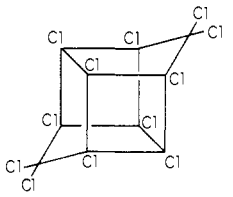
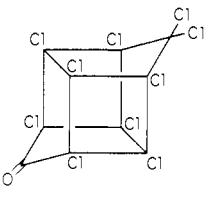
tion presently available, we suggest the following structures for I and III.



I



III

Acknowledgment.—The authors are indebted to Dr. W. P. Binnie, Physics Department, Purdue University, for the execution and interpretation of the X-ray diffraction studies and to Hooker Electrochemical Company for the support of this work.

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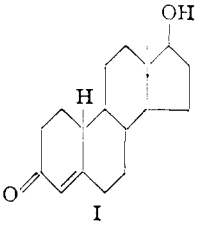
MICROBIOLOGICAL TRANSFORMATIONS OF STEROIDS. XIV.[1] THE PREPARATION OF A TERTIARY HYDROXY-STEROID, 10ξ-HYDROXY-19-NORTESTOSTERONE

Sir:

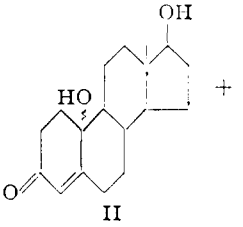
Numerous studies have been done in these laboratories investigating the relationship between steroids of varying structures and the enzymes elaborated by *Rhizopus nigricans* (A.T.C.C.6 227b). Previous results of such studies[2] showed that the major hydroxylation proceeded in the 11α-position, while hydroxylation in the 6β- and 6β,11α-positions occurred only to a minor extent. Although no tertiary hydroxylations had been reported in these earlier studies with *R. nigricans*, other molds were shown to introduce tertiary hydroxyl groups.[3,4,5,6,7]

We now wish to report the preparation of a 10-hydroxy-steroid, namely, 10ξ-hydroxy-19-nortestosterone by the microbiological action of *R. nigricans* on 19-nortestosterone. This mold has thus been found to produce an enzyme which can also hydroxylate a steroid in a tertiary position.

The new steroids were obtained by fermentation and extraction methods previously described[8] using 19-nortestosterone (I)[9] as the substrate and *R. nigricans* as the microörganism.



I



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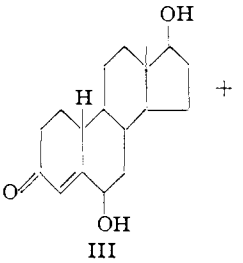
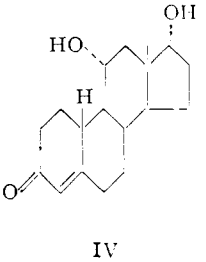


III



IV

After 25 g. of 19-nortestosterone had been subjected to the action of *R. nigricans*, it was possible to isolate from the methylene chloride extract 4.1 g. of 6β-hydroxy-19-nortestosterone (III) by direct crystallization. Chromatography of the liquors over Florisil then afforded, besides small amounts of starting material, three major fractions.[10]

The first of these (from 10% acetone in petroleum ether) gave 0.32 g. of II, m.p. 199–205°; $[\alpha]_D$ +76° (methanol); $\lambda_{max.}^{ethanol}$ 237 mμ (15,025); $\nu_{max.}^{Nujol}$ 3305, 1656, 1622 cm.$^{-1}$; (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.45, 74.52; H, 9.21, 8.77). Tertiary character was indicated for the new hydroxyl group by formation of a 17-monoacetate, m.p. 184–185°, $\nu_{max.}^{Nujol}$ 3375, 1707, 1684, 1625 cm.$^{-1}$, (*Anal.* Calcd. for $C_{20}H_{28}O_4$: C, 72.26; H, 8.49. Found: C, 72.27, 72.81; H, 8.70, 8.63) and by oxidation to a hydroxydiketone (VI), m.p. 198–201°, $\lambda_{max.}^{ethanol}$ 235.5 (14,025); $\nu_{max.}^{Nujol}$ 3410, 1718 cm.$^{-1}$. Its location near the chromophore in ring A was suggested by the hypsochromic shift in the ultraviolet[11] and by acid-catalyzed dehydration of II to estradiol. The structure was confirmed by chemical synthesis. Treatment of 17β-hydroxy-5(10)-estren-3-one[9] with osmium tetroxide followed by sodium sulfite afforded 10-hydroxy-19-nortestosterone (II) identical to that obtained by the microbiological procedure.

From the second fraction (15% acetone) was obtained an additional 0.75 g. of 6β-hydroxy-19-nortestosterone (III), m.p. 217–219°, $[\alpha]_D$ −63° (methanol), $\lambda_{max.}^{alcohol}$ 238 mμ (13,875), $\nu_{max.}^{Nujol}$ 3320, 1654, 1620 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.64; H, 9.30). It readily formed a diacetate (VII), m.p. 137–138°, $\lambda_{max.}^{ethanol}$ 236 mμ (13,550), $\lambda_{max.}^{Nujol}$ 1731, 1724, 1694, 1630, 1240 cm.$^{-1}$. On oxidation, 4-estrene-3,6,17-trione (VIII), m.p. 155–57°, 254 mμ (9,550),

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(10) We are grateful to J. Mejeur, H. Triemstra, J. R. Heald, G. Staffen and H. Woltersom for technical assistance, to W. A. Struck and his group for microanalyses and rotations, and to Dr. J. L. Johnson and his group for infrared and ultraviolet measurements.

(11) Similar changes have been noted for 6β-hydroxy- and 6β-acetoxy-Δ^4-3-ketosteroids, I. Dorfman, *Chem. Rev.*, **50**, 47 (1953).

$\nu_{max.}^{Nujol}$ 1734, 1688, 1664, 1600 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.22; H, 7.74) was obtained.

Alkaline rearrangement[12] of III afforded a 17β-hydroxyestrane-3,6-dione (IX), m.p. 145–146°, $[\alpha]_D$ −14° (methanol), $\nu_{max.}^{Nujol}$ 3290, 1715, 1704 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 75.12; H, 9.23).

From the third fraction (20% acetone) was obtained 1.1 g. of 11α-hydroxy-19-nortestosterone (IV), m.p. 167–168°,[13] $[\alpha]_D$ −46° (chloroform), $\lambda_{max.}^{alcohol}$ 242 mμ (15,475), $\nu_{max.}^{Nujol}$ 3345, 1650, 1610 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{26}O_3$: C, 74.44; H, 9.03. Found: C, 74.37; H, 8.95). On acetylation it gave a diacetate (X), m.p. 190.5–191.5°, $[\alpha]_D$ −39.6° (chloroform). Oxidation afforded 19-noradrenosterone (XI), m.p. 213.5–215°, $[\alpha]_D$ +145° (methanol), $\lambda_{max.}^{alcohol}$ 240 mμ (14,600), $\nu_{max.}^{Nujol}$ 1732, 1698, 1665, 1612 cm.$^{-1}$, (*Anal.* Calcd. for $C_{18}H_{22}O_3$: C, 75.49; H, 7.75. Found: C, 75.09; H, 7.81).

The structure assignment for 11α-hydroxy-19-nortestosterone is based upon (1) molecular rotation data, (2) infrared, ultraviolet and chemical properties of IV and its derivatives, and (3) a consideration of the microbiological data obtained from fermentation of a large number of steroids with *Rhizopus nigricans*.[14]

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(13) A higher melting polymorph, m.p. 185–187°, was subsequently obtained.

(14) Since the preparation of this M.S., F. B. Colton (U. S. Patent 2,729,654) has described the preparation of epimeric 10-hydroxy-19-nortestosterone *via* the epoxide derived from 17β-hydroxy-5(10)-estren-3-one. Although distinguishing constants for the 10–17 diols are not given, one of the 10-hydroxy-10,17-ketones he describes corresponds reasonably well with that derived from the 10,17-diol produced microbiologically.

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ATP[1] FORMATION ACCOMPANYING FORMIMINOGLYCINE UTILIZATION

Sir:

Formiminoglycine (FIG) is formed from 4-aminoimidazole or xanthine by extracts of *Clostridium cylindrosporum*.[2] Washed cell suspensions of *Clostridium acidi-urici* convert FIG to acetic acid and carbon dioxide.[3] Extracts of this organism or of *C. cylindrosporum* have now been obtained which carry out the partial reaction in which FIG is converted to glycine, formic acid, and ammonia. When the extracts of *C. acidi-urici* are treated with Dowex-1 chloride, the activity of the enzyme is dependent on the addition of a boiled extract of the organism; this can be replaced by a number of folic acid derivatives (Table I). Sagers, *et al.*,[7] using a similar preparation, have also reported the activation of this reaction by tetrahydrofolic acid.

TABLE I

STIMULATION OF FORMIMINOGLYCINE DEGRADATION BY PTERIDINE DERIVATIVES

Compound	Pteridine,[a] μmoles/ml.	Activity[b]
No addition	0	1.6
Boiled extract[c]	0.024	3.8
	.24	8.4
Folic acid	.4	3.0
Tetrahydrofolic acid[d]	.9	5.5
Teropterin[e]	.3	6.2
N-10-Formylfolic acid[f]	.14	2.9
N-5-Formyltetrahydrofolic acid[g]	.06	7.1
Diglutamyl-N-10-formylfolic acid[h]	.04	6.8

[a] Calculated from the molar extinction coefficient at the absorption maximum in 0.1 *N* KOH. This was assumed to be 26,000 at 260 mμ for the boiled extract, which on this basis contained 2.4 μmoles per ml. [b] μMoles of FIG utilized in 20 min. at 37° in a system containing 10 μmoles of FIG, 50 μmoles of potassiun phosphate, *p*H 7.0, 0.5 μmole of Na_2S, 2 μmoles of ferrous sulfate, 0.4 ml. of enzyme, and the additions shown, in 1 ml. FIG was determined colorimetrically as described elsewhere.[2] The enzyme was an alumina-ground extract of *C. acidi-urici* treated with Dowex-1 chloride at 0° for 15 min. This preparation contained 21 mg. of protein per ml. [c] Prepared by heating 2.5 g. of lyophilized cells of *C. acidiurici* in 50 ml. of 0.01 *M* KPO_4, *p*H 7, 0.02 *M* cysteine in a boiling water-bath for 5 min. [d] Prepared by Dr. T. Miles from purified folic acid by catalytic reduction.[4] [e] Gift of Dr. H. P. Broquist, Lederle Laboratories, purified by Dr. B. E. Wright. [f] A sample obtained from the Lederle Laboratories and purified as previously described.[5] This was provided by Dr. M. Silverman. [g] A gift of Leucovorin from Dr. H. P. Broquist. [h] A sample isolated from *C. cylindrosporum* by Dr. B. E. Wright.[6]

Dowex treated extracts prepared from lyophilized cells of *C. cylindrosporum* which had been stored for over 2 years at −10° show an additional requirement for ADP and orthophosphate (Table II). UDP, CDP, IDP, and GDP (tested at 2

TABLE II

REQUIREMENTS OF FORMIMINOGLYCINE UTILIZATION

	FIG utilized, μmoles/ml.
Complete system[a]	6.1
Omit N-5-formyltetrahydrofolic acid	1.9
Omit ADP	1.7
Omit P_i[b]	1.6

[a] The complete system contained, per ml., 50 μmoles of potassium phosphate, *p*H 7.0, 10 μmoles of FIG, 1 μmole of ferrous sulfate, 0.5 μmole of 2-mercaptoethanol, 0.2 μmole of N-5-formyltetrahydrofolic acid, 5 μmoles of ADP, an extract of lyophilized cells of *C. cylindrosporum* equivalent to 6 mg. of protein, prepared in maleate buffer and treated with Dowex-1 chloride. Tubes were incubated at 37° for 30 min. [b] The phosphate was replaced by 25 μmoles of maleate buffer, *p*H 6.8, which showed no inhibition.

(1) The following abbreviations have been used: ATP, adenosine triphosphate; ADP, adenosine diphosphate; CDP, cytidine diphosphate; IDP, inosine diphosphate; GDP, guanosine diphosphate; UDP, uridine diphosphate; G-6-P, glucose 6-phosphate.

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