CHEMISTRY LETTERS, pp. 595-598, 1972. Published by the Chemical Society of Japan

# A TOTAL SYNTHESIS OF COENZYME A BY OXIDATION-REDUCTION CONDENSATION

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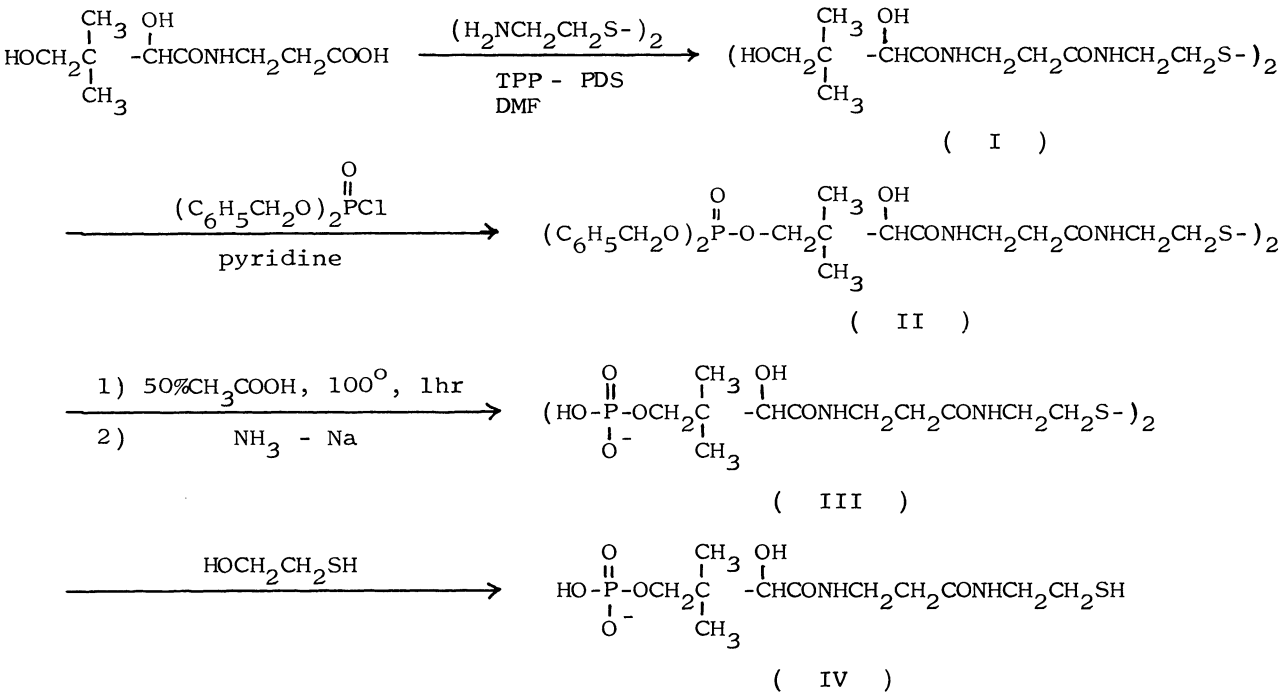
A total synthesis of coenzyme A by utilization of the oxidation-reduction condensation in each important steps is described. Adenosine 2',3'-cyclic phosphate 5'-phosphoromorpholidate and pantethine, important intermediates of coenzyme A, were prepared starting from adenosine 2' (3'-) phosphate and pantothenoic acid in 60% and 80% yields, respectively, mainly by the use of triphenylphosphine and 2,2'-dipyridyl disulfide as coupling reagents.

It has been reported in the previous communications[1-4] that the use of triphenylphosphine and 2,2'-dipyridyl disulfide (TPP-PDS) as the coupling reagents led to the formation of some biologically active peptides and oligonucleotides in good yields. This communication describes the total synthesis of coenzyme A by utilization of the above mentioned oxidation-reduction condensation in each important steps.

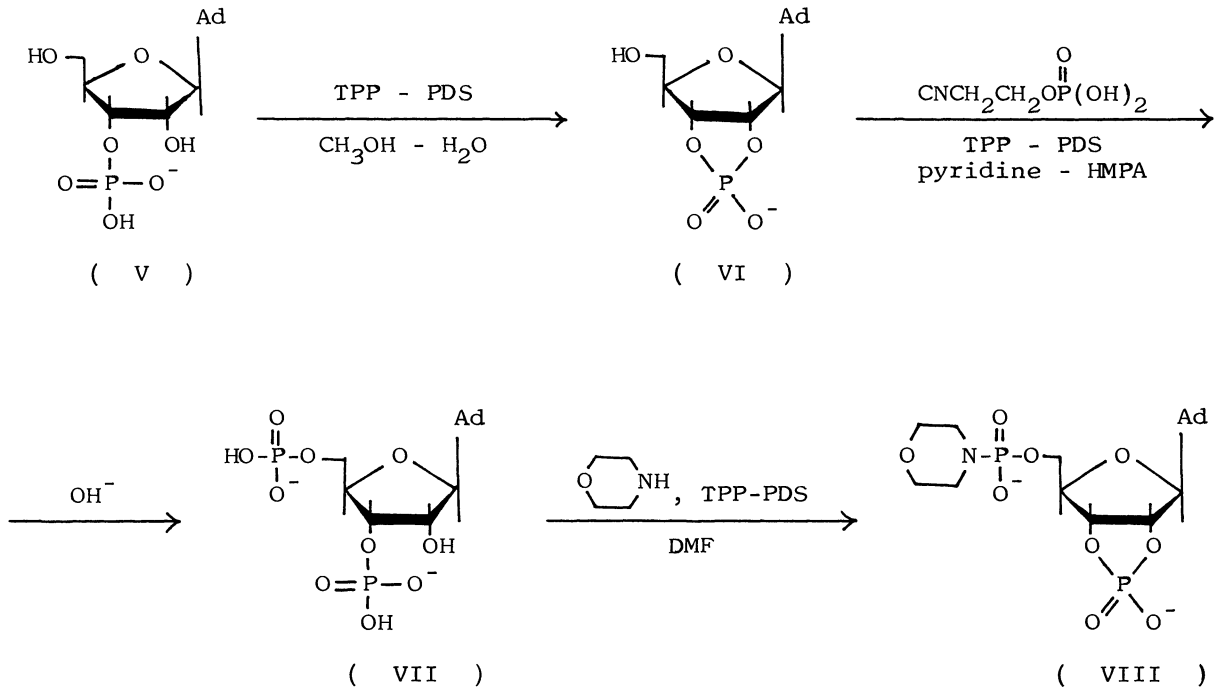
Four independent papers in the total synthesis of coenzyme A have been published, each of which employed in principle the condensation of D-pantetheine derivatives with adenine nucleotides, differing in the procedures of the formations of pyrophosphate[5-7] and cysteamine[8] bonds. The present method is characterized in building up coenzyme A structure using the oxidation-reduction condensation in each important steps as shown in Chart 1.

Two components, adenosine 2',3'-cyclic phosphate 5'-phosphoromorpholidate (VIII) and D-pantetheine-4' phosphate (IV), were prepared by the following procedure: The III was prepared and isolated in 60% yield as its barium salt by treating D-pantethine (I) with dibenzyl phosphorochloridate in anhydrous pyridine according to the ordinary method.[5,6] Found: C, 24.20; H, 4.57; N, 4.83%. Calcd for $C_{22}H_{40}O_{14}N_4S_2P_2Ba_2 \cdot 6H_2O$: C, 24.08; H, 4.75; N, 5.13%. The treatment of D-pantothenic

Synthesis of Pantetheine-4' phosphate




Synthesis of Adenosine 2',3'-cyclic phosphate 5'-phosphoromorpholidate



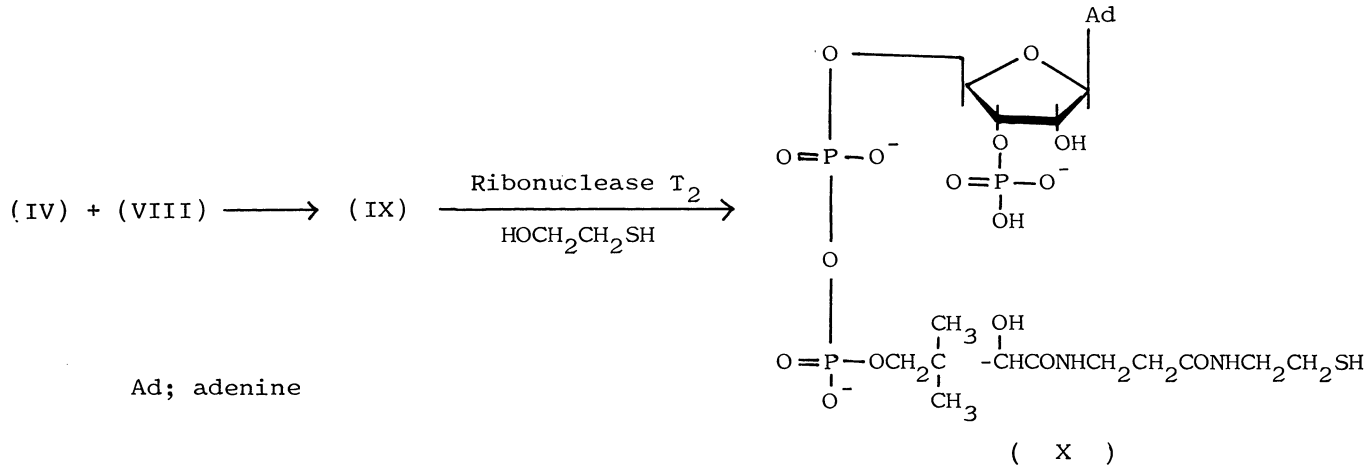



Chart 1.

acid with 0.7 mole equiv of cystamine and one equiv each of TPP-PDS in DMF gave a colorless glass substance I in 80% yield $[\alpha]_D^{20}$ 16.9° (c=3, $H_2O$). Found: C, 47.35; H, 7.86; N, 10.28; S, 11.70%. Calcd for $C_{22}H_{42}O_8N_4S_2$: C, 47.65; H, 7.64; N, 10.11; S, 11.56%. The IV was prepared by reduction of pantethine-4' phosphate (III) with 2-mercaptoethanol. The other component, VIII, was prepared in quantitative yield by the treatment of VII with excess morpholine and 5 equiv each of TPP-PDS in DMF. Found: C, 52.80; H, 7.98; N, 15.20%. Calcd for $C_{48}H_{82}O_{11}N_{12}P_2 \cdot 2H_2O$: C, 52.40; H, 7.88; N, 15.27%. The VII was obtained in 60% yield by treating VI with 2 mole equiv of β-cyanoethyl phosphate and 10 mole equiv each of TPP-PDS in pyridine-HMPA for 2 days at room temperature and successive treatment with alkali. Found: C, 25.21; H, 3.75; N, 15.05%. Calcd for $C_{10}H_{13}O_{10}N_5P_2Li_2 \cdot 2H_2O$: C, 25.03; H, 3.61; N, 14.74%. The VI was obtained in quantitative yield by the treatment of adenosine-2' (or 3') phosphate (V) with 3 equiv each of TPP-PDS in methanol-water solution.

In accordance with Khorana's pyrophosphate formation method, VIII was allowed to react with IV in anhydrous pyridine at room temperature over night. The crude product (IX), obtained by evaporation of the reaction mixture, was incubated with ribonuclease $T_2$[10] at 37° for 4.5 hr in aqueous solution adjusted to PH 4.6 to give crude coenzyme A (X). After the reaction mixture was adjusted to PH 6.0 with ammonium hydroxide and treated with 2-mercaptoethanol, purification was effected by chromatography on DEAE-cellulose column using a liner salt gradient to yield analytically pure coenzyme A (X) (adenosine-phosphorous=1:2.96). Coenzyme A thus obtained was chromatographically and electrophoretically identical with the commercial sample. The total yield was 60% based on VIII. The phosphotransacetylase assay showed its

purity to be 98%. The X was isolated as its trilithium salt. Found: C, 29.00; H, 5.36; N, 11.01%. Calcd for $C_{21}H_{33}O_{16}N_7P_3SLi_3 \cdot 6H_2O$: C, 28.84; H, 5.08; N, 10.98%. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3370, 2940, 1650, 1545, 1475, 1423, 1370, 1240, 1122, 1081, 950, 830, 720, 640, 534. Satisfactory analytical date were obtained for all the compounds described above, which showed only one spot on paper chromatogram.

In conculusion, it is noted that the oxidation-reduction condensation is useful for the preparation of two important intermediates of coenzyme A, namely adenosine 2',3'-cyclic phosphate 5'-phosphoromorpholidate and pantethine, especially in the preparation of pantethine directly from D-pantothenoic acid and cystamine in high yield.

Achnowlegment This work partly supported by a grant from the Mitsubishi Foundation.

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( Received June 5, 1972 )