1,2,3,4,6,7,7-Octachlorobicyclo[2.2.1]-2-heptene.—A mixture of hexachlorocyclopentadiene (54 g., 0.2 mole) and *cis*-1,2-dichloroethylene (20 g., 0.2 mole) was heated for 25 hours in a sealed tube at 200°. The solid brown reaction product was crystallized from methanol to yield 28 g. (38%) of isomeric adducts, m.p. 170–190°.[9]

Anal. Calcd. for $C_7H_2Cl_8$: C, 22.7; H, 0.50. Found: C, 23.0; H, 0.49.

When *trans*-1,2-dichloroethylene was substituted for the *cis* isomer, only 20 g. (26%) of crude product was obtained under the same conditions.

1,2,3,4,7,7-Hexachloro-5,6-dibromobicyclo[2.2.1]-2-heptene.—Hexachlorocyclopentadiene (54 g., 0.2 mole) and 1,2-dibromoethylene (37 g., 0.2 mole) were heated in a sealed tube at 200° for 27 hours. The crude product (20 g.) was decolorized and crystallized from methanol. After three recrystallizations from methanol, there was obtained 15 g. (16%) of product, m.p. 210°.

Anal. Calcd. for $C_7H_2Br_2Cl_6$: total halogen (as chloride), 61.8. Found: total halogen, 61.9.

1,2,3,4,6,7,7-Heptachloro-5-methylbicyclo[2.2.1]-2-heptene.—A mixture of hexachlorocyclopentadiene (68 g., 0.25 mole) and freshly distilled 1-chloropropene (19 g., 0.25 mole) was heated in a sealed tube at 150–160° for 24 hours. Distillation of the reaction products gave 7 g. of 1-chloropropene, b.p. 35° (745 mm.), 38 g. of hexachlorocyclopentadiene (b.p. 83° (3 mm.)) and 33 g. of black residue, which solidified on cooling. Decolorization and crystallization from methanol furnished 6.5 g. of brown crystals, m.p. 165–180°. After several crystallizations from the same solvent, 4.7 g. (7.5%) of product, m.p. 197–198°, was obtained. When the same reactants were heated at 100°, no adduct was formed, while at 200° extensive decomposition took place.

Anal. Calcd. for $C_8H_5Cl_7$: C, 27.4; H, 1.40. Found: C, 28.2; H, 1.34.

1,2,3,4,6,7,7-Heptachloro-5-chloromethylbicyclo[2.2.1]-2-heptene.—Hexachlorocyclopentadiene (273 g., 1 mole) and 1,3-dichloropropene (111 g., 1 mole) were heated in a 1-liter flask, equipped with a reflux condenser, at 130° for 24 hours. Distillation of the reaction products yielded 73 g. of 1,3-dichloropropene (b.p. 106–113° (742 mm.)), 205 g. of hexachlorocyclopentadiene (b.p. 98–107°(7 mm.)) and 86 g. of black residue, which deposited brown crystals on standing. Decolorization and crystallization of this material from methanol gave 55 g. of yellow-brown solid, m.p. 55–65°. Several recrystallizations furnished 14 g. (15%) of colorless adduct, m.p. 78–80°.

Anal. Calcd. for $C_8H_4Cl_8$: C, 25.0; H, 1.04. Found: C, 25.5; H, 1.07.

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[9] The product may represent a mixture of *endo* and *exo* isomers.

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Microbiological Transformation of Steroids. XIII. Oxygenation of 16α,17α-Oxidoprogesterone to 11α-Hydroxy-16α,17α-oxidoprogesterone by *Rhizopus Nigricans* Ehrb. (A.T.C.C. 6227b)[1]

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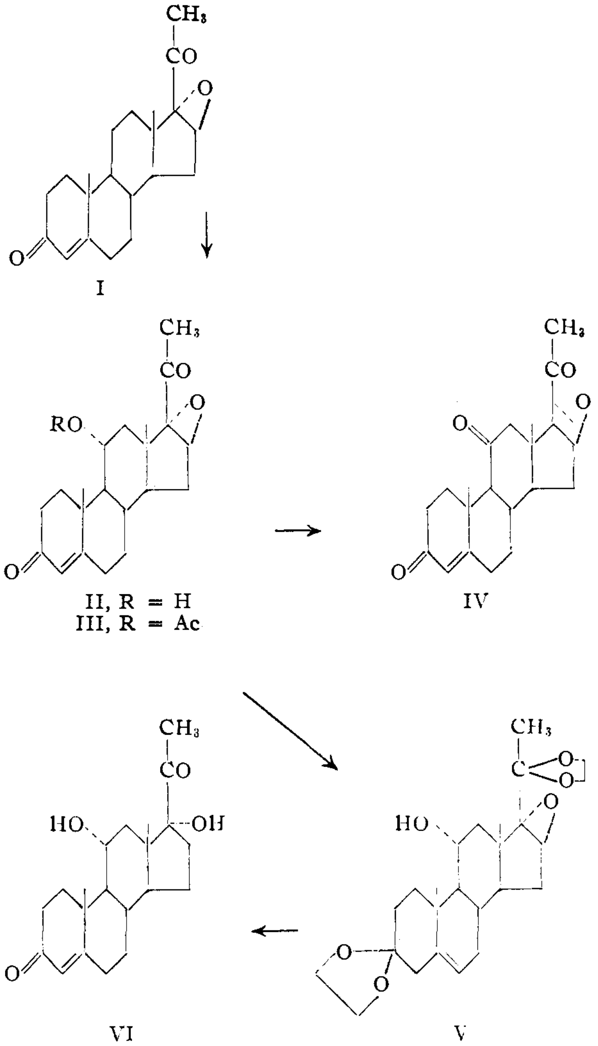
In continuation of our studies on the enzymatic oxygenation of steroids with fungi of the genus *Rhizopus*, we wish to report the introduction of an 11α-hydroxyl group into 16α,17α-oxido-4-pregnene-3,20-dione (I) by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). The substrate I is readily prepared from sapogenins such as diosgenin by the method of Julian, Meyer and Ryden.[2] Upon incubation of I with a 24-hour growth of *Rhizopus nigricans* under conditions similar to those previously defined,[3] 11α-hydroxy-16,17-oxido-4-pregnene-3,20-dione (II) was obtained in 70–75% yield by direct crystallization. Compound II gave, on oxidation with chromium trioxide, 16,17-oxido-4-pregnene-3,11,20-trione (IV). Acetylation of II with acetic anhydride and pyridine yielded 11α-acetoxy-16,17-oxido-4-pregnene-3,20-dione (III). The latter compound has been synthesized chemically by the Schering group in a six-step synthesis from 3α,11α-diacetoxypregnan-20-one.[4] A comparison of the physical constants of our compound [m.p. 225–26°, [α]D +113° (CHCl$_3$)] to those of the Schering compound [m.p. 217–18°, [α]D +112.9° (CHCl$_3$)] would suggest that these two compounds are identical. However, the infrared spectra of the two



I

II, R = H
III, R = Ac

IV

VI

V

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[1] Paper XII of this series, THIS JOURNAL, **76**, 4050 (1954).

[2] P. L. Julian, E. W. Meyer and I. Ryden, *ibid.*, **72**, 367 (1950).

[3] D. H. Peterson, *et al.*, *ibid.*, **74**, 5933 (1952).

[4] H. L. Herzog, C. C. Payne, M. E. Tully and E. B. Hershberg, *ibid.*, **75**, 5751 (1953).

compounds have never been directly compared to each other.

In order to prove unambiguously the structure of our bioconversion product, the following sequence of reactions was employed.

Treatment of II with ethylene glycol in benzene solution in the presence of *p*-toluenesulfonic acid gave the 3,20-bis-(ethylene ketal) (V).[5] Reductive opening of the epoxide ring in compound V followed by acidic hydrolysis gave 11α,17α-dihydroxyprogesterone (VI) which was in all details identical to the compound obtained by enzymatic hydroxylation of 17α-hydroxyprogesterone with *Rhizopus*.[6] This reaction sequence, therefore, provides conclusive proof for the 11α-position of the hydroxyl group in the bioconversion product II and indirectly supports the identity of the acetate III with the above mentioned Schering compound.

Experimental Part

1. **Conversion of 16,17-Oxidoprogesterone (I) to 11α-Hydroxy-16,17-Oxidoprogesterone (II) by Rhizopus nigricans Ehrb. (A.T.C.C. 6227b).**—*Rhizopus nigricans* was grown on 12 liters of a medium containing cerelose (20 g./l.), sodium chloride (10 g./l.) and corn steep (20 g./l.) for 24 hours at an aeration of 1 liter/minute and an agitation of 300 r.p.m. Then 3 g. of substrate I dissolved in 150 ml. of acetone was added and the fermentation continued for another 24 hours under the same conditions of aeration and agitation.

Extraction of the conversion products with methylene dichloride as described previously[3] yielded 6.62 g. of a residue which contained, by papergram analysis, a new compound with a mobility similar to 11α-hydroxyprogesterone.

The extractives were triturated three times with 10-ml. portions of ether. The ether solutions were decanted in each instance and discarded. The crystalline residue obtained by this process (2.79 g., m.p. 228–42°)[7] was dissolved in 60 ml. of methylene dichloride. This solution was concentrated to 15 ml. and diluted with 20 ml. of ether to give 2.31 g. (73.5% yield) of 11α-hydroxy-16,17-oxidoprogesterone (II). A sample was recrystallized twice more from ethyl acetate to a constant melting point of 247–249°; $\lambda_{\max}^{\text{alc.}}$ 242 mμ, *E* 15,300; $[\alpha]^{23}\text{D}$ +136° (*c* 0.82, in chloroform). The infrared spectrum indicated the presence of one hydroxyl group.

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.22; H, 8.19. Found: C, 73.41; H, 8.13.

The acetate III was prepared with acetic anhydride–pyridine at room temperature. From 94 mg. of II, so treated, there was obtained upon recrystallization from ethyl acetate 39.7 mg. of 11α-acetoxy-16,17-oxidoprogesterone, m.p. 225–26°, $[\alpha]^{23}\text{D}$ +113° (*c* 0.52, in chloroform). The infrared spectrum indicated complete acetylation.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.41; H, 7.72.

2. **Oxidation of II to 16,17-Oxido-4-pregnene-3,11,20-trione (IV).**—Compound II (60.2 mg.) was dissolved in 3 ml. of glacial acetic acid. To the chilled solution was added 13.5 mg. of chromium trioxide (calcd. for one oxygen atom: 11.6 mg.) dissolved in 0.26 ml. of water and 3 ml. of glacial acetic acid. The reaction mixture was kept at room temperature for 6 hours. It was diluted with 10 ml. of methanol and 50 ml. of water and extracted twice with ether–methylene dichloride 5:1 (30 ml.). The extracts were washed 4 times with 5% sodium hydroxide and three times with water (10 ml. each time), dried over sodium sulfate and concentrated to give 60.0 mg. of crystals. These were recrystallized from 3 ml. of acetone–Skellysolve B to give a material (49.5 mg.), m.p. 192–195°. After two further recrystallizations from methanol or acetone, the compound showed the following properties: m.p. 192.5–194.5°; $\lambda_{\max}^{\text{alc.}}$ 239 mμ, *E* 15,600; $[\alpha]^{23}\text{D}$ +250° (*c* 0.854, in chloroform). The infrared spectrum confirmed the presence of three keto groups, one of which was conjugated.

Anal. Calcd. for $C_{21}H_{26}O_4$: C, 73.66; H, 7.66. Found: C, 73.79; H, 7.82.

3. **11α-Hydroxy-16,17-oxidoprogesterone-3,20-bis-(ethylene Ketal) (V).**—11α-Hydroxy-16,17-oxidoprogesterone (II) (800 mg.) was dissolved in 90 ml. of dry benzene. To this solution was added 85 mg. of *p*-toluenesulfonic acid and 1 ml. of freshly distilled ethylene glycol. The solution was refluxed for 5 hours. The water was separated by means of a trap. After cooling, the solution was washed three times with 10 ml. of 5% sodium carbonate solution, three times with 10 ml. of water, and dried over sodium sulfate. The benzene solution was chromatographed over 80 g. of Florisil. Ethylene dichloride (two fractions of 140 ml.), ethylene dichloride–acetone 15:1 (three fractions), 12:1 (two fractions), 8:1 and 5:1 (two fractions in each instance) were used as elution solvents.

The fractions eluted by the 15:1 and 12:1 mixtures were combined, dissolved in ethyl acetate, concentrated to a small volume (3 ml.), and diluted with ether (3 ml.) to give 269.0 mg. of crystals, m.p. 178–183°. A sample was recrystallized from ethyl acetate–ether to a constant melting point, 192.5–193.5°; $[\alpha]^{23}\text{D}$ −185° (*c* 0.829, in chloroform). The infrared spectrum was in complete agreement with the proposed structure (V) showing absorption bands at 3548 cm.$^{-1}$ for hydroxyl, at 1673 cm.$^{-1}$ for the 5,6-double bond, and at 1131, 1118, 1092 and 1054 cm.$^{-1}$ for the C–O bonds.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 70.09; H, 8.36.

4. **11α,17α-Dihydroxyprogesterone (VI) from Compound V.**—Compound V (158 mg.) was dissolved in 15 ml. of benzene (dried over sodium). This solution was added dropwise to 100 mg. of lithium aluminum hydride dissolved in 10 mg. of dry ether. After the addition was completed the vigorously stirred solution was refluxed for 0.5 hour. The lithium aluminum hydride was decomposed by adding methanol dropwise. The solution was acidified with 10% sulfuric acid and extracted with ether. The extracts were washed once with 5% sodium carbonate solution and three times with water, dried over sodium sulfate, and concentrated to give 135.5 mg. of a partially crystalline residue. This was dissolved in 15 ml. of methanol and 2 ml. of 10% sulfuric acid. The solution was allowed to stand at room temperature overnight; then it was adjusted with sodium carbonate to *p*H of 8, diluted with water and extracted with chloroform. The chloroform extracts were washed twice with water, dried over sodium sulfate, and concentrated to give 100.0 mg. of an oily residue which crystallized from ethyl acetate solution. Two recrystallizations from ethyl acetate (2 ml.) and ether gave 85 mg. of crystals, m.p. 218–220°. The identity of this compound with 11α,17α-dihydroxyprogesterone was established through the infrared spectra, the mobilities on several papergram systems, $[\alpha]\text{D}$ +74° (in chloroform) and the mixture melting point.

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[5] For the assignment of the double bond to the 5,6-position see: E. Fernholz and H. E. Stavely, Abstracts of the 102nd Meeting of The American Chemical Society, Atlantic City, N. J., September 8–12, 1941, p. 39M; R. Antonucci, S. Bernstein, D. Giancola, M. Heller, R. Lenhard, R. Littell, K. J. Sax and J. H. Williams, Abstracts of the 4th Meeting-in-miniature of the New York Section of The American Chemical Society, New York, N. Y., 1952, p. 36; G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

[6] H. C. Murray and D. H. Peterson, U. S. Patent 2,602,769 (July 8, 1952); J. Fried, R. W. Thoma, J. R. Gerke, J. E. Herz, M. N. Donin and D. Perlman, THIS JOURNAL, **74**, 3962 (1952); P. D. Meister, *et al.*, *ibid.*, **75**, 416 (1953). For a laborious chemical preparation of this compound see: J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, *ibid.*, **75**, 1277 (1953).

[7] All melting points were taken on a Fisher–Johns block and are uncorrected.