to 6.55 g. of α-diethylaminomethyl-γ-phenyl-γ-butyrolactone methiodide, a thick, yellow oil separated. After standing for a day, the mixture was extracted with ether, and the ether layer was dried over sodium sulfate. The product was distilled in an atmosphere of nitrogen and weighed 2.40 g. (83%), b.p. 124–126° (0.3 mm.), $n^{25}D$ 1.5547. The lactone exhibited characteristic bands in the infrared at 5.68, 6.01 and 6.23 μ.

Anal. Calcd. for $C_{11}H_{10}O_2$: C, 75.84; H, 5.79. Found: C, 75.98; H, 5.95.

The pyrazoline derivative VI was obtained by the addition of ethereal diazomethane to a solution of the methylene lactone in ether. After the solvent had spontaneously evaporated, the orange oil remaining subsequently crystallized. Two crystallizations from aqueous acetic acid afforded colorless needles which melted with decomposition at 98.5–99.5°.

Anal. Calcd. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.60. Found: C, 66.54; H, 5.76.

The cysteine addition product VII was prepared through the addition of 0.5 g. of the lactone II in 1 ml. of ethanol to a solution of an equimolar amount of cysteine in 1.5 ml. of water adjusted to *p*H 6.5. The precipitate, which formed immediately, was removed by filtration after standing for 1 hr. and was recrystallized from ethanol, yielding crystals melting at 171–173° dec.

Anal. Calcd. for $C_{14}H_{17}NO_4S$: C, 56.90; H, 5.80. Found: C, 56.44; H, 5.67.

Hydrogenation of II.—One gram of α-methylene-γ-phenyl-γ-butyrolactone (II) in 100 ml. of glacial acetic acid was reduced over 300 mg. of 10% palladium-on-carbon at an initial hydrogen pressure of 38 lb. The calculated pressure drop for one molar proportion of hydrogen was 11.2 lb., whereas the observed value at the point where reduction ceased was 13.7 lb. After removal of the catalyst and evaporation of the solvent by distillation *in vacuo* at 40°, the product, an orange oil, was taken up in ether and distilled, b.p. 134–138° (2.0 mm.).

For the comparison with the hydrogenation product, authentic α-methyl-γ-phenyl-γ-butyrolactone was required. This previously reported[5] substance was obtained conveniently by the condensation of styrene oxide and diethyl methylmalonate followed by the alcoholic base cleavage of the unisolated α-carbethoxy-α-methyl-γ-phenyl-γ-butyrolactone. To a solution of 0.25 mole of the sodium salt of diethyl methylmalonate in 150 ml. of absolute ethanol was added dropwise with stirring during a one hour period an equivalent amount of styrene oxide. After standing overnight, during which time the cleavage[2] to α-methyl-γ-phenyl-γ-butyrolactone was completed, the clear, yellow solution was chilled to 15° and neutralized to litmus with cold glacial acetic acid. After removal of the excess alcohol under reduced pressure and at room temperature, 150 ml. of water was added and the oily layer which separated was removed from the aqueous phase. After extraction of the water solution with ether, the combined ether extracts were dried over sodium sulfate. A forerun boiling at 98–105° (1.0 mm.) was discarded, and the main fraction then distilled at 134–138° (0.9 mm.) (37 g., 91%).

The infrared spectra of authentic lactone and the product obtained by the hydrogenation of II were, within experimental error, identical. To confirm the identity of the two lactones, each was converted to the solid α-methyl-γ-hydroxy-γ-phenylbutyramide. The ammonolysis was accomplished by allowing a solution of the lactone in ethanolic ammonia (anhydrous) to stand for 24 hours. The mixture then was cooled in ice and the precipitated amide was removed by filtration. Crystallization from benzene–petroleum ether (b.p. 60–68°) afforded material with a m.p. 162.5–163° dec., which was not raised on subsequent crystallization from benzene–methanol.

Anal. Calcd. for $C_{11}H_{15}NO_2$: C, 68.37; H, 7.82. Found: C, 68.66; H, 7.85.

The mixed melting point of amides prepared separately from the two lactones was not depressed.

Ozonization of II.—One gram of II, dissolved in 50 ml. of glacial acetic acid, was subjected at room temperature to the action of excess ozone. The ozonide was decomposed with zinc dust and glacial acetic acid, after which the product was steam distilled, the distillate being collected in water. From the distillate, 0.76 g. (45%) of dimedone adduct of formaldehyde was obtained; the mixed melting point with authentic material was not depressed.

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(5) O. Mumm and K. Brodersen, *Ber.*, **56**, 2295 (1923).

COMMUNICATIONS TO THE EDITOR

A NEW CLASS OF BIOLOGICALLY ACTIVE CORTICOIDS

Sir:

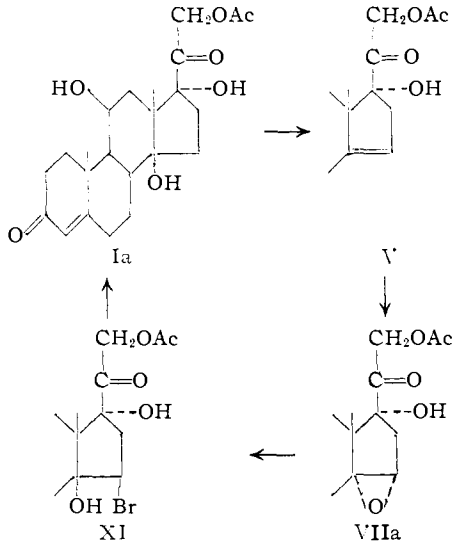
We wish to report the preparation of a new class of corticosteroids which display varying degrees of glucocorticoid activity. The compounds described are derivatives of hydrocortisone and cortisone functionally substituted in the D-ring. Introduction of the various nuclear substituents was readily effected by chemical transformations of a dihydroxylated product obtained from the microbiological oxygenation of 11-desoxy-17α-hydroxycorticosterone.[1] This O_6-compound, shown below to be Δ⁴-pregnene-11β,14α,17α,21-tetrol-3,20-dione (14α-hydroxyhydrocortisone) (I), is itself more active than hydrocortisone acetate in the thymus involution assay[2] and has been found to be an active anti-inflammatory agent in rheumatoid arthritis.[3]

Preliminary formulation of (I), m.p. 241–242°, $[\alpha]_D$ +183° (EtOH), λ_{max}^{alc} 241 mμ (log ε 4.23), Found: C, 66.5, H, 7.93; as a tertiary-hydroxylated derivative of hydrocortisone arose from two observations: (a) acetylation of (I) with acetic anhydride–pyridine afforded only a *monoacetate* (Ia), m.p. 211–212°, $[\alpha]_D$ +147° (EtOH), λ_{max}^{alc} 241 mμ (log ε 4.19), Found: C, 65.7; H, 7.70; and (b) this monoacetate (Ia) underwent oxidation with chromic anhydride yielding a dehydro compound (IIa), m.p. 262–263°, $[\alpha]_D$ +237° (dioxane), λ_{max}^{alc} 237 mμ (log ε 4.22), Found: C, 66.1; H, 7.21; the 21-alcohol (II) of which had m.p. 232–233° dec., $[\alpha]_D$ +210° (dioxane), λ_{max}^{alc} 237 mμ (log ε 4.20), Found: C, 66.8; H, 7.53; which displayed typical six-membered ring ketone absorption in the

(1) (a) G. M. Shull, D. A. Kita, J. W. Davisson, U. S. Patent 2,658,023, November 3, 1953; (b) G. M. Shull and D. A. Kita, THIS JOURNAL, **77**, 763 (1955).

(2) Pharmacological activities were determined by Dr. S. Y. P'an who will report on his findings in a forthcoming publication.

(3) Reports on the clinical investigations of the anti-inflammatory activity of (I) will appear elsewhere.



infrared region. These reactions unequivocally demonstrated the presence in (I) of a non-acetylatable secondary alcohol grouping (11β-hydroxyl) as well as a tertiary hydroxyl function.

With the elucidation of the gross structural features of (I), allocation of the tertiary hydroxyl function to the 14α-position followed from the exact parallelism observed between the chemical reactions of (I) and those of authentic Δ^4-pregnene-14α,17α,21-triol-3,20-dione (14α-hydroxy-Compound S).[4] Demonstration of the presence of the 14α-hydroxyl substituent allowed complete formulation of (I) as Δ^4-pregnene-11β,14α,17α,21-tetrol-3,20-dione and of (II) as Δ^4-pregnene-14α,17α,21-triol-3,11,20-trione.[5]

Treatment of (Ia) with *p*-toluenesulfonic acid in refluxing benzene resulted in the selective removal of the tertiary hydroxyl group to give $\Delta^{4,14}$-pregnadiene-11β,17α,21-triol-3,20-dione acetate (V), m.p. 253–255° dec., $[\alpha]_D$ +116° (dioxane), λ_{max}^{alc} 242 mμ (log ϵ 4.21); Found: C, 68.5; H, 7.56; which was oxidized with chromic acid to $\Delta^{4,14}$-pregnadiene-17α,21-diol-3,11,20-trione acetate (VI), m.p. 200–201°, $[\alpha]_D$ +121° (dioxane), Found: C, 68.9; H, 6.86. Oxidation of (V) with excess monoperphthalic acid gave Δ^4-14α,15α-oxidopregnene-11β,17α,21-triol-3,20-dione acetate (VIIa), m.p. 229–230°, $[\alpha]_D$ +144° (dioxane), λ_{max}^{alc} 240 mμ (log ϵ 4.21); Found: C, 65.7; H, 7.14; the 21-alcohol (VII) of which had m.p. 225–226°, $[\alpha]_D$ +159° (dioxane), λ_{max}^{alc} 239 mμ (log ϵ 4.23); Found: C, 66.6; H, 7.45. As expected, (VIIa) was oxidized with chromic acid to the corresponding Δ^4-14α,15α-oxidopregnene-17α,21-diol-3,11,20-trione acetate (VIII), m.p. 184–186°, $[\alpha]_D$ +186° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.22); Found: C, 66.3; H, 6.80.

D-Ring halogen-substituted derivatives of (I) were prepared by the same method utilized in the 11-desoxy series.[4] When the oxido derivatives (VIIa) and (VIII) were treated with hydrogen chloride in chloroform at 0°, the products were Δ^4-15β-chloropregnene-11β,14α,17α,21-tetrol-3,20-dione acetate (IX), m.p. 174–175°, $[\alpha]_D$ +110° (dioxane), λ_{max}^{alc} 240 mμ (log ϵ 4.22), Found: C, 60.0; H, 6.93; Cl, 7.82; and Δ^4-15β-chloropregnene-14α,17α,21-triol-3,11,20-trione acetate (X), m.p. 233–234° dec., $[\alpha]_D$ +106° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.21), Found C, 60.9; H, 6.42; Cl, 7.91; respectively.

The reaction of (VIII) with hydrogen bromide in chloroform at −15° afforded Δ^4-15β-bromopregnene-14α,17α,21-triol-3,11,20-trione acetate (XI), m.p. 207–209° dec., $[\alpha]_D$ +118° (dioxane), λ_{max}^{alc} 237 mμ (log ϵ 4.21), Found: C, 55.4; H, 5.87. On similar treatment the 11β-hydroxylated epoxide (VIIa) resulted in a markedly unstable bromohydrin (Δ^4-15β-bromopregnene-11β,14α,17α,21-tetrol-3,20-dione acetate) (XII), m.p. 129–131° dec., which could be converted back to (Ia) by dehalogenation with Raney nickel,[6] thereby establishing the stereochemistry of the above-mentioned epoxides and halohydrins.

(4) B. M. Bloom, E. J. Agnello and G. D. Laubach, in preparation.

(5) The structures assigned to (I) and (II) were further substantiated by oxidative side-chain cleavage experiments of (I) with chromic acid and sodium bismuthate. The chromic acid product (III) had the molecular formula $C_{19}H_{24}O_4$, m.p. 283–285° dec., $[\alpha]_D$ +208° ($CHCl_3$), λ_{max}^{alc} 236.5 mμ (log ϵ 4.20), Found: C, 72.3; H, 7.88; and infrared spectrum consistent with its formulation as Δ^4-androsten-14α-ol-3,11,17-trione. Sodium bismuthate cleavage of (I) afforded a product (IV), $C_{19}H_{26}O_4$, which exhibited m.p. 224–226°, $[\alpha]_D$ +169° (dioxane), +186° ($CHCl_3$), λ_{max}^{alc} 241 mμ (log ϵ 4.20), Found: C, 72.0; H, 8.28; and infrared spectrum consistent with its structural assignment as Δ^4-androstene-11β,14α-diol-3,17-dione.

(6) P. L. Julian, *et al.*, THIS JOURNAL, **72**, 5145 (1950).

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BIOSYNTHESIS OF NUCLEIC ACID GUANINE: THE ENZYMIC CONVERSION OF INOSINE-5′-PHOSPHATE TO XANTHOSINE-5′-PHOSPHATE[1]

Sir:

The work of several investigators implicates inosine-5′-phosphate as a precursor of nucleic acid purines.[2,3] We wish to report its conversion to xanthosine-5′-phosphate.

Sonic extracts of *Aerobacter aerogenes*, strain 1033, were precipitated with half-saturated acidic ammonium sulfate, dialyzed, and treated with protamine sulfate. The protamine supernate reduced DPN but not TPN in the presence of inosine-5′-phosphate, but not in the presence of inosine or hypoxanthine. The reaction, followed spectrophotometrically at 340 mμ, proceeded without lag under anaerobic conditions at *p*H 8.3 and required NH_4^+ and cysteine for maximum activity. A fourfold purification was achieved by collecting the protein precipitated between 20–30% ammonium sulfate saturation.

To obtain sufficient product for characterization, an extract of glycerol-grown cells was used and the DPNH reoxidized by dihydroxyacetone through the glycerol dehydrogenase present. The reaction

(1) This work was supported in part by an institutional grant to Harvard University from the American Cancer Society, by a research grant (G-3554) from the U. S. Public Health Service, and by funds received from the Eugene Higgins Trust.

(2) R. G. Greenberg, THIS JOURNAL, **74**, 6307 (1952).

(3) J. M. Buchanan and M. P. Schulman, *J. Biol. Chem.*, **202**, 241 (1953).