N_2 for 6 hr. during which evolution of NH_3 was observed. The reaction mixture was concentrated *in vacuo* to dryness, the residue was dissolved in acetone (100 ml.), and the insoluble material was filtered off. Evaporation of the solvent gave the crude thiazoline (Ⅳ) as pale yellow oil (6.5 g.). The crude product was dissolved in acetone (4 ml.) and isopropyl ether (100 ml.) was added to give an oily precipitate, which after decantation of the supernatant was collected and dried *in vacuo*. The dried material (5.8 g.) was dissolved in methyl ethyl ketone (10 ml.), mixed well with "Celite" (10 g.) and dried. The mixture was placed on the top of the chromatocolumn which was prepared from "Celite (535)" (500 g.) and H_2O (500 ml.) saturated with methyl ethyl ketone–methyl isobutyl ketone (1:1). The column was eluted with methyl ethyl ketone–methyl isobutyl ketone (1:1) saturated with H_2O. Total 57 fractions of each 50 ml. were collected. The residue (4.4 g.) obtained from fraction Nos. 37～50 was practically pure. A portion of the residue was subjected to molecular distillation (bath temperature 100°; pressure 10^{-4} mm. Hg) to give an analytical sample of Ⅳ, hygroscopic, colorless viscous oil. $[\alpha]_D^{23}$ +23°(c=1.22, H_2O). UV λ_{max}^{EtOH} mμ (ε): 231 (2670), 246.5 (2370). $\lambda_{max}^{N\,HCl}$: 265 mμ (ε 4930). IR $\nu_{max}^{liq.film}$ cm^{-1}: 3360, 1662, 1653, 1648, 1635, 1540, 1532, 1212, 1140, 1112, 1076, 1042, 982, 917. *Anal.* Calcd. for $C_{11}H_{20}O_3N_2S$: C, 50.74; H, 7.74; N, 10.76. Found: C, 50.82; H, 7.75; N, 10.59.

The picrate, oxalate and hydrochloride of Ⅳ were not obtained in crystalline state, the derivatives were unstable and decomposed readily.

16) E.E. Snell, F.L. Wittle: Methods in Enzymology, Ⅲ, 925 (1957).

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b) A solution of 2-(2-aminoethyl)-2-thiazoline (Ⅲa) (4.35 g.) (see below) and D-pantolactone (4.14 g.) in CH_3OH (10 ml.) was heated under N_2 at 50～55° for 6.5 hr. After evaporation of the solvent, the residue was dissolved in acetone (5 ml.) and isopropyl ether (130 ml.) was added gradually to separate an oil, which was collected, washed with isopropyl ether and dried *in vacuo*. Concentration of the mother liquor afforded an additional crop. Total 8.0 g. of the crude product was obtained. A portion of the product was dissolved in methyl ethyl ketone and the solution was filtered through a column of charcoal-Celite (1:3). The residue obtained from the filtrate was distilled by molecular distillation to give a pure sample. The IR spectrum of this sample was identical with that described above.

D-Pantetheine (VI)——The following procedure was carried out in the stream of N_2. A solution of the crude thiazoline (Ⅳ) (6.5 g.) in 0.1*N* CH_3COOH (130 ml.) was set aside at room temperature for 24 hr. during which a maximal ultraviolet absorption at 250 mμ disappeared. The reaction mixture was concentrated *in vacuo* giving a thick syrup which was dissolved in H_2O (100 ml.). The solution was passed through a column of "Amberlite IR4B (OH^-)" (3 ml.) and then a column of "Amberlite IRC 50 (H^+)" (7 ml.) over a period of 3 hr. to remove acidic and basic impurities. The columns were washed with H_2O (100 ml.) and the combined effluent was evaporated *in vacuo* to give pantetheine (6.8 g.) as a viscous oil. For further purification, the oil (1 g.) was dissolved in C_2H_5OH (1 ml.), ether (40 ml.) was added, and the mixture was set aside overnight. The separated oil was collected, and dried *in vacuo* over P_2O_5 for 3 days to give an analytical sample. $[\alpha]_D^{23}$ +12.2° (c=3.45, H_2O) (reported[17] +12.9°). IR $\nu_{max}^{liq.film}$ cm^{-1}: 3300, 2550, 1650, 1535, 1073, 1040. *Anal.* Calcd. for $C_{11}H_{22}O_4N_2S$: C, 47.46; H, 7.97; N, 10.06. Found: C, 47.58; H, 8.39; N, 9.40.

Treatment of the thiazoline (Ⅳ) (6.5 g.) in H_2O (100 ml.) with "Amberlite IRC 50 (H^+)" (30 ml.) at room temperature for 20 hr. afforded the crude pantetheine in 91.8% yield, and heating the thiazoline (Ⅳ) (6.5 g.) in H_2O (150 ml.) at refluxing temperature for 7 hr. gave the crude pantetheine in 83.7% yield.

D-Pantethine (VIII)——a) To a solution of pantetheine (Ⅵ) (6.8 g.) in H_2O (60 ml.) were added 10% NH_4OH solution (2 ml.) and $FeSO_4 \cdot 7H_2O$ (10 mg.). The mixture was cooled in ice-water and aqueous 3.77% H_2O_2 solution was added with stirring until the red-purple color of the mixture turned to pale yellow; 10 ml. of 3.77% H_2O_2 solution was consumed. The resulting solution was brought to room temperature and passed successively through a column of "Amberlite IRC 50 (H^+)" (7 ml.) and of "Amberlite IR 4B(OH^-)" (1 ml.). The columns were washed with H_2O (100 ml.). The combined effluent was evaporated below 60°, the residue in C_2H_5OH (6 ml.) was mixed with isopropyl ether (60 ml.) and the mixture was set aside in an ice-box overnight. The precipitated product was collected and dried *in vacuo* over P_2O_5 at room temperature for 3 days to give 6.3 g. of Ⅷ. $[\alpha]_D$ +18° (c=2.16, H_2O).

For further purification, the product (5 g.) in H_2O (50 ml.) was filtered through a column of a mixture of "Amberlite IR 120 (H^+)" and "Amberlite IRA 410 (OH^-)" (1:1; 60 ml.) and the column was washed with H_2O (600 ml.). The eluate was concentrated and the residue was dissolved in CH_3OH (5 ml.) and mixed with $CH_3COOC_2H_5$ (200 ml.) to precipitate an oil which was collected and dissolved in H_2O. Concentration of the solution and drying the residue *in vacuo* over P_2O_5 at 55° for 8 hr. gave a pure sample of pantethine (Ⅷ), as colorless glass. $[\alpha]_D^{23}$ +17.1° (c=3.2, H_2O) (reported +15°,[18] +16±1°,[13b] +17.7°[19]). UV: λ_{max}^{EtOH} 246～248 mμ (ε 500) (inflexion). IR ν_{max}^{KBr} cm^{-1}: 3320, 1658, 1640, 1547, 1530, 1075, 1037. *Anal.* Calcd. for $C_{22}H_{42}O_8N_4S_2$: C, 47.63; H, 7.64; N, 10.10. Found: C, 47.55; H, 7.71; N, 9.85.

b) **From D-Pantothenonitrile (II) without Isolation of the Intermediates.** To a solution of sodium isopropoxide prepared from Na (3.8 g.) and iso-PrOH (60 ml.) was added a solution of cysteamine hydrobromide (26.1 g.) in iso-PrOH (24 ml.). The mixture was stirred thoroughly to liberate the amine, and the nitrile (Ⅱ) (30 g.) was then added. The mixture was heated under reflux for 7 hr. and cooled. Removal of NaBr by filtration and evaporation of the solvent *in vacuo* afforded the crude thiazoline (Ⅳ) (46.2 g.). This was dissolved in H_2O (90 ml.) and *N* oxalic acid solution (25.5 ml.) was added. The solution was heated at 60° for 3 hr., while the pH of the solution changed from 5.1 to 3.63. The solution was then adjusted to pH 8.3 with 10% NH_4OH solution and $FeSO_4 \cdot 7H_2O$ (10 mg.) was added.

To the stirred and cooled solution (0～10°) was added dropwise aqueous 3.5% H_2O_2 solution until the reaction mixture gave no further color with sodium nitroprusside reagent; 75 ml. of 3.5% H_2O_2 solution was consumed. After being brought to room temperature the reaction mixture was passed through a column of a mixture of "Amberlite IR 120 (H^+)" (90 ml.) and "Amberlite IRA 410 (OH^-) (90 ml.) over a period of 1 hr. The column was washed with H_2O (1.8 L.). The eluted solution was evaporated *in vacuo* below 60° and the residue was dried *in vacuo* at 55° for 8 hr. to give 32.2 g. of Ⅷ; yield 77.2%. The IR spectrum was identical with that described above. No impurities were detected by IR spectrum (no band at 1780 cm^{-1} of lactone, at 2250 cm^{-1} of C≡N), paper chromatography and thin-layer chromatography.

c) To a solution of pantothenic acid (1.31 g.) and cystamine (0.46 g.) in anhyd. pyridine (10 ml.) was added dicyclohexylcarbodiimide (1.23 g.). The mixture was stirred at room temperature for 6 hr. and then

17) J. Baddiley, E. M. Thain: J. Chem. Soc., **1952**, 800.
18) R. E. Bowman, J. F. Cavalla: *Ibid.*, **1954**, 1171.
19) M. Viscontini, K. Adank, N. Merking, K. Ehrhardt, P. Karrer: Helv. Chim. Acta, **37**, 375 (1954).

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set aside for 30 hr. The separated crystals were filtered off and the filtrate was concentrated *in vacuo* to dryness. The residue was shaken with H_2O (10 ml.) and $CHCl_3$ (5 ml.), and the aqueous layer was washed twice with $CHCl_3$ (each 5 ml.) and passed through a column of "Amberlite IR 4B(OH^-)" (5 ml.) and then a column of "Amberlite IRC 50 (H^+)" (5 ml.). The columns were washed with H_2O (40 ml.) and the combined effluent was evaporated *in vacuo* and the residue was dried *in vacuo* at 55° to give VIII (0.56 g.). The IR spectrum and Rf values of paper chromatography of this sample were identical with that described above.

Paper Chromatography——Paper chromatography by the ascending technique was done of Toyo filter paper No. 50, using following solvents: solvent I, BuOH saturated with H_2O; solvent II, methyl ethyl ketone saturated with H_2O; solvent III, methyl ethyl ketone-methyl isobutyl ketone (1:1) saturated with H_2O. Compounds containing -SH and -S-S were detected on chromatograms with nitroprusside spray after KCN spray. Compounds containing amino or amido functions were detected with ninhydrin spray followed by heating.

The possible impurities on the synthetic D-pantethine were all detectable by this method and their Rf values are shown in Table I and are approximated to the nearest values.

Table I. Rf Values of Compounds

Compound	Rf		
	Solvent I	Solvent II	Solvent III
Pantethine	0.65	0.35	
Pantetheine	0.70	0.66	
Pantothenonitrile	0.64	0.88	0.67
2-(2-Pantamidoethyl)-2-thiazoline	0.77	0.84	0.43
Pantothenic Acid	0.40		
Cysteamine	0.01		
Cystamine	0~0.14		
Alethine	0~0.12		
β-Alanine	0.025		

Quantitative Assay of Pantethine——Method I. Determination of disulfide by the method of Siggia and Edsberg.[14] To about 0.3 mmole of pantethine in H_2O (50 ml.) was added conc. H_2SO_4 (25 ml.) under cooling (below 60°). The solution was titrated with standard 0.1*N* bromate-bromide solution at 45~50° until a yellow color persisted during 5 min. A blank was run on H_2O to correct for the excess bromine needed for the end point. The results are shown in Table II.

Method II. Colorimetric determination of pantolactone after hydrolysis of pantethine to pantolactone. The procedure employed was the same as described for determination of panthenol and pantothenates by Wollish and Schmall[15] except that D-pantolactone, instead of D-pantothenate, was used as a reference standard. The results are shown in Table II.

Table II.

Sample	Purities (%)	
	Method I	Method II
1 Pantethine	98.0	102.1
2 〃	98.3	101.0
3 〃	100.0	101.0
4 D-Ca Pantothenate	—	100.3
5 Cysteine	99.1	—

2-(2-Aminoethyl)-2-thiazoline (IIIa)——A solution of cysteamine (8.7 g.) and 3-aminopropionitrile (Ia) (7.88 g.) in iso-PrOH (20 ml.) was refluxed under N_2 for 5 hr. After evaporation of the solvent, the residue was distilled under reduced pressure to give a colorless oil (6.71 g.), b.p_2 70~74°.

To the oil in iso-PrOH (20 ml.) was added "Amberlite IRA 410 (OH^-)" (15 ml.) and the mixture was stirred and filtered. The filtrate was concentrated and the residue was redistilled to afford a pure sample of IIIa, b.p_2 75~76°. UV λ_{max}^{EtOH} mμ (ε): 232 (2470), 244 (2140), $\lambda_{max}^{N\ HCl}$ mμ (ε): 211 (2330), 266 (3890). IR $\nu_{max}^{liq.\ film}$ cm^{-1}: 3340, 3270, 1629, 1595, 1195, 995.

This compound is rather unstable and changes into resinous product.

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Dihydrochloride : Prepared by bubbling anhyd. HCl into a solution of Ⅲa in ether. Needles (from CH_3OH–ether), m.p. 138～141°. *Anal.* Calcd. for $C_5H_{12}N_2SCl_2$: C, 29.56; H, 5.95; N, 13.79. Found : C, 29.58; H, 5.92; N, 13.64.

2-(2-Formamidoethyl)-2-thiazoline (IIIb)——A solution of cysteamine (8.1 g.) and 3-formamidopropionitrile (9.35 g.) in iso-PrOH (30 ml.) was refluxed under N_2 for 9 hr. and then concentrated *in vacuo*. The residue was distilled under reduced pressure giving Ⅲb (14.2 g.), $b.p_{0.15}$ 145～147°.

Re-distillation afforded an analytical sample, $b.p_2$ 162～163°, colorless liquid, UV λ_{max}^{EtOH} mμ (ε) : 232 (2310), 245 (2075); $\lambda_{max}^{N\ HCl}$: 265 mμ (ε 4855). IR $\nu_{max}^{liq.\ film}$ cm^{-1} : 3270, 3040, 1696, 1680, 1670, 1633, 1550, 1538, 1206, 979. *Anal.* Calcd. for $C_6H_{10}ON_2S$: C, 45.54; H, 6.37; N, 17.70. Found : C, 45.01; H, 6.68; N, 17.11.

Picrate : Yellow leaflets (from CH_3OH), m.p. 108～109° (decomp.). *Anal.* Calcd. for $C_{12}H_{13}O_8N_5S$: C, 37.19; H, 3.38; N, 18.08. Found : C, 37.31; H, 3.36; N, 18.27.

2-(2-Acetamidoethyl)-2-thiazoline (IIIc)——A solution of 3-acetamidopropionitrile (2.24 g.) and cysteamine (1.15 g.) in C_2H_5OH (10 ml.) was refluxed under N_2 for 5 hr. After evaporation of the solvent the residue was poured into H_2O, acidified with 10% HCl solution and shaken with $CHCl_3$. The aqueous layer was separated, made alkaline with K_2CO_3 and extracted with $CHCl_3$. The residue obtained from the $CHCl_3$ extract was distilled under reduced pressure to give Ⅲc (2.3 g.) as colorless liquid, $b.p_3$ 146～148°. UV λ_{max}^{EtOH} mμ (ε) : 233 (3100), 248 (2840).

Picrate : Yellow plates (from CH_3OH), m.p. 159～160°. *Anal.* Calcd. for $C_{13}H_{15}O_8N_5S$: C, 38.91; H, 3.77; N, 17.45. Found : C, 38.73; H, 4.02; N, 16.98.

Alethine Dihydrochloride (VIIa)——a) A solution of the aminoethylthiazoline (Ⅲa) (3.75 g.) in H_2O (7.5 ml.) was adjusted to pH 4.9 with *N* HCl solution and heated at 60° for 4 hr. The resulting solution was adjusted to pH 7.3 with dil. $NaHCO_3$ solution and 3.52% H_2O_2 solution was added dropwise until the reaction mixture gave no color with nitroprusside reagent. After removal of the solvent, the product was extracted with hot C_2H_5OH (50, 30, and 20 ml.). To the extract was added an ethanolic 2*N* HCl solution (17.4 ml.) and the mixture was concentrated *in vacuo* to dryness to leave a solid (4.04 g.) which was recrystallized from CH_3OH to afford alethine dihydrochloride (2.76 g.), m.p. 220～222°, identical with an authentic specimen. (reported[18] m.p. 221～222°).

b) A solution of diformylalethine (Ⅶb) (0.35 g.) in a methanolic *N* HCl solution (2.2 ml.) was allowed to stand at room temperature for 24 hr. The separated dihydrochloride (Ⅶa) was filtered, and concentration of the filtrate gave an additional crop. Total yield was 0.35 g. Recrystallization from CH_3OH afforded a pure sample, m.p. and mixed m.p. 220～222°.

N-Formylalethein (Vb)——A solution of the formamidoethylthiazoline (Ⅲb) (1.45 g.) in H_2O (6 ml.) was adjusted to pH 5.1 with *N* oxalic acid solution, heated under N_2 at 60° for 2 hr., and cooled. The solution was filtered successively through a column of "Amberlite IR 4B(OH^-)" (1 ml.), "Amberlite IR 120 (H^+)" (1 ml.) and "Amberlite IR 4B (OH^-)" (1 ml.). The filtrate was evaporated *in vacuo* and the residue (1.53 g.) was crystallized from ether to give Vb, hygroscopic needles, m.p. 59～61°. *Anal.* Calcd. for $C_6H_{12}O_2N_2S$: C, 40.90; H, 6.86; N, 15.90. Found : C, 41.24; H, 6.89; N, 15.49.

N,N′-Diformylalethine (VIIb)——A solution of the formamidoethylthiazoline (Ⅲb) (14.2 g.) in H_2O (60 ml.) was adjusted to pH 5.1 with *N* HCl solution, heated at 60° for 2 hr. and cooled. The resultant solution (pH 2.1) was adjusted to pH 8.4 with dil. NH_4OH solution and 3.5% H_2O_2 solution was dropped with stirring until a sample of the solution no longer colored the sodium nitroprusside reagent. The reaction mixture was percolated through a column of a mixture of "Amberlite IR 120 (H^+)" (80 ml.) and "Amberlite IRA 410 (OH^-)" (80 ml.). Evaporation of the effluent and crystallization of the residue (11.6 g.) from CH_3OH afforded Ⅶb, prisms, m.p. 137～139°. *Anal.* Calcd. for $C_{12}H_{22}O_4N_4S_2$: C, 41.11; H, 6.32; N, 15.99. Found : C, 41.01; H, 6.58; N, 15.97.

N,N′-Diacetylalethine (VIIc)——A solution of the acetamidoethylthiazoline (Ⅲc) (1.0 g.) in H_2O (30 ml.) was refluxed for 8 hr. To the cooled solution was added 3.5% H_2O_2 solution until the reaction mixture gave no color with nitroprusside reagent. The separated Ⅶc was filtered (0.46 g., m.p. 204～206°). Concentration of the mother liquor and crystallization of the product from C_2H_5OH gave an additional crop (0.37 g.). Recrystallization from C_2H_5OH afforded an analytical sample, leaflets, m.p. 204～206° (reported[20] m.p. 208～209°). *Anal.* Calcd. for $C_{14}H_{26}O_4N_4S_2$: C, 44.42; H, 6.92; N, 14.81. Found : C, 44.62; H, 7.28; N, 14.84.

S-Benzoylpantetheine (IX)——Pantetheine (1.4 g.) was dissolved in a buffered solution (pH 9.0) prepared from 0.2*M* NaOH solution (8.6 ml.) and 0.2*M* H_3BO_3 and 0.2*M* KCl solution (20 ml.). To the stirred solution at 20～25° were added alternately benzoylchloride (0.77 g.) and 2*N* NaOH solution (2.75 ml.) during 10 min. To maintain the pH 8～8.5, 2*N* NaOH solution was then added, and stirring was continued for 10 min. After addition of NaCl, the reaction mixture was extracted with $CH_3COOC_2H_5$ (4 times, total 120 ml.). The extract was washed with H_2O, dried and evaporated. The residue (2.02 g.) was crystallized

20) D. S. Tarbell, D. P. Cameron : J. Am. Chem. Soc., 78, 2731 (1956).

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from $CH_3COOC_2H_5$ (2 ml.) to afford the crude benzoate (1.10 g.) melting at 108~113°. The mother liquor was chromatographed over neutral Al_2O_3 (20 g.). The fraction eluted by $CH_3COOC_2H_5$ was discarded, and the fraction eluted by $CH_3COOC_2H_5$–CH_3OH (9:1) was crystallized from $CH_3COOC_2H_5$ to give an additional crop (0.29 g.). Recrystallization of the combined product from $CH_3COOC_2H_5$ gave the benzoate as needles (1.24 g.), m.p. 115~117°. Further recrystallization gave the analytical sample, m.p. 116~117°, identical with an authentic sample. $[\alpha]_D^{19}$ +32.9° (c=1.15, C_2H_5OH) (reported m.p. 116°,[22] 117~117.5°;[23] $[\alpha]_D$ +31±4° (C_2H_5OH),[21] $[\alpha]_D^{20}$ +21.2° (dioxane)[22]). UV λ_{max}^{EtOH} mμ (ε): 239 (10,880), 267 (8,030). IR ν_{max}^{KBr} cm^{-1}: 3400, 3290, 3100, 1658, 1652, 1627, 1565, 1535, 1212, 1078, 1023, 912. *Anal.* Calcd. for $C_{18}H_{26}O_5N_2S$: C, 56.52; H, 6.85; N, 7.33. Found: C, 56.29; H, 6.65; N, 7.41.

For large scale preparation, it was preferable to acidify the reaction mixture before extraction with $CH_3COOC_2H_5$ in order to decompose the borate adduct.

Adduct of S-Benzoylpantetheine and Sodium Borate——To a solution of S-benzoylpantetheine (2 g.) in H_2O (200 ml.) were added a solution of boric acid (0.324 g.) in H_2O (16 ml.) and then aq. NaOH solution (0.212 g. in 2.5 ml.). Addition of NaCl (50 g.) to the solution separated crystals which were filtered and dried *in vacuo* at room temperature (3.3 g.). The crystals were dissolved in C_2H_5OH, insoluble materials were filtered off and to the filtrate was added $CH_3COOC_2H_5$ to precipitate the adduct. This was isolated, washed with $CH_3COOC_2H_5$ and dried *in vacuo* at room temperature, m.p. 142~144° (decomp.). $[\alpha]_D^{25}$ +43.4° (c=0.67, C_2H_5OH). *Anal.* Calcd. for $C_{36}H_{48}O_{10}N_4S_2BNa \cdot 2H_2O$: C, 52.04; H, 6.31; N, 6.74. Found: C, 52.18; H, 6.51; N, 7.10.

When an aqueous solution of the adduct was acidified, or shaken with $CH_3COOC_2H_5$, the adduct decomposed giving the benzoate.

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Summary

A novel synthesis of pantethine (Ⅷ) from 3-aminopropionitrile (Ⅰ) was established through the intermediate formation of thiazoline derivative (Ⅳ) followed by hydrolysis thereof.

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22) E. Felder, D. Pitré: Gazz. chim. ital., 88, 401 (1958); C. A., 53, 17917 (1959).

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