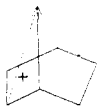
symmetrically. The 7-norbornenyl cation may be represented by (VI). It reacts with solvent



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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