[CONTRIBUTION FROM THE LABORATORIES OF G. D. SEARLE AND CO.]

2-Hydroxy-$\Delta^{1,4}$-3-keto Steroids

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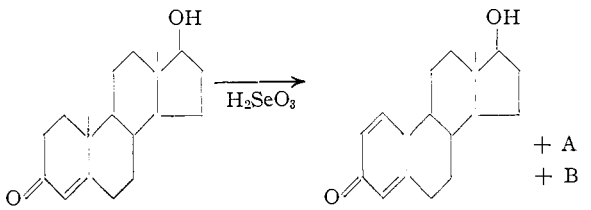
In addition to 1-dehydrotestosterone and seleno-1-dehydrotestosterone, the selenious acid oxidation of testosterone in *t*-butyl alcohol yielded 2,17β-dihydroxyandrosta-1,4-diene-3,17-dione. A structure is proposed for seleno-1-dehydrotestosterone. Other 2-hydroxy-$\Delta^{1,4}$-3-keto steroids have been prepared.

In a recent note Ringold, Rosenkranz and Sondheimer[1] reported that when testosterone (I) is treated with selenium dioxide in refluxing benzene containing a little water, 1-dehydrotestosterone (II) can be isolated in a 35% yield. During the course of an investigation, using conditions developed by Wettstein[2] for the dehydrogenation of Δ^4-3-keto steroids to the corresponding $\Delta^{1,4}$-3-keto steroids in good yields, testosterone (I) was treated with selenious acid in refluxing *t*-butyl alcohol containing a little acetic acid. There was formed in



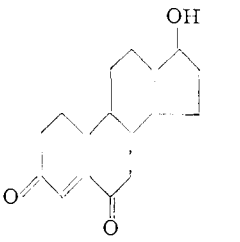
I II

addition to a 53% yield of 1-dehydrotestosterone (II), a 2% yield of a compound A, m.p. 206–208°, which analyzed for $C_{19}H_{26}O_3$, and a 24% yield of a compound B, m.p. 273–275°, reported recently[3] to analyze for $C_{19}H_{25}O_2Se$.

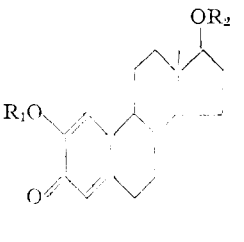
The structural features of compound A were clarified by means of its spectral data. Its ultraviolet spectrum in methanol showed a λ_{max} 254 mμ (ϵ 15,400) with a small shoulder at 284 mμ, indicating that oxidation most probably occurred at position two or six in testosterone.[4] In the infrared spectrum of A in a KBr pellet, sharp maxima appeared at 2.87, 2.99 and 6.12 μ, characteristic of *two hydroxyl groups* and a *conjugated unsaturated ketone*, respectively. Evidence for the former was obtained when A yielded a diacetate, m.p. 205–206°, λ_{max} 247.5 mμ (ϵ 16,400), on treatment with acetic anhydride in pyridine. A maximum at 5.67 μ in the infrared spectrum of A diacetate and conversion of A to its monomethyl ether, m.p. 226–227°, λ_{max} 254 mμ (ϵ 16,000), furnished evidence for an enolic hydroxyl group.

Structure III, 17β-hydroxyandrost-4-ene-3,6-dione,[5] was rejected because (1) the infrared spectrum of III[6] showed only one hydroxyl group, and (2) the diacetate or monomethyl ether of III would be expected to absorb in the vicinity of 280 mμ.[4]

The consideration that the oxidation product was represented by IV had precedents in the conversion of cholestan-3-one to cholestane-2,3-dione by oxidation with selenium dioxide,[7] and was supported by the ultraviolet spectrum of its diacetate and monomethyl ether, which were formulated as V and VI, respectively. When A was refluxed in



III



IV, R_1,R_2 = H
V, R_1,R_2 = Ac
VI, R_1 = Me, R_2 = H

ethanol with *o*-phenylenediamine, it yielded a quinoxaline derivative. This evidence gave further support for structure IV. Confirmation of structure IV by synthesis followed simply, when oxidation of 2α,17β-dihydroxyandrost-4-en-3-one[8] with bismuth trioxide in acetic acid[9] led to a product in 55% yield whose infrared spectrum in a KBr pellet was indistinguishable from that of A.

The third product in the selenium dioxide oxidation, B, has been investigated recently by K. Florey and A. R. Restivo. They did not suggest a structure but reported the following experimental results and conclusions. The ultraviolet spectrum of B showed maxima at 245 mμ (ϵ 10,800), 257 mμ (ϵ 10,700) and 307 mμ (ϵ 1170).[10] The infrared spectrum in a Nujol mull indicated maxima at 2.87 μ (hydroxyl) and 6.12, 6.20 and 6.28 μ ($\Delta^{1,4}$-3-keto). On treatment with acetic anhydride in pyridine, B yielded a monoacetate having essentially the same spectral characteristics as B. Hydrolysis of B acetate with aqueous alcoholic base regenerated B. When B acetate was heated with acetic anhydride containing *p*-toluenesulfonic acid, it underwent the diene–phenol rearrangement to give a phenol acetate containing selenium, which could be hydrolyzed to its phenolic derivative. The spectral data on B and its transformation products, they concluded, reflected the presence of selenium as a part of a $\Delta^{1,4}$-3-keto moiety. Compound B was inert to refluxing dilute sulfuric acid or base. Also, when B acetate was treated with acetic acid containing aqueous hydrogen

(1) H. J. Ringold, G. Rosenkranz and F. Sondheimer, *J. Org. Chem.*, **21**, 239 (1956).

(2) Ch. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956).

(3) K. Florey and A. R. Restivo, *J. Org. Chem.*, **22**, 406 (1957).

(4) L. Dorfman, *Chem. Revs.*, **53**, 47 (1953).

(5) A. Butenandt and B. Riegel, *Ber.*, **69B**, 1163 (1936).

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

(7) E. T. Stiller and O. Rosenheim, *ibid.*, 353 (1938).

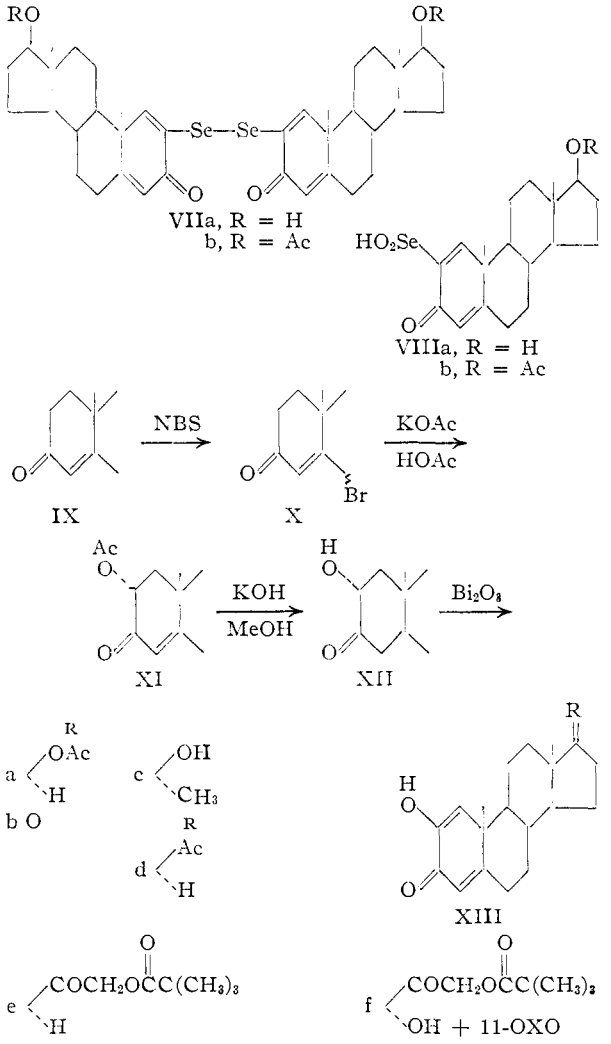
(8) F. Sondheimer, St. Kaufman, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

(9) W. Rigby, *J. Chem. Soc.*, 793 (1951).

(10) The maximum is actually a very broad band extending from 245 mμ to 269 mμ with ϵ = 10,700.

peroxide, a compound analyzing for $C_{21}H_{28}O_5Se$ was isolated and presumed to be a hydrated selenoxide; upon treatment with aqueous bisulfite the hydrated selenoxide regenerated B acetate.

In light of the aforementioned findings, one is led to conclude that the chemical transformations of B can be interpreted on the basis of the structure VIIa. The structure is in agreement with the



spectral data for B and B acetate and its dienone–phenol rearrangement product. An ebullioscopic molecular weight determination in chloroform yielded the value 700 ± 35 which is in accord with the proposed structure. As a dialkyl diselenide, with selenium attached at position two of the dienone nucleus, VIIa would be expected to be inert to dilute acid or base.[11] On the basis of the conversion of dialkyldiselenides to seleninic acids with aqueous hydrogen peroxide,[12] VIIb would be oxidized to the seleninic acid VIIIb and not to a selenoxide. That the hydrated selenoxide was indeed an acid was evident when the compound dissolved in aqueous bicarbonate with evolution of gas, and when VIIa also yielded a seleninic acid (pK_a = 5.5, neut. equiv., 407) upon oxidation with aqueous hydrogen peroxide. Finally, the regeneration of VIIb by treatment of VIIIb with aqueous bisulfite finds precedent in the reduction of *m*-nitrophenylseleninic acid to *m*-nitrophenyl diselenide by aqueous bisulfite,[13] and the reduction of selenious acid to selenium and selenide by sulfur dioxide.[14]

In order to evaluate the consequences of the new structural modification in steroids on physiological activity, efforts were directed toward the preparation of other 2-hydroxy-$\Delta^{1,4}$-3-keto steroids. To this end the oxidation of 2α-hydroxy-Δ^4-3-keto steroids with bismuth trioxide in acetic acid was chosen as the most desirable approach. The preparation of 2α-hydroxyandrost-4-ene-3,17-dione (XIIb) and 2α-hydroxyprogesterone (XIId) has been described.[8,15] The previously unknown 2-hydroxy derivatives XIIa, c, e, and f were readily available from their parent steroids by the straightforward sequence IX → X → XI → XII introduced by Sondheimer and co-workers.[8,15] In the synthesis of XIIIe, f to prevent the oxidation of the C_{17}-side chain by bismuth trioxide, the C_{21}-hydroxyl had to remain acylated. Such protection was rendered by beginning with the 21-trimethylacetate of 11-desoxycorticosterone and cortisone and quantitatively removing the more readily hydrolyzable acetate group in XIe, f with potassium hydroxide in methanol.

Thus in the manner described above the 2-hydroxy-1-dehydro analogs of testosterone acetate, androst-4-ene-3,17-dione, 17-methyltestosterone, progesterone, 11-desoxycorticosterone trimethylacetate and cortisone 21-trimethylacetate were made available. Each of the analogs has a maximum at 252–254 mμ (ϵ 15,000) in the ultraviolet, due to the 2-hydroxy-$\Delta^{1,4}$-3-keto chromophore. Their molecular rotations indicate that the change in molecular rotation associated with the conversion of a Δ^4-3-keto steroid to its 2-hydroxy-$\Delta^{1,4}$-3-keto analog is strongly negative.

Compounds IV and XIIIa, b, c showed some anabolic activity as measured by the ani levator method[16] and no androgenic activity as measured by the increase in weight of the seminal vesicle and prostate. In the gonadotropin inhibition assay in rabbits, XIIId had 40% the activity of progesterone; XIIId also reversed the effect of desoxycorticosterone (DOC) on urinary Na/K ratio of adrenolectomized rats. In the cotton granuloma assay, modified for daily systematic treatment, XIIIf was inactive at 2 mg.[17,18]

(11) An alternate structure of B, with a –Se–Se– bridge between the one positions of the 1-dehydrotestosterones, resulting from the attack by selenium at the other unoxidized position of ring A in testosterone was rejected. Such a compound should be susceptible to conjugate attack by OH^- at position one with concomitant loss of Se_2^{--} to give a selenium free steroid.

(12) (a) B. Rathke, *Ann.*, **152**, 216 (1869); (b) F. Wohler and J. Dean, *ibid.*, **97**, 6 (1856); (c) A. Fredga, *J. prakt. Chem.*, [2] **123**, 143 (1929).

(13) F. L. Pyman, *J. Chem. Soc.*, **115**, 166 (1919).

(14) N. V. Sidgwick, "The Chemical Elements and Their Compounds," Clarendon Press, Oxford, 1950, p. 972.

(15) G. Rosenkranz, O. Mancera and F. Sondheimer, THIS JOURNAL, **77**, 146 (1955).

(16) E. Eisenberg and G. S. Gordon, *J. Pharmacol. Exptl. Therap.*, **99**, 38 (1950).

(17) L. D. Hershberger and D. W. Calhoun, *Endocrinol.*, **60**, 153 (1957).

(18) The author is indebted to F. J. Saunders, L. H. Hershberger and C. Kagawa of the Biological Research Staff, G. D. Searle and Co., and to G. Pincus of the Worcester Foundation for Experimental Biology for the biology reported above.

Experimental

Melting points were determined on a Fisher–Johns block and are corrected. The ultraviolet spectra were taken in methanol. The analytical data were determined by R. T. Dillon and his staff of G. D. Searle and Co.

Oxidation of Testosterone with Selenious Acid.—To a solution of 14.4 g. (0.05 mole) of testosterone in 300 ml. of *t*-butyl alcohol containing 3 ml. of acetic acid was added 6.5 g. (0.05 mole) of selenious acid. The mixture was refluxed with stirring for five hours. An additional 2.0 g. of selenious acid was added and the mixture was refluxed for another 16 hours. The mixture was cooled and filtered. The filtrate was concentrated *in vacuo* and the residue was dissolved in 125 ml. of methylene chloride. After the solution was filtered, the filtrate was washed with water, several portions of aqueous sodium bicarbonate, dried over sodium sulfate and concentrated *in vacuo*. The residue was dissolved in benzene. Upon slow cooling, crystallization ensued and the mixture was filtered. After the crude product, **seleno-1-dehydrotestosterone**,[3] was collected and dried, it weighed 2.75 g. and melted at 265–270°. The mother liquors were concentrated *in vacuo* and the residue was chromatographed on silica gel. The column was eluted with benzene containing increasing amounts of ethyl acetate. When the column was eluted with 1:4 ethyl acetate–benzene, earlier fractions yielded 0.84 g. of crude material which melted at 170–175°. Several crystallizations of the crude product from acetone–Skellysolve B yielded 300 mg. of 2,17β-dihydroxyandrosta-1,4-dien-3-one which had the following physical properties, m.p. 207–209°, λ_{max} 254 mμ (ϵ 15,400), $[\alpha]_D$ −20.1° ($CHCl_3$); λ_{max} (KBr) 2.87, 2.99 and 6.12 μ (broad).

Anal. Calcd. for $C_{19}H_{26}O_3$: C, 75.5; H, 8.69. Found: C, 75.37; H, 8.74.

A solution of 50 mg. of 2,17β-dihydroxyandrosta-1,4-dien-3-one and 50 mg. of *o*-phenylenediamine in 7 ml. of absolute ethanol was refluxed for one hour. The solution was concentrated *in vacuo* and the residue was extracted with hot water. Crystallization of the residue from ether–Skellysolve B gave a crude quinoxaline melting at 224–228°. Crystallization of the crude product from ether–Skellysolve B gave an analytical sample, m.p. 226–227°; λ_{max} 224.5 (ϵ 27,800), 267 (ϵ 24,300), 347 (ϵ 16,000) and 362 (ϵ 15,300).

Anal. Calcd. for $C_{25}H_{30}ON_2$: C, 80.17; H, 8.07. Found: C, 79.76; H, 8.30.

When the column was eluted with 1:4 ethyl acetate–benzene, later fractions yielded 8.75 g. of crude 1-dehydrotestosterone which melted at 155–165°. Crystallization of the crude product from acetone–ether yielded 7.7 g. of material which melted at 165–168°. Elution of the column with 3:7 ethyl acetate–benzene yielded an additional 1.88 g. of **seleno-1-dehydrotestosterone** which melted at 255–265°.

2,17β-Dihydroxyandrosta-1,4-dien-3-one (IV) (Alternate Synthesis).—To a solution made up of 4.4 g. of 2α-hydroxytestosterone in 20 ml. of acetic acid was added 2.7 g. of bismuth trioxide. The mixture was heated at 100° with stirring for 15 minutes. Another 2.7 g. of bismuth oxide was added, and the heating was continued for another 45 minutes. After the mixture was filtered, 200 ml. of water and 200 ml. of ethyl acetate were added to the filtrate. The organic layer was separated, washed with 150 ml. of water, 75 ml. of saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated. The crystalline residue was crystallized from acetone to give 2.0 g. (45%) of colorless crystals which melted at 204–208°. Concentration and cooling of mother liquors yielded an additional 0.4 g. (10%) of crude product which melted at 196–200°. Crystallization of the crude material from acetone gave an analytical sample, which melted at 208–210°, and was identical in a mixture melting point and infrared spectrum with that of 2,17β-dihydroxyandrosta-1,4-dien-3-one obtained above.

2,17β-Dihydroxyandrosta-1,4-dien-3-one Diacetate (V).—To a solution of 300 mg. of 2,17β-dihydroxyandrostan-1,4-dien-3-one made up in 1.5 ml. of pyridine was added 0.5 ml. of acetic anhydride. The solution was warmed on a steam-bath for 15 minutes, cooled to 0°, and diluted with water. After needles precipitated, the mixture was filtered. The product was washed with water and dried. It weighed 380 mg. (99%) and melted at 200–206°. An analytical sample which was prepared by crystallization of the product from ether–Skellysolve B had the following physical properties, m.p. 205–206° λ_{max} 247.5 mμ (ϵ 16,750), $[\alpha]_D$ +20.2° ($CHCl_3$), λ_{max} (KBr) 5.67, 5.78, 5.99, 6.08 and 6.12 μ.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.47; H, 7.82. Found: C, 71.56; H, 7.70.

17β-Hydroxy-2-methoxyandrosta-1,4-dien-3-one (VI).—To a solution of 41 mg. of potassium in 30 ml. of *t*-butyl alcohol was added 300 mg. of 2,17β-dihydroxy-androsta-1,4-dien-3-one and 1 ml. of methyl iodide. The solution was refluxed for one-half to one hour. The mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in acetone, and the solution was filtered, concentrated, diluted with ether–Skellysolve B, and cooled. After crystallization ensued, the mixture was filtered. The product weighed 225 mg. (71%) and melted at 218–221°. An analytical sample which was prepared by crystallization of the crude product from acetone had the following physical properties, m.p. 226–227°, λ_{max} 254 mμ (ϵ 16,000), $[\alpha]_D$ −34.8° ($CHCl_3$).

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 76.0; H, 8.83. Found: C, 76.18; H, 8.78.

2,17β-Dihydroxyandrosta-1,4-dien-3-one 17-Acetate (XIIIa).—To a solution of 11.6 g. (30 mmol.) of 2β-hydroxytestosterone diacetate[8] in 350 ml. of methanol under nitrogen was added 29.6 ml. of 1.01 *M* potassium hydroxide in methanol. After four minutes 30 ml. of methanol containing 30 mmol. of water was added. After another four minutes, the solution was acidified with 42.5 ml. of 1.06 *N* acetic acid. When the solution was concentrated and cooled, crystallization ensued. The mixture was filtered, and the product was washed with a little methanol and dried. The product weighed 7.85 g. (76%) and melted at 190–203°. To a solution of 3.0 g. (8.7 mmol.) of the hydrolysis product in 15 ml. of acetic acid was added 1.85 g. of bismuth trioxide. The mixture was heated for 15 minutes at 100° with stirring. Another 1.85 g. of bismuth trioxide was added and the mixture was heated for another 45 minutes. The mixture was cooled and filtered, and the filtrate was diluted slowly with swirling with 125 ml. of water. After crystallization was complete, the mixture was filtered and the product was washed with water, aqueous bicarbonate, and dried. The product weighed 2.8 g. (94%) and melted at 185–203°. Crystallization of the crude product from acetone–Skellysolve B gave an analytical sample, m.p. 193–195°, λ_{max} 253 mμ (ϵ 17,100), $[\alpha]_D$ +3.8° ($CHCl_3$).

Anal. Calcd. for $C_{21}H_{28}O_4$: C, 73.23; H, 8.19. Found: C, 73.27; H, 7.94.

2-Hydroxypregnan-1,4-diene-3,20-dione (XIIId).—To a solution of 2.90 g. of 2α-hydroxyprogesterone made up in 12 ml. of acetic acid was added 1.6 g. of bismuth trioxide. The mixture was heated at 100° for 15 minutes with stirring. Another 1.6 g. of bismuth trioxide was added and the mixture was heated for another 45 minutes. The mixture was cooled, filtered, and the filtrate was diluted slowly with swirling with 125 ml. of water. The mixture was filtered and the product was collected and dried; it weighed 2.1 g. (72%) and melted at 180–187°. Crystallization of the crude product from acetone–Skellysolve B gave an analytical sample, m.p. 189–190°, λ_{max} 253.5 mμ (ϵ 14,500), $[\alpha]_D$ +124° ($CHCl_3$).

Anal. Calcd. for $C_{21}H_{28}O_3$: C, 76.79; H, 8.59. Found: C, 76.50; H, 8.31.

2α-Hydroxy-17-methyltestosterone 2-Acetate (XIc).—To a solution of 4.0 g. (12.5 mmol.) of 17-methyltestosterone in 250 ml. of carbon tetrachloride was added 2.45 g. (13.8 mmol.) of N-bromosuccinimide. The mixture was refluxed with stirring and illuminated by infrared light for 45 minutes. The reaction mixture was filtered and cooled to 0°. When the crystalline product precipitated the mixture was filtered and the product was washed with carbon tetrachloride. The product, which weighed 3.5 g. (74%) and melted at 120–122° (dec. 125°), was refluxed for four hours with 12 g. of potassium acetate in 75 ml. of acetic acid. Then the solution was concentrated *in vacuo*, and the residue was dissolved in ethyl acetate and water. The organic layer was separated, washed with water and aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated *in vacuo*. Trituration of the residue with ether gave crystals which melted at 188–190° and weighed 500 mg. (15%). Crystallization of the crude material from acetone–Skellysolve B gave an analytical sample, m.p. 199°, λ_{max} 214 mμ (ϵ 15,600), $[\alpha]_D$ +57.5° ($CHCl_3$).

Anal. Calcd. for $C_{22}H_{32}O_4$: C, 73.30; H, 8.95. Found: C, 73.25; H, 9.00.

2,17β-Dihydroxy-17-methylandrosta-1,4-dien-3-one (XIIIc).—A solution of 500m g. (1.39 mmol.) of 2α-hydroxy-17-methyltestosterone 2-acetate in 4 ml. of methanol under nitrogen was treated for four minutes with 2.78 ml. of 1 *M* potassium hydroxide in methanol. Then to the solution 2.78 ml. of methanol containing 2.78 mmol. of water was added. After four minutes the solution was acidified with 4.0 ml. of 1.06 *M* acetic acid. The solution was concentrated *in vacuo* and the residue was dissolved in ethyl acetate. The organic layer was washed with water, dried over sodium sulfate, and concentrated. The crystalline residue which melted at 150–155° was dissolved in 3 ml. of acetic acid and heated at 100° with 250 mg. of bismuth trioxide for 15 minutes and another 200 mg. of bismuth trioxide for 45 minutes. The mixture was filtered and the filtrate was dissolved in ethyl acetate and water. The organic layer was separated, washed with water and aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated *in vacuo*. The crystalline residue was crystallized from ether–Skellysolve B to give colorless crystals which melted at 172–173° and weighed 190 mg. (40%). Crystallization of the crude material from ether–Skellysolve B gave an analytical sample, m.p. 178–180°, λ_{max} 254 mμ (ε 14,300), $[\alpha]_D$ −32.9° ($CHCl_3$).

Anal. Calcd. for $C_{20}H_{28}O_3$: C, 75.91; H, 8.92. Found: C, 75.89; H, 8.88.

2-Hydroxyandrosta-1,4-diene-3,17-dione (XIIIb).—To a solution of 11.35 g. (37.6 mmol.) of 2α-hydroxyandrosta-4-ene-3,17-dione in 50 ml. of acetic acid was added 12.1 g. (26.0 mmol.) of bismuth trioxide. The mixture was heated at 100° with stirring for 30 minutes. Another 6.0 g. of bismuth trioxide was added and the mixture was heated for another 30 minutes. Then 1 g. of charcoal was added and the mixture was filtered. The filtrate was diluted with 500 ml. of water and extracted with five 150-ml. portions of chloroform. The organic layers were combined, washed with water and aqueous bicarbonate, and dried over sodium sulfate. The chloroform was concentrated *in vacuo*, and the residue was crystallized from ether–Skellysolve B to give 6.5 g. (57%) of product which melted at 145–146°. Concentration and cooling of mother liquors yielded another 1.25 g. (11%). Crystallization of the crude material from acetone–Skellysolve B gave an analytical sample, m.p. 157–158°, λ_{max} 253 mμ (ε 14,200), $[\alpha]_D$ +36° ($CHCl_3$).

Anal. Calcd. for $C_{19}H_{24}O_3$: C, 75.97; H, 8.05. Found: C, 76.17; H, 8.16.

2α,21-Dihydroxypregn-4-ene-3,20-dione 2-Acetate 21-Trimethylacetate (XIe).—A solution of 1.24 g. of the desoxycorticosterone trimethylacetate and 588 mg. of N-bromosuccinimide in 250 ml. of carbon tetrachloride was refluxed for one hour, cooled, and filtered. The filtrate was concentrated *in vacuo* at 50°. The crystalline product was triturated with Skellysolve B, and the mixture was filtered. The crude product was dried at 40°, weighed 1.2 g., and melted at 100° dec. It was refluxed for four hours with 4.0 g. of potassium acetate in 120 ml. of acetic acid. The solution was concentrated *in vacuo* and the residue dissolved in ethyl acetate and water. The organic layer was separated, washed with water and aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated *in vacuo*. Trituration of the residue with 50% ether–Skellysolve B gave crystals which melted at 210–220° and weighed 200 mg. (16%). Crystallization of the crude product from acetone–Skellysolve B gave an analytical sample, m.p. 227–229°, λ_{max} 241 mμ (ε 16,190), $[\alpha]_D$ +144° (dioxane).

Anal. Calcd. for $C_{23}H_{40}O_6$: C, 71.15; H, 8.53. Found: C, 70.97; H, 8.74.

2α,21-Dihydroxypregn-4-ene-3,20-dione 21-Trimethylacetate (XIIe).—To a solution of 1.98 g. (4.2 mmol.) of 2α,21-dihydroxypregn-4-ene-3,20-dione 2-acetate 21-trimethylacetate in 200 ml. of methanol and 50 ml. of methylene chloride under nitrogen was added 4.55 ml. of 1.01 *M* KOH in methanol. After four minutes 4.6 ml. of methanol containing 4.6 mmol. of water was added. After another four minutes the mixture was acidified with 6.5 ml. of 1.06 *M* aqueous acetic acid. The methylene chloride was distilled *in vacuo* and the solution was diluted with water. The mixture was filtered, and the product was washed with water, and dried. The product weighed 1.73 g. (100%) and melted at 195–205°. Crystallization of the crude product from acetone–Skellysolve B gave an analytical sample, m.p. 221–223°, λ_{max} 241 mμ (ε 13,300), $[\alpha]_D$ +169° (dioxane).

Anal. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90. Found: C, 72.51; H, 8.92.

2,21-Dihydroxypregna-1,4-diene-3,20-dione 21-Trimethylacetate (XIIIe).—To a warm solution made up of 1.85 g. of 2α,21-dihydroxypregn-4-ene-3,20-dione 21-trimethylacetate in 20 ml. of acetic acid was added 1.34 g. of bismuth trioxide. The mixture was heated at 100° with stirring for 15 minutes and then another 1.34-g. portion of bismuth oxide was added. After an additional 45-minute heating period, charcoal was added and the mixture was filtered. On dilution of the filtrate with a small amount of water, crystals precipitated. The mixture was filtered. After the product was washed with water and dried, it weighed 1.2 g. (28%) and melted at 220–222°. Recrystallization of the crude product from acetone–Skellysolve B gave an analytical sample, m.p. 225–226°, λ_{max} 253.5 mμ (ε 15,600), $[\alpha]_D$ +90° (dioxane).

Anal. Calcd. for $C_{26}H_{36}O_5$: C, 72.86; H, 8.47. Found: C, 72.65; H, 8.55.

2α,17α,21-Trihydroxypregn-4-ene-3,11,20-trione 2-Acetate 21-Trimethylacetate (XIf).—To a refluxing solution composed of 14.7 g. of cortisone 21-trimethylacetate in 500 ml. of chlorobenzene, 350 ml. of carbon tetrachloride and 34.3 ml. of 10% pyridine in carbon tetrachloride was added with stirring 6.45 g. of N-bromosuccinimide. The mixture was refluxed with stirring for ten minutes with exposure to infrared light. Then the organic solution was washed rapidly with warm water and dried over sodium sulfate. The carbon tetrachloride and about 300 ml. of the chlorobenzene were distilled *in vacuo*. Then, while the container was scratched and the solution was diluted with Skellysolve B, a crystalline precipitate appeared. The mixture was filtered, and the product was washed with carbon tetrachloride, Skellysolve B and dried *in vacuo*. The crude product, which weighed 14.5 g. and melted at 143–145° dec., was refluxed with 56 g. of potassium acetate in 1.4 l. of acetic acid for four hours. The solution was concentrated *in vacuo* and the residue dissolved in ethyl acetate and water. The organic layer was separated, washed several times with water and with aqueous sodium bicarbonate, dried over sodium sulfate, and concentrated *in vacuo*. The oily residue was dissolved in ether. When crystallization ensued the solution was cooled overnight. The first crop of material weighed 1.1 g. and consisted mainly of cortisone 21-trimethylacetate. A second crop obtained by concentration and cooling the mother liquors yielded 3.5 g. of needles which melted at 245–255°. This second crop yielded on crystallization from methanol 2.1 and 0.5 g. (13%) of 2α-acetoxycortisone 21-trimethylacetate, m.p. 257–261°, λ_{max} 238 mμ (ε 15,400), $[\alpha]_D$ +178° (dioxane).

Anal. Calcd. for $C_{28}H_{38}O_8$: C, 66.91; H, 7.62. Found: C, 66.84; H, 7.51.

2α,17α-Trihydroxypregn-4-ene-3,11,20-trione 21-Trimethylacetate (XIIf).—To a solution of 2.02 g. (4.0 mmol.) of 2α-acetoxycortisone 21-trimethylacetate in 15 ml. of methanol and 10 ml. of methylene chloride under nitrogen was added 5.2 ml. of 0.813 *M* potassium hydroxide in methanol. After four minutes 4.1 ml. of methanol containing 4 meq. of water was added. The product began to separate during the reaction. After 30 minutes, the mixture was acidified with 6.3 ml. of 1 *M* aqueous acetic acid. The mixture was filtered. After the product was washed with water and dried, it weighed 1.16 g. (63%) and melted at 270–280°. Crystallization of the crude product from acetone gave cotton-like needles, m.p. 276–278°, λ_{max} 237 mμ (ε 12,600), $[\alpha]_D$ +196° (dioxane).

Anal. Calcd. for $C_{26}H_{36}O_7$: C, 67.8; H, 7.87. Found: C, 67.9; H, 7.94.

2,17α,21-Trihydroxypregna-1,4-diene-3,11,20-trione 21-Trimethylacetate (XIIIf).—Using a mixture of 1.06 g. of 2α-hydroxycortisone 21-trimethylacetate, two 1.1-g. portions of bismuth oxide and 75 ml. of acetic acid, the procedure for the oxidation of 2α,21-dihydroxypregn-4-ene-3,20-dione 21-trimethylacetate with bismuth oxide was followed exactly. The crystalline product which weighed 850 mg. was chromatographed on silica gel. The desired product, which was eluted with 15% ethyl acetate in benzene, weighed

400 mg. (40%) and melted at 260–265°. Several crystallizations of crude product from acetone–Skellysolve B gave an analytical sample, m.p. 263–265°, λ_{max} 252.5 mμ (ε 12,100), $[\alpha]_D$ +161° (dioxane).

Anal. Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.47. Found: C, 68.20; H, 7.27.

Oxidation of Seleno-1-dehydrotestosterone with Hydrogen Peroxide to (VIIIa).—To 300 mg. of seleno-1-dehydrotestosterone in 3 ml. of acetic acid was added 0.5 ml. of 30% aqueous hydrogen peroxide. The solution was kept at room temperature for 20 minutes and then diluted with 50 ml. of water. Upon cooling at 5° overnight, colorless needles appeared. The mixture was filtered and the product was washed with cold water, and dried; weight 200 mg., m.p. 174–178°, λ_{max} 252 mμ (ε 10,600), $[\alpha]_D$ −62.5° ($CHCl_3$), pK_a 5.5 (equivalent weight 407).

Anal. Calcd. for $C_{19}H_{26}O_4Se$: C, 57.43; H, 6.60. Found: C, 57.14; H, 6.77.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, HARVARD UNIVERSITY]

The Structure of Mangostin[1]

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Mangostin is shown to be 1,3,6-trihydroxy-7-methoxy-2,8-di-(3-methyl-2-butenyl)-xanthone (X).

Mangostin is the yellow coloring matter obtained from various parts of the mangosteen tree (*Garcinia mangostana, Guttiferae*). It was isolated first by Schmid in 1855 from the fruit hulls[3]; it has subsequently been obtained from the bark and dried sap.[4] The last is by far the richest source, yielding 30–50% of mangostin. It was early claimed that mangosteen hulls surpass cinchona bark as a febrifuge and they and the bark also have been used in the treatment of dysentery,[5] but there have been no reports on the pharmacological properties of mangostin itself.

Mangostin is a bright yellow, optically inactive, phenolic, crystalline material, m.p. 182–183°. Although a number of workers have investigated its chemistry,[3,4,6–13] there has been continuing disagreement over its empirical formula and molecular weight, leading in turn to a range of proposed molecular formulas. The most recently advocated formula, $C_{23}H_{24}O_6$, was advanced by Murakami[12] as a result of analyses of derivatives of mangostin containing additional elements. On this basis, Murakami concluded from his own and earlier work that mangostin had the following functional groups: two double bonds susceptible to hydrogenation, one methoxyl group, and three hydroxyl groups, two of which readily could be methylated and a third which could be acetylated, but methylated only with great difficulty. In addition, Dragendorff[4] had observed that mangostin and dimethylmangostin form complexes with boron pyroacetate ("boroacetic anhydride")[14] and deduced that it must contain a carbonyl function in a β-relationship to the unreactive hydroxyl group.

The first significant degradative work was carried out by Dragendorff[4,10] who found that isovaleric acid and an amyl alcohol were obtained on fusion of mangostin with potassium hydroxide. He also observed that oxidation of mangostin or dimethylmangostin gave α-hydroxyisobutyric acid, while oxidation of tetrahydromangostin with chromic acid gave isocaproic acid. Ozonolysis of dimethylmangostin yielded acetone peroxide and a major fragment which he formulated as $C_{15}H_{14}O_6$ or $C_{22}H_{20}O_9$. Subsequently, Yamashiro[11] repeated the basic fusion of mangostin and obtained acetic acid, oxalic acid, isovaleric acid, phloroglucinol and a yellow, phenolic compound, which on renewed basic fusion gave phloroglucinol and isovaleric acid. Shortly thereafter, Murakami[12] investigated the cleavage of mangostin in ethanolic potassium hydroxide at 170–180°; he isolated isoamyl alcohol, a 3,5-dihydroxy-2-methoxyisopentenylbenzene (I) and 2-methyl-2-hepten-6-ol (II). In the light of his isolation of I, he reformulated Yamashiro's phenol as a $C_{19}H_{18}O_6$ compound and assigned to it the structure III. On the basis of this assignment, the isolation of II, and other observations, he proposed the structure IV for mangostin.[15]

It appeared to us that the evidence available did not justify the formulation of mangostin as IV and that, indeed, there were substantial grounds for doubting the correctness of this structure. Thus: (i) the yellow color, and more generally, the ultraviolet spectrum[4] of mangostin were inconsistent with IV; (ii) the question of the molecular formula of

(1) In part, from the Ph.D. Thesis of George H. Stout, Harvard University, 1956; a preliminary account of part of this work has appeared previously: P. Yates and G. H. Stout, *Chemistry & Industry*, 1392 (1956).

(2) National Science Pre-doctoral Fellow, 1954–1955; General Electric Rice Fellow, 1955–1956.

(3) W. Schmid, *Ann.*, **93**, 83 (1855).

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(9) J. Dekker, *ibid.*, **43**, 727 (1924).

(10) O. Dragendorff, *Ann.*, **487**, 62 (1931).

(11) S. Yamashiro, *Bull. Chem. Soc. Japan*, **7**, 1 (1932).

(12) M. Murakami, *Proc. Imp. Acad. (Tokyo)*, **7**, 254, 311 (1931); *Ann.*, **496**, 122 (1932); *J. Chem. Soc. Japan*, **53**, 150, 162 (1932).

(13) A. Tschirch and E. Stock, "Die Harze," Borntraeger Verlag, Berlin, 3rd ed., Vol. II, 1936, p. 1562.

(14) O. Dimroth and T. Faust, *Ber.*, **54**, 3020 (1921); O. Dimroth, *Ann.*, **446**, 97 (1926).

(15) A structure based on a C_{24}-formulation for mangostin which is referred to in two subsequent discussions[16,17] appears to result from a misreading of Murakami's proposal and to have no basis in fact.

(16) F. Mayer and A. H. Cook, "The Chemistry of Natural Coloring Matters," Reinhold Publ. Corp., New York, N. Y., 1943, p. 249.

(17) R. Robinson, "The Structural Relations of Natural Products," Clarendon Press, Oxford, 1955, p. 45.