277; $\lambda_{max}^{pH\,4}$ mμ : 268; $\lambda_{max}^{pH\,1}$ mμ : 263, 278 (identical with those reported by Cavalieri and Bendich[7]). Paper chromatography : Rf 0.26 (1-butanol-water, 86:14).

Thus a versatile method of introduction of hydroxyl group to the 8-position of purine nucleosides was established. The application of this method to the various nucleosides is in progress in this laboratory.

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A Total Synthesis of Coenzyme A *via* Thiazoline Intermediate*1

In the previous paper,[1] the authors described a novel synthesis of D-pantethine (IIIa), one of the biosynthetic intermediates of coenzyme A from D-pantothenic acid,[2] which comprises the interim formation of thiazoline derivative from D-pantothenonitrile (Ia) followed by acid hydrolysis thereof (hereafter referred to as the thiazoline method). This method seems to be applicable to the syntheses of other metabolites from D-pantothenic acid including coenzyme A itself. The present communication deals with the syntheses of coenzyme A and dephospho coenzyme A by the thiazoline method.

Three independent papers on the total synthesis of coenzyme A[3~5] have so far been published, each of which employed in principle the condensation of D-pantetheine derivatives with adenine nucleotides, differing in the procedure for pyrophosphate bond formation. The present method is characterized in building up aletheine moiety in coenzyme A structure in the last step of the synthetic route.

The key intermediates used in the present experiment are P^1-adenosine-3′-phosphate-5′P^2-D-pantothenonitrile 4′-pyrophosphate (Ib) for coenzyme A, and P^1-adenosine-5′P^2-D-pantothenonitrile 4′-pyrophosphate (Ic) for dephospho coenzyme A. For the preparation of Ib and Ic, it is essential at the outset to establish the synthesis of D-pantothenonitrile 4′-phosphate (V). D-Pantothenonitrile (Ia) was treated with dibenzyl phosphorochloridate in anhydrous pyridine to furnish a syrupy substance (IV), $[\alpha]_D^{23}$ +19.1° (EtOH), in 73.2% yield. Catalytic hydrogenation of IV over palladium-charcoal afforded V, $[\alpha]_D^{25}$ +11.4° (H_2O), IR ν_{max}^{KBr} cm^{-1} : 2250 (C≡N), in 69% yield as its barium salt ($C_9H_{15}O_6N_2PBa \cdot 2H_2O$).

*1 A part of this communication was read at the Kanto Branch Meeting of the Pharmaceutical Society of Japan, May, 1965, Tokyo.

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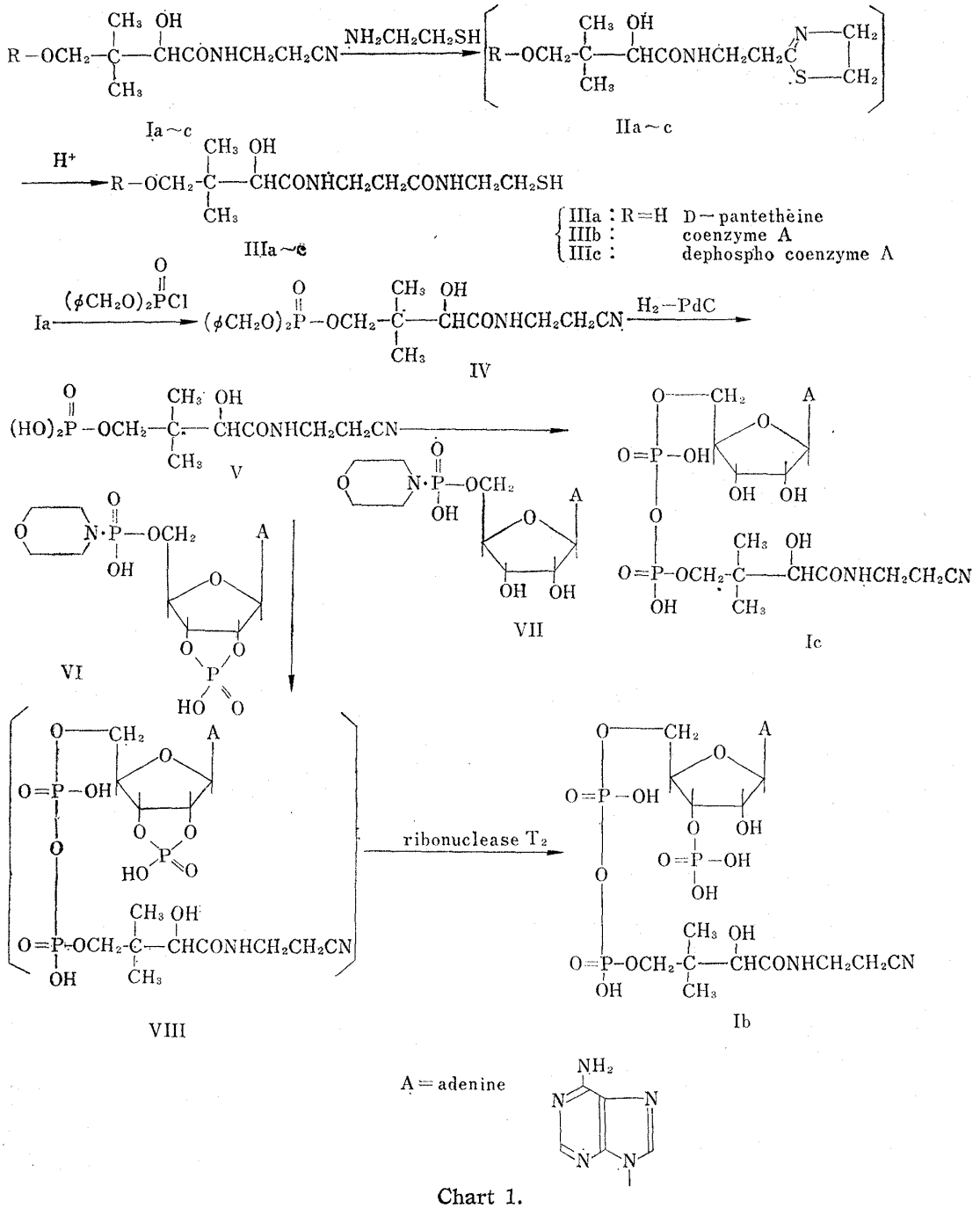

Chart 1.

The presence of 4′-phosphate bond was confirmed by conversion of V into D-pantetheine 4′-phosphate through the thiazoline intermediate.

In accordance with Khorana's pyrophosphate formation method,[3] D-pantothenonitrile 4′-phosphate (V) was allowed to react with adenosine 2′,3′-cyclic phosphate 5′-phosphoromorpholidate (VI) in anhydrous pyridine at room temperature overnight. The crude product (VIII) obtained by evaporation of the reaction mixture was incubated with partially purified ribonuclease T_2[4,6] at 37° for 3.5 hours in aqueous solution adjusted to pH 4.6 to give Ib (adenosine-phosphorus=1:3.06, IR ν_{max}^{KBr} cm^{-1} : 2250 (C≡N)) in 61.8% yield as its trilithium salt ($C_{19}H_{27}O_{15}N_7P_3Li_3 \cdot 6H_2O$).

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According to the thiazoline method,[1] Ib was refluxed in methanolic solution with 5 equivalents of cysteamine in nitrogen for 7 hours, and the resulting thiazoline intermediate (IIb), 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 coenzyme A (IIIb) in 78.5% yield after passage through Dowex 50(H^+) column, neutralization with lithium hydroxide, and reduction with 2-mercaptoethanol followed by precipitation with acetone from methanolic solution. When assayed by the modified phosphotransacetylase system,[*2,7] the content of coenzyme A in this sample was 80%. Further purification was effected by chromatography on DEAE-cellulose column using a linear salt gradient to yield analytically pure coenzyme A (IIIb) (adenosine-phosphorus=1:3.03). Coenzyme A thus obtained was chromatographically and electrophoretically identical with the commercial sample as well as the synthetic sample to be described below. The total yield was 29.7% based on Ib. The phosphotransacetylase assay showed its purity to be 102.5~107%.

For comparison with the above result, Khorana's original procedure was followed exactly, using VI and D-pantetheine 4′-phosphate prepared by the thiazoline method, but it was found very difficult in separating coenzyme A from iso-coenzyme A. The use of ribonuclease T_2 for hydrolysis of 2′,3′-cyclic phosphate bond was very effective in improving the yield to 37.8% and the purity of coenzyme A to 104~107%.

Prior to the above synthesis of coenzyme A, the thiazoline method had been applied to the synthesis of dephospho coenzyme A as a preliminary experiment. V was condensed with VII to give Ic (adenosine-phosphorus=1:2.00, IR ν_{max}^{KBr} cm^{-1}: 2250 (C≡N)) in 52.5% yield as the dilithium salt ($C_{19}H_{27}O_{12}N_7P_2Li_2 \cdot 4H_2O$). Then, Ic was converted through thiazoline intermediate (IIc) to dephospho coenzyme A (IIIc) (adenosine-phosphorus=1:1.89) in 64.0% yield as the dilithium salt ($C_{21}H_{33}O_{13}N_7P_2SLi_2 \cdot 5H_2O$). Dephospho coenzyme A thus obtained had no activity in the phosphotransacetylase system.

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

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*2 Coenzyme A was assayed according to Stadtman with phosphotransacetylase which was prepared from *Escherichia coli* B, with a minor modification. The procedure for the preparation of this enzyme from *E. coli* B will be reported in detail elsewhere.

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