Anal. Calcd. for $C_{28}H_{32}PBr$: C, 70.14; H, 6.73. Found: C, 69.85; H, 6.70.

(7-Hydroxy-3,7-dimethyl-2-octenyl)triphenylphosphonium Bromide (XXIII).—(3,7-Dimethyl-2,6-octadienyl)triphenylphosphonium bromide (XXII) (479 g.) was placed in water (5 l.) and stirred at reflux temperature for 20 hr. The reaction mixture was allowed to cool overnight to room temperature and the product was extracted with methylene chloride. The solution was concentrated *in vacuo* to a small volume, and the residue was induced to crystallize by the addition of ethyl acetate. After cooling in a refrigerator overnight, the product was filtered off and recrystallized from methylene chloride and ethyl acetate. There was obtained 447 g. (90%) of XXIII, m.p. 194°.

Anal. Calcd. for $C_{28}H_{34}OPBr$: C, 67.60; H, 6.89. Found: C, 67.84; H, 6.84.

1,1'-Dihydroxy-1,2,1',2'-tetrahydrolycopene XXV.—By the same procedure described for the preparation of XX, there was obtained from XXIII (1091 g.) and crocetin dialdehyde (VIII) (296 g.), 315 g. (55%) of XXV, m.p. 190°, after recrystallization from pyridine. The absorption spectrum had maxima at 515, 482 and 456 mμ (in chloroform); 503, 470, and 444 mμ (in petroleum ether); $E^{1\%}_{1\,cm}$ (470 mμ) 3150.

Anal. Calcd. for $C_{40}H_{60}O_2$: C, 83.86; H, 10.56. Found: C, 83.94; H, 10.68.

Dipalmitate XXVI.—1,1'-Dihydroxy-1,2,1',2'-tetrahydrolycopene (XXV) (28.6 g.), pyridine (200 ml.), and palmitoyl chloride (50 ml.) were placed in a 1-l. flask and stirred under an atmosphere of nitrogen at 70–75° for 4 hr. The reaction mixture was diluted with methyl alcohol (500 ml.) and cooled for 24 hr. in a refrigerator. The product, which crystallized as a dark red solid, was filtered off under an atmosphere of nitrogen, and washed successively with water and cold methyl alcohol. After recrystallization from acetone, there was obtained 36.8 g. (70%) of XXVI, m.p. 118°; absorption maxima at 499, 468, and 440 mμ (in petroleum ether); $E^{1\%}_{1\,cm}$ (468 mμ) 1690.

Anal. Calcd. for $C_{72}H_{120}O_4$: C, 82.38; H, 11.52. Found: C, 82.44; H, 11.85.

***trans*-Lycopene (XXVII).**—A mixture of pyridine (50 ml.), phosphorus oxychloride (5.0 ml.), and XXV (2.0 g.) was stirred for 1 hr. under an atmosphere of nitrogen at 90–95°. The cooled reaction mixture was diluted with ethyl alcohol (100 ml.) and stored overnight in a refrigerator. The product was filtered off and recrystallized from benzene, giving 1.2 g. (64%) of lycopene (XXVII), m.p. 171°. The absorption spectrum had maxima at 507 mμ, $E^{1\%}_{1\,cm}$ 3010; 475 mμ, $E^{1\%}_{1\,cm}$ 3370; 447 mμ, $E^{1\%}_{1\,cm}$ 2230.

Acknowledgment.—We wish to thank Dr. A. Steyermark and his staff for the microanalyses and Dr. F. Forrester for the ultraviolet and infrared spectra.

The Microbiological Hydroxylation of Solasodine[1]

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The steroidal alkaloid solasodine has been hydroxylated by the fungus *Helicostylum piriforme* to yield 9α-hydroxysolasodine, 11α-hydroxysolasodine, 7β-hydroxysolasodine, and probably 7ξ,11α-dihydroxysolasodine. The proofs for their structures are discussed.

The microbial hydroxylation of steroids has been a most powerful tool for the introduction of hydroxyl groups into the steroid molecule, and it has been extensively applied to the C_{19} and C_{21} steroids of adrenal and sex hormonal origin.[3,4] More recently the technique has been extended to the cardiac lactones.[5,6]

We wish now to report the successful hydroxylation of the steroidal alkaloid, solasodine,[7] one of the more important members of this family of alkaloids. The incubation of solasodine (I) with the fungus *Helicostylum piriforme* (A.T.C.C. 8992) resulted in the formation of 9α-hydroxysolasodine (IIa), in amounts ranging from 30 to 35%. Two other monohydroxysolasodines, the 11α-hydroxy, IIIa, and the 7β-hydroxy compound IV were isolated in lesser yields (*ca.* 1% each). A smaller amount (*ca.* 0.5%) of a fourth component with analysis for a dihydroxysolasodine V also was isolated and is tentatively ascribed a 7ξ,11α-dihydroxy structure.

The identity of IIa as 9α-hydroxysolasodine was established by its conversion to 9α-hydroxyprogesterone (IX) by utilizing the degradative procedure previously reported from our laboratory.[8] Careful acetylation of IIa afforded the hydroxy O,N-diacetyl derivative IIb, which was submitted to isomerization by brief treatment with glacial acetic acid, and, without isolation of the unsaturated pseudoderivative, oxidized with chromium trioxide in acetic acid and hydrolyzed with potassium hydroxide in *t*-butyl alcohol[9] to afford a dihydroxy-5,16-pregnadien-20-one (VIa). Catalytic reduction (10% palladium on barium sulfate in ethyl acetate) of the 16-dehydroacetyl derivative VIb to the 16,17-dihydro compound VIIIb, and Oppenauer oxidation of the free alcohol VIIIa afforded the crude hydroxyprogesterone, the purified product of which proved to be identical (melting point, mixture melting point, and infrared spectrum) with an authentic specimen of 9α-hydroxyprogesterone (IX).[10]

During the course of these transformations the pregnadiendiolone acetate (VIb) was also converted into 3β,9α-dihydroxy-5α-pregnan-20-one acetate (VII) by catalytic reduction with Adams' catalyst and oxidation of the resulting C-20 hydroxyl moiety with Kiliani's oxidant.[11]

Compound IIIa was established as 11α-hydroxysolasodine by a similar series of transformations which involved acetylation to the O,O,N-triacetyl derivative

(1) A preliminary account of portions of this work was published in *J. Org. Chem.*, **26**, 4181 (1961).

(2) Visiting Scientist (1960–1962), National Institutes of Health.

(3) J. Fried, R. W. Thoma, D. Perlman, J. E. Herz, and A. Bowman, "Recent Progress in Hormone Research," Vol. XI, Academic Press, Inc., New York, N. Y., 1955, p. 149.

(4) T. H. Stoudt, "Advances in Applied Microbiology," Vol. 2, Academic Press, Inc., New York, N. Y., 1960, p. 183.

(5) A. Gubler and Ch. Tamm, *Helv. Chim. Acta*, **41**, 297, 301, 1762 (1958); **42**, 239, 473 (1959).

(6) H. Nawa, M. Uchibayashi, T. Kamiya, T. Yamano, H. Arai, and M. Abe, *Nature*, **184**, 469 (1959).

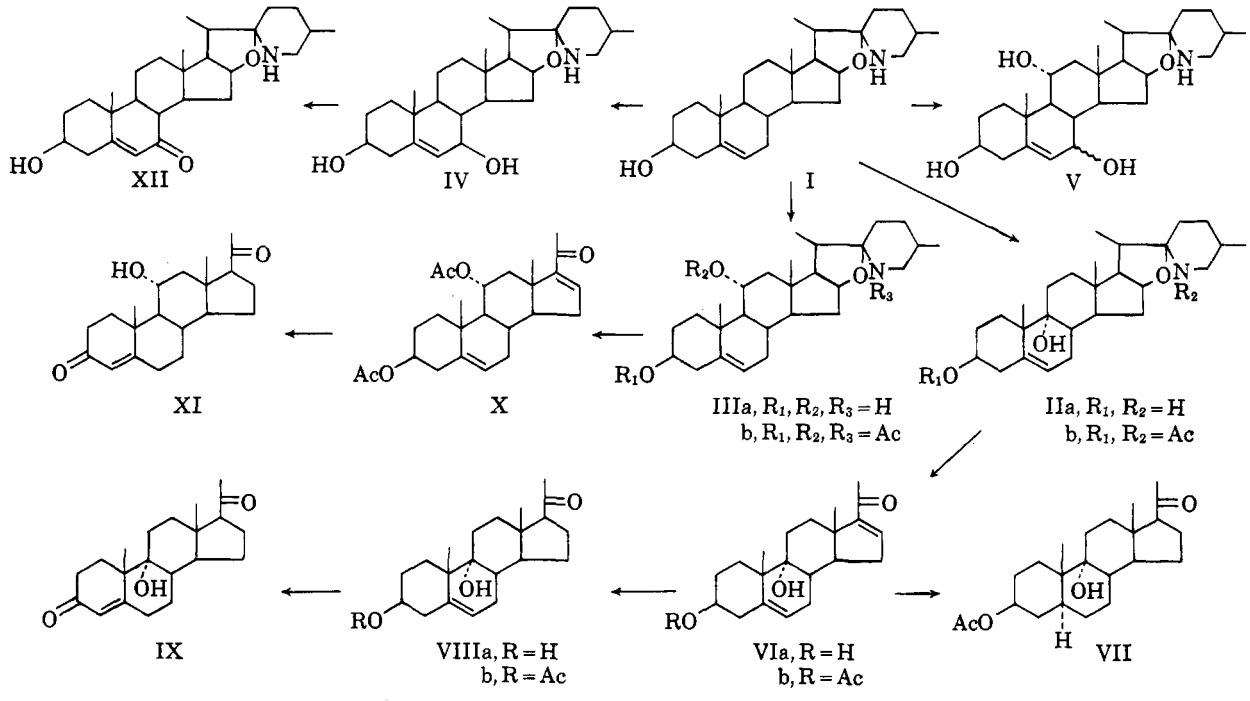
(7) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p. 853.

(8) Y. Sato, N. Ikekawa, and E. Mosettig, *J. Org. Chem.*, **24**, 893 (1959).

(9) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.*, **77**, 5665 (1955).

(10) We are indebted to Dr. J. Fried of the Squibb Institute for Medical Research, New Brunswick, N.J., for an authentic specimen of this compound.

(11) H. Kiliani, *Ber.*, **46**, 676 (1913). A solution of 53 g. of chromium trioxide and 80 g. of concentrated sulfuric acid in 400 g. of water.



IIIb followed by pseudomerization, oxidation, and hydrolysis (in acetic acid) to the diacetate of 3β,11α-dihydroxy-5,16-pregnadien-20-one (X).[12] Saponification of the diacetate X to the free alcohol, and partial reduction (5% palladium on charcoal in ethyl acetate) to the 16,17-dihydro derivative followed by an Oppenauer oxidation resulted in the formation of 11α-hydroxyprogesterone (XI).[13]

The structure of substance IV was deduced from the fact that IV was very readily converted into the α,β-unsaturated carbonyl derivative, 7-oxosolasodine (XII) (λ_{max}^{alc} 238 mμ, log ε 3.85; λ_{max}^{chlf} 5.99, 6.12, 6.23 μ) by allylic oxidation with manganese dioxide in chloroform at room temperature. The β-configuration is proposed on the basis of the molecular rotation data (ΔMD +115).[14]

The sparingly formed dihydroxysolasodine V upon acetylation affords the O,O,O,N-tetraacetyl derivative readily. In view of the fact that the fungus *Helicostylum piriforme* hydroxylates tomatidine (5,6-dihydro-25-epimer of solasodine) to yield 7α,11α-dihydroxytomatidine[1] and diosgenin (oxo analog of solasodine) to form 7β,11α-dihydroxydiosgenin,[15] we tentatively assign the 7ξ,11α-positions to the secondary hydroxyl moieties in the triol V. Lack of material has prevented its further study.

Solasodine in recent years has achieved considerable significance[16] in the production of biologically active steroid hormones. The hydroxylation of solasodine further enhances the usefulness of this alkaloid as a starting material for these purposes.

(12) This compound subsequently was compared with an authentic specimen and proven to be identical. We thank Dr. Otto Halpern of Syntex, S.A., Mexico City, for providing us with an authentic sample.

(13) D. H. Peterson and H. C. Murray, *J. Am. Chem. Soc.*, **74**, 1871 (1952).

(14) ΔMD = MD (7ξ-hydroxysolasodine) − MD (solasodine); S. Lieberman and D. K. Fukushima [*ibid.*, **72**, 5211 (1950)] list ΔMD (7β-OH) = +183 and ΔMD (7α-OH) = −194.

(15) S. Hayakawa and Y. Sato, *J. Org. Chem.*, **27**, 704 (1962).

(16) See Tagungberichte no. 27, "Chemie und Biochemie der Solanum-Alkaloide," Deutsche Akademie der Landwirtschaftswissenschaften zu Berlin, 1961.

Experimental[17]

The fungus *Helicostylum piriforme* (A.T.C.C. 8992) was cultivated for 48 hr. at 28° in a medium (pH 4.5) consisting of 2% peptone, 0.3% corn steep liquor, and 5% technical dextrose in tap water. In a typical run 40 mg. of solasodine in 2 ml. of ethanol was then introduced into the flask containing 250 ml. of the aforementioned medium and fungus and incubated for another 24 hr. The mycelium was removed by filtration and the filtrate made basic for extraction with chloroform. Thus in a 10-flask run, 400 mg. of solasodine yielded 420 mg. of residue which, when triturated with acetone, gave crude 9α-hydroxysolasodine (yield 30–35%). Repeated crystallizations from methanol, acetone, and finally aqueous ethanol afforded plates of **9α-hydroxysolasodine**; m.p. 214–217°; $[\alpha]^{20}D$ −138° (chloroform); $\lambda_{max}^{CHCl_3}$ 2.81, 2.95 μ (OH and NH).

Anal. Calcd. for $C_{27}H_{43}O_3N$: C, 75.62; H, 10.09. Found: C, 75.62; H, 10.11.

The mother liquors, after removal of the 9α-hydroxysolasodine, were combined, evaporated to dryness, and chromatographed on alumina (Woelm, neutral, activity I) with the following eluents: 0.1, 0.2, 0.3, 0.5, 1, 3, and 5% methanol in ether. Each fraction was tested by paper chromatography in a system consisting of 30% propylene glycol as the stationary phase, and 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, drying, and spraying again with a saturated solution of antimony trichloride in nitrobenzene. Warming on a hot plate at about 100° brings out the color. The fractions which possessed the same mobility and color were pooled. The eluates with 0.2–0.3% methanol in ether gave a reddish violet coloration with antimony trichloride and yielded needles of **11α-hydroxysolasodine** (*ca.* 1%) from acetone–pentane; m.p. 200–203°; $[\alpha]^{20}D$ −110° (chloroform); $\lambda_{max}^{CHCl_3}$ 2.79, 2.93 μ (OH and NH). An analytical specimen, recrystallized from benzene and then from acetone–pentane, melted at 202–204°.

Anal. Calcd. for $C_{27}H_{43}O_3N$: C, 75.48; H, 10.09. Found: C, 75.52; H, 9.98.

Fractions eluted with 0.5–1% methanol in ether gave a violet color with antimony trichloride on the paper chromatogram and proved to be 9α-hydroxysolasodine.

The 1–5% methanol in ether eluates were evaporated to dryness and chromatographed on Florisil with the following solvent mixtures: 5, 10, 20, 30, 40, and 50% acetone in chloroform and straight acetone. The 10–50% acetone in chloroform eluates,

(17) 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. Anne H. Wright of this laboratory.

which gave a green coloration with antimony trichloride, afforded slightly impure **7β-hydroxysolasodine**. Further purification was achieved by repeated silica gel chromatography (20% acetone in chloroform) until only one spot was exhibited by thin layer chromatography (silica gel G, heptane–pyridine–ethyl acetate, 5:3:2 v./v.). Crystallization from ethyl acetate yielded crystals (yield, 1%) of m.p. 244–247° dec.; $[\alpha]^{20}D$ −109.4° (chloroform).

Anal. Calcd. for $C_{27}H_{43}O_3N$: C, 75.48; H, 10.09. Found: C, 75.78; H, 9.89.

The more polar fractions from the previous purification, which gave a blue spot on the paper chromatogram with antimony trichloride, were collected and subjected to silica gel chromatography. The fractions, eluted with acetone, were crystallized from methanol several times until homogeneity was achieved, as indicated by thin layer chromatography (silica gel G, methanol–chloroform–water, 30:70:0.02 v./v.). The **dihydroxysolasodine** (0.5%) obtained in this manner melted at 256–259°; $[\alpha]^{20}D$ −43.1° (chloroform).

Anal. Calcd. for $C_{27}H_{43}O_4N$: C, 72.77; H, 9.73. Found: C, 72.87; H, 9.85.

O,N-Diacetate of 9α-Hydroxysolasodine (IIb).—A solution of 9α-hydroxysolasodine (1.470 g.), 15 ml. of pyridine, and 1.24 ml. of acetic anhydride was refluxed for 1 hr. and poured on ice. The mixture was made basic with aqueous ammonia and saturated with sodium chloride. After 1 hr. the product was collected and chromatographed over alumina (Woelm neutral, activity III). The benzene and 5% ether in benzene eluate yielded the diacetate (715 mg.) which crystallized as rods from methanol; m.p. 196–198°; $[\alpha]^{20}D$ −75.3° (chloroform); $\lambda_{max}^{CS_2}$ 2.82 (OH), 5.77 (OAc), 6.03 μ (N–Ac).

Anal. Calcd. for $C_{31}H_{47}O_5N$: C, 72.48; H, 9.22. Found: C, 72.66; H, 9.42.

3β,9α-Dihydroxy-5,16-pregnadien-20-one (VIa) and Its Acetate (VIb).—The O,N-diacetate IIb (571 mg.) was added in small portions to boiling acetic acid (10 ml.) and refluxed for 15 min. After cooling and addition of 20 ml. of acetic acid, the solution was oxidized with dropwise addition of 226 mg. of chromic anhydride in 15 ml. of 80% aqueous acetic acid. During the addition of the oxidant the mixture was cooled (15°) and after the addition allowed to stand at room temperature for 1 hr. with stirring. Water and a small amount of sodium sulfite was added, and the mixture was saturated with sodium chloride for extraction with ether. The crude dried residue from the ethereal extract was solvolyzed for 2 hr. in a mixture of 2.5 g. potassium hydroxide, 22.5 ml. of *t*-butyl alcohol, and 2.5 ml. of water while stirring. The chloroform extract of the hydrolysis yielded a semicrystalline diene which was chromatographed on Florisil. The eluates from 5% acetone in chloroform gave 164 mg. of plates, m.p. 225–228°. Thin layer chromatography (silica gel G, cyclohexane–ethyl acetate, 1:1 v./v.) indicated, however, a trace impurity of less polar nature still present in the compound. It was, therefore, reacetylated and submitted to alumina chromatography. The hexane and benzene eluates yielded needles from acetone–pentane which melted at 181–183°; $[\alpha]^{20}D$ −70.3° (chloroform); $\lambda_{max}^{CS_2}$ 2.82 (OH), 5.76 (OAc), 5.98 μ (α,β-unsaturated carbonyl).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 74.27; H, 8.81.

A sample (21.7 mg.) of the acetate VIb was stirred with 0.2 ml. of 50% potassium hydroxide solution in 2 ml. of *t*-butyl alcohol for 140 min. at room temperature. After addition of water and neutralization with dilute hydrochloric acid, the mixture was extracted with chloroform. It afforded plates from acetone–pentane which melted at 227–229°; $[\alpha]^{20}D$ −70.5° (chloroform); $\lambda_{max}^{CHCl_3}$ 2.82, 2.92 (OH), 6.01, 6.29 μ (Δ16-20-one).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.64; H, 9.08.

3β,9α-Dihydroxy-5α-pregnan-20-one Acetate (VII).—Eighty-eight milligrams of the diene acetate VIb was dissolved in 8 ml. of acetic acid with 71 mg. of Adams' platinum oxide catalyst and hydrogenated at room temperature and atmospheric pressure. After 1 hr., 3 moles of hydrogen were absorbed. The reduction product was dissolved in 8 ml. of acetone and oxidized with Kiliani's reagent.[11] After 10 min. the reaction mixture was diluted with water and the resulting precipitate was filtered, washed with water, and dried. The benzene and ether eluates from the alumina chromatography (Woelm neutral, activity I) of the oxidation product, upon crystallization from acetone, yielded 25 mg. of prisms; m.p. 190–192.5°; $[\alpha]^{20}D$ +63.2° (chloroform); $\lambda_{max}^{CS_2}$ 2.78, 2.83 (OH), 5.76 (OAc), 5.85 μ (20-ketone).

Anal. Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.52; H, 9.39.

3β,9α-Dihydroxy-5-pregnen-20-one (VIIIa) and Acetate (VIIIb).—The diene acetate VIb (80.6 mg.) was dissolved in 5 ml. of ethyl acetate with 68.5 mg. of 10% palladium–barium sulfate catalyst and hydrogenated at room temperature for 30 min., at which time the uptake of hydrogen ceased. When the reduced product was crystallized from methanol–ether and then from benzene, 65.8 mg. of prisms [m.p. 227–229°; $[\alpha]^{20}D$ −20.7° (chloroform); $\lambda_{max}^{CHCl_3}$ 2.82 (OH), 5.78 (OAc), 5.88 μ (20-ketone)] were obtained.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 74.05; H, 9.39.

The acetate VIIIb (222.4 mg.) was dissolved in a mixture of 2 ml. of 50% potassium hydroxide and 20 ml. of *t*-butyl alcohol and stirred for 40 min. at about 40°. During the hydrolysis the rate was checked at 10 min. intervals by thin layer chromatography (silica gel G, acetic acid–toluene–water, 50:50:1 v./v.). The results indicated that the hydrolysis was complete in 30 min. The chloroform extract of the neutralized hydrolysis mixture, upon crystallization from acetone and then methanol–ether, yielded 122 mg. of plates; m.p. 202–204°; $[\alpha]^{20}D$ −15.6° (chloroform); $\lambda_{max}^{CHCl_3}$ 2.80, 2.92 (OH), 5.88 μ (20-ketone). A second crop (49 mg.) of m.p. 194–200° was obtained from the mother liquor.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 76.06; H, 9.70.

9α-Hydroxyprogesterone (IX).—Hydroxypregnenolone (VIIIa, 70 mg.) was dissolved in a mixture of 1.2 ml. of cyclohexanone and 15 ml. of toluene, and then, to ensure dryness, 5 ml. of toluene was distilled. Freshly distilled aluminum isopropoxide (78.6 mg.) was added to the mixture and refluxed for 15 min. Thin layer chromatography (silica gel G, ethyl acetate saturated with water) indicated that the oxidation was complete in 10 min. The reaction mixture was diluted with water, acidified with dilute hydrochloric acid, and extracted with chloroform. The crude extract was submitted to silica gel chromatography and the 2% acetone in chloroform eluate crystallized from acetone–pentane. Plates (27.7 mg.) of m.p. 188–190.5°, which agreed in properties with an authentic specimen of 9α-hydroxyprogesterone, were obtained.

11α-Hydroxysolasodine Triacetate (IIIb).—11α-Hydroxysolasodine (301 mg.) was treated with 2.8 ml. of pyridine and 0.37 ml. of acetic anhydride and refluxed for 1 hr. Following the crystallization from methanol–water and from acetone–pentane, the product was submitted to alumina chromatography (Woelm, neutral, activity I). The ether eluate when crystallized from acetone–pentane gave 114.8 mg. of needles; m.p. 197–200°; $[\alpha]^{20}D$ −50.8° (chloroform); $\lambda_{max}^{CS_2}$ 5.78 (OAc) and 6.03 μ (NAc). The mother liquor yielded a second crop (32.2 mg.) of needles, m.p. 194–198°.

Anal. Calcd. for $C_{33}H_{49}O_6N$: C, 71.32; H, 8.89. Found: C, 71.05; H, 8.66.

3β,11α-Dihydroxy-5,16-pregnadien-20-one Diacetate (X).—The triacetate IIIb (128 mg.) was isomerized in 5 ml. of acetic acid and oxidized with 50 mg. of chromium trioxide in 2.5 ml. of 80% acetic acid. The product was worked up in the manner described for VIa. However, the hydrolytic removal of the side chain was effected by refluxing in 13 ml. of acetic acid for 2 hr. After removal of the acetic acid *in vacuo*, the crude substance was chromatographed over alumina (Woelm neutral, activity III) and the fractions eluted with 20% and 50% benzene in pentane and pure benzene were collected. The substance (41 mg.) crystallized as rods from acetone–pentane; m.p. 144–147°; $[\alpha]^{20}D$ −66.8° (chloroform); $\lambda_{max}^{CHCl_3}$ 5.80 (OAc), 6.01 and 6.29 μ (Δ16-20-ketone). The compound agreed in properties with an authentic specimen of X.

11α-Hydroxyprogesterone (XI).—A crude batch (118 mg.) of isomerized and oxidized IIIb was hydrolyzed with a mixture of 0.5 g. of potassium hydroxide, 0.5 ml. of water, and 4.5 ml. of *t*-butyl alcohol for 2 hr. with stirring. The crude diendiolone was chromatographed on Florisil and the fractions, eluted with 5–10% acetone in dichloromethane, yielded 25.6 mg. of 3β,11α-dihydroxy-5,16-pregnadien-20-one; m.p. 215–218°; $\lambda_{max}^{CHCl_3}$ 2.78 and 2.91 (OH), 6.00 and 6.28 μ (Δ16-20-ketone).

The foregoing diendiolone (22.2 mg.) was dissolved in 5 ml. of ethyl acetate with 4.5 mg. of 5% palladium–carbon and hydro-

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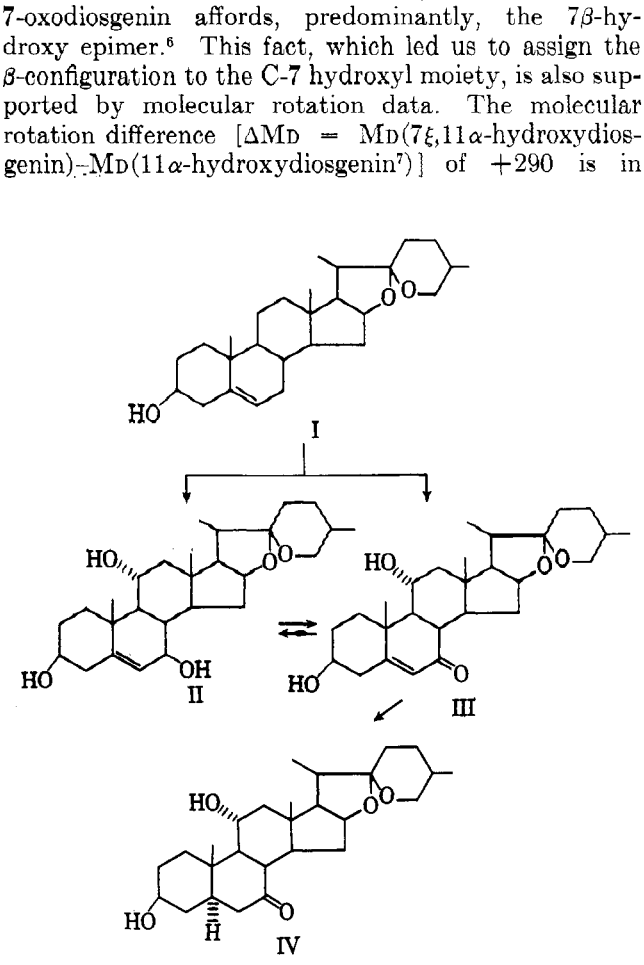
The steroidal sapogenin diosgenin has been hydroxylated by the fungus *Helicostylum piriforme* to yield 7β,11α-dihydroxydiosgenin and 11α-hydroxy-7-oxodiosgenin. Proofs for their structure are given.

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



(1) A preliminary account of this work was published in *J. Org. Chem.*, **27**, 704 (1962).

(2) Visiting Scientist (1960–1962), National Institutes of Health.

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