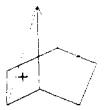
symmetrically. The 7-norbornenyl cation may be represented by (VI). It reacts with solvent



VI

stereospecifically; complete retention of configuration was observed in the hydrolysis of the dibromide (III) to the alcohol, and in the acetolysis of 7-norbornenyl toluenesulfonate (V).

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MICROBIOLOGICAL TRANSFORMATION OF STEROIDS. I. $\Delta^{1,4}$-DIENE-3-KETOSTEROIDS

Sir:

It has become a problem of importance[1,2] to devise efficient techniques for the introduction of Δ^1-unsaturation in cortisone (I)[3] and cortisol (II) since it has been shown that $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,11,20-trione (III) and $\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione (IV) are considerably more potent anti-inflammatory agents than the natural corticosteroids. We wish to report that I may be converted to III and II may be converted to IV by the action of *Corynebacterium simplex* (A.T.C.C. 6946). Either I or II, dissolved in methanol, was added to shake flasks containing a 24-hour culture of *C. simplex* in a nutrient medium of 0.1% Difco yeast extract buffered at *p*H 7. The mixture was shaken at 28° for 3–24 hours. Extraction of the resultant broth with chloroform, followed by evaporation to a residue and crystallization from acetone, afforded excellent yields of III or IV, respectively. Compounds III and IV, obtained in this way, were identical in every respect with samples prepared by purely chemical means.[4] By similar microbiological procedures we have also prepared $\Delta^{1,4}$-pregnadiene-17α,21-diol-3,20-dione (V) [m.p. 246–249° dec., $[\alpha]^{25}D$ + 76° ($CHCl_3$), $\lambda_{max}^{methanol}$ 244 mμ (ϵ = 15,900), λ_{max}^{Nujol} 3.05 μ (OH), 5.80 μ (20-carbonyl), 6.0, 6.16 and 6.22 μ ($\Delta^{1,4}$-diene-3-one),[5] found: C, 73.56; H, 8.40], $\Delta^{1,4}$-pregnadiene-11β,21-diol-3,20-dione (VI) [m.p. 227.5–230.5° dec., $[\alpha]^{25}D$ +173° (methanol), $\lambda_{max}^{methanol}$ 243 mμ (ϵ = 14,300), λ_{max}^{Nujol} 2.88 and 2.97 μ (OH), 5.88 μ (20-carbonyl), 6.07, 6.20 and 6.25 μ ($\Delta^{1,4}$-diene-3-one), found: C, 73.49; H, 8.12], $\Delta^{1,4}$-pregnadiene-21-ol-3,20-dione 21-acetate (VII)[6] (m.p. 202–204°, $[\alpha]^{25}D$ +143° (chloroform), +152° (ethanol) $\lambda_{max}^{methanol}$ 243 mμ (ϵ = 15,800, λ_{max}^{Nujol} 2.93 μ (OH), 5.72 and 5.80 μ (20-carbonyl, 21-acetate interaction), 6.01, 6.16 and 6.23 μ ($\Delta^{1,4}$-diene-3-one) 8.06 μ (C-O-C of acetate), found: C, 74.46; H, 8.24], and 9α-fluoro-$\Delta^{1,4}$-pregnadiene-11β,17α,21-triol-3,20-dione (IX) [m.p. 265–269° dec., $[\alpha]^{25}D$ +111° (ethanol), $\lambda_{max}^{methanol}$ 239 mμ (ϵ = 14,800), found: C, 64.22; H, 7.51. Calcd. for $C_{21}H_{27}O_5F \cdot CH_4O$: C, 64.37; H, 7.61].

In addition to the recently noted, enhanced glucocorticoid activity of the 21-acetate of IX[7] we wish to report that IX and its 21-acetate possess intense mineralocorticoid action,[8] of the order of the parent fluorinated steroid, 9α-fluoro-4-pregnene-11β,17α,21-triol-3,20-dione.[9]

In subsequent reports we will describe in greater detail the chemistry and microbiology of these and related transformations, and the biochemical studies of the previously undescribed Δ^1-unsaturated derivatives of the known natural and synthetic steroid hormones.

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ISOLATION FROM URINE AND SYNTHESIS OF TETRAHYDROCORTISONE GLUCURONOSIDE

Sir:

It is generally agreed that 3α,17α,21-trihydroxypregnane-11,20-dione (tetrahydrocortisone) is the most abundant adrenocortical steroid metabolite excreted by man, and that it is present in urine largely as a glucuronoside. Because of the general interest in this conjugate and the recent evidence that its synthesis can be accomplished *in vitro*[1] we wish to report its recovery from urine in a relatively pure state and the synthesis and characterization of its tetraacetyl methyl ester.

Eight 250-mg. doses of free tetrahydrocortisone in aqueous alcohol were given orally to a man at half hourly intervals. The urine which was collected during this period and the twelve-hour interval that followed was acidified and extracted with butanol. The butanol extract was washed with water, neutralized with aqueous sodium carbonate and concentrated *in vacuo*. The crude product which separated weighed 2.92 g. and contained 1.45 g. of the desired sodium glucuronosidate as determined by analysis based on the method of Porter and Silber.[2] Four hundred milligrams of

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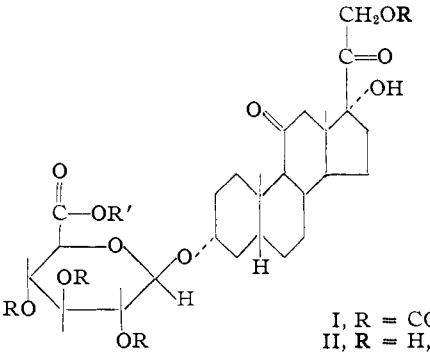
this product was distributed in a 50-tube Craig all-glass countercurrent machine using the system ethyl acetate–butanol–0.01 *N* HCl (90:10:100) and the method of single withdrawal. The upper phases emerging between the 85th and 120th transfers were pooled. Evaporation of the solvents gave 177 mg. of the glucuronoside which was 95% pure as determined by the above method of analysis. In this experiment 47% of the administered tetrahydrocortisone was accounted for as the glucuronoside of this purity. Since it was not possible to crystallize the purified glucuronoside, this and other similar lots were treated with diazomethane followed by acetic anhydride and pyridine to give, after adsorption chromatography, needles from methanol which melted at 208–209° uncor. $[\alpha]^{29}D$ +39.3° (7.62 mg. in 2.40 ml. $CHCl_3$). *Anal.* Found: C, 60.00; H, 7.01; CH_3CO, 23.87.

The purified glucuronoside was hydrolyzed by beef liver β-glucuronidase to give the calculated amount of tetrahydrocortisone as judged by the above method of analysis. Similar hydrolysis of a larger amount of the purified glucuronoside followed by acetylation and partition chromatography[3] of the neutral fraction showed that tetrahydrocortisone diacetate was the only recoverable steroid, m.p. 232.5–233.5° uncor., and indistinguishable from an authentic sample by mixed melting point and infrared spectroscopy. *Anal.* Found: C, 66.82; H, 8.00.

An additional 10 to 15% of the administered tetrahydrocortisone was accounted for as a glucuronoside of β-cortolone.[4] The crude glucuronoside obtained from tubes 12 through 25 following the countercurrent distribution was hydrolyzed with β-glucuronidase. The recovered neutral fraction was acetylated and chromatographed (3) to give 3α,20β,21-triacetoxy-17α-hydroxypregnane-11-one (β-cortolone triacetate) (4) m.p. 205–206° uncor. *Anal.* Found: C, 65.81; H, 8.25. Through the courtesy of Dr. T. F. Gallagher its identity was established by mixed melting point with an authentic sample and by infrared spectroscopy.

The synthesis of 3α-(triacetyl-β-D-glucuronoside methyl ester)-21-acetoxy-17α-hydroxypregnane-11,20-dione (I) was carried out by coupling tetra-



I, R = $COCH_3$, R′ = CH_3
II, R = H, R′ = H

hydrocortisone-21-monoacetate with α-bromo-triacetyl-glucuronic acid methyl ester in chloroform in the presence of silver carbonate to give I, m.p. 209–212° (from methanol); $[\alpha]^{30}D$ +37.8° ± 1.5° (14.0 mg. in 1.00 ml. of $CHCl_3$). *Anal.* Calcd. for $C_{36}H_{50}O_{15}$: C, 59.82; H, 6.92. Found: C, 59.75; H, 7.11. On admixture with the same derivative prepared from the glucuronoside isolated from urine the melting point was not depressed, and the infrared spectra of the two derivatives were identical. The structure of the naturally occurring glucuronoside as deduced from the method of synthesis, optical rotation and hydrolysis with β-glucuronidase must be that of II, which may be designated 3α-(β-D-glucuronoside), 17α,21-dihydroxypregnane-11,20-dione.

This investigation was supported by research grants A-272 and C-2210 from The National Institute of Arthritis and Metabolic Diseases and The National Cancer Institute, of the National Institutes of Health, Public Health Service, and an Institutional Grant to the College of Physicians and Surgeons by the American Cancer Society. The authors wish to thank Dr. Karl Pfister, Merck and Company, and Dr. Robert H. Levin of The Upjohn Company for the tetrahydrocortisone used in this work.

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