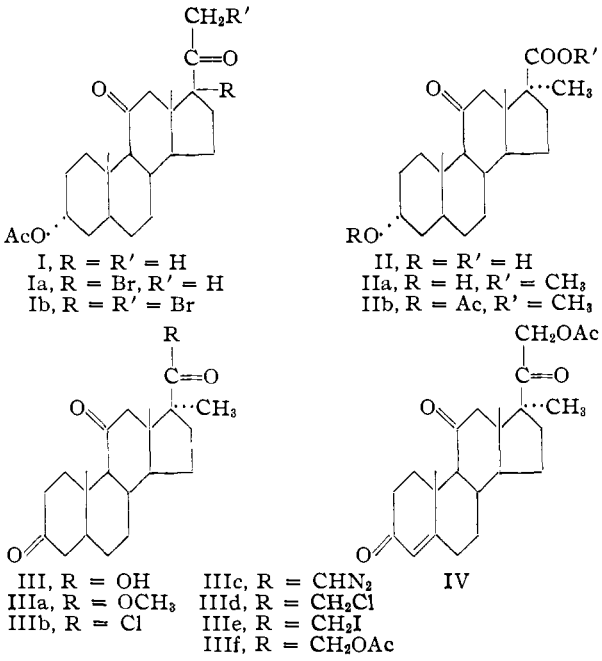

I, R = R′ = H
Ia, R = Br, R′ = H
Ib, R = R′ = Br

II, R = R′ = H
IIa, R = H, R′ = CH_3
IIb, R = Ac, R′ = CH_3

III, R = OH
IIIa, R = OCH_3
IIIb, R = Cl
IIIc, R = CHN_2
IIId, R = CH_2Cl
IIIe, R = CH_2I
IIIf, R = CH_2OAc

IV

C, 51.85; H, 6.24; Br. 29.82] and the starting material I were isolated. The crude, not easily separable bromination product was subjected to a rearrangement of the Aston–Greenburg type[10a,b,c] and, after debromination and reacetylation, methyl 3α-acetoxy-11-keto-17α-methyletianate (IIb) [m.p. 184°, $[\alpha]^{25}D$ 63.7° (*c* 0.982, $CHCl_3$); calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.99. Found: C, 71.21; H, 8.80] obtained in approximately 40% yield from the neutral fraction of the reaction product. From the acid fraction the hydroxy acid II [m.p. 285–286°, $[\alpha]^{25}D$ 29.5° (*c* 1.099, dioxane); calcd. for: $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.34; H, 9.04] was isolated. The pure monobromide Ia gave under similar conditions a higher yield of ester IIb and acid II. Refluxing of IIb with methanolic potassium hydroxide gave the hydroxy ester IIa [m.p. 165°, $[\alpha]^{22}D$ 41.5° (*c* 1.012, $CHCl_3$); calcd. for $C_{22}H_{34}O_4$: C, 72.80; H, 9.45. Found: C, 72.78; H, 9.30], also obtained upon methylation of acid II and easily reacetylated to the ester IIb. Prolonged treatment of the latter with methanolic potassium hydroxide in a sealed tube at 170° gave a high yield of the free acid II. Oxidation of acid II with chromic acid afforded the keto acid III [m.p. 288.5°, $[\alpha]^{22}D$ 45.1° (*c* 0.941, dioxane); calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.68; H, 8.75. Yield 85–90%], further characterized by its methyl ester IIIa [m.p. 185°, $[\alpha]^{23}D$ 49.8 (*c* 1.002, $CHCl_3$); calcd. for $C_{22}H_{32}O_4$: C, 73.27; H, 8.95. Found: C, 73.49; H, 8.81] which was also obtained by chromic acid oxidation of IIa. Acid III was transformed to its chloride IIIb with oxalyl chloride, using Reichstein's modification[11] of Wilds' method.[12] The crude acid chloride reacted with diazomethane, giving the diazo ketone IIIc, which, upon decomposition with hydrochloric acid, yielded the chloroketone IIId [m.p. 151°, $[\alpha]^{25}D$ 48.1° (*c* 0.890, $CHCl_3$); calcd. for $C_{22}H_{31}O_3Cl$: C, 69.73; H, 8.25; Cl, 9.36. Found: C, 69.93; H, 8.38; Cl, 9.32. Yield from III 65–70%]. The chloride IIId was converted to the iodide IIIe and thence, using a method previously described,[1,8a] with silver acetate in boiling pyridine, in the presence of small amounts of acetic anhydride and under nitrogen, to the ketol acetate IIIf [m.p. 191.5–192.5°, $[\alpha]^{24}D$ 45.9° (*c* 1.051, $CHCl_3$); calcd. for $C_{24}H_{34}O_5$: C, 71.61; H, 8.51. Found: C, 71.67; H, 8.44. Yield from IIId 65–70%]. Introduction of the Δ^4-double bond, according to Kendall's procedure,[13] through the 4-bromide (m.p. 163–164°) and the Δ^4-3-semicarbazone (m.p. 210–215°) of IIIf, gave 11-dehydro-17α-methylcorticosterone acetate (IV) [m.p. 157–158°, $[\alpha]^{24}D$ 170° (*c* 0.79, $CHCl_3$); λ_{max}^{EtOH} 237 mμ (log ϵ 4.44); $\nu_{max}^{CHCl_3}$ 1750 and 1720 cm.$^{-1}$ (21-acetoxy-20-ketone doublet); 1710 cm.$^{-1}$ (11-ketone); 1670 and 1620 cm.$^{-1}$ (Δ^4-3-ketone doublet); calcd. for $C_{24}H_{32}O_5$: C, 71.97; H, 8.05. Found: C, 72.23; H, 7.96. Yield from IIIf approximately 60%].

The adrenal cortical activity exhibited by the new hormone analog, 11-dehydro-17α-methylcorticosterone acetate, will be the subject of a separate communication.

I wish to express sincere thanks to the Canadian Life Insurance Officers Association for financial support; to the Ciba Company Limited, Basel, Switzerland, for the generous gift of starting material, to Dr. E. H. Venning, Royal Victoria Hospital, Montreal, for the biological tests; to Dr. R. N. Jones, National Research Council, Ottawa, for the infrared spectral analysis; and to Dean J. B. Collip for his continued encouragement. The author also wishes to express his appreciation to Mr. J. F. Alicino, Metuchen, New Jersey, who carried out the micro analyses.

(10) (a) J. G. Aston and R. B. Greenburg, THIS JOURNAL, **62**, 2590 (1940). (b) See also Al. Faworsky, *J. prakt. Chem.*, [2] **88**, 658 (1913). (c) Comparable rearrangements of 17-bromo-20-ketosteroids have been described by R. E. Marker and R. B. Wagner [THIS JOURNAL, **64**, 216, 1273 (1942)]; Pl. A. Plattner, H. Heusser and S. F. Boyce [*Helv. Chim. Acta*, **31**, 603 (1948)]; H. Heusser, Ch. R. Engel, P. Th. Herzig and Pl. A. Plattner [*ibid.*, **33**, 2229 (1950)].

(11) F. Reber, A. Lardon and T. Reichstein, *ibid.*, **37**, 45 (1954). A. Lardon and T. Reichstein, *ibid.*, **37**, 388, 443 (1954).

(12) A. L. Wilds, U. S. Patent 2,538,611. A. L. Wilds and C. H. Shunk, THIS JOURNAL, **70**, 2427 (1948). Compare also R. Adams and L. H. Ulich, *ibid.*, **42**, 599 (1920).

(13) W. F. McGuckin and E. C. Kendall, *ibid.*, **74**, 5811 (1952).

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FORMATION OF A NEW DINUCLEOTIDE FROM COZYMASE BY ENZYMIC DESTRUCTION OF THE "ONIUM" LINKAGE

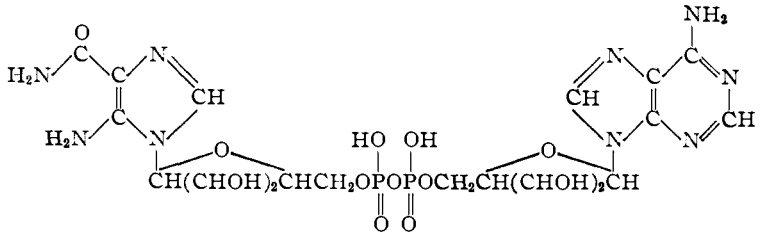
Sir:

Recently it has been shown[1] that certain enzymatically catalyzed syntheses derive their energy from the reduction of quaternary ammonium or sulfonium salts, rather than from the usual mechanism of cleavage of energy-rich phosphate esters. One such "onium" salt, *viz.*, cozymase (DPN) was proposed as a suitable substrate from which to derive dinucleotides by this mechanism. We wish to record the realization of such a reaction. The substrate was DPN and the acceptor amine was

(1) D. W. Woolley, *Nature*, **171**, 323 (1953).

4-amino-5-carboxamido-imidazole, the known precursor of purines.[2] The enzyme was soluble, highly purified, beef-spleen DPNase. The reaction proceeded to completion according to the equation

The Imidazole + DPN ⟶ Nicotinamide + H^+ +



The dinucleotide was isolated in relatively pure form, and its structure established.

Although DPNases from animal sources have not previously been separated from cellular particles,[3,4] it was found possible to do so by treatment of washed beef-spleen cell fragments with isoamyl alcohol plus desoxyribonucleic acid. The solubilized enzyme was then purified by conventional methods (45 U./mg. protein).

The dinucleotide was formed by incubation of 33 units of this enzyme with 26.1 micromoles of DPN and 3000 micromoles of the imidazole at *p*H 7.5 in 10 ml. for 16 hr. at 37°. It was then isolated by paper electrophoresis (4°, 0.2 *M* ammonium acetate, *p*H 5.0). Its mobility toward the anode was 2.2 times that of DPN, whereas for the free imidazole it was 0, and for the nicotinamide-free hydrolytic product of DPN it was 2.5. Yield was 29 per cent. of the DPN, R_F 0.26 in the ethanol-acetic acid system[4] and 0.56 in the isoamyl alcohol K_2HPO_4 system,[5] both at 4°. It gave the test for diazotizable amines[6] and, when analyzed quantitatively, showed a ratio of imidazole to adenine to ribose to phosphate of 1.0:1.1:2.0:1.9.

The structure of the new dinucleotide was further established by cleavage with Kornberg's pyrophosphatase.[7] The products were isolated by electrophoresis and shown to be adenosine-5′-phosphate and a mononucleotide, the 5′-phosphate of the amino-carboxamidoimidazole riboside. This compound has been postulated by Greenberg[8] and by Buchanan[9] as the precursor of inosinic acid in pigeon liver and in bacteria. Mobility relative to DPN was 1.9, for adenosine-5′-phosphate was 1.6. It had R_F 0.69 in the isoamyl alcohol K_2HPO_4 system.[5]

These results strongly suggest that inosinic acid and its derivatives could arise in living things through the action of DPNase with this new dinucleotide as an intermediate. Such a pathway could be an alternative to the one viewed by Greenberg[8] or even the one he has been investigating.

The conversion of DPN to this new dinucleotide can be clearly distinguished from the exchange reactions of DPN with isoniazide and with β-acetyl-pyridine which have been described by Zatman, *et al.*[10] In these exchange reactions, (a) the energy rich quaternary linkage is not lost; (b) no H^+ is formed; and (c) the susceptibility to DPNase attack is not lost. On the contrary, in the new reaction, the quaternary linkage is lost, a H^+ is formed and the product is no longer attacked by DPNase. Similar features which distinguish between base exchange without loss of "onium" linkage and destruction of this linkage have been discussed fully in the case of thianinase.[1]

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D. W. WOOLLEY

RECEIVED DECEMBER 27, 1954

(2) W. Shive, W. W. Ackermann, M. Gordon, M. E. Getzendaner and R. E. Eakin, THIS JOURNAL, **69**, 725 (1947).

(3) S. G. A. Alivisatos and O. F. Denstedt, *Science*, **114**, 281 (1951).

(4) L. J. Zatman, N. O. Kaplan and S. P. Colowick, *J. Biol. Chem.*, **200**, 197 (1953).

(5) C. E. Carter, THIS JOURNAL, **72**, 1466 (1950).

(6) A. C. Bratton and E. K. Marshall, Jr., *J. Biol. Chem.*, **128**, 537 (1939).

(7) A. Kornberg and W. E. Pricer, Jr., *ibid.*, **182**, 763 (1950).

(8) G. R. Greenberg, *J. Biol. Chem.*, **190**, 611 (1951).

(9) W. J. Williams and J. M. Buchanan, *ibid.*, **202**, 253 (1953).

(10) L. J. Zatman, N. O. Kaplan, S. P. Colowick and M. M. Ciotti, *J. Biol. Chem.*, **209**, 453 (1954).

(11) Damon Runyon Memorial Fellow.

ISOLATION OF HOMOGENEOUS MELANOCYTE STIMULATING HORMONE FROM HOG PITUITARY GLAND

Sir:

The melanocyte stimulating hormone[1] (MSH), elaborated by the pituitary gland, darkens the skin of many chordates,[2] including man.[3] Although some investigators[4,5] suggest that MSH is not a distinct substance but an adrenocorticotropin, evidence is good that the major portion of MSH activity is distinct from other pituitary hormones.[2] We wish to report the isolation of a fraction homogeneous to electrophoresis and to countercurrent distribution possessing the main MSH activity of the pituitary gland.

One hundred g. of acetone dried hog posterior pituitary powder[6] (4–6 × 10^7 MSH u./g.)[7] mixed with 250 ml. acetone and 1 l. acetic acid, was heated to 50° for 10 min. and centrifuged. The residue was separated and re-extracted. The combined extracts (1900 ml.) were mixed with acetone (950 ml.) and 10 ml. saturated NaCl.[8] The supernate obtained by centrifugation was mixed with petroleum ether (5400 ml.) and allowed to stand overnight at −5°. The precipitate was acetone washed and then dried *in vacuo*. Sixteen to 18 g. of product

(1) Melanocyte stimulating hormone has been referred to also as melanophore hormone, melanophore dilating principle, intermedin, etc.

(2) H. Waring and F. W. Landgrebe in "The Hormones," edited by G. Pincus and K. V. Thimann, Academic Press, Inc., New York, N. Y., Vol. 2, 1950, p. 427.

(3) A. B. Lerner, K. Shizume and I. Bunding, *J. Clin. Endocrinol. & Metab.*, **14**, 1463 (1954).

(4) P. H. Bell, THIS JOURNAL, **76**, 5565 (1954).

(5) N. G. Brink, G. E. Boxer, V. C. Jelinek, F. A. Kuehl, Jr., J. W. Richter and K. Folkers, *ibid.*, **75**, 1960 (1953).

(6) We are grateful to the Armour Laboratories and Wilson & Company for supplying us with a total of 3 kg. of hog posterior pituitary powder.

(7) An MSH unit is defined by K. Shizume, A. B. Lerner and T. B. Fitzpatrick, *Endocrinology*, **54**, 553 (1954).

(8) F. W. Landgrebe and K. A. Munday, *Quart. J. Exp. Physiol.*, **39**, 11 (1954).