$C_{29}H_{42}O_2$: C, 82.6; H, 10.0. Found: C, 82.4; H, 10.0) is oxidized to II by lead dioxide in ether. Compound III has an OH stretching band in the infrared at 2.74 μ and three intense bands between 6.1 and 6.5 μ . Methanol adds to the quinone methide to yield the bisphenol I, R is OCH₃, m.p. 160–161°. *Anal.* Calcd. for $C_{80}H_{46}O_3$: C, 79.5; H, 10.2. Found: C, 79.3; H, 10.0, 10.3.

Compound II is sensitive to traces of strong acid in hydroxylic or hydrocarbon solvents. The radical is converted in methanol with a trace of toluenesulfonic acid to I, R is OCH_3 and 3,3',5,5'tetra-*tert*-butyl-4,4'-diphenoquinone, m.p. 239– 240°. Base transforms II to a different radical which reacts rapidly with oxygen. These reactions will be described later.

Compound II is being investigated as a standard for electron magnetic resonance measurements.

EMERYVILLE RESEARCH CENTER

Shell Development Company Galvin M. Coppinger Emeryville, California

Received December 6, 1956

SOME NEW ACTIVE CORTICOSTEROIDS

Sir:

The enhancement of the glucocorticoid¹ and antiarthritic² activities of prednisone (I)³ and prednisolone (II)⁴ relative to cortisone (III) and cortisol (IV), led us to introduce further unsaturation into cortical hormones. To this end, we have prepared 6-dehydroprednisone (V) and 6-dehydroprednisolone (VI).

Bromination of the 21-acetate of I with Nbromosuccinimide⁵ gave 6-bromo-1,4-pregnadiene-17 α ,21-diol-3,11,20-trione 21-acetate (VII) (m.p. 185–188° (dec.); $[\alpha]^{25}$ D +173° (dioxane); λ_{max}^{EtOH} 245 m μ (ϵ = 16,300); found: Br, 16.54). Dehydrobromination of VII in refluxing collidine gave 1,4,6-pregnatriene-17 α ,21-diol-3,11,20-trione, (V) 21-acetate (m.p. 225–228°; $[\alpha]^{25}$ D +265° (dioxane); λ_{max}^{EtOH} 222 m μ (11,400), 255 (10,300), 297 (12,100); λ_{max}^{Nujol} 2.96 μ (OH), 5.76 (20 C=O, 21-OAc), 5.85 (11 C=O), 6.02 (3 C=O), 6.21, 6.31 ($\Delta^{1.4.6}$); found: C, 69.18; H, 6.73). The 21-acetate of V also was obtained by dibromination of the 21-acetate of III in acetic acid, followed by dehydrobromination with collidine.

An alternate procedure was the microbiological dehydrogenation of 6-dehydrocortisone (VIII)⁵

 (a) H. L. Herzog, A. Nobile, S. Tolksdorf, W. Charney, E. B. Hershberg, P. L. Periman and M. M. Pechet, *Science*, **121**, 176 (1955);
(b) H. L. Herzog, C. C. Payne, M. A. Jevnik, D. Gould, E. L. Shapiro, E. P. Oliveto and E. B. Hershberg, THIS JOURNAL, **77**, 4781 (1955).

(2) J. J. Bunim, M. M. Pechet and A. J. Bollet, J. Am. Med. Assoc., **157**, 311 (1955).

(5) Cf. V. R. Mattox, E. L. Woroch, G. A. Fleischer and E. C. Kendall, J. Biol. Chem., 197, 261 (1952).

(6) Cf. W. Charney, D. Sutter, C. Federbush, M. Gilmore, H. L. Herzog, M. J. Gentles, M. E. Tully and E. B. Hershberg, to be published; T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. J. Kirschmann, V. Marlatt and R. Miller, Arch. Biochem. Biophys., 59, 304 (1955).

or its 21-acetate with *Bacillus sphaericus*,⁶ leading directly to V, two forms (m.p. 235° (dec.); 225° (dec.); $[\alpha]^{25}$ D +246° (dioxane); λ_{max}^{MeOH} 222 μ (11,100), 255 (9,900), 296 (11,700); $\lambda_{max}^{CHBr_3}$ 2.79, 2.88 μ (OH), 5.85 (11, 20 C=O), 6.06 (3 C=O), 6.13, 6.24, 6.31 ($\Delta^{1,4,6}$); found: C, 70.83; H, 6.81).

6-Dehydrocortisol (IX) (m.p. 239–241°; $[\alpha]^{25}$ D +177° (dioxane); λ_{\max}^{MeOH} 283 m μ (24,900); λ_{\max}^{Nujol} 2.97 μ (OH), 5.84 (20 C=O), 6.11 (3 C=O), 6.20, 6.33 ($\Delta^{4,6}$); found: C, 69.86; H, 8.06) was prepared from the 3-semicarbazone 21-acetate of VIII⁵ by formation of the 3,20-bis-semicarbazone 21-acetate of VIII⁷ (darkens 250° , m.p. $>320^{\circ}$; ^{Nujol} 2.89, $\lambda_{\max}^{\text{MeOH}}$ 242 m μ (14,500), 301 (40,200); $\lambda_{\max}^{\text{Nuj}}$ 2.98, 3.05 μ (OH, NH), 5.79 (ester C=O), 5.86 (11 C==O), 5.99 (amide C==O), 6.35 ($\Delta^{4,6}$), 7.99 (ester C-O-C); found: N, 16.46), reduction with (codi m borohydride to the 3,20-bis-semicarbazone of IX (darkens 260°, m.p. > 320°; λ_{max} 236 m μ (11,100), 298 (37.400); $\lambda_{\text{max}}^{\text{Nujol}}$ 2.93, 3.06 μ (OH, NH), 5.99 (amide C=O), 6.40 ($\Delta^{4,6}$); found: N, 16.27), and cleavage with pyruvic acid and p-toluenesulfonic acid. Microbiological dehydrogenation of IX with B. sphaericus gave 1,4,6-pregnatriene-11 β ,17 α ,21-triol-3,20-dione (VI) (m.p. 239–243°; $[\alpha]^{25}D$ +100° (dioxane); λ_{max}^{MeOH} 222 m μ (11,600), 256 (9,600), 298 (12,200); λ_{max}^{Nujol} 2.85, 2.93, 3.00 μ (OH), 5.84 (20 C==O), 6.09 (3 C==O), 6.20, 6.27, 6.34 ($\Delta^{1,4,6}$); found: C, 70.25; H, 7.26).

The structure is supported by the preparation of 1,4,6-androstatriene-3,11,17-trione (X) (m.p. 215° (dec.); $[\alpha]^{25}D + 309^{\circ}$ (acetone); $\lambda_{max}^{MeOH} 222 \text{ m}\mu$ (11,300), 255 (9,900), 296 (11,900); $\lambda_{max}^{Nujol} 5.73 \mu$ (17 C=O), 5.84 (11 C=O), 6.06 (3 C=O), 6.23, 6.34 ($\Delta^{1,4,6}$); found: C, 77.00; H, 6.67), both from degradation of V with sodium bismuthate^{1b,8} and from 1,4-androstadiene-3,11,20-trione^{1b} by reaction with N-bromosuccinimide⁵ to give 6-bromo-1,4-androstadiene-3,11-20-trione (m.p. 168° (dec.); $[\alpha]^{25}D + 197^{\circ}$ (dioxane); $\lambda_{max}^{MeOH} 243 \text{ m}\mu$ (15,300); $\lambda_{max}^{Nujol} 5.74 \mu$ (17 C=O), 5.85 (11 C=O), 6.01 (3 C=O), 6.19, 6.24 ($\Delta^{1,4,6}$); found: Br. 22.13), followed by dehydrobromination with collidine.

Animal assay of V and VI showed potencies as given in the table compared to cortisone (III = 1).

Eosinopenia⁹ Thymus Involution⁹ Liver Glycogenesis⁹

V	1	2.1	2.2
VI	1.9	2.5	4.5

The electrolyte excretion pattern using V and VI was the same as with I and $II.^{10}$ Preliminary

(7) Cf. E. P. Oliveto, R. Rausser, L. Weber, E. Shapiro, D. Gould and E. B. Hershberg, THIS JOURNAL, 78, 1736 (1956).

(8) C. J. W. Brooks and J. K. Norymberski, *Biochem. J.*, **55**, 371 (1953).

(9) For methods, see S. Tolksdorf, M. L. Battin, J. W. Cassidy, R. M. MacLeod, F. H. Warren and P. L. Perlman, *Proc. Sec. Exper. Biol. Med.*, **92**, 207 (1956).

(10) The biological testing was carried out by Carole Rice, Alexandra D. Stephenson, James A. Truan and Felix H. Warren. We appreciate the aid of Lawrence Finckenor, Herbert Gerber and Merl Steinberg in preparing quantities of V and VI for testing. DATTE COTT

clinical evaluation indicates that V and VI have activities similar to I and II.

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SYNTHESIS OF 6-(1,2-DICARBOXYETHYLAMINO)-9- β -D-RIBOFURANOSYLPURINE AND THE STRUC-TURE OF ADENYLOSUCCINIC ACID¹

Sir:

The recent isolation² from mouse and rabbit livers of adenylosuccinic acid (AMPS) supports evidence from enzymatic studies^{3,4} that AMPS is an intermediate in the biosynthesis of adenylic acid (AMP) from inosinic acid. Carter and Cohen^{5a} assigned to AMPS the structure 6-(1,2dicarboxyethylamino) - 9 - ribofuranosylpurine - 5'phosphate on the basis of its physical properties, enzymatic reactions, and its acid degradation to authentic 6-(1,2-dicarboxyethylamino)-purine^{5b}.

6-(1,2-Dicarboxyethylamino)-9-β-D-ribofuranosylpurine (I) has been synthesized by an unambiguous 6-Methylmercapto-9-β-D-ribofuranosylroute. purine⁶ (1.68 \times 10⁻³ mole), dl-aspartic acid (1.68 \times 10⁻² mole), NaOH (3.02 \times 10⁻² mole), and water (7 cc.) were refluxed for 20 hours; methyl mercaptan was evolved; HCl $(3.02 \times 10^{-2} \text{ mole})$ was added. Paper chromatograms of the solution run in solvent B7 were sprayed to detect acidic components⁸ and *cis*-glycol systems⁹; I was identified as an ultraviolet light-absorbing spot which reacted positively in both tests. The solution was chromatographed on Dowex-1 (formate) (100 cc.). Elution with water (2 1.) followed by 0.2 N formic acid $(1.6 \ 1.)$ removed aspartic acid and ultraviolet light-absorbing by-products. Evaporation at 0.5 mm. of a 1 N formic acid eluate gave crude I as a gum (153 mg., 24%). Rechromatography at 3° on Dowex-1 (formate) (60 cc., height 17 cm.), employing gradient elution (2 N formic acid in reservoir; mixer volume, 1 liter), effected elution

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service, Grant No. C-471, and the Atomic Energy Commission, Contract No. AT(30-1)-910.

(2) W. K. Joklik, Biochem. Biophys. Acta, 22, 211 (1956).

(3) R. Abrams and M. Bentley, THIS JOURNAL, 77, 4179 (1955).

(4) I. Lieberman, ibid., 78, 251 (1956).

(5) (a) C. E. Carter and L. H. Cohen, ibid., 77, 499 (1955); (b) C. E. Carter, Fed. Proc., 15, 230 (1956); (c) C. E. Carter and L. H. Cohen, J. Biol. Chem., 222, 17 (1956).

(6) A. Hampton, J. J. Biesele, A. E. Moore, and G. B. Brown, THIS JOURNAL, 78, 5695 (1956).

(7) R_f values (ascending solvents, Schleicher and Schuell No. 597 paper): (A) *n*-butanol 50, acetic acid 20, water 30, *n*-butyl acetate 30 (0.40); (B) *n*-butanol 50, acetic acid 25, water 25 (0.59); (C) 5% Na2HPO4, iscamyl alcohol (C. E. Carter, THIS JOURNAL, 72, 1466 (1950)) (0.90).

(8) The reagent was a 0.1% solution of brom thymol blue in 0.02~NNaOH.

(9) J. G. Buchanan, C. A. Dekker and A. G. Long, J. Chem. Soc., 8162 (1950).

of a non-glycosidic substance¹⁰ just prior to I. The product (75 mg.) was chromatogramed on paper in solvent A7 to remove traces of an unidentified nucleoside $(R_f \ 0.27)$. Elution with methanol followed by crystallization from methyl cyanide gave a white powder which in 2 cc. of ethanol deposited microplates of I (32 mg., m.p. 235-245° dec.) after 4 days at 25°. Calcd. for $C_{14}H_{17}N_5O_8$: C, 43.86; H, 4.47; N, 18.27. Found¹¹. C, 43.81; H, 4.54; N, 18.20. Paper chromatography⁷ and paper electrophoresis¹² showed no other components. Potentiometric titration revealed ionizing groups of $pK_{\rm B}$ 2.2 (±0.1), 5.1 (±0.1), and a third of intermediate pK_a ; λ_{max} (pH 0.1) 268 m μ (A_M 15,500 ± 1000), (ρ H 8.2) 269 m μ $(A_{\rm M} 17,600 \pm 1000)$. AMPS possesses very similar spectroscopic and ionization constants.5c

Treatment of AMPS13 with phosphatases14 yielded a nucleoside¹⁵ (λ_{max} 269 m μ at pH 8.2) indistinguishable from I by paper chromatography⁷ or electrophoresis.12

AMPS was heated in $0.1 N H_2SO_4$ for 4 hours at 100°. The solution was neutralized with ammonia and chromatogramed on paper in four solvent systems,¹⁶ together with a similar hydrolysate from AMP and samples of D-ribose-5'-phosphate, D-ribose, and Na_2HPO_4 . Duplicates from each hydrolysate yielded evidence for much D-ribose-5'phosphate,^{17,18} traces of D-ribose¹⁷ and of inorganic phosphate.¹⁸ AMPS reacted as an unsubstituted cis-glycol in the periodate test.⁹

These findings afford strong evidence that AMPS is the 5'-phosphate of I, in agreement with the structure previously proposed.^{5a} The author thanks Drs. George Bosworth

Brown and C. E. Carter for valuable discussions.

(10) This material, λ_{max} 276 in 0.1 N HCl, was probably the aglycone^{5b} of I and could be detected in the presence of I on chromatograms run in pyridine-water (65:35).

(11) Analysis by J. F. Alicino, Metuchen, N. J.

(12) Migration distances towards the anode (Whatman 3MM paper, 800 volts, 0.04 M buffer) were: pH 4.7 (acetate-HCl buffer), 9.5 cm. in 1 hr.; pH 7.3 (phosphate), 10.5 cm. in 50 min.; pH 10.1 (glycine-NaOH), 8.5 cm. in 35 min.

(13) Kindly provided by Dr. C. E. Carter.

(14) Crudesnake venom phosphatases at pH 8.1, or human prostatic phosphatase at pH 5.4 caused complete conversion of AMPS in 24 hours at 37°

(15) Purified by paper chromatography in solvent B.7

(16) *n*-Butanol-acetic acid-water (50:20:35); 1% aqueous $(NH_4)_2SO_4$ -isopropyl alcohol (1:2) (N. Anand, V. M. Clark, R. H. Hall, and A. R. Todd, J. Chem. Soc., 3665 (1952)); acetone-30% acetic acid (1:1) (S. Burrows, F. S. M. Grylls and J. S. Harrison, Mature, **170**, 800 (1952); pyridine-ethyl acetate-water (1:2:2) (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

(17) S. M. Partridge, Nature, 164, 443 (1949).

(18) C. S. Hanes and F. A. Isherwood, ibid., 164, 1107 (1949).

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A CLEAVAGE REACTION INVOLVING *a*-METHYL-STYRENE OXIDE¹

Sir:

It has been found that α -methylstyrene oxide (1,2-epoxy-1-methylethylbenzene) when allowed to

(1) This work was supported by the Air Research and Development Command under contract No. (AF 18(600)787) with the Ohio State University Research Foundation.