stretched in the transition state of the catalyzed reaction and that the catalysis involves proton transfer.

From the results described above, the catalysis of FKI hydrolysis by amino group may be considered as general–base catalysis and proceed through the following kinetically indistinguishable transition states which may be preceded by one or more pre–equilibrium steps (Chart 1).

Namely, amino group (B) may react as general base in the intramolecular catalysis of FKI hydrolysis. Nitrogen atom with higher electron density may attract a proton to make a hydrogen bonding, while the base may stretch the other hydrogen bonding between oxygen and itself (I). In the step from (II) to (III), proton transfer may be involved by the base. And the bond cleavage between nitrogen (hydrazide) and carbon (ketone) may occur as the final stage of the hydrolysis.

The above considerations suggest that in the case of hydrazone with a poor leaving group relative to the attacking amino reagent the transition state reflects the expulsion of this group from a species which resembles a tetrahedral addition compound, while with a relatively good leaving group the rate-limiting step reflects principally nucleophilic attack on the hydrazone group and is sensitive to the nucleophilic reactivity of the attacking reagent. In the former case proton transfer becomes an important, or even a necessary, part of the reaction to aid expulsion of the attacking group. It is furthermore suggested that the step from (II) to (III) may be the rate–limiting one, since the decrease of catalytic rate constant in deuterium oxide solvent was found.[14]

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Investigations on Pantothenic Acid and Its Related Compounds. XXIV. Chemical Studies. (11).[1] Chemical Synthesis of Coenzyme A Analogs of a Modified Pantetheine Moiety

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In parallel with the synthetic works on coenzyme A (CoA) analogs,[1,3] the approach to the mechanism of enzymatic transacetylation with phosphotransacetylase is in progress by the biochemical research group in this laboratory, during which the importance of pantothenic acid moiety of CoA has been noticed concerning the interaction between CoA and the enzyme. The requirement for the suitable analogs led to initiate the present work. The chemical and trivial names of the synthesized analogs are shown in Table I together with their structures in the simplified forms. The trivial names were given by putting the eliminated moiety with added prefix de- in accordance with the precedent of 3′-de-phospho-CoA.

In the previous work,[4] the thiazoline method developed by us was successfully applied for the total synthesis of CoA from P^1-adenosine 3′-phosphate 5′-P^2-D-pantothenonitrile 4′-

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[1] Part XXIII: M. Shimizu, O. Nagase, S. Okada, and Y. Hosokawa, *Chem. Pharm. Bull.* (Tokyo), **18**, 313 (1970).

[2] Location: *Minamifunabori-cho, Edogawa-ku, Tokyo, 132, Japan.*

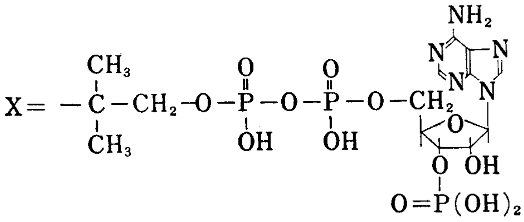
[3] M. Shimizu, O. Nagase, Y. Hosokawa, and H. Tagawa, *Tetrahedron*, **24**, 5241(1968).

[4] M. Shimizu, O. Nagase, S. Okada, Y. Hosokawa, H. Tagawa, Y. Abiko, and T. Suzuki, *Chem. Pharm. Bull.* (Tokyo), **15**, 655 (1967).

Table I. Trivial Names, Chemical Names and Partial Structures of the Synthesized Analogs

Compd.	Trivial name	Chemical name	Partial structure
VIII	CoA(L)	[A]-L-pantetheine-[B]	$X\text{-}\overset{OH(L)}{C}H\text{-}CONH\text{-}CH_2CH_2\text{-}CONH\text{-}CH_2CH_2SH$
IVa	de-cysteamine-CoA(L) nitrile	[A]-L-pantothenonitrile-[B]	$X\text{-}\overset{OH(L)}{C}H\text{-}CONH\text{-}CH_2CH_2CN$
IVb	keto-CoA	[A]-2′-ketopantetheine-[B]	$X\text{-}\overset{O}{\overset{\Vert}{C}}\text{-}CONH\text{-}CH_2CH_2\text{-}CONH\text{-}CH_2CH_2SH$
IVc	de-cysteamine-CoA	[A]-D-pantothenic acid-[B]	$X\text{-}\overset{OH}{C}H\text{-}CONH\text{-}CH_2CH_2COOH$
IVd	de-cysteamine-CoA amide	[A]-D-pantothenamide-[B]	$X\text{-}\overset{OH}{C}H\text{-}CONH\text{-}CH_2CH_2\text{-}CONH_2$
IVe	de-aletheine-CoA amide	[A]-D-pantoylamide-[B]	$X\text{-}\overset{OH}{C}H\text{-}CONH_2$
IVf	de-β-alanine-CoA	[A]-N-D-pantoyl cysteamine-[B]	$X\text{-}\overset{OH}{C}H\text{-}CONH\text{-}CH_2CH_2SH$

[A]=P¹-adenosine 3′-phosphate 5′-P²-
[B]=4′-pyrophosphate



pyrophosphate (de-cysteamine-CoA nitrile), which was obtained by the condensation of D-pantothenonitrile 4′-phosphate with adenosine 2′,3′-cyclic phosphate 5′-phosphoromorpholidate followed by the specific fission of cyclic phosphate with ribonuclease T_2. The synthesis of CoA-(L) (VIII) has been performed from de-cysteamine-CoA(L) nitrile (IVa) according to the same principle. L-Pantothenonitrile 4′-phosphate (IIa L-form) was obtained by phosphorylation with 2-cyanoethyl phosphate of L-pantothenonitrile (VIIa L-form). The latter compound synthesized from L-pantolactone (V L-form) and 3-aminopropionitrile has the same melting point and the revers sign of $[\alpha]_D$ with the D-isomer.

The other analogs have been synthesized by a combination of the methods of Moffatt and Khorana,[5] for the formation of pyrophosphate bond, and Michelson,[6] for 2′,3′-cyclic phosphate

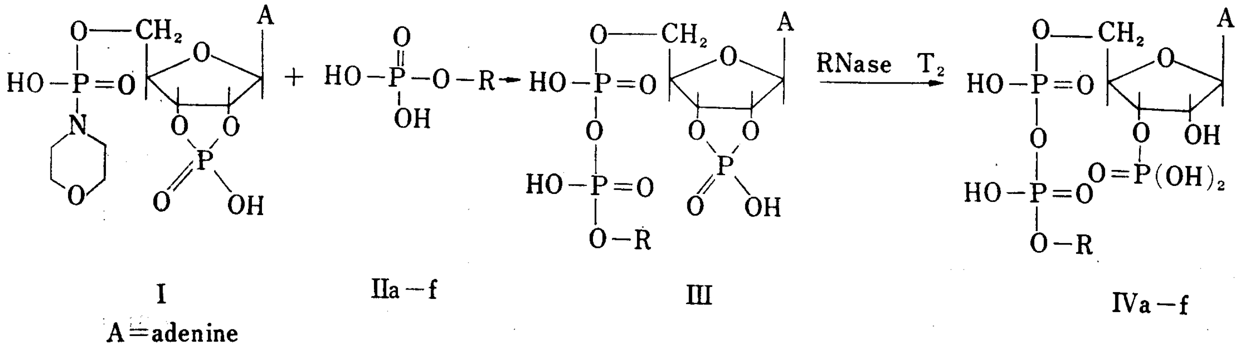


Chart 1

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5) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **81**, 1265 (1959); **83**, 663 (1961).
6) A.M. Michelson, *Biochim. Biophys. Acta*, **50**, 605 (1961); **93**, 71 (1964).

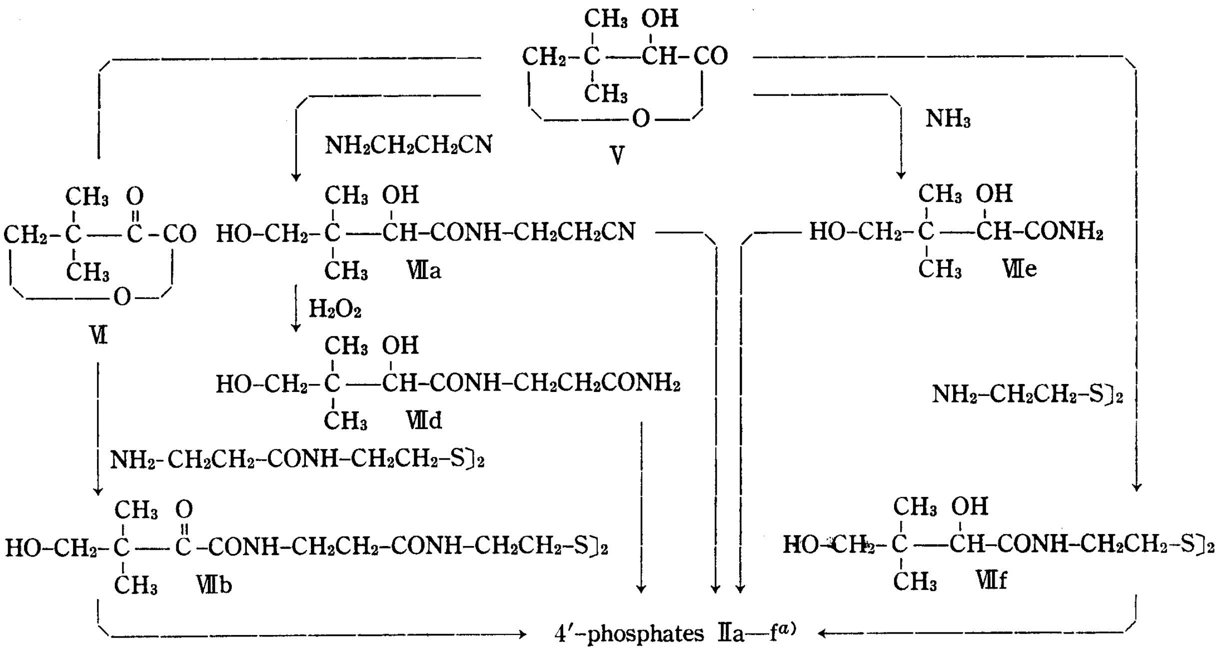

Chart 2

a) a—f of II correspond to a—f of VII, respectively.

TABLE II. Physical Constants and Analytical Data of CoA-analogs and Intermediates

Compd.	Yield (%)	$[\alpha]_D^{25}$ ($c=2.0$, H_2O)	IR ν_{max}^{KBr} (cm^{-1}) amide	IR ν_{max}^{KBr} (cm^{-1}) PO_3^{2-}		$Rf^{a)}$ I	$Rf^{a)}$ II	Formula	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
IIa(L)	48.0	−10.6	1650, 1530	1085,	980	.38	.45	$C_9H_{15}O_6N_2PBa \cdot H_2O$	24.93	3.95	6.46	24.88	4.04	6.26
IIb	52.4		1650, 1540	1090,	980	.28	.43	$C_{22}H_{36}O_{14}N_4P_2S_2Ba_2 \cdot 3H_2O$	25.53	4.09	5.41	25.68	4.52	5.47
IId	58.3	+12.1	1660, 1560	1090,	980	.26	.31	$C_9H_{17}O_7N_2PBa \cdot H_2O$	23.93	4.22	6.20	23.66	4.47	5.26
IIe	21.1	+13.4	1670	1090,	980	.31	.35	$C_6H_{12}O_6NPBa \cdot 3H_2O$	17.30	4.36	3.36	17.13	4.04	3.73
IIf	32	+17.8	1650, 1530	1090,	1000	.27	.42	$C_{16}H_{30}O_{12}N_2P_2S_2Ca_2 \cdot 2H_2O$	28.06	5.01	4.09	27.90	5.28	3.94
			amide	PO_2^-	P–O–P	III	IV							
VIII	52.3		1650, 1546	1250	958	.28	.12	$C_{21}H_{33}O_{16}N_7P_3SLi_3 \cdot 8H_2O$	27.13	5.31	10.55	27.29	5.03	9.98
IVa(L)	58.2		1650, 1538	1245	955	.22	.09	$C_{19}H_{27}O_{15}N_7P_3Li_3 \cdot 7H_2O$	27.38	4.96	11.77	27.08	5.09	11.64
IVb	39.7		1650, 1540	1245	955	.30	.13	$C_{21}H_{31}O_{16}N_7P_3SLi_3 \cdot 9H_2O$	26.68	5.22	10.37	26.79	5.05	10.40
IVc	57.7		1650, 1550	1245	955	.20	.02	$C_{19}H_{27}O_{17}N_6P_3Li_4 \cdot 4H_2O$	28.37	4.39	10.45	28.45	4.76	9.37
IVd	54		1660, 1550	1250	960	.19	.10	$C_{19}H_{29}O_{16}N_7P_3Li_3 \cdot 8H_2O$,	26.25	5.22	11.38	26.38	5.08	10.54
IVe	65.7		1660	1243	960	.19	.10	$C_{16}H_{24}O_{15}N_6P_3Li_3 \cdot 6H_2O$	25.21	4.76	11.03	24.91	4.64	10.90
IVf	46.1		1650, 1540	1245	960	.25	.11	$C_{18}H_{28}O_{15}N_6P_3SLi_3 \cdot 6H_2O$	26.29	4.90	10.22	26.44	4.65	9.83

a) Paper chromatography was performed on Toyo Roshi No. 51A by the ascending technique.
solvent system: I, PrOH–NH_4OH–H_2O (6:3:1); II, BuOH–AcOH–H_2O (5:2:3); III, EtOH–0.5N $AcONH_4$ buffer (pH 3.8) (5:2); IV, EtOH–1N $AcONH_4$ buffer (pH 7.5) (5:2)

fission, as shown in Chart 1. The derivatives of pantoic acid used were obtained as shown in Chart 2. Phosphorylation of the derivatives (VIIa—f) including 2′,2″-diketopantethine⁷⁾ was effected with 2-cyanoethyl phosphate and N,N′-dicyclohexylcarbodiimide (DCC). It has been confirmed by the previous work⁸⁾ that the present phosphorylating agent gives exclusively 4′-phosphates in the pantoic acid derivatives. The synthesis of pantothenic acid 4′-phosphate (IIc) has been already reported.⁹⁾

The properties and constants of the CoA analogs thus obtained are shown in Table II. The experimental results and discussion on the interaction between phosphotransacetylase and CoA using CoA analogs will be reported elsewhere in the near future.

Exprimental

L-Pantothenonitrile (VIIa)——A mixture of L-pantolactone (1.30 g) and 3-aminopropionitrile (0.70 g) was heated at 50° for 3.5 hr and then recrystallized from AcOEt to give VIIa (934 mg, 46.7%), mp 82.5—84°, prisms, $[\alpha]_D^{26}$ $-28.5°$ (c=2.0, H_2O). *Anal.* Calcd. for $C_9H_{16}O_3N_2$: C, 53.98; H, 8.06; N, 13.99. Found: C, 54.31; H, 8.01; N, 13.75.

D-Pantothenamide (VIId)[10]——A slight alkaline solution of D-pantothenonitrile (2.0 g) in 3% H_2O_2 (40 ml) was stirred at room temperature for 9 hr and then H_2O_2 was removed by several evaporations with water *in vacuo*. The residue was treated with IR 120 (H^+) and IRA 410 (OH^-), precipitated from MeOH solution with addition of ether, and dried *in vacuo* over P_2O_5 to yield a viscous syrup of VIId (1.83 g, 83.8%), $[\alpha]_D^{25}$ $+25.7°$ (c=2.1, H_2O). *Anal.* Calcd. for $C_9H_{18}O_4N_2$: C, 49.52; H, 8.31; N, 12.84. Found: C, 48.96; H, 8.63; N, 12.42.

General Procedure for the Synthesis of Pantothenic Acid 4′-Phosphate Analogs (IIa—f)——A mixture of pantothenic acid analog (4 mmoles), pyridinium salt of 2-cyanoethyl phosphate (6 mmoles) and DCC (6 mmoles) in anhydrous pyridine (15 ml) was stirred at room temperature for 1—2 days. Water (10 ml) was added and after 1 hr, dicyclohexylurea was filtered off. The filtrate was evaporated *in vacuo*, the residue dissolved in water, and the solution neutralized with 0.2M $Ba(OH)_2$. After evaporation of the solvent, the residue was dissolved in MeOH (10 ml) and the insoluble material was removed by filtration. Evaporation of the filtrate *in vacuo* gave the residue, which was crystallized from EtOH or a mixture of MeOH and AcOEt to afford the barium salt of pantothenic acid analog 4′-(2-cyanoethyl) phosphate. It was dissolved in water (3 ml) followed by addition of 4N NaOH (3 ml) with ice cooling and stirred at 0° for 30 min. The mixture was acidified with addition of an aqueous slurry of IR 120 (H^+) and applied to the top of a column of IR 120 (H^+) (7 ml). The effluent was neutralized with $Ba(OH)_2$ and evaporated *in vacuo* to give the barium salt of II, which was purified by precipitation from MeOH–acetone or H_2O–EtOH.

General Procedure for the Synthesis of CoA Analogs (IVa—f)——A mixture of the pyridinium salt of II (0.8 mmole) and the bis-(4-morpholine N,N′-dicyclohexylcarboxamidinium) salt of adenosine 2′,3′-cyclic phosphate 5′-phosphoromorpholidate⁵⁾ (I, 0.4 mmole) was rendered anhydrous by evaporation of added pyridine, dissolved in anhydrous pyridine (30 ml), and left at room temperature overnight. After evaporation of the solvent *in vacuo*, the residue was dissolved in acetate buffer (pH 4.5, 2 ml) followed by addition of partially purified RNase $T_2$⁴⁾ (2000 units) and incubated at 25° for 2 hr. The mixture was chromatographed on a column (2 × 19 cm) of DEAE–Sephadex A–25 (Cl^-), which was eluted with a linear gradient method from 0.003N HCl (2 liters) in a mixing chamber to 0.15M LiCl in 0.003N HCl (2 liters) in a reservoir at flow rate of 1.5 ml per min. The main peak containing IV was adjusted to pH 4.5 with LiOH and evaporated to dryness *in vacuo*. LiCl was removed by dissolving the residue in MeOH and precipitating with 20 volumes of acetone. Drying over P_2O_5 *in vacuo* gave the trilithium salt of IV as a white powder.

L-CoA (VIII)——The preparation from de-cysteamine-CoA nitrile (L-form) (244 mg, 0.3 mmole) and cysteamine (127 mg, 1.65 mmoles) *via* thiazoline intermediate was performed by the same manner as with CoA⁴⁾ and methyl-CoA³⁾ except that chromatography was carried out on a column (1.55 × 25 cm) of DEAE–Sephadex A–25 (Cl^-) using a linear salt gradient of 0.003N HCl (2 liters) and 0.3N LiCl in 0.003N HCl (2 liters).

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7) O. Nagase, Y. Hosokawa, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **17**, 398 (1969).
8) Y. Hosokawa, M. Tomikawa, O. Nagase, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **17**, 202 (1969).
9) S. Okada, O. Nagase, and M. Shimizu, *Chem. Pharm. Bull.* (Tokyo), **15**, 713 (1967).
10) M.B. Moore, U.S. Patent 2369839 (1945).