biological oxidation of 6α,9α-difluorohydrocortisone[5b] with *S. roseochromogenus* gave the 16α-hydroxy derivative IV (m.p. 247–251° [α]D +58° (dioxane), $\lambda_{\max}^{\text{EtOH}}$ 234 mμ, log ε 4.18. Found for $C_{21}H_{28}F_2O_6$: C, 61.61; H, 6.92).

In preliminary assays, these values based on thymolytic and anti-inflammatory activity were found[12] (hydrocortisone = 1, 9α-fluoro-16α-hydroxyprednisolone = 5[12]): I = 5; II = 4; III = 20; IV = 15. I through IV exhibited marked excretion of sodium.[12]

[12] All assays were carried out in adrenalectomized rats, the salt assays without sodium chloride load and the anti-inflammatory assays by cotton pellet implant. For anti-inflammatory and thymolytic activities, essentially equal for this series of compounds, the compounds were administered orally. We wish to thank Dr. R. I. Dorfman, The Worcester Foundation for Experimental Biology, for the bioassays.

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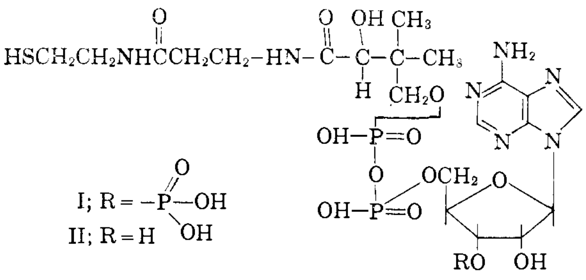
THE TOTAL SYNTHESIS OF COENZYME A

Sir:

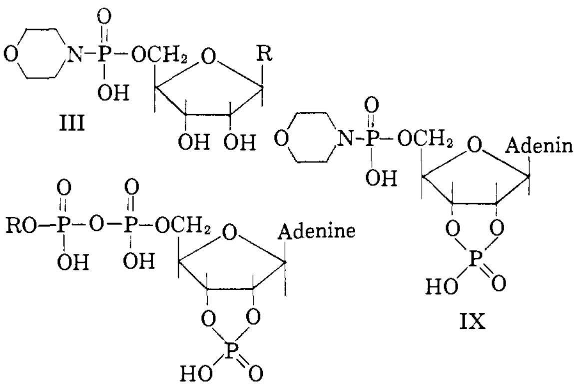
Since its discovery some twelve years ago,[1] Coenzyme A (I) has been the focus of intensive chemical and biochemical research and the substance is now known to occupy a central position as a mediator of biosynthetic reactions. We wish to record the total chemical synthesis of this complex substance.



The basic approach used for the synthesis of the pyrophosphate bond, a major problem in the synthesis of I, is that which was described recently for the synthesis of other nucleotide coenzymes.[2] However, we now wish to report that nucleoside-5′ phosphoromorpholidates (III) are superior to the unsubstituted phosphoramidates because of their high solubility in organic solvents and greater reactivity.

In initial experiments, the reaction of adenosine-5′ phosphoromorpholidate (III, R = adenine) with DL-pantetheine-4′ phosphate[3] gave "dephospho"-Coenzyme A (II) containing a racemic pantoyl group in 63% yield. The product which was homogeneous by a number of criteria had the correct phosphorus:adenosine:sulfhydryl ratios.

The key intermediate used for the synthesis of Coenzyme A itself was IV, which was prepared in 98% yield by the reaction of adenosine-2′(3′),5′-diphosphate[4] with morpholine and dicyclohexylcarbodiimide. The reaction of IV with DL-pantetheine-4′ phosphate in anhydrous pyridine for 15 hr. at room temperature gave V, which was purified by ion exchange chromatography and characterized. More directly, the synthetic mixture was treated with 0.1*N* hydrochloric acid to open the cyclic phosphate ring, then with 2-mercaptoethanol and the products then chromatographed on an ECTEOLA cellulose column. Coenzyme A and its 2′-phosphate isomer (*iso*-Coenzyme A) were eluted together in 50% yield. The product was chromatographically and electrophoretically homogeneous and identical with natural sulfhydryl-Coenzyme A. It had a ratio of P:Adenosine:SH of 2.98:1.00:1.06. When assayed by the phosphotransacetylase system,[5] the sample had 33%[6] Coenzyme A activity. This result is in the expected range in view of the fact that DL-pantetheine-4′ phosphate had been used and that the cyclic phosphate ring opened to give roughly equal mixture of the two isomers.



V, R = Pantetheine

Finally, the reaction of IV with D-pantetheine-4′ phosphate[7] followed by a work-up as above gave a product which on rechromatography on ECTEOLA cellulose largely resolved into two peaks. The first peak (*iso*-Coenzyme A) had 4%[6] enzymic activity and gave mostly adenosine-2′,5′-diphosphate after digestion with crude venom. The second peak showed 86% Coenzyme A activity and gave after degradation with venom mainly adenosine-3′,5′ diphosphate.

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[1] F. Lipmann, "Les Prix Nobel," Stockholm, 1954, p. 151.

[2] J. G. Moffatt and H. G. Khorana, THIS JOURNAL, **80**, 3756 (1958).

[3] Prepared by modification of procedure of J. Baddiley and E. M. Thain, *J. Chem. Soc.*, 1610 (1953).

[4] Prepared in 60% yield by an improvement of the method of F. Cramer, G. W. Kenner, N. A. Hughes and A. R. Todd, *ibid.*, 3297 (1957).

[5] E. R. Stadtman in "Methods in Enzymology," Vol. I, Academic Press Inc., New York, N. Y., 1955, p. 596.

[6] As against a Pabst preparation which is regarded as 75% active.

[7] Prepared in 44% yield by improvement of the procedure of Baddiley and Thain (ref. 3).