Table I shows the result obtained with this system. The function of coenzyme A in numerous biochemical reactions originates in acylation of its mercapto group. Therefore, it is very interesting that the structural modifications on cysteamine moiety caused hindrance or disappearance of coenzyme function and had a tendency to reduce the activity of coenzyme A when present with the latter.

Table I. Activity of CoA Analogs in the Phosphotransacetylase System

	Unit assayed as CoA	Assay (%) of added CoA
α-CH_3-CoA	0.63 0.55	
β-CH_3-CoA	0.0 0.0	
Carboxy-CoA	0.0 0.0	
CoA 2.1 units plus α-CH_3-CoA	2.50	93
plus β-CH_3-CoA	1.64	78
plus Carboxy-CoA	1.87	89
CoA 4.1 units plus α-CH_3-CoA	4.36	92
plus β-CH_3-CoA	2.93	71.5
plus Carboxy-CoA	3.60	88

38 mμmoles (12 units equivalent to CoA) of each CoA analog was assayed for CoA activity by the phosphotransacetylase method. And 2.1 and 4.1 units of CoA were assayed in the presence of 38 mμmoles of each of CoA analogs.

Satisfactory analytical data were obtained for all the compounds described above, which showed only one spot on paper chromatogram or paper electropherogram. Full paper on this work will be published in the near future.

The authors express their grateful acknowledgments to Dr. J. Shinoda, Chairman of the Board of Directors, and Dr. Ishiguro, President of this Company, for their unfailing encouragements.

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Synthesis of Guano-coenzyme A*[1]

In parallel with the preceding experiment,[1] the authors attempted to replace adenine in coenzyme A structure with guanine. This communication deals with the synthesis and activity in the phosphotransacetylase system of guano coenzyme A (P^1-guanosine 3′-phosphate 5′-P^2-D-pantetheine 4′-pyrophosphate).

*1 A part of this work was read at the 9th Symposium on the Chemistry of Natural Products, Japan (Osaka, October 15, 1965).

1) M. Shimizu, O. Nagase, Y. Hosokawa, H. Tagawa, Y. Abiko, T. Suzuki : This Bulletin, **14**, 681 (1966).

According to the previous experience in the total synthesis of coenzyme A,[2] the authors employed the Moffatt-Khorana method[3] for pyrophosphate bond formation and the Michelson method[4] for opening the 2′,3′-cyclic phosphate bond. Guanosine 2′,3′-cyclic phosphate 5′-phosphoromorpholidate (I) in dimethylformamide was allowed to react with D-pantetheine 4′-phosphate (II) in anhydrous pyridine at room temperature overnight. The crude product (III) obtained by evaporation of the reaction mixture was incubated

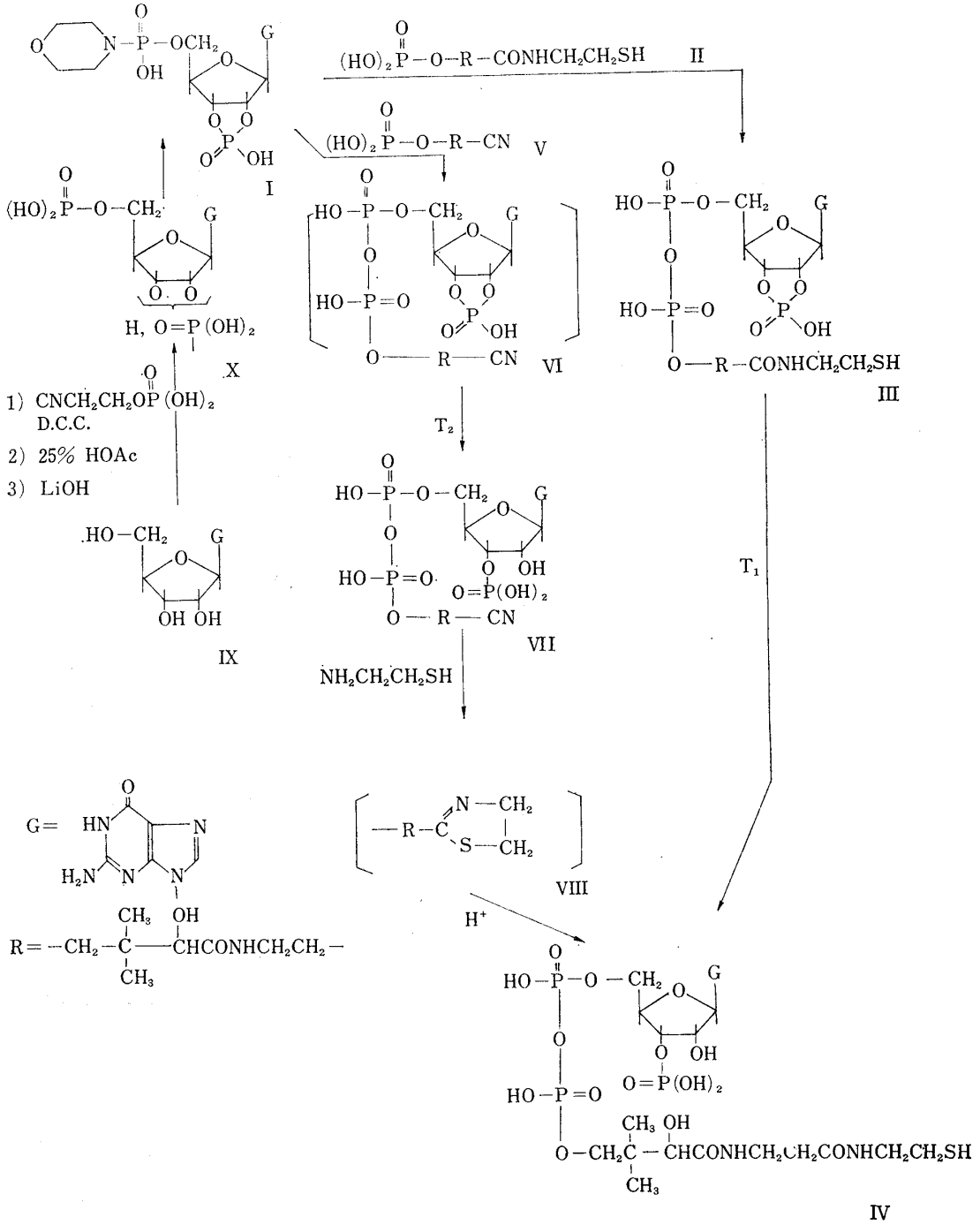

Chart 1.

2) M. Shimizu, O. Nagase, S. Okada, Y. Hosokowa, H. Tagawa: This Bulletin, **13**, 1142 (1965).
3) J.G. Moffatt, H.G. Khorana: J. Am. Chem. Soc., **81**, 1265 (1959); **83**, 663 (1961).
4) A.M. Michelson: Biochim. Biophys. Acta, **50**, 605 (1961); **93**, 71 (1964).

with partially purified ribonuclease T_1[5] at 37° for 13 hours in aqueous solution adjusted to pH 7.5. By column chromatography on DEAE-cellulose(Cl^-) using a linear salt gradient elution, neutralization with lithium hydroxide, reduction with β-mercaptoethanol, and precipitation with acetone from methanolic solution, an analytically pure sample of guano-coenzyme A (Ⅳ) (guanosine-phosphorus=1:3.13) was obtained in 23.6% yield as its trilithium salt ($C_{21}H_{33}O_{17}N_7P_3SLi_3 \cdot 17H_2O$). The structure of this compound was confirmed by paper chromatographic detection of guanosine 3′,5′-diphosphate in alkaline hydrolyzate and of D-pantetheine 4′-phosphate in acidic hydrolyzate.

Applicability of the thiazoline method[1,2] developed by the present authors was also checked in this case. The key compound (Ⅶ), P^1-guanosine 3′-phosphate 5′-P^2-D-pantothenonitrile 4′-pyrophosphate, was prepared in 26.9% yield by the reaction of I with D-pantothenonitrile 4′-phosphate[2] (Ⅴ) as described above and opening of 2′,3′-cyclic phosphate bond with ribonuclease T_2.[6,7] The use of T_2 was based on the more rapid velocity for hydrolysis than T_1. This compound (Ⅶ) ($C_{19}H_{27}O_{16}N_7P_3Li_3 \cdot 6H_2O$) showed a band for nitrile at 2250 cm^{-1} in its infrared spectrum. Ⅶ suspended in methanol was refluxed with 5.5 equivalents of cysteamine in nitrogen for 12 hours, and the resultant thiazoline intermediate (Ⅷ), without purification, was hydrolyzed in aqueous solution adjusted to pH 5.0 with hydrochloric acid at 60° for 5 hours to give a crude guano-coenzyme A (Ⅳ). After purification as described above, a pure sample was obtained in 7.18% yield from Ⅶ. Owing to insolubility of the starting material (Ⅶ) in methanol, the reaction time was forced to be prolonged for completion of ring closure. Therefore, the unsatisfactory yield by the thiazoline method was thought to be due to concomitant decomposition of pyrophosphate bond during the reaction. In this case only the disulfide form was found in the effluent from column chromatography, which was different from the case of the synthesis of coenzyme A. Guano-coenzyme A seems to be more easily oxidized than coenzyme A.

With regard to the preparation of I, experimental data deserve mention here. Guanosine (Ⅸ) in dimethylformamide was phosphorylated by standing with 6 equivalents of 2-cyanoethyl phosphate and 12 equivalents of dicyclohexylcarbodiimide (DCC) in pyridine at room temperature for 4 days to give guanosine 2′(3′),5′-diphosphate[4] (X) ($C_{10}H_{13}O_{11}N_5P_2Li_2 \cdot 3H_2O$) (guanosine-phosphorus=1:2.06) in 48% yield after stepwise elution through ion exchanger column. By refluxing X in aqueous *tert*-BuOH with morpholine and DCC, I ($C_{48}H_{82}O_{12}N_{12}P_2 \cdot H_2O$) (guanosine-phosphorus=1:2.05) was obtained in 90% yield as 4-morpholine-N,N′-dicyclohexylcarboxamidinium salt.[8]

As shown in Table I, guano-coenzyme A was inactive in the assay system using phosphotransacetylase. Therefore, the adenine moiety in coenzyme A seems to carry

Table I. Activity of Guano-CoA in the Phosphotransacetylase System

	Unit assayed as CoA	Assay (%) of added CoA
Guano-CoA	0.0 0.0	—
CoA 2.1 units plus Guano-CoA	2.26	108
CoA 4.1 units plus Guano-CoA	4.48	110

38 mμmoles (12 units equivalent to CoA) of guano-CoA was assayed for CoA activity with or without added CoA (2.1 or 4.1 units).

5) K. Sato-Asano: J. Biochem. (Tokyo), **46**, 31 (1959).
6) M. Naoi-Tada, K. Sato-Asano, F. Egami: J. Biochem. (Tokyo), **46**, 757 (1959); T. Uchida, F. Egami: Progress of Ribonucleic Acid Research, **3**, 59 (1964).
7) G. W. Rushizky, H. A. Sober: J. Biol. Chem., **238**, 371 (1963).
8) cf. J. G. Moffatt, H. G. Khorana: J. Am. Chem. Soc., **83**, 649 (1961).

the subsidiary function in enzymatic reaction. It is notable that guano-coenzyme A has a tendency to increase the activity of coenzyme A when present with it.

Satisfactory analytical values were obtained for all the compounds described above, which showed only one spot on paper chromatogram or paper electropherogram. Detailed report on this work will be published in the near future.

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

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