genated at room temperature. The hydrogen absorption ceased after an uptake of 1 mole. Dihydroxy-5-pregnen-20-one (18.6 mg.) crystallized as prisms from ether; m.p. 178–182°; $\lambda_{max}^{CHCl_3}$ 2.77 and 2.89 (OH) and 5.86 μ (20-ketone).

A portion (16.2 mg.) of the reduced compound was submitted to Oppenauer oxidation with 5 ml. of toluene and 0.3 ml. of cyclohexanone. After removal of 3 ml. of toluene by distillation, 14 mg. of freshly distilled aluminum isopropoxide was added to the solution and refluxed for 30 min. The reaction mixture was then acidified with dilute hydrochloric acid and extracted with ether. The crude residue from the ethereal extract was submitted to paper chromatography (40% propylene glycol and toluene saturated with propylene glycol). After 4 hr. of development the compound was eluted by the usual preparative paper chromatographic technique after detection by the ultraviolet scanner (2537 Å.). In this manner 4.7 mg. of 9α-hydroxyprogesterone, which crystallized from methanol with a m.p. of 165–168°, was isolated. The properties (infrared spectrum, melting point and mixture melting point) of this compound agreed with an authentic sample.

7-Oxosolasodine (XII).—One hundred milligrams of compound IV was dissolved in 10 ml. of chloroform and treated with 1 g. of active manganese dioxide. The slurry was stirred for 8 hr., at the end of which the mixture was analyzed by thin layer chromatography (chloroform–methanol–water, 8:2:0.2 v./v.) and found to consist of approximately 80% oxidized product and 20% starting material. The manganese oxide was filtered off and the filtrate evaporated to dryness. The residue (64 mg.) was chromatographed on alumina (Woelm neutral, activity III) and the 20–30% ether in benzene eluates collected. Upon crystallization from ethyl acetate, plates (36 mg.) of m.p. 198–201 were obtained. An analytical sample melted at 200–203.5°; $[\alpha]^{20}D$ −154.5° (chloroform); $\lambda_{max}^{C_2H_5OH}$ 238 mμ (log ϵ 3.85); $\lambda_{max}^{CHCl_3}$ 2.74, 2.79 and 2.94 (OH), 5.99, 6.12 and 6.23 μ (Δ^5-7-ketone).

Anal. Calcd. for $C_{27}H_{41}O_3N$: C, 75.83; H, 9.66. Found: C, 76.09; H, 9.93.

Microbiological Transformation of Diosgenin[1]

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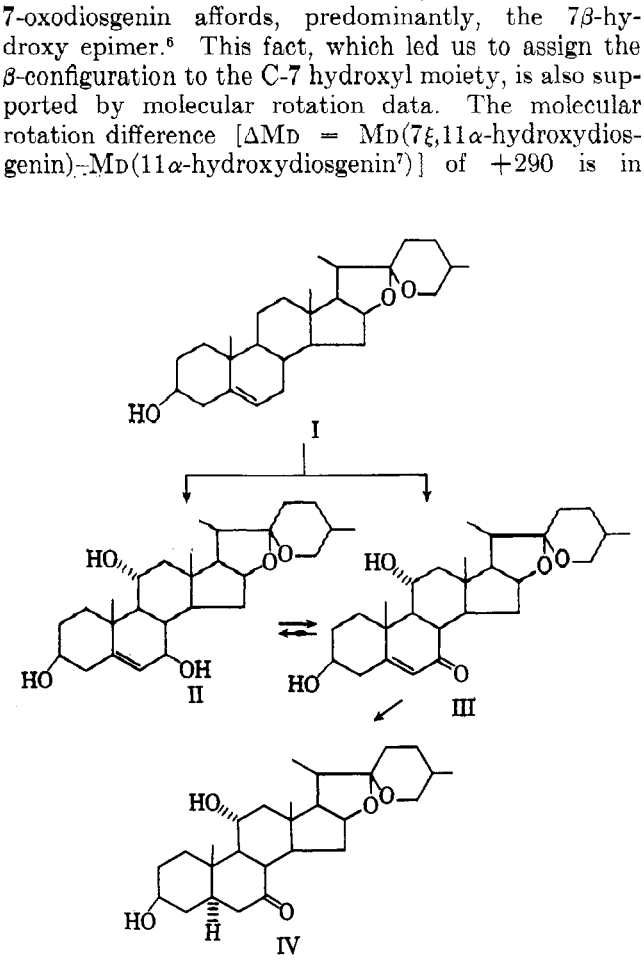
The steroidal sapogenin diosgenin has been hydroxylated by the fungus *Helicostylum piriforme* to yield 7β,11α-dihydroxydiosgenin and 11α-hydroxy-7-oxodiosgenin. Proofs for their structure are given.

With the widespread use of microorganisms for transformations of the steroid molecule, most classes of steroids in recent years have succumbed to microbial attack.[3] One of the few which have so far withstood microbiological hydroxylation are the steroidal sapogenins. Mininger, *et al.*,[4] attempted to hydroxylate the commonly occurring sapogenins with a number of microorganisms and concluded that the steroidal sapogenins are not readily hydroxylated.

We now report our successful hydroxylation of the steroidal sapogenin, diosgenin. The incubation of diosgenin (I) with the fungus *Helicostylum piriforme* (A.T.C.C. 8992) resulted in the formation of 7β,11α-dihydroxydiosgenin (II, 10–15%) and 11α-hydroxy-7-oxodiosgenin (III, 5–10%).

The structures of II and III were deduced from the following data. Oxidation of dihydroxydiosgenin, II, with manganese dioxide in chloroform at room temperature readily converted II into an α,β-unsaturated carbonyl derivative, identical in properties with compound III which had been isolated directly from the fermentation of diosgenin. The hydroxyoxo derivative, III, in turn, upon catalytic reduction (palladium on charcoal, acetic acid) afforded the 5,6-dihydro derivative, 11α-hydroxy-7-oxotigogenin (IV) whose properties were in agreement with an authentic specimen of 22-isoallospirostan-3β,11α-diol-7-one.[5] Compound III, furthermore, was easily reduced to the original dihydroxydiosgenin, II, in preponderant amounts with lithium aluminum hydride. It has been shown that the lithium aluminum hydride reduction of 7-oxodiosgenin affords, predominantly, the 7β-hydroxy epimer.[6] This fact, which led us to assign the β-configuration to the C-7 hydroxyl moiety, is also supported by molecular rotation data. The molecular rotation difference [ΔM_D = M_D(7ξ,11α-hydroxydiosgenin) − M_D(11α-hydroxydiosgenin[7])] of +290 is in



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general agreement with the data of Ringold, *et al.*,[8] for a 7β configuration.

In addition to II and III, a very small amount of a third component which resembled substance IV in its spectral, paper, and gas chromatographic behavior was isolated. However, due to lack of material and difficulties involved in purification, further characterization of the compound has been set aside.

As an extension of these studies we have sought to hydroxylate tigogenin, the 5,6-dihydro derivative of diosgenin. In contrast to our findings with dihydrosolasodine,[9] in the steroidal alkaloid field where some enhancement in hydroxylation is observed, the reduction of the Δ^5 bond to the 5α-dihydro derivative in the steroidal sapogenins apparently suppresses hydroxylation. Several attempts at hydroxylation of tigogenin resulted in the recovery of starting material.

Experimental[10]

Fermentation[11].—The medium was prepared by mixing 20 g. of peptone, 3 g. of corn steep liquor, 50 g. of technical dextrose, and 1000 ml. of tap water and the pH adjusted to 4.5. Two 100-ml. batches of the sterile medium in 500-ml. erlenmeyer flasks were inoculated with *Helicostylum piriforme* (A.T.C.C. 8992) and agitated for 2 days at 28° on a platform shaker. Diosgenin (10 mg.) in 1 ml. of ethanol was then introduced into each flask and shaking continued for 30 hr.

Isolation of 7β,11α-Dihydroxydiosgenin (II), 11α-Hydroxy-7-oxodiosgenin (III), and 11α-Hydroxy-7-oxotigogenin (IV).—The contents of the flasks were filtered through a bed of Celite to remove the mycelium and the filtrate was extracted with chloroform. The extract was evaporated to dryness to give a semicrystalline residue. In a ten-flask run the yield of the residue was about 102 mg. from 100 mg. of diosgenin. It was triturated with acetone to give crude 7β,11α-hydroxydiosgenin (yield, 10–15%), which was chromatographed on Florisil, using the following solvent mixtures: 10, 20, and 30% acetone in chloroform. Each fraction was tested by paper chromatography using 30% propylene glycol as the stationary phase and a mixture of toluene–dioxane (7:3 v./v.) as the mobile phase. The compounds were detected by spraying the paper first with a 1% ethanolic cinnamic aldehyde solution, followed by a saturated solution of antimony trichloride in nitrobenzene and warming over a hot plate. The fractions which gave a green coloration with antimony trichloride (mostly 20% acetone in chloroform eluates) were combined, crystallized from methanol, and then from methanol–ether to afford prisms of 7β,11α-dihydroxydiosgenin (II), m.p. 263–266°; $[\alpha]^{20}D$ −47.0° (C_2H_5OH); λ_{max}^{Nujol} 2.96 μ (OH).

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.55; H, 9.75.

The mother liquors from the crude preparations of dihydroxydiosgenin II were combined, evaporated to dryness, and chromatographed over silica gel with the following solvent mixtures: 10, 20, and 30% acetone in dichloromethane. Each fraction was tested by paper chromatography as described before, and the fractions (mainly from elutions with 20 and 30% acetone in dichloromethane), which gave the same yellow coloration with antimony trichloride and were visible under ultraviolet light, were combined. Upon crystallization from methanol, plates of 11α-hydroxy-7-oxodiosgenin (III, 5–10%), m.p. 221–225°; $[\alpha]^{25}D$ −137.1° (C_2H_5OH); $\lambda_{max}^{C_2H_5OH}$ 239 mμ (ε 4.07); $\lambda_{max}^{CHCl_3}$ 2.78, 2.90 μ (OH), 5.95, 6.10 and 6.21 μ (Δ^5-7-one) were obtained.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.08; H, 8.78.

In the course of the silica gel chromatography, fractions less polar than III which gave a yellow coloration with antimony trichloride but invisible under ultraviolet radiation, were detected. Accordingly, the earlier fractions and the mother liquors of III were combined, evaporated to dryness, and chromatographed on alumina (Woelm neutral, activity I). Elution with 0.5 and 1% methanol in ether gave a small amount of a substance (>0.1%) which crystallized from acetone–pentane and melted at 218–223°. This compound possessed the same retention time (gas–liquid chromatography, 1% SE-30, 225°[12]) and R_f (paper chromatography) as 11α-hydroxy-7-oxotigogenin, but its infrared spectra were not quite identical. Scarcity of material prevented its further purification.

Manganese Dioxide Oxidation of 7β,11α-Dihydroxydiosgenin to 11α-Hydroxy-7-oxodiosgenin (III).—7β,11α-Dihydroxydiosgenin (II, 15.8 mg.) was dissolved in 15 ml. of chloroform with 140 mg. of manganese dioxide[13] and stirred for 6 hr. at room temperature. The precipitate was filtered and the filtrate was evaporated to dryness. The residue, which partially crystallized from acetone, afforded 5 mg. of starting material II. The mother liquor was evaporated to dryness and submitted to preparative paper chromatography using the solvent system described previously. The compound, eluted from the paper chromatogram, crystallized as plates (4.6 mg.) from methanol and melted at 215–220°. A recrystallized sample agreed in properties (melting point, mixture melting point, and infrared spectrum) with a specimen of 11α-hydroxy-7-oxodiosgenin isolated directly from the incubation mixture.

Lithium Aluminum Hydride Reduction of 11α-Hydroxy-7-oxodiosgenin (III) to 7β,11α-Dihydroxydiosgenin (II).—To a solution of 5 ml. of ether, 5 ml. of tetrahydrofuran, and 30 mg. of III was added, dropwise, a solution of 10 mg. of lithium aluminum hydride in 5 ml. of ether. After refluxing the reactants for 2 hr., 1 ml. of acetone, 5 ml. of N-sulfuric acid, and 10 ml. of water were added to the solution and the reaction mixture thoroughly extracted with ether. The extract yielded 30 mg. of a semicrystalline residue, which was recrystallized from methanol, to give 3.1 mg. of prisms, m.p. 258–262°. The alumina chromatography of the mother liquor afforded an additional 6.8 mg. of prisms of m.p. 258–262°. The substance was identical (melting point and infrared spectrum) with a sample of II isolated directly from the incubation mixture.

The Catalytic Reduction of 11α-Hydroxy-7-oxodiosgenin (III) to 11α-Hydroxy-7-oxotigogenin (IV).—A solution of 67 mg. of III in 6 ml. of ethyl acetate was hydrogenated over 50 mg. of 5% palladium on carbon. After 20 min. the uptake of hydrogen (1.04 moles) ceased, and the product was submitted to alumina (Woelm, neutral, activity I) chromatography. Elutions with 1 and 2% methanol in ether yielded 8.6 mg. of needles (from ethyl acetate), m.p. 234–237°; $[\alpha]^{20}D$ −125° ($CHCl_3$); $\lambda_{max}^{CHCl_3}$ 2.75, 2.88 (OH), 5.82 μ (7-ketone). A second crop (12.9 mg.) of needles of m.p. 232–236° also was obtained. The compound proved to be identical in properties (melting point, mixture melting point, and infrared spectrum) with an authentic specimen of 11α-hydroxy-7-oxotigoenin.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.75; H, 9.76.

(8) The authors in ref. 6 list −156 for Δ^5-22-isospirosten-3β,7α-diol and +211 for Δ^5-22-isospirosten-3β,7β-diol.

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(10) Melting points were taken on the Kofler block and are uncorrected. Microanalyses were performed by the Microanalytical Services Unit of this laboratory under the direction of Mr. Harold G. McCann. The infrared spectra were taken on the Model 21 Perkin-Elmer infrared spectrometer by Mr. H. K. Miller and Mrs. A. H. Wright of this laboratory.

(11) We are deeply indebted to Mr. D. L. Rogerson, Jr., of the National Heart Institute for carrying out a large-scale tank fermentation of diosgenin for us.

(12) We thank Dr. H. M. Fales of the National Heart Institute for running gas chromatograms of these substances.

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