tures from a parallel arrangement (*syn* for the *cis* and *anti* for the *trans*) and hence similar isotope effects.[8]

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(8) No more than a qualitative judgment is possible, for the extent to which C-1 approaches sp^2-hybridizationin the transition state is unknown. Models suggest that the arrangement is more nearly parallel for the *trans* isomer, in agreement with the slightly larger isotope effect.

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3,4-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione. An Intermediate in the Microbiological Degradation of Ring A of Androst-4-ene-3,17-dione

Sir:

Previous studies have shown that one degradative pathway of androst-4-ene-3,17-dione (I) by microorganisms may be envisaged as follows[1,2]: androst-4-ene-3,17-dione (I) → 9α-hydroxyandrost-4-ene-3,17-dione (II) or androsta-1,4-diene-3,17-dione (III) → 3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (IV) → 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indandione (V) ⇢ CO_2 + H_2O.

As large-scale fermentations have failed to yield intermediates which gave an insight as to the mechanism of conversion of IV into V, an alternate approach to this problem was undertaken. It is well documented that before a benzene ring is opened by means of bacterial enzymes which degrade aromatic compounds, two hydroxyl groups must first be introduced into the nucleus.[3,4] It has also been shown that, in some bacteria, the methyl group of *p*-cresol must be first oxidized to a carboxyl group before hydroxylation could take place on the aromatic ring.[5] Since the rate-determining step in the over-all breakdown of aromatic compounds by bacteria is usually the hydroxylation step, it would seem desirable to prepare 3,19-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VI), 2,3-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VII), and 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (VIII). The availability of these compounds would then allow us to evaluate which one, if any, of these compounds is the likely intermediate in the fission of the aromatic ring.

When 6,19-oxidoandrost-4-ene-3,17-dione[6] was exposed to *Nocardia restrictus* (ATCC 14887), 9α-hydroxy-6,19-oxidoandrost-4-ene-3,17-dione was obtained, m.p. 260–266° dec., $[\alpha]^{26}D$ +79°, λ_{max}^{alc} 240 mμ (ε 11,900), λ_{max}^{Nujol} 2.86, 5.78, 6.02, and 6.18 μ. When the epoxide was treated with zinc dust, 9α,19-dihydroxyandrost-4-ene-3,17-dione was obtained, m.p. 256.5–258.5°, $[\alpha]^{29}D$ +166°, λ_{max}^{alc} 245 mμ (ε 14,700), λ_{max}^{Nujol} 2,86, 2.95, 5.79, 6.08, and 6.21 μ. The latter compound was aromatized by reaction with *N. restrictus* in the presence of phenazine methosulfate to give VI, m.p. 140–141°, $[\alpha]^{25}D$ +89°, λ_{max}^{alc} 278 mμ (ε 2000), λ_{max}^{KBr} 2.98, 5.78, 5.84, 6.20, 6.31, and 6.66 μ. However, VI was only metabolized at a rate equal to IV by *N. restrictus* as measured by their rate of disappearance.

The simplest procedure that we could visualize for the preparation of VII would appear to involve the introduction of a 9α-hydroxyl group into 2-hydroxyandrosta-1,4-diene-3,17-dione; the resulting vinylog of a β-hydroxy ketone should then undergo reverse aldolization to yield VII.[7] However, when 2,17β-dihydroxyandrosta-1,4-dien-3-one[8] was exposed to *N. restrictus*, the major product obtained was 2ξ,9α-dihydroxyandrost-4-ene-3,17-dione, m.p. 240–243°, λ_{max}^{alc} 243 mμ (ε 12,000), $[\alpha]^{35}D$ +9°, λ_{max}^{Nujol} 2.90, 5.78, 6.05, and 6.20 μ. In an attempt to block the hydrogenase activity of this organism, 2-methoxy-17β-hydroxyandrosta-1,4-dien-3-one[9] (IX) was prepared with a view to obtaining 2-methoxy-3-hydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (X) *via* an analogous 9α-hydroxylation procedure. When IX was exposed to *Nocardia corallina*[10] (ATCC 13259), surprisingly a phenolic compound with all the characteristics of 2-methoxy-3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (XI) was obtained, m.p. 185–188°, $[\alpha]^{25}D$ +135°, λ_{max}^{alc} 273 mμ (ε 900), λ_{max}^{KBr} 2.95, 5.78, 5.85, 6.15, 6.25, and 6.65 μ; n.m.r.[11] peaks τ 8.83 (3 H, one tertiary CH_3), 7.72 (3 H, one CH_3 on aromatic ring), 6.17 (3 H, one aromatic OCH_3), 4.62 and 3.58[12] (2 H, two phenolic OH), and 3.71 (1 H, one aromatic H). The catechol structure was further confirmed by its instability to base and a quinone was obtained after its oxidation by means of Ag_2O or mushroom tyrosinase. Thus, the synthesis of VII has lost its relevance to the problem since it appeared that VIII might be a more likely intermediate in the aromatic cleavage reaction.

Treatment of 9α,17β-dihydroxyandrost-4-en-3-one[13] (XII) with alkaline H_2O_2 resulted in the formation of 9α,17β-dihydroxy-4,5-oxidoandrostan-3-one (XIII), m.p. 197–198.5°, $[\alpha]^{26}D$ −66°, λ_{max}^{Nujol} 2.78, 2.86, and 5.85 μ. The epoxide was opened by its reaction with a sulfuric–acetic acid mixture to yield 4,9α,17β-trihydroxyandrost-4-en-3-one (XIV), m.p. 227–229°, $[\alpha]^{29}D$ +42°, λ_{max}^{alc} 278 mμ (ε 12,700), λ_{max}^{Nujol} 2.87, 6.03, and 6.14 μ. When XIV was incubated with frozen cells of *N. restrictus* in the presence of phenazine methosulfate, VIII was obtained, m.p. 164–165°, $[\alpha]^{28}D$ +149°, λ_{max}^{alc} 282 mμ (ε 1950), $\lambda_{max}^{CHCl_3}$ 2.86, 3.00, 5.76, 5.92, 6.19, 6.26, and 6.69 μ.[14]

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(3) S. Dagley, P. J. Chapman, D. T. Gibson, and J. M. Wood, *Nature*, **202**, 775 (1964).
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(6) H. Ueberwasser, K. Heusler, J. Kalvoda, Ch. Meystre, P. Wieland, G. Anner, and A. Wettstein, *Helv. Chim. Acta*, **46**, 344 (1963).
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(8) P. N. Rao and L. R. Axelrod, *J. Am. Chem. Soc.*, **82**, 2830 (1960).
(9) J. S. Baran, *ibid.*, **80**, 1687 (1958).
(10) Among the 9-hydroxylating organisms tested (*N. restrictus*, *Pseudomonas testosteroni*, *Bacterium cyclooxydans*, and *Mycobacterium rhodochrous*), this *Nocardia corallina* gave the best yield of the product.
(11) All n.m.r. spectra were determined on a Varian Associates recording spectrometer (A-60) at 60 Mc. in deuterated chloroform. Chemical shifts are reported in τ-values (p.p.m.) [G.V.D. Tiers, *J Phys. Chem.*, **62**, 1151 (1958)].
(12) These two peaks disappeared after the addition of HCl.
(13) C. J. Sih, *J. Org. Chem.*, **26**, 4716 (1961).
(14) Satisfactory analyses have been obtained on all the new compounds cited herein. We thank Mr. Joseph Alicino, Metuchen, N. J., for the analyses.

When VIII was exposed to cell-free extracts of *N. restrictus* it was very readily converted into V, m.p. 98–100°, identical (mixture melting point and infrared spectrum) with an authentic specimen[2] of 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indandione. Since, under similar conditions, IV and VI were only slowly converted into V by these same extracts, VIII appears to be involved as an intermediate in the main pathway of this degradation. Similar conversions have been obtained with other microorganisms[10] indicating that this is a general pathway of steroid degradation.[15]

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On the Mechanism of Ring A Cleavage in the Degradation of 9,10-Seco Steroids by Microorganisms

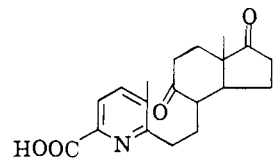
Sir:

In our previous communication[1] we have shown that 3,4-dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione (I) was readily transformed into 3aα-H-4α-[3′-propionic acid]-7aβ-methylhexahydro-1,5-indandione (II) by cell-free extracts of *Nocardia restrictus* (ATCC 14887). We now wish to present evidence for the structure of other intermediates and to define the reaction sequence involved in the cleavage of the steroid A ring.

In a typical experiment, 300 mg. of I was incubated with cell-free extracts of *N. restrictus* in the presence of Fe^{2+}. After acidification, the reaction mixture was extracted overnight with ether. The residue obtained from the ethereal extract was chromatographed over a silicic acid column to yield 62 mg. of II and 40 mg. of an oil (III) whose properties were consistent with 2-oxo-4-ethylbutyrolactone. Its ultraviolet spectrum in acid showed an absorption peak at 228 mμ which shifted to 262 mμ in base. Its infrared spectrum (KBr pellet) showed bands at 3.02, 5.75, 5.82, and 6.04 μ. Its n.m.r. spectrum[2] showed bands at τ 8.97 (3 H, triplet, J = 8 c.p.s., CH_3—CH_2), 8.22 and 8.37 (2 H, quartets, J = 6.5, 2 c.p.s., CH_3—CH_2—CH), 5.13 (1 H, sextet, J = 7, 2.5 c.p.s.; CH_2—CHOR—CH), 3.83 (1 H, doublet, J = 2 c.p.s. vinyl proton coupled with adjacent H), and 2.80 (1 H, enolic OH). The structure of III was conclusively established as 2-oxo-4-ethylbutyrolactone as follows: III was found to be identical (infrared spectrum, n.m.r. spectrum, and chromatographic behavior) with a sample of (±)-2-oxo-4-ethylbutyrolactone prepared by the published route from sodium diethyloxaloacetate and propionaldehyde.[3] A crystalline 2,4-dinitrophenylhydrazone of III was obtained, m.p. 168–171°, whose infrared and mass spectrum[4] were also found to be identical with those of a synthetic sample. However, the actual product of the enzymatic reaction is probably 2-oxo-4-hydroxycaproic acid (IV) rather than III, for III was not further metabolized by the cell-free extract. Also, III and IV have been shown to be readily interconvertible, depending on the hydrogen ion concentration of the surrounding medium.

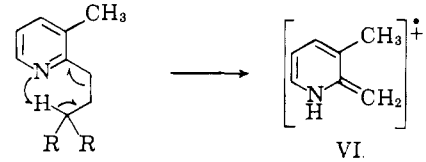
When the cell-free extract was treated with Sephadex G-25, a preparation was obtained which also readily metabolized I. For each mole of I present, 1 mole of O_2 was consumed and no CO_2 was evolved. During the reaction a yellow compound accumulated (λ_{max} 392 mμ (pH 13) and 315 mμ (pH 1.0)) which was slowly converted to II. This type of ultraviolet spectrum is reminiscent of those given by α-hydroxymuconic semialdehyde, α-hydroxy-γ-carboxymuconic semialdehyde, and 2,6-diketonon-4-enedioic acid.[5,6] Because of the extreme instability of this yellow intermediate, it was treated directly with NH_4OH to yield an amorphous pyridine compound[7] (V), m.p. 94–97°, λ_{max}^{alc} 273 mμ (ε 4700), λ_{max}^{KBr} 2.97, 5.78, and 5.86 μ; n.m.r. peaks at τ 8.82 (3 H, one tertiary CH_3), 7.52 (3 H, one CH_3 on pyridine ring), and 2.74 and 2.16 (2 H, aromatic protons).

Strong evidence for the assigned structure V was found in its mass spectrum. While there was no parent



V

ion at *m/e* 329, there was a prominent (M − CO_2) peak at *m/e* 285.[8] The base peak in the spectrum, *m/e* 107, is assigned to structure VI. The virtual absence of a



VI

peak at *m/e* 106 eliminates from consideration pyridines having the hydrindanedione substituent at C-3, C-4, or C-5,[9] since these should give rise to a prominent fragment VII.[10,11] Other prominent peaks at *m/e* 120 (VIII) and 55 (IX) also lend support to structure V.

(1) C. J. Sih, S. S. Lee, Y. Y. Tsong, and K. C. Wang, *J. Am. Chem. Soc.*, 87, 1385 (1965).

(2) All n.m.r. spectra were determined on a Varian Associates recording spectrometer (A-60) at 60 Mc. in either deuterated chloroform or carbon tetrachloride. Chemical shifts are reported in τ-values (p.p.m.) [G.V.D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958)].

(3) A. Rossi and H. Schinz, *Helv. Chim. Acta*, 31, 473 (1948).

(4) The mass spectra of all samples were taken on a CEC-103 C mass spectrometer operating at 70-v. ionization voltage and employing a heated glass inlet system at 250°.

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(7) The ring fission product of protocatechuic acid by the action of a 4,5-oxygenase was identified as α-hydroxy-γ-carboxymuconic semialdehyde. The latter compound was characterized by reaction with ammonia to yield 2,4-lutidinic acid (see ref. 5). Similarly, the ring fission product of β-phenylpropionate was characterized by an analogous reaction (see ref. 6).

(8) Decarboxylation of V probably occurred in the inlet system (250°).

(9) These would arise if oxidation of I occurred between C-2 and C-3.

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