Further elution of the silica gel column with ether gave after crystallization from acetone–petroleum ether 155 mg. of starting material, m.p. 201–203°.

B.—The 15α-ol VIII (710 mg.) was treated with 750 mg. of chromium trioxide in 30 ml. of pyridine as in the above experiment. The solid resulting from removal of the extracting solvent was chromatographed on silica gel. Elution by ether–benzene (1:1) followed by crystallization from acetone–petroleum ether gave 41 mg. of VI, m.p. 173–174°, ultraviolet and infrared absorption spectra identical to that of the above experiment.

Further elution of the silica gel column with ether gave after recrystallization from acetone–petroleum ether 101 mg. of VIII, m.p. 194–196°.

15α,17α,21-Trihydroxy-4-pregnene-3,20-dione (VIIa).—Six grams of Reichstein's substance S was fermented in the same type of apparatus described for the preparation of Ia using a medium consisting of corn steep liquor, 2%; soy bean meal (Staley extracted soy grits), 1%; dextrin, 1%; potassium hydrogen phosphate, 1%; sodium chloride, 1%; calcium carbonate, 0.01%; *p*H 5.0. An inoculum (400 ml.) of 72-hour old mycelial of *Hormodendrum olivaceum* (ATCC 13596) was used and the mixture was incubated at 21° and aerated with 0.75 l. of sterile air per liter of medium per minute for 72 hours when the substance S in 100 ml. of ethanol was added. Fermentation was continued for 53 hours when paper chromatographic analysis indicated almost complete disappearance of substance S. The work-up was done as in the preparation of Ia. The residue from the methylene chloride extraction was crystallized from acetone–petroleum ether to afford 1.82 g. of VIIa, m.p. 182–188°. Recrystallization from acetone gave a m.p. 227–230°, $[\alpha]^{25}D$ +146° (methanol); λ_{max}^{EtOH} 241 mμ (ε 16,400); ν_{max}^{KBr} 3472, 1715, 1664, 1626, 1109, 1044 cm.$^{-1}$.

Anal. Calcd. for $C_{21}H_{30}O_5$ (362.45): C, 69.58; H, 8.34. Found: C, 69.70; H, 8.34.

15α,21-Diacetoxy-17α-hydroxy-4-pregnene-3,20-dione (VIIb).—Acetylation of 100 mg. of VIIa in 6 ml. of pyridine with 2 ml. of acetic anhydride gave after recrystallization from acetone–petroleum ether 70 mg. of VIIb, m.p. 199.5–200.5°, $[\alpha]^{25}D$ +132° (chloroform), λ_{max}^{EtOH} 240 mμ (ε 16,800); ν_{max}^{KBr} 3320, 1745, 1655, 1631 (shoulder) and 1240 cm.$^{-1}$.

Anal. Calcd. for $C_{25}H_{34}O_7$ (446.52): C, 67.24; H, 7.68; OAc, 19.3. Found: C, 67.50; H, 7.84; OAc, 19.0.

15α-Hydroxy-4-androstene-3,17-dione (VIII).—A mixture of 250 mg. of VIIa was stirred for 20 minutes with 4.0 g. of sodium bismuthate and 25 ml. of glacial acetic acid. The mixture then was worked up as in the preparation of V. The final residue was slurried with cold ether to give 28 mg. of VIII, m.p. 179–181°. Recrystallization from acetone–petroleum ether gave a m.p. of 194–196°, $[\alpha]^{24}D$ +206° (methanol), λ_{max}^{EtOH} 240 mμ (ε 16,600); ν_{max}^{KBr} 3545, 1735, 1675 and 1623 cm.$^{-1}$.

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.63; H, 8.69.

PEARL RIVER, N. Y.

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins LVII. 11α-Hydroxylation of Tigogenin Derivatives[2]

BY HAROLD E. KENNEY, SAMUEL SEROTA, ELMER A. WEAVER AND MONROE E. WALL

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Tigogenin (I) was converted to the corresponding 16-dehydro-20-ketopregnene (II) which in turn gave the 16α,17α-epoxide III and thence the 3-ketone IV. Microbiological hydroxylation of IV gave the 11α-hydroxy steroid V which in three steps gave 17α-hydroxy-5α-pregnane-3,11,20-trione (VIII).

This Laboratory has been engaged in the investigation of sources for a variety of steroidal sapogenins[3a,b,c,d] and in studies of the utilization of these compounds and their derivatives. In contrast to diosgenin which is the source for a variety of important steroidal hormones,[4] little work has appeared since Marker's classical researches[5] on the use of the saturated 5β- or 5α-sapogenins for hormonal intermediates. In a previous paper we have discussed the preparation of 11-oxygenated-5β-pregnanes derived from sarsasapogenin or smilagenin.[6] The present report deals with the preparation of 11-oxygenated 5α-derivatives derived from tigogenin. This sapogenin is available in particularly high quantity and purity from *Yucca peninsularis*.

The starting point for the various preparations was 3β-hydroxy-5α-16-pregnen-20-one (II) available from tigogenin (I) *via* the standard side chain degradation procedure used at this Laboratory.[7]

Epoxidation with alkaline hydrogen peroxide[8] gave 16α,17α-epoxy-3β-hydroxy-5α-pregnan-20-one (IIIa), a small portion of which was characterized as the known 3β-acetate IIIb.[9] The major portion of III without purification was oxidized with chromium trioxide–pyridine reagent[10] to give 16α,17α-epoxy-5α-pregnane-3,20-dione (IV), a compound for which surprisingly we could not find a literature reference. The structure of IV is established from its method of preparation, infrared spectrum, analysis and microbial hydroxylation to the known 16α,17α-epoxy-11α-hydroxy-5α-pregnane-3,20-dione (V).[11] The reaction sequence II → IV proceeded in high yield giving better than 80% IV under non-isolation conditions.

Microbiological hydroxylation of IV to the 11α-hydroxy compound Va was carried out with *Rhizopus nigricans* or *Aspergillus ochraceus*. Both of these organisms have been widely used for the 11α-hydroxylation of a number of steroids.[12]

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture. Article not copyrighted.

(2) Previous paper in this series, Steroidal Sapogenins, LVI, S. G. Levine and M. E. Wall, THIS JOURNAL, **82**, 1444 (1960).

(3) (a) M. E. Wall, *et al.*, *J. Am. Pharm. Assoc., Sci. Ed.*, **43**, 1 (1954); (b) **43**, 503 (1954); (c) **44**, 438 (1955); (d) **46**, 653 (1957).

(4) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publ. Corp., New York, N. Y., 1959, p. 661.

(5) R. E. Marker, *et al.*, THIS JOURNAL, **69**, 2167 (1947).

(6) H. E. Kenney, E. A. Weaver and M. E. Wall, *ibid.*, **80**, 5568 (1958).

(7) M. E. Wall, H. E. Kenney and E. S. Rothman, *ibid.*, **77**, 5665 (1955).

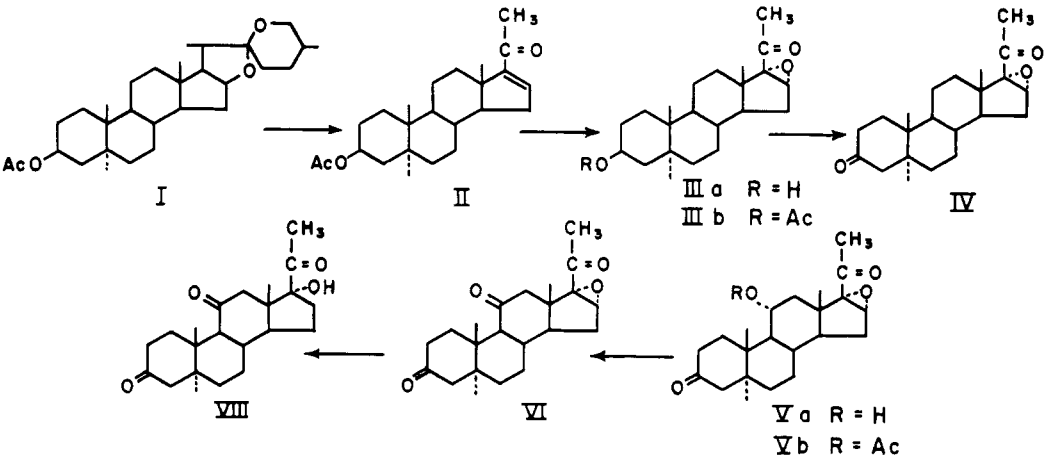
(8) P. L. Julian, *et al.*, *ibid.*, **71**, 756, 3574 (1949); **72**, 5145 (1950).

(9) R. E. Marker, E. M. Jones and E. L. Wittbecker, *ibid.*, **64**, 468 (1942).

(10) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(11) J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, *ibid.*, **75**, 1277 (1953).

(12) For a comprehensive recent review of this subject *cf.* S. H. Eppstein, P. D. Meister, H. C. Murray and D. H. Peterson, *Vitamins and Hormones*, **14**, 359 (1956).



In connection with the microbiological hydroxylation of IV there are several points which should be noted. For successful hydroxylation it was necessary to keep the stock cultures in active vigorous condition by repeated transfers on a 5% malt medium in a stationary bottle provided with a closure to give good aeration.[13] All transformation reactions were carried out using vegetative growths produced by submerged culture on a rotary shaker. Growths which took longer than 24 hours to reach a saturated cell population were considered unsatisfactory. Hydroxylation of the steroid then was conducted by a procedure which we term "dilution culture." In this method, an aliquot of the culture is diluted with several volumes of distilled water, and, after addition of the substrate (IV), incubation is continued. The dilution method has several advantages, particularly for small scale work. Among other things it has been found that foaming problems disappear and the extraction and purification of the hydroxylated substrate is greatly facilitated.

Using *Rhizopus nigricans* the conversion of IV to Va took place in 60% yield. With *Aspergillus ochraceus*, yields were lower due in part to formation of polyhydroxylated steroids. Acylation of Va in pyridine–acetic anhydride gave the known 11α-acetate Vb.[11] Oxidation with chromium trioxide in acetic acid at low temperature converted V to the trione, 16α,17α-epoxy-5α-pregnane-3,11,20-trione (VI) which apparently has not been previously prepared. Proof of structure of VI is based on the method of preparation, analytical and physical constants in agreement with the proposed structure and formation of a dioxime, VII, with one unreactive carbonyl group. In addition, treatment of VI with hydrobromic acid in glacial acetic acid followed by hydrogenation of the crude bromohydrin in the presence of a palladium–calcium carbonate catalyst gave the known 17α-hydroxy-5α-pregnane-3,11,20-trione (VIII).[14] Lack of material has prevented us from working on the further elaboration of VI or VIII to cortical hormones, although a variety of attractive routes are currently available.[4]

(13) E. A. Weaver, T. C. Cordon and H. J. John, *Mycologia*, **15**, 307 (1953).

(14) A. S. Meyer, O. G. Rodgers and G. Pincus, *Acta Endocrinol.*, **16**, 293 (1954).

Experimental[15]

16α,17α-Epoxy-3β-hydroxy-5α-pregnane-20-one, (IIIa).—A solution of 30.0 g. of 3β-hydroxy-5α-16-pregnen-20-one (II)[6] in 2400 ml. of methanol was cooled to 5°. To this solution was added successively 72 ml. of a 4 *N* sodium hydroxide solution and 136 ml. of 30% hydrogen peroxide.[7] The mixture was allowed to stand 23 hours at 5° and then slightly acidified with 18 ml. of glacial acetic acid. Four volumes of water was added and the precipitate filtered, washed with hot water and then recrystallized from methanol to give 27.7 g. of IIIa (90%), m.p. 175–180° (lit.[8a,b] gives m.p. 181–182°). Compound IIIa was further characterized as the 3β-acetate IIIb, square crystals from acetone, m.p. 190–191° (lit.[8a,b] gives m.p. 186–187°). The infrared spectra of both IIIa and IIIb show a strong band at 1710 cm.$^{-1}$ (20-ketone) and bands near 900 and 850 cm.$^{-1}$ which may be due to the 16α,17α-oxide moiety.

16α,17α-Epoxy-5α-pregnane-3,20-dione (IV).—Compound IIIa, 10.0 g. in 100 ml. of pyridine was treated with a suspension of chromium trioxide prepared by sifting *slowly* 10.0 g. of chromium trioxide into 100 ml. of pyridine at 5°.[10] The mixture was allowed to stand overnight at room temperature, and then transferred to a separatory funnel with ether and water. Dilute hydrochloric acid and sodium bisulfite were successively shaken with the ethereal suspension until all the chromium was reduced. Several extractions with ether followed by the usual work-up gave 9.5 g. of IV in good purity, m.p. 208–210° (yield 95%). The analytical sample was obtained as plates from ether; m.p. 210–211°, $[\alpha]^{25}D$ +87.5°; infrared spectrum shows absence of hydroxyl bands, a strong band at 1712 cm.$^{-1}$ (3,20-carbonyl) and bands near 900 and 850 cm.$^{-1}$ (16,17-epoxide).

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.09; H, 9.43. Found: C, 75.98, H, 9.01.

16α,17α-Epoxy-11α-hydroxy-5α-pregnane-3,20-dione (Va).—A four to six day old growth of *Rhizopus nigricans* Ehrb (A.T.C.C. 6227b), grown at 28° as previously described, was used as a source of inoculum. The air-permeable cap on the culture bottle was replaced with a sterile solid cap and the bottle shaken by hand for about 5 minutes to dislodge the spores and to disperse the mycelial mat. This growth was used as the inoculum on a 2% volume to volume basis. An abundant mycelial growth was produced in 18 hours by submerged culture in the medium described in reference 12, page 422. A 50-ml. volume of this abundant growth was added to a 4-liter serum bottle containing 450 ml. of distilled water. A 0.2-g. sample of compound IV in 20 ml. of propylene glycol was next added to the mixture with agitation. The medium was aerated at 0.1 liter per minute for 24 hours on a rotary shaker having a 2-inch stroke and a speed of 220 r.p.m. Ambient air temperature was 28° during the incubation period.

(15) Unless stated otherwise, infrared spectra were measured in carbon bisulfide solution, concentration 10 g./liter; optical rotations in chloroform solutions in 2-dm. tubes at concentrations of approximately 20 mg./ml. We wish to thank R. B. Kelly for carbon and hydrogen analyses.

The final *p*H was 4.10. The entire ferment was extracted seven times with one-quarter volumes of chloroform to give 0.310 g. of crude product. Elution from a Florisil column with benzene gave 0.12 g. of crystalline product, m.p. 200–203° (yield 60%). Recrystallization from benzene gave a product with m.p. 205–207°, $[\alpha]^{25}D$ + 49.0 (lit.[11] gives m.p. 201–203°, $[\alpha]D$ +49.0°), infrared spectrum shows a band at 3600 cm.$^{-1}$ (hydroxyl), strong band at 1712 cm.$^{-1}$ (3,20-carbonyl) and bands near 900 and 850 cm.$^{-1}$ (epoxide).

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.52; H, 8.39.

16α,17α - Epoxy - 11α - acetoxy - 5α - pregnane - 3,20 - dione (Vb).—Acetylation of Va with acetic anhydride in pyridine in the usual manner at room temperature gave Vb, crystals from methanol, m.p. 169–170.5°, $[\alpha]^{25}D$ +12.5 (lit.[11] gives m.p. 170–172°, $[\alpha]D$ +32°), infrared spectrum shows absence of hydroxyl, strong band at 1735 cm.$^{-1}$ (acetate), strong bands at 1712 cm.$^{-1}$ (3,20-carbonyl) and bands near 900 and 850 cm.$^{-1}$ (epoxide).

16α,17α - Epoxy - 5α - pregnane - 3,11,20 - trione (VI).—A solution of 0.2 g. of compound V in 5 ml. of glacial acetic acid was cooled to 14° and treated with a precooled solution of 0.1 g. of chromium trioxide in 2 ml. of 50% glacial acetic acid, which was added dropwise to the solution of V. The solution was allowed to come to room temperature over a period of 10 minutes, six volumes of water added and the resultant silvery plates filtered; yield 0.17 g., m.p. 180–190°. Crystallization from acetone gave the analytical sample, plates, m.p. 189–190.5°, $[\alpha]^{25}D$ +125°; infrared spectrum shows absence of hydroxyl bands, a broad strong band at 1710 cm.$^{-1}$ (3,11,20-carbonyl) and bands near 900 and 850 cm.$^{-1}$ (epoxide).

16α,17α - Epoxy - 5α - 3,11,20 - trione 3,20 - Dioxime (VII).—A solution of 0.1 g. of compound VI was prepared in 0.36 ml. of pyridine. To this solution were added 1.56 ml. of 95% ethanol and 0.1 g. of hydroxylamine hydrochloride. The solution was refluxed one hour. After dilution with water the product was given an ethereal extraction. The residue from ether was not crystalline.

Anal. Calcd. for $C_{21}H_{30}O_4N_2$: N, 7.48. Found: N, 7.54.

17α - Hydroxy - 5α - pregnane - 3,11,20 - trione (VIII).—A solution of 0.16 g. of the triketo-epoxide VI in 7.75 ml. of glacial acetic acid was cooled to 15°. To this solution was added 3.25 ml. of a solution prepared by adding 1 ml. of 48% hydrobromic acid to 5 ml. of glacial acetic acid. The mixture was allowed to stand at room temperature overnight. The solvent then was removed *in vacuo* at room temperature. The residue of crude bromohydrin was without purification dissolved in 25 ml. of 90% methanol and catalytically hydrogenated at 4 atmospheres pressure in the presence of 0.1 g. of a 10% palladium–calcium carbonate catalyst for 5 hours. Filtration of the catalyst, removal of the solvent *in vacuo*, and recrystallization from methanol gave plates, m.p. 262–263°, $[\alpha]^{25}D$ +60° (lit.[14] gives m.p. 259–262°, $[\alpha]D$ +44°); infrared spectrum (potassium bromide disk) shows a strong, bonded band at 3370 cm.$^{-1}$ (17-hydroxyl), a strong, broad band 1705–1690 cm.$^{-1}$ (3,11,20-carbonyl) and absence of the 900 and 850 cm.$^{-1}$ doublet.

[CONTRIBUTION FROM THE CHEMICAL RESEARCH AND DEVELOPMENT DIVISION OF THE SCHERING CORP.]

Synthesis of 17α-Halo Analogs of Corticoids. 17α-Chloro- and 17α-Fluoro-4-pregnen-21-ol-3,11,20-trione Acetates

BY HERSHEL L. HERZOG, MARGARET JEVNIK GENTLES, HELEN M. MARSHALL AND E. B. HERSHBERG

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17α-Bromopregnan-3α-ol-11,20-dione acetate has been transformed into 17α-chloro-4-pregnen-21-ol-3,11,20-trione acetate and 17α-fluoro-4-pregnen-21-ol-3,11,20-trione acetate. The substitution of 17α-halogen for 17α-hydroxyl diminishes adrenocorticoid activity markedly as measured by the eosinophil test in the mouse.

In view of the interesting and therapeutically worthwhile effects which have been achieved sometimes by introduction of halogen, particularly fluorine, into corticosteroids,[1] we chose to study the effect of replacement of the 17α-hydroxyl group in cortisone by halogen. While methods have been described for the introduction of bromine at 17- which were conceivably applicable to chlorine,[2] no satisfactory system has been reported for introducing fluorine at this position. Since fluorine seemed to us the most interesting function, we investigated possible new routes for its introduction at 17-.

A recent Upjohn patent[3] contains a description of the synthesis of what was considered to be a 17,20-epoxy-20-cyano steroid, from the action of potassium cyanide in aqueous ethanol on 17α-bromopregnan-3α-ol-11,20-dione acetate (V). We felt that such a cyanoepoxide would afford any desired 17α-halo-20-ketosteroid by the action of the appropriate halogen acid.[3a] Accordingly, the procedure of the patent was repeated and the isolated products studied.

As it turned out, the reaction of cyanide ion with V is quite complex. Careful chromatography on Florisil of the water-precipitated, neutral fraction from the reaction, run essentially as described by Lincoln and Hogg, afforded four crystalline products of the same empirical formula (I, II, III and IV). All four displayed C–O–C bands, combined or split acetate and 11-ketone carbonyl bands, and nitrile bands in their infrared spectra. In addition, II and IV showed hydroxyl bands, while I and III were hydroxyl-free.

The action of hydrogen bromide in acetic acid on the principal product (I) afforded a crystalline bromohydrin (X), one mole of hydrogen bromide being taken up. The infrared spectrum of X displayed a hydroxyl band in addition to the bands observed initially in I. The stability of X was quite remarkable. Refluxing in acetone in the presence of *p*-toluenesulfonic acid or refluxing in xylene effected no change, X being recovered in

(1) J. Fried and E. F. Sabo, THIS JOURNAL, **79**, 1130 (1957); J. E. Herz, J. Fried, P. Grabowich and E. F. Sabo, *ibid.*, **78**, 4814 (1956); A. Bowers and H. J. Ringold, *ibid.*, **80**, 4423 (1958).

(2) Ch. R. Engel and H. Jahnke, *Can. J. Biochem. Physiol.*, **35**, 1047 (1957). Prof. Engel has kindly advised us that he and Dr. R. Deghenghi have independently carried out the synthesis of 17α-bromo- and 17α-chloro-4-pregnen-21-ol-3,11,20-trione 21-acetate.

(3) F. H. Lincoln and J. A. Hogg, U. S. Patent 2,813,860 (Nov. 19, 1957).

(3a) ADDED IN PROOF.—Professor Gilbert Stork has kindly informed us that he and his collaborators have described a general synthesis of α-haloketones from cyanoepoxides; G. Stork, W. S. Worrall and J. J. Pappas, THIS JOURNAL, in press.