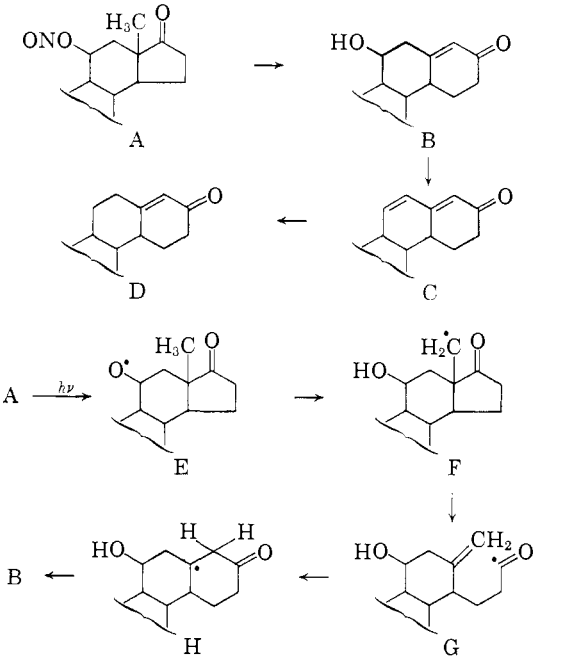
structure B; m.p. 269–273°; $[\alpha]_D$ +134° (pyr.); λ_{max}^{MeOH} 241 mμ, ε 33,500; $\lambda\lambda^{Nujol}$ 3.00, 6.00, 6.10, 6.20 μ) by its infrared and ultraviolet spectra and by chemical transformations. Compound I was acetylated readily with acetic anhydride in pyridine at room temperature to give the 11-acetate (m.p. 161–165°; $[\alpha]_D$ +148°; λ_{max}^{MeOH} 239 mμ, ε 34,200; $\lambda\lambda^{Nujol}$ 5.78, 6.00, 6.16, 8.00 μ). Dehydration of I with perchloric acid in methanol gave 18-nor-D-homo-4,11,13(17a)-androstatriene-3,17-dione (partial structure C; m.p. 189–192°; $[\alpha]_D$ −88°; $\lambda\lambda_{max}^{MeOH}$ 238 mμ, ε 15,200 and 280 mμ, ε 29,000; $\lambda\lambda^{Nujol}$ 6.00, 6.06, 6.20, 6.32 μ) which was hydrogenated to the known[6] 18-nor-D-homo-4,13(17a)-androstadiene-3,17-dione (partial structure D; m.p. 196–199°; $[\alpha]_D$ +49; λ_{max}^{MeOH} 242 mμ, ε 33,600; $\lambda\lambda^{Nujol}$ 6.01, 6.18 μ; reported[6] m.p. 196°; $[\alpha]$ +47°; $\lambda_{max}^{alc.}$ 243 mμ, ε 33,000) using palladium on calcium carbonate catalyst in benzene.[7]

Analogously, 1,4-androstadiene-11β-ol-3,17-dione,[8] 11β-hydroxyestrone 3-acetate[9] and androstane-3β,11β-diol-17-one 3-acetate[10] were converted to the corresponding 11-nitrites (respectively m.p. 109–111°; $[\alpha]_D$ +179°; λ_{max}^{MeOH} 239 mμ, ε 16,400; $\lambda\lambda^{Nujol}$ 5.72, 5.76, 6.00, 6.10, 6.22 μ; m.p. 176–178°; $[\alpha]_D$ +93; $\lambda\lambda_{max}^{MeOH}$ 268 mμ, ε 1,340 and 275 mμ, ε 1,200; $\lambda\lambda^{Nujol}$ 5.65, 5.72, 6.06, 6.20, 6.67, 8.3 μ and m.p. 134–136°; $[\alpha]_D$ +69°; $\lambda\lambda^{Nujol}$ 5.78, 6.08, 6.12, 8.12 μ) which were photolyzed to give, respectively, 18-nor-D-homo-1,4,13(17a)-androstatriene-11β-ol-3,17-dione (II; m.p. 254–257°; $[\alpha]_D$ +35° (pyr.); λ_{max}^{MeOH} 243 mμ, ε 32,600; $\lambda\lambda^{Nujol}$ 2.97, 6.02, 6.12, 6.18 μ), 18-nor-D-homo-1,3,5(10),-13(17a)-estratetraene-3,11β-diol-17-one 3-acetate (III; m.p. 181–183°; $[\alpha]_D$ +117° (pyr.); λ_{max}^{MeOH} 237 mμ, ε 19,500; $\lambda\lambda^{Nujol}$ 2.96, 5.70, 5.95, 6.15, 6.28, 6.68, 8.1 μ) and 18-nor-D-homo-13(17a)-androstene-3β,11β-diol-17-one 3-acetate[11] (IV; m.p. 165–168°; $[\alpha]_D$ −3° (pyr.); λ_{max}^{MeOH} 241 mμ, ε 15,200; $\lambda\lambda^{Nujol}$ 2.90, 5.75, 5.99, 6.12, 8.0 μ). Compound II was converted to the 11-acetate (m.p. 199–201°; $[\alpha]_D$ +80°; λ_{max}^{MeOH} 240 mμ, ε 32,000; $\lambda\lambda^{Nujol}$ 5.77, 6.00, 6.15, 6.24), and dehydrated to give 18-nor-D-homo-1,4,11,13(17a)-androstatetraene-3,17-dione (m.p. 200–202°; $[\alpha]_D$ −193°; $\lambda\lambda_{max}^{MeOH}$ 240 mμ, ε 15,700 (shoulder) and 278 mμ, ε 34,000; $\lambda\lambda^{Nujol}$ 5.99, 6.16, 6.21, 6.29 μ).

Further substantiation of partial structure B for the photolysis product is furnished by the following reactions. Treatment of compound IV with perchloric acid or potassium hydroxide in methanol gave 11,13(17a)-androstadiene-3β-ol-17-one (m.p. 255–258°; $[\alpha]_D$ −46°; λ_{max}^{MeOH} 284 mμ, ε 27,800; $\lambda\lambda^{Nujol}$ 2.98, 6.08, 6.18, 6.32 μ) which was converted to the 3-benzoate (m.p. 220–223°; $[\alpha]_D$ −22°; $\lambda\lambda_{max}^{MeOH}$ 238 mμ, ε 14,200 and 283 mμ, ε 23,800; reported[12] m.p. 223°; $[\alpha]_D$ −25°; λ_{max}^{MeOH} 283 mμ, ε 25,900), and hydrogenated to yield 18-nor-D-homo-13(17a)-androstene-3β-ol-17-one (m.p. 201–204°; $[\alpha]_D$ −36°; λ_{max}^{MeOH} 240 mμ, ε 16,200; $\lambda\lambda^{Nujol}$ 2.96, 6.05, 6.17 μ; reported[13] m.p. 194–196°.

Recently a free radical rearrangement which is formally of the Wagner–Meerwein type has been observed[14]; we believe that the rearrangement reported here is another instance of this reaction type. A plausible mechanism is indicated in partial structures E to H, the intermediate primary



radical F rearranging via the open chain intermediate G to the tertiary radical H, which then may lose a hydrogen atom to a radical species in the system to give the final product B.

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(11) This compound was isolated from the crude photolysis product by partition chromatography and fractional crystallization.

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CORRECTION OF THE STRUCTURE OF A URINARY METABOLITE OF ALDOSTERONE[1]

Sir:

The major urinary metabolite of aldosterone, measured as the triacetoxy derivative, has been used to estimate the rate of secretion of the hormone in man.[2,3] The structure 3α,18,21-trihydroxy-11,20-diketopregnane, previously[4] assigned to the metabolite, however, was that of a contaminating

(1) This work was aided by Grant No. P-274 from the American Cancer Society, and by grants from the National Institutes of Health and the John and Mary R. Markle Foundation.

(2) S. Ulick, J. H. Laragh, and S. Lieberman, *Trans. Assoc. Amer. Phys.*, **71**, 225 (1958).

(3) C. Flood, D. S. Layne, S. Ramcharan, E. Rossipal, J. F. Tait, and S. A. Tait, *Acta Endocrinologica*, **36**, 237 (1961).

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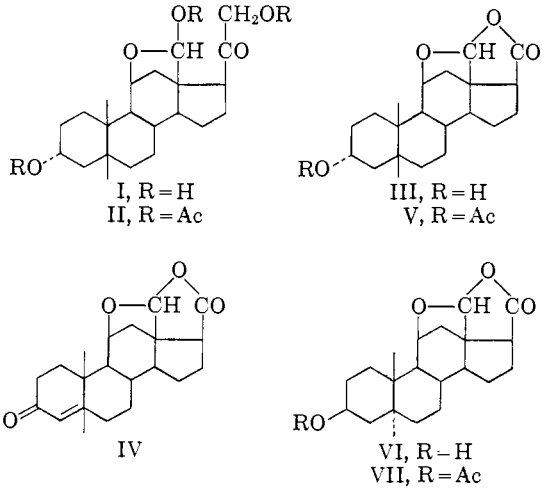
substance, a metabolite of 18-hydroxycorticosterone.[5] We wish to report the correct structure of the aldosterone metabolite as the 3α,5β isomer of tetrahydroaldosterone (I).

Tetrahydroaldosterone (3α,5β) was prepared using a soluble[6,7] enzyme fraction. Male rat livers were homogenized in acetone at −15°, the homogenate was filtered and the residue dried at room temperature in vacuum. The acetone powder was suspended in buffer solution[8] and the filtrate incubated with *d*-aldosterone-21 monoacetate[9] in the presence of TPNH[10] under nitrogen at 37°. Extraction and chromatography of the incubation mixture revealed only one substance (I), other than unchanged substrate, which reduced blue tetrazolium.[11]

Tetrahydroaldosterone (I) was isolated from extracts of combined incubates by chromatography on paper (ethylene dichloride–ethylene glycol, R_{Aldo} = 0.3[12] and on Celite (toluene–ethyl acetate 9:1-methanol:water 1:1, R_{Aldo} = 0.6) as a white amorphous solid, melting range 107–114,°[13] $[\alpha]^{24}D$ + 50° ($CHCl_3$ (C = 0.9), $\lambda_{max}^{CH_2Cl_2}$ 2.78, 5.88 (weak) μ; C, 69.00; H, 8.88. Acetylation with acetic anhydride in pyridine gave a triacetoxy[14] derivative (II) R_{DOCA} = 1.1 in methylcyclohexane–formamide, = 1.3 in methylcyclohexane–methanol:water 4:1, $\lambda_{max}^{CS_2}$ 5.71, 5.77μ, which like aldosterone[15] readily lost one acetoxy group when treated with dilute acetic acid.



Oxidation of tetrahydroaldosterone (I) with periodic acid yielded the theoretical amount of formaldehyde[16] and a monohydroxy γ-lactone (III), m.p. 252–254°, $\lambda_{max}^{CH_2Cl_2}$ 2.88, 5.64 μ, C, 72.46; H, 8.46. This lactone (III) also was obtained from aldosterone etiolactone (IV)[17] using the enzyme preparation described above. Acetylation yielded the monoacetoxy dervative[14] (V), R_{An} = 0.9 in methylcyclohexane–formamide, $\lambda_{max}^{CS_2}$ 5.60, 5.78 μ. There was a single band of simple contour at 8.08 μ which was characteristic of equatorial 3-acetoxy steroids.[18] The 3α,5β configuration was assigned to lacetone III by excluding the other possible 3-equatorial isomer. Hydrogenation of aldosterone etiolactone (IV) over platinum oxide in acetic acid gave the known 3β,5α, lactone VI,[17,19] m.p. 241–243°, which depressed the melting point of lactone III. The infrared spectra of the pair of isomeric lactones (III and VII) and of their acetoxy derivatives (V and VII) differed in the fingerprint region. Only lactone VII formed an insoluble digitonide.

The metabolite of aldosterone (3.8 mg.), isolated as described[4] from the urine (17 day pool) of a patient with cirrhosis of the liver, was compared with tetrahydroaldosterone (3α,5β). Three derivatives (II, III, V) of the isolated and the synthetic steroid were prepared. Both ketols as well as their corresponding derivatives had identical infrared spectra and chromatographic running rates. Both ketols also were shown to be identical by double isotope techniques. Following the administration of *d*-aldosterone-7-H^3, the major radioactive moiety was isolated from the glucuronide fraction of urine and mixed with tetrahydroaldosterone (3α,5β)-4-C^{14}, and derivatives II, III and V of the doubly labeled mixture were prepared. The H^3:C^{14} ratios of the ketol and its derivatives agreed within 5%.

(5) The separation of the metabolite of 18-hydroxycorticosterone from the aldosterone metabolite and the confirmation of its structure by synthesis of the γ-lactone obtained by periodate oxidation will be reported separately, S. Ulick and K. Kusch. In certain states of hyperaldosteronism such as cirrhosis of the liver and maligant hypertension, the secretion of 18-hydroxycorticosterone as measured by the excretion of this metabolite was greater than 4.0 mg. per day, exceeding that of aldosterone.

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(7) J. T. August, *Biochem. Biophys. Acta*, **48**, 203 (1961).

(8) *p*H 7.4 containing these concentrations of salts in m. moles per liter: NaCl 110, KCl 4.6, $MgSO_4$ 1.0, Na_2HPO_4 22.

(9) We wish to express our appreciation to Dr. C. H. Sullivan, Ciba Pharmaceutical Products, for providing the *d*-aldosterone-21-monoacetate used in this work.

(10) Generated from TPN (triphosphopyridine nucleotide) by the coupled oxidation of glucose-6-phosphate in the presence of glucose-6-phosphate dehydrogenase.

(11) When cortisol was incubated with the same enzyme preparation, under the same conditions, the only product was 3α,17α,11β,21-tetrahydroxy-20-ketopregnane.

(12) R values refer to the distance migrated by the sample on paper chromatogram relative to the reference steroid. Abbreviations: Aldo = aldosterone, DOCA = desoxycorticosterone acetate, An = 4-androstene-3,17-dione.

(13) Melting points (uncorrected) were determined on a micro hot stage. Only the hydroxyl and carbonyl regions of the infrared are reported here. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories.

(14) The acetate number was determined with H^3-labeled acetic anhydride. The number of moles of steroid present in the sample was determined from its C^{14} content. The c.p.m. C^{14} per mole for tetrahydroaldosterone and its derivative was determined from the specific activity of aldosterone-4-C^{14} used as a substrate in the incubation.

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VINYL AZENE CHEMISTRY: FORMATION OF AZACYCLOPROPENE

Sir:

As a continuation of our studies of the chemistry of the monovalent nitrogen species, azenes,[1,2] the