Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.32; H, 10.88.

A mixed melting point determination of XIa with the lower melting polymorphic form of dihydrocholegenin, m.p. 155–157°, gave a m.p. of 148–153° accompanied by resolidification and remelting at 173–178°. Their infrared spectra were completely different.

The triacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as a colorless oil, $[\alpha]^{20}D$ +23°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.67; H, 9.35. Found: C, 70.49; H, 9.39.

16,22-Epoxycoprostane-3β,26-27-triol (epimer of XIa) was obtained as needles from ether–petroleum ether, m.p. 155–157°, $[\alpha]^{20}D$ +2°.

Anal. Calcd. for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.72; H, 10.79.

The triacetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as needles from dilute methanol, m.p. 93–95°, $[\alpha]^{20}D$ +3°.

Anal. Calcd. for $C_{33}H_{52}O_7$: C, 70.67; H, 9.35. Found: C, 70.56; H, 9.45.

16,22-Epoxycoprostan-3α-ol (XII). a. From XIb.—A mixture of 100 mg. of 16,22-epoxycoprostane-3α,26,27-triol (XIa), 5 ml. of pyridine and 160 mg. of *p*-toluenesulfonyl chloride was allowed to stand overnight at room temperature. The light yellow solution was poured into ice and water, and the oily precipitate was extracted with ether. The ethereal solution was washed with cold 5% hydrochloric acid, water, 2% sodium bicarbonate solution, water and dried over sodium sulfate. The solution was evaporated *in vacuo* to an oil which was dissolved in 30 ml. of ether and 2 ml. of 1 *M* solution of lithium aluminum hydride in ether was added and mixture was refluxed for 2 hr. The reaction mixture was cooled and after addition of several drops of ethyl acetate, followed by water, it was treated with 10 ml. of 6 *N* hydrochloric acid. The ether layer was separated and washed with 10% bicarbonate solution, water, dried over sodium sulfate and evaporated to dryness *in vacuo*. The oily residue was chromatographed over benzene–petroleum ether (60–70°) 9:1 washed alumina. The fraction eluted with benzene–chloroform 9:1 crystallized from methanol to give 26 mg. of needles, m.p. 143–145°, $[\alpha]^{20}D$ +6°, γ^{CS_2} 3590 cm.$^{-1}$ (hydroxyl). No hydrocarbon was obtained.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.60; H, 11.65.

b. From IIIb.—A mixture of 110 mg. of 16,22-epoxycoprostane-3α,26-diol 3α-acetate-26 tosylate (IIIb), 50 ml. of ether and 3 ml. of 1 *M* solution of lithium aluminum hydride in ether was refluxed for 1.5 hr. The lithium aluminum hydride mixture worked up as in **a** above yielded 80 mg. of XII as needles from dilute methanol, m.p. 146–147.5°, $[\alpha]^{20}D$ +7°, identical with the material obtained from procedure **a** above.

Anal. Calcd. for $C_{27}H_{46}O_2$: C, 80.54; H, 11.52. Found: C, 80.44; H, 11.60.

The acetate (acetic anhydride–pyridine, 18 hr., 25°) was obtained as needles from dilute methanol, m.p. 91–93°, $[\alpha]^{20}D$ +22°.

Anal. Calcd. for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.05; N, 10.88.

Acknowledgments.—Microanalyses are by the Analytical Service Laboratory of this Institute under the direction of Dr. William C. Alford. Infrared spectra were determined by Mr. H. K. Miller of this Laboratory.

BETHESDA 14, MD.

[CONTRIBUTION FROM THE NATURAL PRODUCTS AND INDUSTRIAL MICROBIOLOGY DEPARTMENTS, SCHERING CORPORATION]

Some Substances Derived from Ruscogenin

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RECEIVED MARCH 5, 1959

The conversion of ruscogenin into 1β-hydroxylated pregnane derivatives is described. An analog corresponding to Compound S is found to be identical with a product obtained by microbiological hydroxylation of the latter.

Ruscogenin, a steroidal sapogenin, was isolated from *Ruscus aculeatus* L. by Sannié and Lapin and found to possess the structure of diosgenin plus an additional hydroxyl group.[1a–d] The latter was at first assigned to C-19,[1b–c] thus making the genin an interesting potential starting material for the synthesis of 19-norsteroids, but subsequent work showed the hydroxyl group in question to be located at C-1. Burn, Ellis and Petrow[2] submitted a mixture of ruscogenin and neoruscogenin[3] to Oppenauer oxidation, and isolated a ring A dienone having the 25L stereochemistry. Nussbaum, *et al.*,[4] starting with ruscogenin diacetate proper,[5] found a corresponding oxidation product to be identical with a dienone prepared from diosgenone (25D). Analogous dienones were prepared in the pregnane[2] and androstane[6] series, so that assignment of the hydroxyl group to C-1 became quite certain.[7]

The configuration of the substituent in question was established to be β, as suggested by its rotatory contribution[8] and perhaps on biogenetic grounds.[9] This was proved by the work of the Searle group who found that Δ^5-androstene-1ζ,3β,17β-triol derived from ruscogenin differed from an authentic 1α-isomer, prepared from a 1α,2α-epoxide by reduction with lithium aluminum hydride in the manner of Tamm,[9] but was identical with 1β-

(1) (a) Ch. Sannié and H. Lapin, *Compt. rend.*, **241**, 1498 (1955); (b) H. Lapin and Ch. Sannié, *Bull. soc. chim. France*, **22**, 1552 (1955); (c) Ch. Sannié and H. Lapin, *ibid.*, **22**, 1556 (1955); (d) Ch. Sannié, H. Lapin, F. Eloy and L. Cogolludo Sanchez, *Bull. soc. chim. biol.*, **39**, 301 (1957).

(2) D. Burn, B. Ellis and V. Petrow, *Proc. Chem. Soc.*, **119** (1957).

(3) The 25D and 25L isomers, respectively; see ref. 2 and H. Lapin, *Compt. rend.*, **244**, 3065 (1957).

(4) A. L. Nussbaum, F. E. Carlon, D. Gould, E. P. Oliveto, E. B. Hershberg, M. L. Gilmore and W. Charney, THIS JOURNAL, **79**, 4814 (1957).

(5) Again we wish to thank Dr. Lapin for supplying us with both sapogenins.

(6) W. R. Bonn, F. Colton and R. Pappo, THIS JOURNAL, **79**, 3920 (1957).

(7) Dr. Lapin concurs with this assignment; see his article quoted in ref. 3, and H. Lapin, *Bull. soc. chim. France*, **24**, 1237 (1957).

(8) D. Burn, B. Ellis and V. Petrow, *J. Chem. Soc.*, 795 (1958).

(9) See ref. 6, footnote 11.

isomer derived from a 1-ketone prepared in the same laboratory.[10,11]

Ruscogenin thus shares a 1β-hydroxyl with certain other steroidal sapogenins recently described by Japanese workers: tokorogenin[12] and rhodeasapogenin.[13] Because of the facile degradation of sapogenins, it seemed advantageous to make use of ruscogenin as a starting material for the synthesis of novel compounds related to physiologically active substances, in the manner of acovenosigenin,[14] with the possible aim of preparing ring A dienones of the corticoid type presently used in therapy.

In our previous communication[4] we reported the preparation of the hydroxylated progesterone derived from ruscogenin.[15] In this present paper we wish to record the details of this preparation, those describing the conversion of the genin to its ring A-dienone (25D), as well as certain other transformations leading to more highly oxygenated derivatives in the direction of corticoid structures.

Fig. 1 summarizes the reactions under discussion. Ruscogenin diacetate (Ia) was selectively hydrolyzed at C-3 in acid[16] and subjected, without isolation, to Oppenauer oxidation. The resulting dienone II also was prepared by dehydrogenation of diosgenone (III)[17] with selenium dioxide.[18,19]

Oxidative degradation of the diacetates of either ruscogenin or neoruscogenin[20] followed by saponification furnished 1β,3β-dihydroxy-$\Delta^{5,16}$-pregnadiene-20-one (IV).[21] Hydrogenation of the double bond at C-16 was carried out in the conventional manner, but oxidation to the progesterone analog VIa was accomplished by means of *Flavobacterium dehydrogenans*,[22] which did not result in elimination of the sensitive β-hydroxyl group.[23]

The cortical side chain was elaborated using conventional means. Treatment of IV with alkaline hydrogen peroxide gave 1β,3β-dihydroxy-16α,17α-oxido-Δ^5-pregnen-20-one (VII), and the epoxide ring of the derived diacetate was opened with hydrogen bromide to the bromohydrin VIII. The latter was not characterized, but debrominated directly to give 1β,3β,17α-trihydroxy-Δ^5-pregnen-20-one 1,3-diacetate (IX) in reasonable yield. The next step, however, involving tribromination at positions 5,6 and 21, regeneration of the double bond with simultaneous exchange in the side chain by means of sodium iodide, and final acetoxylation, was greatly disappointing. Thus, from 428 mg. of IX, only 43.5 mg. of the desired 1β,3β,-17α,21-tetrahydroxy-Δ^5-pregnen-20-one 1,3,21-triacetate (X) was obtained. This material was characterized only by melting point, infrared spectrum, and a positive color reaction with red tetrazolium (indicative of the dihydroxy-acetone side chain). However, its structure was established

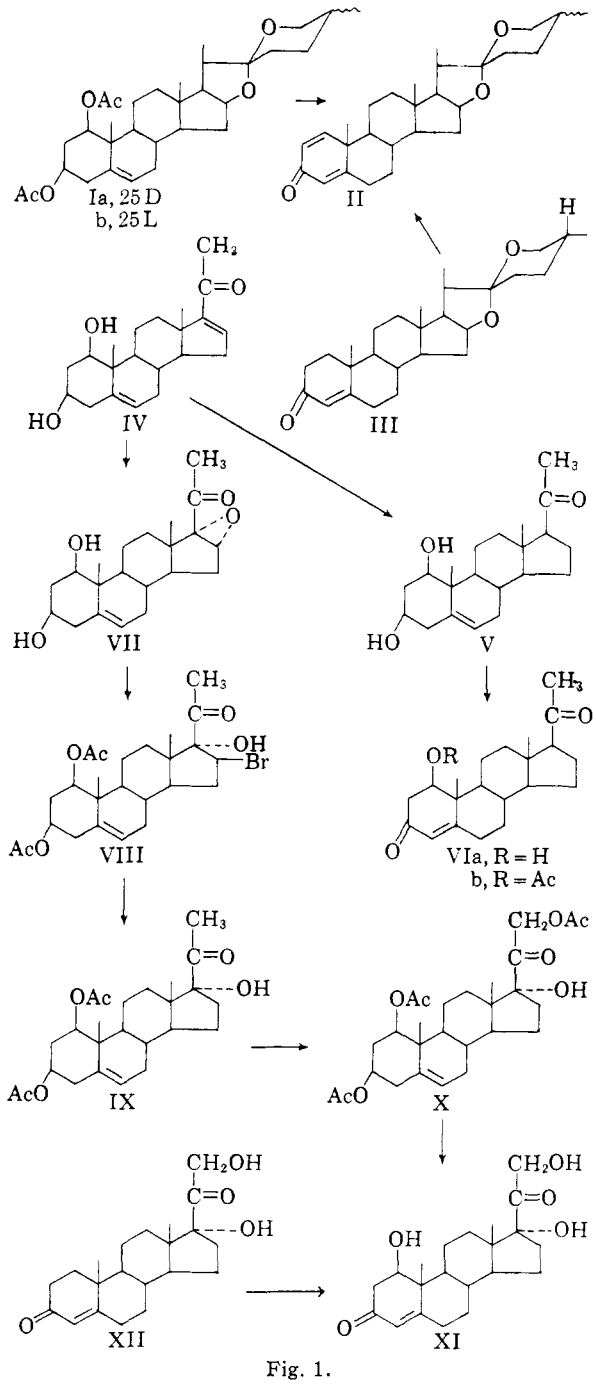

Fig. 1.

(10) R. M. Dodson, A. H. Goldkamp and R. D. Muir, THIS JOURNAL, **79**, 3921 (1957).

(11) Dr. J. N. Shoolery of Varian Associates was kind enough to examine the nuclear magnetic resonance spectrum of ruscogenin diacetate and ascribes the absorption bands to equatorial acetates, thus concurring with a 1β-assignment.

(12) K. Morita, *Pharm. Bull. (Japan)*, **5**, 494 (1957).

(13) H. Nawa, *Chem. and Pharm. Bull.*, **6**, 255 (1958).

(14) For leading references, see W. Schütt and Ch. Tamm, *Helv. Chim. Acta*, **41**, 1730 (1958).

(15) The same compound was also reported by Burn, *et al.*, in their full paper (see ref. 8).

(16) E. P. Oliveto, C. Gerold, L. Weber, H. E. Jorgenson, R. Rausser and E. B. Hershberg, THIS JOURNAL, **75**, 5486 (1953).

(17) F. Sondheimer, C. Amendolla and G. Rosenkranz, *ibid.*, **75**, 5932 (1953).

(18) T. Miki and Y. Hara, *Pharm. Bull. (Japan)*, **4**, 421 (1956).

(19) The same compound was prepared by the English workers (ref. 2) from tigogenone by an alternate route.

(20) *Inter alia* R. E. Marker and E. Rohrmann, THIS JOURNAL, **61**, 3592 (1939); D. H. Gould, H. Staeudle and E. B. Hershberg, *ibid.*, **74**, 3685 (1952).

(21) The yield from the 25L-isomer is superior to that of the 25D-isomer. This is not unreasonable if one postulates that the conditions are inadequate for complete pseudomerization of the latter; see M. E. Wall and S. Serota, *ibid.*, **79**, 6481 (1957).

(22) C. Arnandi, *Zentr. Parasitenk.*, **105**, 352 (1942).

(23) Burn, *et al.* (ref. 8), used *Corynevacterium mediolanum* to obtain the same compound.

by subsequent microbiological conversion with *Flavobacterium dehydrogenans*, resulting in an enzymatic Oppenauer oxidation and furnishing 1β,17α,21-trihydroxy-Δ⁴-pregnene-3,20-dione (XI). This latter substance turned out to be identical with a material produced by direct microbiological hydroxylation of Compound S, using *Rhizoctonia ferrugena*.[24] The present correlation establishes the configuration of the microbiologically introduced hydroxyl as β.

Microbiological introduction of a hydroxyl group at C-1 of 11-oxygenated corticoids already has been reported.[25]

Experimental[26]

Δ¹,⁴-20α,22β,25D-spirostadien-3-one[27] (II). A. From Ruscogenin Diacetate (Ia).—Ruscogenin diacetate, 1 g. (kindly supplied by Dr. Lapin), was dissolved in chloroform, 10 ml. To this solution, 35 ml. of methanol containing 2.1 ml. of concentrated hydrochloric acid and 3.5 ml. of water were added, and the resulting solution was allowed to stand at room temperature for 24 hours. Water (120 ml.) was then added, and the resulting suspension kept in the refrigerator for another 24 hours. It was then extracted with chloroform, and the resulting extract was washed with sodium bicarbonate solution and water. After drying and concentration, crystallization from benzene–petroleum ether gave 718 mg. of material, m.p. 185–195°. Of this, 20 mg. was withdrawn for the determination of infrared spectrum and optical rotation. The infrared spectra showed the appearance of a hydroxyl group (2.88 μ); the relative intensity of the bands ascribed to acetate had markedly decreased. Recrystallization from 90% ethanol gave a product, m.p. 190–197°, $[\alpha]_D$ −94.3° ($CHCl_3$), but further purification was not possible. Therefore, the entire crystalline material (698 mg.) was combined and dissolved in toluene, 35 ml., and cyclohexanone, 5.3 ml. Aluminum isopropoxide (1.75 g.) was added, and the mixture refluxed for 10 hours. After overnight standing at room temperature, it was washed with dilute hydrochloric acid, water, bicarbonate solution and water again. The resulting solution was subjected to steam distillation and extracted with ether. Crystallization from ether gave 158.6 mg. (20%) of material possessing m.p. 185–194°. An analytical sample was prepared by recrystallization from hexane, m.p. 198–202°, $[\alpha]^{23}D$ −73.8°, ϵ_{244}^{max} 16,000; λ^{Nujol} 6.00, 6.14, 6.21 (dienone) 10.18, 10.86, 11.12, 11.55, where 11.12 > 10.86 (25D configuration). Infrared examination of the mother liquors revealed the presence of at least another 30% of dienone.

Anal. Calcd. for $C_{27}H_{38}O_3$: C, 78.98; H, 9.33. Found: C, 78.64; H, 9.35.

B. From Diosgenone (III).—Diosgenone, 2 g., was dissolved in 40 ml. of *t*-amyl alcohol and 8 ml. of glacial acetic acid. Selenium dioxide (540 mg.) was added, and the suspension refluxed for 4 hours. After overnight standing at room temperature it was extracted with methylene chloride, the extract washed with water, sodium hydroxide solution (5%), and again water to neutral reaction. The washed extract was dried and chromatographed on Florisil. Elution with benzene gave large amounts of starting material contaminated with a small quantity of selenium-containing substance.[28] Subsequent benzene–ether eluates gave 69.5 mg. of the desired dienone, m.p. 185–190°. Recrystallization from hexane gave the dienone; m.p. 198–202°, no depression upon admixture with material from A, $[\alpha]^{23}D$ −77°, ϵ_{244}^{max} 16,800; infrared spectrum identical with that of substance prepared from ruscogenin diacetate.

1β,3β-Dihydroxy-Δ⁵,¹⁶-pregnadien-20-one (IV). A. From Ruscogenin Diacetate (Ia).—Genin diacetate, 600 mg., was dissolved in butyric anhydride, 1.5 ml., and refluxed for 8 hours. The solution then was cooled to 75°, 3 ml. of acetic acid added, and the mixture further cooled to 15°. An oxidizing solution, 0.24 g. of chromium trioxide in 2 ml. of water, was added over a 25-minute period, care being taken to keep the temperature below 18°. Excess oxidizing agent was destroyed by the addition of 0.1 g. of sodium bisulfite in 2.5 ml. of water, and partial hydrolysis accomplished by distilling off 4.5 ml. and then continuing boiling the mixture under reflux for a total of 4 hours. The solution was cooled to room temperature, extracted with methylene chloride and concentrated to dryness. Total hydrolysis was accomplished by dissolving the residue in 4.5 ml. of *t*-butyl alcohol, adding 0.26 g. of sodium hydroxide in 2.3 ml. of water and refluxing for one hour. The reaction mixture was acidified to *p*H 6 and extracted with *t*-butyl alcohol–benzene (4:1). The extract was dried and concentrated *in vacuo*. Chromatography over Florisil gave, from the benzene–ether eluates, the desired product. Crystallization from acetone gave 55.5 mg., m.p. 228–232°, ϵ_{240}^{max} 9,100; λ at 2.93, 5.83 (acetone of solvation) 6.00 and 6.28. Recrystallization from benzene gave a product free of solvation; λ at 2.82, 6.06 and 6.28.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.69; H, 8.98.

B. From Neoruscogenin Diacetate (Ib).—The degradation was carried out on 600 mg. as described above, except that a larger volume (2.5 ml.) of butyric anhydride was used; yield 106 mg., m.p. 230–233°, infrared spectrum identical with that of the material obtained from ruscogenin.

C. From a Genin Diacetate Mixture.—The degradation was carried out on 600 mg. of a mixture of roughly equal parts of the ruscogenin and neoruscogenin diacetates; yield 86 mg., m.p. 228–235°.

1β,3β-Dihydroxy-Δ⁵-pregnen-20-one (V).—1β,3β-Dihydroxy-Δ⁵,¹⁶-pregnadien-20-one (IV), 120.7 mg., was dissolved in 3 ml. of freshly distilled pyridine and hydrogenated over 41 mg. of palladium–charcoal (5%) which had been prehydrogenated. After the uptake of one mole of hydrogen, the catalyst was filtered off and the solution concentrated to dryness *in vacuo*. Chromatography on Florisil gave, in the benzene–ether eluates, 55.5 mg. of the desired product, m.p. 195–198°, no selective absorption in the ultraviolet between 220 and 300 mμ; λ^{Nujol} at 2.96, 3.03, 5.83 and 5.92 μ; λ^{CHCl_3} at 2.97, 5.87; $[\alpha]^{23}D$ +22.4°.

Anal. Calcd. for $C_{21}H_{32}O_3$: C, 75.86; H, 9.70. Found: C, 75.49; H, 9.37.

1β-Hydroxy-Δ⁴-pregnene-3,20-dione (1β-Hydroxyprogesterone) (VIa).—A medium containing 10 g. of Difco yeast extract, 4.49 g. of KH_2PO_4 and 8.83 g. of Na_2HPO_4 per liter of tap water (*p*H 6.8) was prepared, and forty 300-ml. erlenmeyer flasks were charged with 100 ml. of this medium. Each flask was inoculated with a 1% inoculum of a 72-hour shake culture of *Flavobacterium dehydrogenans*. Growth was permitted to take place, during 45 hours, on a rotary shaker under constant illumination. 1β,3β-Dihydroxy-Δ⁵-pregnen-20-one (V), 20 mg. in 1 ml. of acetone per flask (total 1.2 g.), was then added, and shaking continued for 80 hours. The pooled broth was extracted with chloroform, the extract concentrated to dryness and chromatographed over 40 g. of Florisil. Benzene–ether mixtures eluted a crystalline material, which was recrystallized from methylene chloride–isopropyl ether to give 438 mg., m.p. 152–153°. An analytical sample showed m.p. 154–156°, $[\alpha]^{23}D$ +142.2°; λ^{max} at 2.98 (OH), 5.84 (20-ketone), 6.01 (3-ketone), 6.19 (Δ⁴-double bond) μ; ϵ_{241}^{max} 14,000, after 2 hours at 60° in alkaline medium[29] $\epsilon_{244.5}^{max}$ 17,700.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.32; H, 9.15. Found: C, 76.32; H, 9.05.

An acetate (VIB) was prepared in the usual manner and crystallized from ether. The analytical sample had m.p. 174–176°, $[\alpha]^{22}D$ +71.1; λ_{max}^{Nujol} at 5.72, 5.91, 5.95, 6.16, 8.10 μ.

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.16; H, 8.66. Found: C, 73.92; H, 8.78.

(24) G. Greenspan, C. P. Schaffner, W. Charney, H. L. Herzog and E. B. Hershberg, THIS JOURNAL, **79**, 3922 (1957).

(25) W. J. McAleer, M. A. Kozlowski, T. H. Stoudt and J. M. Chemerda, *J. Org Chem.*, **23**, 508 (1958).

(26) All melting points were taken on a Kofler block. Rotations were taken in a 1-dm. tube at a concentration of *ca.* 1% in chloroform. Ultraviolet spectra were taken in methanol. Analyses and spectral data were obtained by the Microanalytical and Physical Chemistry Departments of these laboratories.

(27) For sapogenin nomenclature, see M. E. Wall and S. Serota, THIS JOURNAL, **79**, 6481 (1957).

(28) K. Florey and A. R. Restivo, *J. Org. Chem.*, **22**, 406 (1957); J. S. Baran, THIS JOURNAL, **80**, 1687 (1958).

(29) A. S. Meyer, *J. Org. Chem.*, **20**, 1240 (1955).

1β,3β-Dihydroxy-16α,17α-oxido-Δ⁵-pregnen-20-one (VII).—1β,3β-Dihydroxy-Δ⁵,¹⁶-pregnadien-20-one (IV) (895 mg.) was dissolved in 68 ml. of methanol and cooled to 10°. At this temperature, 1.8 ml. of 4 *N* sodium hydroxide and 3.6 ml. of 30% hydrogen peroxide were added, and the resulting solution was allowed to stand at 5° overnight. It then was neutralized with acetic acid, 100 ml. of water was added, and the volume of the resulting suspension was reduced *in vacuo* to *ca.* 100 ml. Cooling and filtration gave 693 mg., m.p. 190–197°. Recrystallization from benzene gave the analytical sample, m.p. 200–202° (after drying for three hours at 100° *in vacuo*); $[\alpha]^{23}D$ −8.9°; λ^{Nujol} 2.88, 5.90 μ.

Anal. Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73. Found: C, 72.80; H, 8.67.

16β-Bromo-1β,3β,17α-trihydroxy-Δ⁵-pregnen-20-one 1,3-Diacetate (VIII).—The foregoing epoxide (VII, 1.25 g.) was acetylated with pyridine–acetic anhydride and, upon the usual workup (recrystallization from isopropyl ether), gave 1.4 g. of a diacetate, m.p. 158–162. This was dissolved in 50 ml. of glacial acetic acid, 5 ml. of a solution of acetic acid saturated with hydrogen bromide was added, and the reaction was allowed to proceed for 1 hour at room temperature. The solution then was quenched in a large amount of water, the resulting solid was filtered off (1.53 g., dec. 190–195°) and used as such in the next step.

1β,3β,17α-Trihydroxy-Δ⁵-pregnen-20-one 1,3-Diacetate (IX).—Bromohydrin VIII (980 mg.) was dissolved in 25 ml. of tetrahydrofuran, 0.24 ml. of triethylamine was added, and the solution was hydrogenated over 400 mg. of 5% palladium-on-charcoal. After one mole of hydrogen had been absorbed, the catalyst was filtered off, amine hydrobromide was washed out in a separatory funnel, and the organic layer was dried and concentrated *in vacuo*. The residual oil was chromatographed over Florisil, and from the benzene–ether eluates 578 mg. of IX, m.p. 174–179°, was obtained. Recrystallization from isopropyl ether gave an analytical sample, m.p. 179–180°, $[\alpha]^{22}D$ −34.3; λ^{Nujol} at 2.90, 2.95, 5.77, 5.86, 8.10 μ.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39. Found: C, 69.43; H, 8.28.

1β,3β,17α,21-Tetrahydroxy-Δ⁵-pregnen-20-one 1,3,21-Triacetate(X).—The foregoing diacetate IX (428 mg.) was dissolved in 7 ml. of chloroform, and 1.8 ml. of a solution of bromine in chloroform (3.68 g. in 40 ml.) was added. Upon the addition of a small amount of hydrogen bromide in chloroform, the reaction mixture decolorized. After standing for 25 minutes at room temperature, 2 g. of potassium acetate, 7.2 ml. of methanol and 850 mg. of sodium iodide were added, and the mixture stirred at 45° for 45 minutes. Ice-water (20 ml.) was added, and the color of the liberated iodine was discharged with a dilute solution of hydrazine. The aqueous phase was extracted with chloroform, the latter extract was washed with water, dried over potassium acetate, and concentrated to dryness *in vacuo*. Potassium acetate (2 g.), 2 ml. of water and 20 ml. of acetone were added, and the mixture was refluxed for 17 hours. It then was concentrated to a low volume, extracted with chloroform, the extract washed with water and dried over magnesium sulfate. Filtration and concentration *in vacuo* gave an oil which was chromatographed over Florisil (30 g.). Eluates with benzene–ether (1:1) gave 43.5 mg. of crystalline X, m.p. 169–177°, positive color reaction with red tetrazolium; λ^{Nujol} 2.86, 5.78 (broad) μ.

1β,17α,21-Trihydroxy-Δ⁴-pregnene-3,20-dione (XI).—The foregoing triacetate (X, 38 mg.) was subjected to the action of *Flavobacterium dehydrogenans*, as described for the preparation of V. The crude extract was chromatographed over silicic acid,[20] and with 1% methanol in chloroform, 19 mg. of the α,β-unsaturated ketone XI was eluted, m.p. 203–207° (soft. at 180). Its infrared spectrum was identical with XI obtained by a different route.[24]

BLOOMFIELD, N. J.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTHEX, S. A.]

Steroids. CXXIV.[1] Studies in Cyano Steroids. Part I. The Synthesis of a Series of C-6-Cyano Steroid Hormones

BY A. BOWERS, E. DENOT, MARÍA BLANCA SÁNCHEZ, L. M. SÁNCHEZ-HIDALGO AND H. J. RINGOLD

RECEIVED APRIL 13, 1959

The fission of steroid 3β-acetoxy-5α,6α-epoxides and 3-cycloethylene-ketal-5α,6α-epoxides with potassium cyanide has been studied. Dependent upon the conditions the reaction was attended with or without concomitant elimination of the initially formed 5α-hydroxyl group to afford either the 5α-hydroxy-6-cyanide or the Δ⁵-6-cyanide. Under more vigorous conditions a double elimination at C-3 and C-5 occurred to form Δ³,⁵-6-cyanides. Manipulation of appropriate intermediates gave the 6-cyano-Δ⁴-3-ketones, which exist mainly in the enol form. Application of this general method led to the synthesis of the 6α-cyano derivatives of progesterone, 17α-acetoxyprogesterone, testosterone and cortisone.

In 1953, Fried and Sabo made a significant contribution to steroid hormone studies by disproving the then widely held view that structural modification of the adrenal hormones cortisone and hydrocortisone always led to a decrease in biological activity. They demonstrated that introduction of a chlorine[2] or a fluorine[3] atom at C-9(α) significantly enhanced the anti-inflammatory activity of the parent hormone. This finding stimulated considerable interest in modified steroid hormones and prodigious efforts have been made to introduce atoms or groups or further unsaturation at key positions throughout the steroid nucleus.

For both chemical and empirical reasons the position that has attracted the most attention has been C-6 and the earliest modification was the preparation of a series of 6-hydroxy (or acetoxy) and 6-keto steroid hormones. Amongst the more important may be mentioned the 6β-hydroxy analogs of testosterone,[4,5] progesterone,[4,6,7] desoxycorticosterone[4,5,8,9] Reichstein's Compound S[7,10,11] and cortisone.[11] The corresponding C-6 ketones of all the above compounds have also been pre-

(1) Steroids CXXIII, R. Villotti, C. Djerassi and H. J. Ringold THIS JOURNAL, **81**, 4566 (1959).

(2) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953).

(3) J. Fried and E. F. Sabo, *ibid.*, **76**, 1455 (1954).

(4) C. Amendolla, G. Rosenkranz and F. Sondheimer, *J. Chem. Soc.*, 1226 (1954).

(5) J. Romo, G. Rosenkranz, C. Djerassi and F. Sondheimer, *J. Org. Chem.*, **19**, 1509 (1954).

(6) M. Ehrenstein and T. O. Stevens, *ibid.*, **5**, 318 (1940).

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