$C_{29}H_{42}O_2$: 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 OCH_3, m.p. 160–161°. *Anal.* Calcd. for $C_{30}H_{46}O_3$: 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

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SOME NEW ACTIVE CORTICOSTEROIDS

Sir:

The enhancement of the glucocorticoid[1] and antiarthritic[2] 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 N-bromosuccinimide[5] gave 6-bromo-1,4-pregnadiene-17α,21-diol-3,11,20-trione 21-acetate (VII) (m.p. 185–188° (dec.); $[\alpha]^{25}D$ +173° (dioxane); λ_{max}^{EtOH} 245 mμ (ϵ = 16,300); found: Br, 16.54). Dehydrobromination of VII in refluxing collidine gave 1,4,6-pregnatriene-17α,21-diol-3,11,20-trione, (V) 21-acetate (m.p. 225–228°; $[\alpha]^{25}D$ +265° (dioxane); λ_{max}^{EtOH} 222 mμ (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)[6] 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 mμ (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 VIII[5] by formation of the 3,20-bis-semicarbazone 21-acetate of VIII[7] (darkens 250°, m.p. >320°; λ_{max}^{MeOH} 242 mμ (14,500), 301 (40,200); λ_{max}^{Nujol} 2.89, 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 sodium borohydride to the 3,20-bis-semicarbazone of IX (darkens 260°, m.p. > 320°; λ_{max} 236 mμ (11,100), 298 (37.400); λ_{max}^{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-11β,17α,21-triol-3,20-dione (VI) (m.p. 239–243°; $[\alpha]^{25}D$ +100° (dioxane); λ_{max}^{MeOH} 222 mμ (11,600), 256 (9,600), 298 (12,200); λ_{max}^{Nujol} 2.85, 2.93, 3.00μ (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° (acetone); λ_{max}^{MeOH} 222 mμ (11,300), 255 (9,900), 296 (11,900); λ_{max}^{Nujol} 5.73 μ (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-bromosuccinimide[5] to give 6-bromo-1,4-androstadiene-3,11-20-trione (m.p. 168° (dec.); $[\alpha]^{25}D$ +197° (dioxane); λ_{max}^{MeOH} 243 mμ (15,300); λ_{max}^{Nujol} 5.74 μ (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).

	Eosinopenia[9]	Thymus Involution[9]	Liver Glycogenesis[9]
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

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(4) Meticortelone, ® 1,4-pregnadiene-11β,17α,21-triol-3,20-dione.

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

DAVID GOULD
CHEMICAL RESEARCH ELLIOT L. SHAPIRO
DEPARTMENT HERSHEL L. HERZOG
MARGARET J. GENTLES
E. B. HERSHBERG
INDUSTRIAL MICROBIOLOGY WILLIAM CHARNEY
DEPARTMENT MARILYN GILMORE
BIOCHEMISTRY DEPARTMENT SIBYLLE TOLKSDORF
SCHERING CORPORATION MILTON EISLER
BLOOMFIELD, N. J. PRESTON L. PERLMAN
MASSACHUSETTS GENERAL HOSPITAL
BOSTON, MASSACHUSETTS MAURICE M. PECHET

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SYNTHESIS OF 6-(1,2-DICARBOXYETHYLAMINO)-9-β-D-RIBOFURANOSYLPURINE AND THE STRUCTURE OF ADENYLOSUCCINIC ACID[1]

Sir:

The recent isolation[2] 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-(1,2-dicarboxyethylamino) - 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 route. 6-Methylmercapto-9-β-D-ribofuranosylpurine[6] (1.68×10^{-3} mole), *dl*-aspartic acid (1.68×10^{-2} mole), NaOH (3.02×10^{-2} mole), and water (7 cc.) were refluxed for 20 hours; methyl mercaptan was evolved; HCl (3.02×10^{-2} mole) was added. Paper chromatograms of the solution run in solvent B[7] were sprayed to detect acidic components[8] and *cis*-glycol systems[9]; 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 l.) followed by 0.2 *N* formic acid (1.6 l.) 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 of a non-glycosidic substance[10] just prior to I. The product (75 mg.) was chromatogramed on paper in solvent A[7] 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. Found[11]. C, 43.81; H, 4.54; N, 18.20. Paper chromatography[7] and paper electrophoresis[12] showed no other components. Potentiometric titration revealed ionizing groups of pK_a 2.2 (±0.1), 5.1 (±0.1), and a third of intermediate pK_a; λ_{max} (*p*H 0.1) 268 mμ (A_M 15,500 ± 1000), (*p*H 8.2) 269 mμ (A_M 17,600 ± 1000). AMPS possesses very similar spectroscopic and ionization constants.[5c]

Treatment of AMPS[13] with phosphatases[14] yielded a nucleoside[15] (λ_{max} 269 mμ at *p*H 8.2) indistinguishable from I by paper chromatography[7] 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-ribose[17] 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]

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(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.

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(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: *p*H 4.7 (acetate–HCl buffer), 9.5 cm. in 1 hr.; *p*H 7.3 (phosphate), 10.5 cm. in 50 min.; *p*H 10.1 (glycine–NaOH), 8.5 cm. in 35 min.

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

(14) Crudesnake venom phosphatases at *p*H 8.1, or human prostatic phosphatase at *p*H 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, *Nature*, **170**, 800 (1952)); pyridine–ethyl acetate–water (1:2:2) (S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

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THE SLOAN-KETTERING DIVISION OF
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK 21, NEW YORK ALEXANDER HAMPTON

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A CLEAVAGE REACTION INVOLVING α-METHYLSTYRENE OXIDE[1]

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.