The reaction of coenzyme Q_{10} with ethanol and sodium ethoxide gave a product, m.p. 34.5–35.5°. *Anal.* Found: C, 82.02; H, 10.44. Its n.m.r. spectrum corresponds to the diethoxy homolog (II).

The name ubiquinone was proposed by Morton, *et al.*,[2,3] "for a substance" which melted at 33–34°, 36°, and 41° ("melting points sharp"). These melting points are significantly lower than that for coenzyme Q_{10} (m.p. 48–49°)[5] and correspond closely to those of the diethoxy and the ethoxy homologs. Furthermore, the infrared spectra[1,2] of ubiquinone appear to correspond to the ethoxy derivatives rather than to that of coenzyme Q_{10}; spectra of carbon disulfide solutions were compared.

An infrared band at 10.55 μ for coenzyme Q_{10} is absent from the spectrum of the ethoxy product (I), and the latter has new bands at 10.10 and 11.18 μ. The 8.30, 8.67 and 10.55 μ bands of coenzyme Q_{10} are not in the spectrum of the diethoxy product (II), and the latter exhibits new bands at 8.51, 10.20 and 11.05 μ. The infrared spectrum[1] of the "best fraction" of ubiquinone (SA) appears identical to that of the diethoxy homolog (II). The infrared spectrum[1] for a second component of ubiquinone (SA) shows bands at 10.20 and 11.1 μ and no band at 10.55 μ; this spectrum is different from that of coenzyme Q_{10} and closely resembles that of the ethoxy homolog (I). Morton and co-workers[1] in their isolation of these ubiquinone preparations used hot ethanolic alkali for the saponification of tissues.

The first published description of a crystalline quinone melting at 48–49° was by Crane, *et al.*,[5] in 1957; it was later designated as coenzyme Q_{10}.[6] In 1958, Morton and co-workers[7,8] and Bouman, *et al.*,[9] described the same quinone. These investigators have now used the expression ubiquinone, not as originally defined,[2,3] but synonymously with coenzyme Q_{10}.

(5) F. L. Crane, Y. Hatefi, R. L. Lester and C. Widmer, *Biochim. et Biophys. Acta*, **25**, 220 (1957).

(6) R. L. Lester, F. L. Crane and Y. Hatefi, THIS JOURNAL, **80**, 4751 (1958).

(7) N. I. Fahmy, F. W. Hemming, R. A. Morton, J. Y. F. Paterson, and J. F. Pennock, *Biochem. J.*, **70**, 1P (1958).

(8) R. A. Morton, U. Gloor, O. Schindler, G. M. Wilson, L. H. Chopard-dit-Jean, F. W. Hemming, O. Isler, W. M. F. Leat, J. F. Pennock, R. Rüegg, U. Schwieter and O. Wiss., *Helv. Chim. Acta*, **41**, 2343 (1958).

(9) J. Bouman, E. C. Slater, H. Rudney, and J. Links, *Biochim. et Biophys. Acta*, **29**, 456 (1958).

CONTRIBUTION FROM THE
MERCK SHARP & DOHME   BRUCE O. LINN
RESEARCH LABORATORIES   NELSON R. TRENNER
DIVISION OF MERCK & CO., INC.   CLIFFORD H. SHUNK
RAHWAY, NEW JERSEY   KARL FOLKERS
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STEROIDS. CXVII.[1] 6α-FLUORO-16α-HYDROXY CORTICAL HORMONES

Sir:

Among the most recent advances in the cortical hormone field have been the reversal[2] of salt

(1) Paper CXVI, J. A. Edwards, A. Zaffaroni, H. J. Ringold and C. Djerassi, *Proc. Chem. Soc.*, February (1959).

(2) S. Bernstein, R. H. Lenhard, W. S. Allen, M. Heller, R. Littell, S. M. Stolar, L. I. Feldman and R. H. Blank, THIS JOURNAL, **78**, 5693 (1956); S. Bernstein, M. Heller, R. Littell, S. M. Stolar, R. H. Lenhard and W. S. Allen, *ibid.*, **79**, 455 (1957).

retention of 9α-fluoro steroids by 16α-hydroxylation,[2,3] albeit with somewhat lowered anti-inflammatory activity,[4a,b] the potentiation of activity (with retention of desirable mineral effects) by 16,-17-acetonide formation,[4b] and the potentiation of activity by 6α-fluoro[5a,b] substitution.

We now wish to report the synthesis of a number of representative 6α-fluoro-16α-hydroxy cortical hormone analogs in which the combination of several such substituents has been accomplished.

$\Delta^{5,16}$-Pregnadiene-3β,21-diol-20-one   21-acetate[6] on successive treatment with potassium permanganate[7] and acetone–perchloric acid gave Δ^5-pregnene-3β,16α,17α,21-tetrol-20-one   16,17-acetonide 21-acetate (m.p. 215–216.5°, [α]D +8°.[8] Found for $C_{26}H_{38}O_6$: C, 70.02; H, 8.58; O, 21.74) and thence by means of monoperphthalic acid the 5α,6α-oxide (m.p. 195–196°, [α]D ±0°. Found for $C_{26}H_{38}O_7$: C, 67.42; H, 8.16; O, 23.96). Fission with boron trifluoride[9] in ether–benzene yielded the 5α-hydroxy-6β-fluoro compound (m.p. 224–226°, [α]D +30°. Found for $C_{26}H_{39}FO_7$: C, 64.10; H, 8.13; F, 4.11) which was converted to 6α-fluoro-16α-hydroxy-"S"-16,17-acetonide-21-acetate (m.p. 295–296°, [α]D +104°, λ_{max}^{EtOH} 236 mμ, log ϵ 4.19. Found for $C_{26}H_{35}FO_6$: C, 66.74; H, 7.62; F, 4.26) by chromium trioxide oxidation followed by treatment with anhydrous hydrogen chloride in acetone. Cleavage of the acetonide function with 60% formic acid and then saponification gave 6α-fluoro-16α-hydroxy-"S" (m.p. 228–230°, [α]D +64° (dioxane). Found for $C_{21}H_{29}FO_5$: C, 66.04; H, 7.81) which on incubation with bovine adrenals[10] yielded 6α-fluoro-16α-hydroxyhydrocortisone (I) (m.p. 233–235°, λ_{max}^{EtOH} 237 mμ, log ϵ 4.18. Found for $C_{21}H_{29}$-FO_6: C, 63.47; H, 7.42). Alternatively, I was prepared by fermentation of 6α-fluorohydrocortisone[5] with *Streptomyces roseochromogenus*, Rutgers Collection No. 3689.[3] The 16,17-acetonide 21-acetate of I, II (m.p. 261–263°, [α]D +135°, λ_{max}^{EtOH} 237 mμ, log ϵ 4.18. Found for $C_{26}H_{35}FO_7$: C, 65.08; H, 7.13) furnished on oxidation with selenium dioxide[11] 6α-fluoro-16α-hydroxy-prednisolone 16,17-acetonide 21-acetate (III) (m.p. 267–269° [α]D +97°, λ_{max}^{EtOH} 241 mμ, log ϵ 4.16. Found for $C_{26}H_{33}FO_7$: C, 65.78; H, 7.12). Further, micro-

(3) R. W. Thoma, J. Fried, S. Bonanno and P. Grabowich. *ibid.*, **79**, 4818 (1957).

(4) (a) S. Bernstein. *Rec. Progress in Hormone Res.*, **14**, 1 (1958); (b) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo. THIS JOURNAL, **80**, 2338 (1958).

(5) (a) A. Bowers and H. J. Ringold, *ibid.*, **80**, 4423 (1958); (b) J. A. Hogg, G. B. Spero, J. L. Thompson, B. J. Magerlein, W. P. Schneider, D. H. Peterson, O. K. Sebek, H. C. Murray, J. C. Babcock, R. L. Pederson and J. A. Campbell, *Chemistry and Industry*, 1002 (1958).

(6) J. S. Buck and R. O. Clinton, U. S. Patent 2,678,932.

(7) B. Ellis, F. Hartley, V. Petrow and D. Wedlake, *J. Chem. Soc.*, 4383 (1955).

(8) Melting points are uncorrected. Unless stated otherwise rotations were determined in chloroform.

(9) H. B. Henbest and T. I. Wrigley, *J. Chem. Soc.*, 4765 (1957); A. Bowers and H. J. Ringold, *Tetrahedron*, **3**, 14 (1958).

(10) H. Zaffaroni. H. J. Ringold, G. Rosenkranz, F. Sondheimer, G. H. Thomas and C. Djerassi, THIS JOURNAL, **80**, 6110 (1958).

(11) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *J. Org. Chem.*, **21**, 239 (1956); C. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); S. A. Szpilfogel, T. A. P. Posthumus, M. S. De Winter and D. A. van Dorp, *Rec. trav. chim.*, **75**, 475 (1956); K. Florey and A. R. Restivo, *J. Org. Chem.*, **22**, 406 (1957).

biological oxidation of 6α,9α-difluorohydrocortisone[5b] with *S. roseochromogenus* gave the 16α-hydroxy derivative IV (m.p. 247–251° [α]D +58° (dioxane), λ_{max}^{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]

RESEARCH LABORATORIES
SYNTEX, S. A.
APDO. POSTAL 2679
MEXICO, D. F.

J. S. MILLS
A. BOWERS
C. CASAS CAMPILLO
CARL DJERASSI
H. J. RINGOLD

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.


I; R = -P(=O)(OH)(OH)
II; R = H
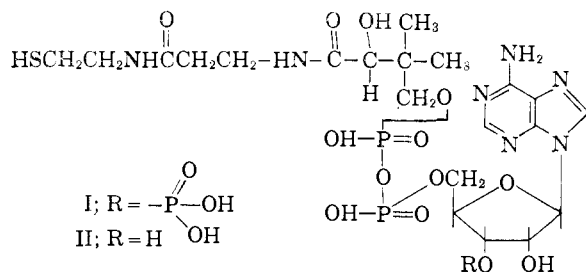
The basic approach used for the synthesis of the pyrophosphate bond, a major problem in the synthesis of I, is that which was described recently for the synthesis of other nucleotide coenzymes.[2] However, we now wish to report that nucleoside-5′ phosphoromorpholidates (III) are superior to the unsubstituted 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.

(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).

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
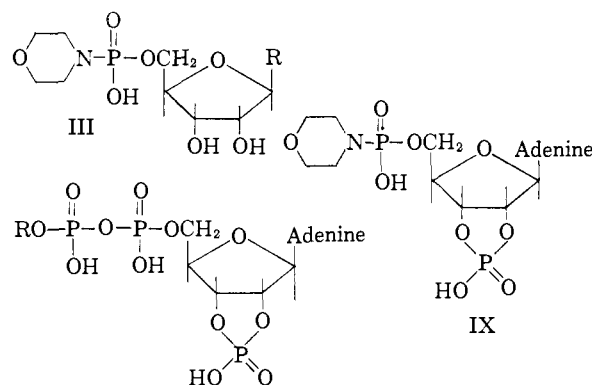
V, R = Pantetheine

characterized. More directly, the synthetic mixture was treated with 0.1N 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.

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.

BRITISH COLUMBIA RESEARCH COUNCIL
AT THE UNIVERSITY OF BRITISH COLUMBIA
VANCOUVER 8, B. C., CANADA

J. G. MOFFATT
H. G. KHORANA

(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).