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Structural Modification of Coenzyme A by Synthesis *via* Thiazoline Intermediate*1

A number of studies on the structural modification of various coenzymes were reported recently.[1] As for coenzyme A, however, studies were limited to those of 3′-dephospho-coenzyme A,[2] iso-coenzyme A[2] and seleno-coenzyme A,[3] which were shown to be inactive in the phosphotransacetylase system. In the preceding communication,[4] the authors reported a total synthesis of coenzyme A *via* thiazoline intermediate. This synthetic route prompted structural modification of cysteamine moiety of coenzyme A. This communication deals with the synthetic data and activity of these modified compounds in the phosphotransacetylase system.

(I) α-Methyl- (IVa) and β-Methyl-coenzyme A (IVb)

The key intermediate used in the present experiment is the same as that in the preceding communication; P^1-adenosine 3′-phosphate 5′-P^2-D-pantothenonitrile 4′-pyrophosphate (Ia). According to the thiazoline method already described,[4] Ia as trilithium salt was refluxed in methanolic solution with 5 equivalents of *rac*-α-methylcysteamine[5] (2-amino-1-propanethiol) (IIa) or *rac*-β-methylcysteamine[6] (1-amino-2-propanethiol) (IIb) in nitrogen for 6~6.5 hours. The respective thiazoline intermediates (IIIa, b), without purification, was hydrolyzed in aqueous solution adjusted to pH 4.7 with hydrochloric acid at 60° for 3.5 hours to give the crude α-methyl-coenzyme A (IVa) (P^1-adenosine 3′-phosphate 5′-P^2-N-D-pantothenoyl-2-amino-1-propanethiol 4′-pyrophosphate) or β-methyl-coenzyme A (IVb) (P^1-adenosine 3′-phosphate 5′-P^2-N-D-pantothenoyl-1-amino-2-propanethiol 4′-pyrophosphate) after passage through Dowex 50(H^+) column, neutralization with lithium hydroxide, and reduction with 2-mercaptoethanol followed by precipitation with acetone from methanolic solution. Furthur purification was effected by chromatography on DEAE-cellulose column using a linear salt gradient to afford analytically pure α-methyl-coenzyme A (IVa) ($C_{22}H_{35}O_{16}N_7P_3SLi_3 \cdot 10H_2O$) (adenosine-phosphorus=1:3.00) in 32.3% yield or β-methyl-coenzyme A (IVb) ($C_{22}H_{35}O_{16}N_7P_3SLi_3 \cdot 7H_2O$) (adenosine-phosphorus=1:3.02) in 37.2% yield. The presence of 3′-phosphate in both compounds was confirmed by paper chromatography of the alkaline hydrolyzate showing a spot for adenosine 3′,5′-diphosphate.

In addition to the above synthesis, α-methyl- and β-methylpantethine, the disulfide form of the partial structure of IVa and IVb, were synthesized in 66% and 83% yield by the thiazoline method.[7]

(II) Carboxy-coenzyme A (IVc) and 3′-Dephospho-carboxy-coenzyme A (IVd)

Biochemical examination on the biosynthesis of coenzyme A from pantothenic acid necessitated the preparation of two compounds with carboxyl group on cysteamine moiety in coenzyme A and 3′-dephospho-coenzyme A. The thiazoline method was also applied

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

1) A. F. Wagner, K. Folkers: "Vitamins and Coenzymes," (1962). Interscience Publishers, Inc., New York.
2) J. G. Moffatt, H. G. Khorana: J. Am. Chem. Soc., **81**, 1265 (1959); **83**, 663 (1961).
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6) S. Gabriel, E. Leupold: *Ibid.*, **31**, 2838 (1898); W. Mylius: *Ibid.*, **49**, 1096 (1916).
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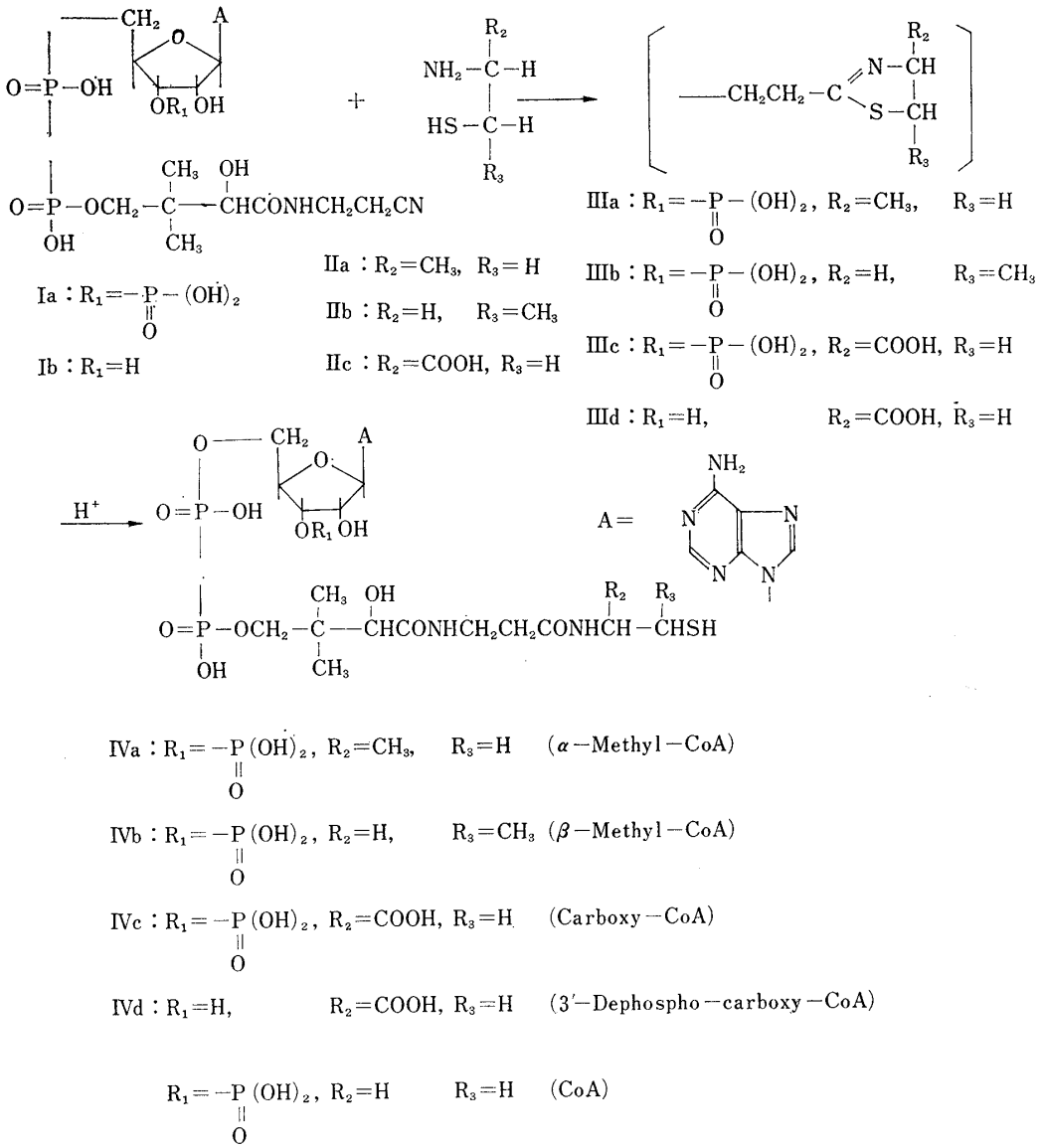

Chart 1.

in this case. Ia was refluxed in methanolic solution with 6 equivalents of L-cysteine monosodium salt (IIc), which was prepared from L-cysteine monohydrochloride monohydrate and 2 equivalents of sodium methoxide.

The successive treatments for thiazoline-ring opening and purification were the same as described above. Analytical values of pure carboxy-coenzyme A (IVc) obtained in 28.9% yield corresponded to $C_{22}H_{32}O_{18}N_7P_3SLi_4 \cdot 20H_2O$ (adenosine-phosphorus=1:3.06). Using P^1-adenosine 5'-P^2-D-pantothenonitrile 4'-pyrophosphate[4] (Ib) as the key compound, 3'-dephospho-carboxy-coenzyme A (IVd) ($C_{22}H_{32}O_{15}N_7P_2SLi_3 \cdot 5H_2O$) (adenosine-phosphorus =1:2.18) was obtained in 25.6% yield. Paper chromatogram of acidic hydrolyzate of IVc and IVd revealed the presence of 4'-phosphopantothenoyl-cysteine.

(III) Activity of the Compounds described above as Coenzyme A in the Phosphotransacetylase System

The assay system of coenzyme A activity can be established using the stable preparation of phosphotransacetylase isolated from *Escherichia coli* B according to Stadtman.[8]

8) E.R. Stadtman, A. Kornberg: J. Biol. Chem., **203**, 47 (1953).

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.

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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).