Carbon 2 of guanine and the methylene carbon of glycine are incorporated equally into nucleic acid adenine and into histidine (Exp. 3, 6, 9), suggesting that they enter a pool from which 1-carbon units are drawn for the biosynthesis of the precursors of purines and of histidine.[7]

This assumption gains support from the results obtained in mutant HP-1, a strain with an unusually high guanine requirement which is spared by histidine. Here carbon 2 of guanine is incorporated into adenine and into the amidine carbon of histidine without dilution (Exp. 10–12), indicating that it is the only available source of the 1-carbon units required for the formation of these compounds; it would seem that in this mutant the guanine requirement reflects a genetic block which prevents the production of these 1-carbon units from other sources.

The experiments presented here suggest that the conversion of guanine to adenine occurs through the replacement of the amino-substituted carbon 2 of guanine by a single carbon unit. An alternative possibility, the reduction of carbon 2 of guanine by a pathway not involving reactions (1) and (2) followed by interchange with single carbon units, is not rigorously excluded but appears less likely because it would not account for the equal incorporation of carbon 2 of guanine into adenine and histidine.

(7) The transfer of carbon 2 of guanine to the amidine carbon of histidine has recently been observed in *Lactobacillus casei;* C. Mitoma and E. E. Snell, *Proc. Nat. Acad. Sci.*, **41**, 891 (1955).

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RECEIVED JANUARY 27, 1956

AN INVESTIGATION OF THE CHLOROCARBON, $C_{10}C_{12}$, M.P. 485° AND THE KETONE, $C_{10}Cl_{10}O$, M.P. 349°

Sir:

A chlorocarbon, $C_{10}Cl_{12}$ (I), m.p. 485°, has been obtained from the reaction of 1,1,2,3,3,4,5,5-octachloropentene and aluminum chloride[1] and directly from hexachlorocyclopentadiene (II) by reaction with aluminum chloride in boiling methylene chloride or carbon tetrachloride.[1,2,3] When II was treated with sulfur trioxide, and the product hydrolyzed, a ketonic material, $C_{10}Cl_{10}O$ (III), m.p. 349°,[3] resulted. Compound III was originally thought to be perchloro-3a,4,7,7a-tetrahydro-4,7-methanoindene. When compound III was treated with phosphorus pentachloride, compound I was obtained.[3] It was initially thought that compound I was perchloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (IV), the "Diels–Alder" dimer of compound II. Recently however, the Diels–Alder dimer (IV) of II has been prepared[4]; thus the compound described as compound I, m.p. 485°, is not the Diels–Alder dimer. The high m.p. of I contrasts sharply with that of IV, m.p. 221° and with 1,2,3,-3a,4,5,6,7,7a,8-decachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene (V), m.p. 215°, both of which give good yields of their respective monomers on thermal degradation. In contrast, the chlorocarbon (I) undergoes pyrolysis only at very high temperatures (500° or above) giving largely carbonaceous material and chlorine with only a small amount of II. Compound IV can be isomerized to I by aluminum chloride. Neither I nor III contain a carbon–carbon double bond. No addition of chlorine, bromine, or other reactive species has been noted. The infrared spectra of I and III, in contrast to the spectra of IV and V, showed no absorption in the 6–7 μ region where the carbon–carbon double bond is known to absorb unless a selection rule is in operation because of the presence of certain elements of symmetry in the molecule.[5] Ultraviolet absorption spectra are not subject to such selection rules. The ultraviolet spectrum of I contrasts sharply with the spectra of IV, 1,2,3,-4,5,6,7,7-octachlorobicyclo[2.2.1]hept-2-ene and octachlorocyclopentene which absorb in the region above 210 mμ, a characteristic of highly chlorinated cyclic monoenes and dienes.[6] The ketone III shows a maximum of 307 mμ which is ascribed to an unconjugated carbonyl group,[7] but no maxima in the "double bond" region. This is in contrast to the spectrum of octachloro-3a,4,7,7a-tetrahydro-7,7-methanoindene-1,8-dione, the structure of which has been established.[7]

Compound I is unaffected by the following: zinc dust and hydrochloric acid, acetic acid, or methanol; silver nitrate and ethanol for long periods; alkaline reagents such as potassium hydroxide in methanol or lithium aluminum hydride in ether; oxidizing agents such as ozone, potassium permanganate, chromic acid, sulfur trioxide, sulfuric acid, nitric acid or nitric acid with selenium. Compound III does not undergo the haloform reaction which would be expected for a dichloromethylene group adjacent to a carbonyl group. The presence of a bridged carbonyl is excluded by the fact that III does not lose carbon monoxide on heating to 200°. Compound III fails to exhibit olefinic properties. The presence of a reactive carbonyl group is demonstrated by the preparation of several hemi-ketals, thiohemiketals, and amine adducts. However, III fails to react with Caro's acid or hydrazoic acid. To meet the requirements of the formula $C_{10}Cl_{12}$ and, excluding the carbonyl double bond, $C_{10}Cl_{10}O$, the structures must contain six rings and/or double bond. Preliminary X-ray diffraction studies on I indicate a highly symmetrical molecule crystallizing in the cubic system or in an arrangement closely approximating the cubic system.

From the preceding evidence, aromatic systems, aliphatic olefins and acetylenes and alicylic olefins seem to be excluded. The only remaining possibility is a "caged" structure possessing a total of six saturated rings. On the basis of the informa-

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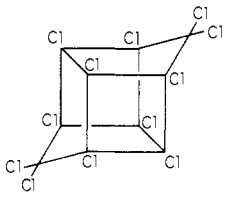
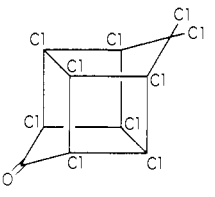
tion presently available, we suggest the following structures for I and III.



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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RECEIVED JANUARY 18, 1956

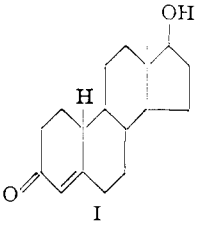
MICROBIOLOGICAL TRANSFORMATIONS OF STEROIDS. XIV.[1] THE PREPARATION OF A TERTIARY HYDROXY-STEROID, 10ξ-HYDROXY-19-NORTESTOSTERONE

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 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.



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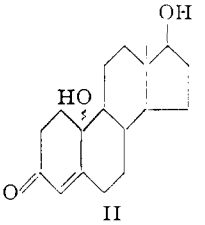
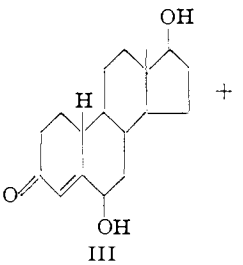
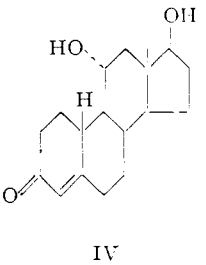


II



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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