ric acid, but no color with ferric chloride; R_f values: 0.97 (*m*-cresol–acetic acid–water 24:1:25), 0.95 (60% acetic acid); specific rotation, 0.3196 g. of subst., 50 ml. of 60% acetone, 2.2-dm. tube: α_D −1°, $[\alpha]^{14}D$ −71.1°.

Anal. Calcd. for $C_{21}H_{22}O_9 \cdot 2H_2O$: C, 55.50; H, 5.72; H_2O, 7.93. Found: C, 55.30; H, 5.57; water of crystallisation (sample dried for 10 hours *in vacuo* at 80°), 7.99.

Verecundin Acetate.—Two-tenths gram of verecundin was treated with 3 ml. of pyridine and 3 ml. of acetic anhydride in the cold for 24 hours. Cold water was then added and the solidified mass was filtered, washed and recrystallized from methanol. The acetate was obtained as colorless needles of m.p. 191°, yield 0.2 g.

Anal. Calcd. for $C_{31}H_{32}O_{14}$: C, 59.23; H, 5.09. Found: C, 58.88; H, 5.07.

Verecundin Monomethyl Ether.—Two-tenths gram of verecundin in 30 ml. of acetone was added with an ethereal solution of diazomethane prepared from 2 ml. of nitrosomethylurethan. After 24 hours standing, to the ether solution was added 250 ml. of petroleum ether. The precipitate thus obtained was extracted with cold water and then with warm ether. The residue was dissolved in hot water, and then allowed to stand overnight. To the turbid solution, ether was added to clarify the solution, when crystals gradually appeared. The crystals were recrystallized by the above method and colorless needles of m.p. 98° (decomposed at 123°) were obtained. The yield was very scanty. In methanol it gave no coloration with ferric chloride.

Anal. Calcd. for $C_{21}H_{21}O_8(OCH_3)$: OCH_3, 7.17. Found: OCH_3, 7.12.

Hydrolysis.—Verecundin (1.8397 g.), 100 ml. of 5% hydrochloric acid and 70 ml. of acetone were heated on a water-bath for 5 hours. The acetone was then evaporated, and the solution was heated on a flame for 30 minutes. After cooling, the precipitate was filtered; yield 1.0 g.

The mother liquor was extracted with ether. After evaporation of ether, the residue and the aglycone obtained above were recrystallized from dilute methanol to give white needles of pinocembrin of m.p. 198°. The acetate of this aglycone was obtained as colorless needles of m.p. 122°.

The melting point of this aglycone did not show any depression when mixed with authentic pinocembrin.

The mother liquor freed from the aglycone was diluted up to 200 ml. with water. In this solution 0.6335 g. of glucose was found, according to Bertrand's method. If the ratio of pinocembrin to glucose is postulated as 1:1, the theoretical yield of glucose would be 0.7293 g. The residual solution was concentrated in a vacuum desiccator over KOH granules and then examined chromatographically. Glucose was the only sugar found.

Isosakuranin.—After repeated recrystallisation from 70% methanol, isosakuranin melted at 190°. This glycoside proved to be identical with isosakuranin obtained previously[4] as compared chromatographically and by mixed melting test.

Anal. Calcd. for $C_{21}H_{21}O_9(OCH_3)$: OCH_3, 6.92. Found: OCH_3, 6.87.

Acknowledgment.—We wish to thank Dr. Masataka Ohmasa of the Government Forest Experiment Station and Prof. Shizuo Hattori of the University of Tokyo for their advice given during this investigation. We are also indebted to Mr. Teizo Maeda of the Government Forest Experiment Station for supplying the wood material, to Prof. H. Erdtman of the Royal Institute of Technology, Stockholm, for generously sending us a specimen of pinocembrin, and to Mr. H. Matsuda for giving us a specimen of genistein. We are also grateful to Prof. Simon H. Wender of the University of Oklahoma for his kindness in revising our manuscript.

MEGURO, TOKYO

[CONTRIBUTION FROM THE MERCK SHARP AND DOHME RESEARCH LABORATORIES]

Cortical Steroids Substituted at C-12[1]

BY D. TAUB, R. D. HOFFSOMMER AND N. L. WENDLER

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The preparation of a number of corticosterone and 11-dehydrocorticosterone derivatives substituted at C-12 is described.

Striking effects on physiological activity have been produced in the adrenal cortical steroids by introduction of substituents at C-9α.[2] In this paper we wish to report the preparation of analogs of corticosterone and 11-dehydrocorticosterone substituted at the alternate α-position, C-12.[3]

The key intermediate in our synthetic route, 11β,12β-oxido-Δ^4-pregnene-21-ol-3,20-dione acetate (Vb) was obtained in 35–40% yield by a five-step sequence from the known 12α-bromo-11-dehydrocorticosterone acetate (I).[4,5] Conversion of the latter into the corresponding 3,20-disemicarbazone II followed by lithium borohydride reduction gave the 3,20-disemicarbazone of 12α-bromocorticosterone (III).[6] Reduction of the hindered 11-carbonyl group of II without concomitant reductive loss of bromine was the crucial step of the sequence and was best accomplished with excess lithium borohydride in tetrahydrofuran at 0° for 6.5 hr.[7] Removal of the semicarbazide residues

(1) A preliminary account of this work was communicated earlier: D. Taub, R. D. Hoffsommer and N. L. Wendler, THIS JOURNAL, **78**, 2912 (1956).

(2) (a) J. Fried and E. F. Sabo, *ibid.*, **75**, 2273 (1953); (b) **76**, 1455 (1954); (c) J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1068 (1955); (d) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett and M. Tishler, *ibid.*, **77**, 3166 (1955); (e) J. Fried, K. Florey, E. F. Sabo, J. E. Herz, A. R. Restivo, A. Borman and F. M. Singer, *ibid.*, **77**, 4181 (1955); (f) A. Nobile, W. Charney, P. L. Perlman, H. L. Herzog, C. C. Payne, M. E. Tully, M. A. Jevnik and E. B. Hershberg, *ibid.*, **77**, 4184 (1955); (g) J. A. Hogg, F. H. Lincoln, A. H. Nathan, A. R. Hanze, W. P. Schneider, P. F. Beal and J. Korman, *ibid.*, **77**, 4438 (1955); (h) E. Vischer, C. Meystre and A. Wettstein, *Helv. Chim. Acta*, **38**, 1502 (1955).

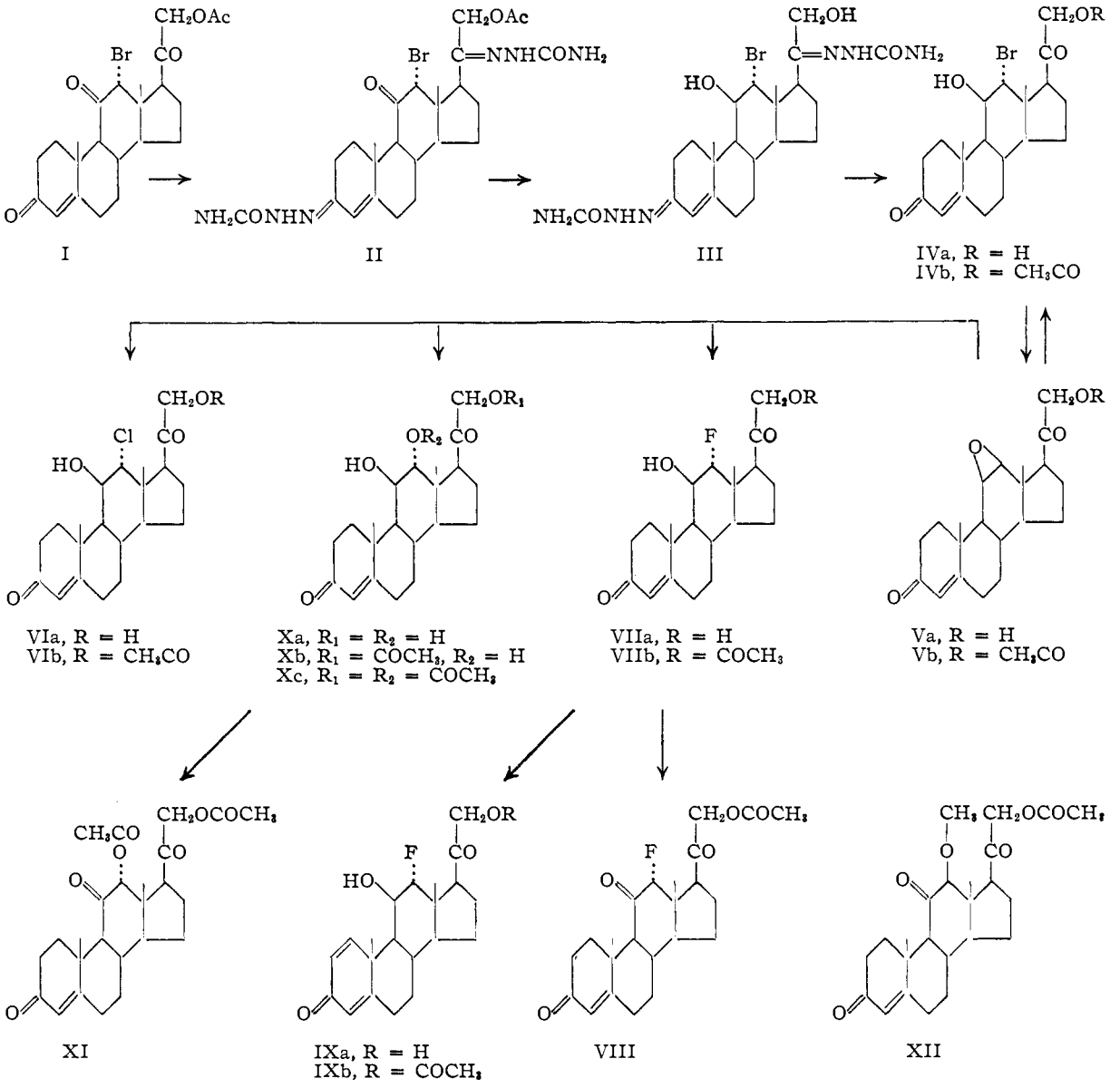
(3) J. E. Herz, J. Fried and E. F. Sabo [THIS JOURNAL, **78**, 2017 (1956)] have communicated the preparation and properties of the 12α-halo derivatives of 11β-hydroxyprogesterone.

(4) V. R. Mattox and E. C. Kendall, *J. Biol. Chem.*, **188**, 287 (1951).

(5) Other starting materials that have been utilized for the preparation of 11β,12β-oxides include the corresponding Δ^{11}-olefins ((a) G. H. Ott and T. Reichstein, *Helv. Chim. Acta*, **26**, 1799 (1943); see also reference 3) and 11α-bromo-12-ketones [(b) J. Schmidlin and A. Wettstein, *ibid.*, **36**, 1241 (1953); (c) J. W. Cornforth, J. M. Osbond and G. H. Phillipps, *J. Chem. Soc.*, 907 (1954)].

(6) The 21-acetate function is cleaved during the reaction.

(7) This procedure is a modification of that utilized by N. L. Wendler, Huang-Minlon and M. Tishler [THIS JOURNAL, **73**, 3818 (1951)] for reduction of the 11-carbonyl group. Under the stated conditions, 15% loss of the 12α-bromo group occurred. Shortening the reaction time resulted in incomplete reduction of the 11-carbonyl function, while lengthening the time or raising the temperature led to increased loss of bromine (see Experimental). By contrast, the 11α-bromo-12-carbonyl system (11α,23ϵ-dibromohecogenin acetate) studied by



of III by the pyruvic acid–aqueous acetic acid procedure[8] gave 12α-bromocorticosterone (IVa), which was converted to the 11β,12β-oxide Vb by potassium hydroxide in methanol[5a,c] followed by acetylation.

It was found that the oxide function of Vb was opened by reagents HX to give products of the diaxial (11β-OH, 12α-X) configuration.[5b,c,9] Conversion of Vb to the bromhydrin IVb, which was identical with a specimen prepared by acetylation of IVa, in turn derived from the authentic 12-bromo-11-ketone I,[4] proved the hydroxyl and bromo groups in IVa and IVb to be at C-11 and C-12, respectively. The ready reversibility of the transformation IV $\rightleftarrows$ V is strong evidence for the *trans* diaxial configuration (11β-OH, 12α-Br) for the bromohydrin grouping. The 11β-configuration for the hydroxyl group is in accord with its generation by lithium borohydride reduction of an 11-carbonyl function and its resistance to acetylation by acetic anhydride–pyridine. Furthermore, the bromine atom in the 11-ketone I must also have the 12α-configuration since I is obtained from IVb by dichromate oxidation. These observations are in agreement with those in the 11-oxygenated 12-bromocholanic acid series which led to similar conclusions.[5a,10]

Reaction of the oxide Vb with concentrated hydrochloric acid–chloroform at 25° gave 12α-chlorocorticosterone 21-acetate (VIb) as well as 12α-chlorocorticosterone (VIa) formed by partial hydrolysis of the acetate function *in situ*. Chro-

Schmidlin and Wettstein (ref. 5b) lost at most 4% bromine when treated with excess lithium borohydride under similar conditions for 10.5 hr.

(8) W. F. McGuckin and E. C. Kendall, THIS JOURNAL, **74**, 5811 (1952).

(9) A. Fürst and Pl. A. Plattner, **Abs. Papers, 12th Int. Cong. Pure and Appl. Chem., New York, N. Y., 1951, p. 409.**

(10) V. R. Mattox, R. B. Turner, B. F. McKenzie, L. L. Engel and E. C. Kendall, *J. Biol. Chem.*, **173**, 283 (1948).

matography on neutral alumina readily separated the two components. 12α-Fluorocorticosterone 21-acetate (VIIb) was obtained by treatment of Vb with hydrogen fluoride in chloroform in the presence of tetrahydrofuran. The conditions used are essentially those of a recent modification by Hirschmann, *et al.*,[11] of Fried and Sabo's procedure for hydrofluorination of the 9β,11β-oxide function.[2b] 12α-Fluorocorticosterone (VIIa) was obtained from its 21-acetate VIIb by methanolysis with sodium methoxide–methanol; under these conditions there was no tendency to reform the 11β,12β-oxide group by loss of hydrogen fluoride. Oxidation of the 11β-hydroxyl group of VIIb by sodium dichromate in acetic acid afforded 12α-fluoro-11-dehydrocorticosterone acetate (VIII). Microbial dehydrogenation of VIIb at positions 1:2 utilizing *Bacillus sphaericus*[12] proceeded without difficulty to give 1-dehydro-12α-fluorocorticosterone (IXa), which was converted to the 21-acetate IXb on acetylation.

Hydrolytic fission of the 11β,12β-oxide Vb with aqueous perchloric acid in dioxane resulted in the formation of 12α-hydroxycorticosterone (Xa). Mild acetylation (acetic anhydride–pyridine 25° for 16 hr.) of the latter gave the 12α,21-diacetate Xc contaminated with a minor amount of a more polar component, probably the 21-monoacetate Xb, readily separable from the diacetate Xc by chromatography. 12α,21-Diacetoxy-11-dehydrocorticosterone (XI) was obtained from Xc by chromic oxide–acetic acid oxidation.

The activities of several of the above compounds and their 9α-substituted analogs in the mouse oral liver glycogen assay relative to hydrocortisone acetate are tabulated below. In this assay corticosterone has 0.4 the activity of hydrocortisone acetate.

Parent steroid	Substituent	12α	9α
11-Dehydrocorticosterone acetate	Br	<0.1	<0.1
Corticosterone acetate	Cl	0.2	
Corticosterone acetate	F	1	1
Corticosterone	F	3.5	3
Corticosterone	OH	<0.1	<0.1
Δ¹-Corticosterone acetate	F	5	
Δ¹-Corticosterone	F	3–10	3–10

The results indicate that 12α-substitution is equivalent to 9α substitution with regard to effect on glucocorticoid activity. With respect to mineralocorticoid activity, the available data indicate the 12α-fluorocompounds to be strong sodium retainers, but probably not as potent as the corresponding 9α-fluoro analogs in the adrenalectomized rat and dog.[13,14] The 11β,12β,-oxide Vb was devoid of both glucocorticoid and mineralocorticoid activity.

(11) R. F. Hirschmann, R. Miller, J. Wood and R. E. Jones, THIS JOURNAL, **78**, 4956 (1956).

(12) T. H. Stoudt, W. J. McAleer, J. M. Chemerda, M. A. Kozlowski, R. F. Hirschmann, V. Marlatt and R. Miller, *Arch. Biochem. and Biophys.*, **59**, 304 (1955). We are indebted to Dr. Stoudt of these laboratories for his aid in this procedure.

(13) J. Herz, J. Fried and E. F. Sabo (ref. 3) found 12α-fluoro-11β-hydroxyprogesterone to have glucocorticoid and mineralocorticoid activity equivalent to that of 9α-fluoro-11β-hydroxyprogesterone.

(14) We are indebted to Drs. Winter, Porter, Stoerk and Watson of the Merck Institute for Therapeutic Research for the physiological assays.

Experimental[15]

3,20-Disemicarbazone of 12α-Bromo-11-dehydrocorticosterone Acetate (II).[16]—12α-Bromo-11-dehydrocorticosterone acetate (I) (23.6 g., 0.0507 mole) was dissolved in 500 ml. of methanol and 120 ml. of dimethylformamide and the air displaced by nitrogen. A slurry of semicarbazide hydrochloride (27.8 g., 0.248 mole) and sodium bicarbonate (15.1 g., 0.180 mole) in 30 ml. of water was added to the stirred reaction mixture, which was refluxed 3.5 hr. and then kept at 45° for 16 hr. The disemicarbazone was precipitated by cooling followed by slow addition of 2.0 l. of 50% saturated aqueous sodium chloride. The chilled precipitate was filtered, washed with water until the washes were free of chloride ion and dried to give 28.7 g. (98%) of 3,20-disemicarbazone II, m.p. >300° (gradually darkened between 250–300°); λ_{max} 269 mμ (30,300), shoulder 245–250 mμ (25,200). The infrared spectrum indicated the presence of the 21-acetate function (λ_{max} 5.78, 8.1 μ). The tetrazolium test for the ketol side chain was negative.

Anal. Calcd. for $C_{25}H_{35}O_5N_6Br$: N, 14.50; Br, 13.80. Found: N, 14.86; Br, 13.67.

3,20-Disemicarbazone of 12α-Bromocorticosterone (III).—The 3,20-disemicarbazone II (27.5 g., 0.0475 mole) was taken to dryness in benzene to remove traces of moisture and was dissolved in 2.25 l. of dry tetrahydrofuran. The air was displaced by nitrogen and the solution cooled to −5°. A solution of 8.3 g. of lithium borohydride in 590 ml. of dry tetrahydrofuran (filtered through Celite) was added during 2.5 hr. to the vigorously stirred steroid solution maintained at −2°. The reaction mixture was kept at 0° or slightly below an additional 4 hr. Following cautious addition of cold aqueous acetic acid (60 ml. of acetic acid and 475 ml. of water) to the stirred reaction mixture at 0°, the tetrahydrofuran was completely removed *in vacuo*. Water (200 ml.) was added and the thick slurry cooled, filtered and washed with water. The crude air-dried reduced disemicarbazone III weighed 26.7 g. (96.5%), m.p. >300° (darkening 200–300°); λ_{max} 268 mμ, $E^{1\%}_{1cm}$ 535, shoulder 240–245 mμ $E^{1\%}_{1cm}$ 404. The infrared spectrum indicated the absence of acetate. Bromide ion analysis of an aliquot of the aqueous mother liquor indicated 15% reductive loss of the 12α-bromine atom.

In a preliminary experiment when the $LiBH_4$ reduction was allowed to proceed 18 hr., 40% of the 12α-bromine atom was lost, and in the chromatography at the 11β,12β-oxide V stage (see below) corticosterone acetate, m.p. 145–146° (identical with authentic material), was obtained as a by-product.

When the reduction was carried out for 3 hr., there was isolated at the end of the sequence a by-product more mobile on alumina than the 11β,12β-oxide Vb. It had m.p. 171–172°, $[\alpha]_D$ + 249°, λ_{max} 238 mμ (16,200); λ^{Nujol}_{max} 5.72, 5.79, 5.85, 6.00 and 6.18 μ.

Anal. Calcd. for $C_{24}H_{32}O_6$: C, 69.20; H, 7.75; OCH_3, 7.45; $COCH_3$, 10.34. Found: C, 69.25; H, 7.71; OCH_3, 8.04; $COCH_3$, 10.70.

This substance provisionally formulated as **12β-methoxy-11-dehydrocorticosterone acetate (XII)** may be obtained directly from 12α-bromo-11-dehydrocorticosterone acetate (I) by treatment with methanolic potassium hydroxide at 25° followed by acetylation.

12α-Bromocorticosterone (IVa).—The total reduced 3,20-disemicarbazone III (26.7 g.) was dissolved in 350 ml. of acetic acid, 60 ml. of pyruvic acid and 130 ml. of water (see ref. 8). After 16 hr. at 25° 1 l. of water was added and the mixture was extracted 5 times with chloroform. The chloroform extract was washed with water, aqueous potassium bicarbonate and saturated sodium chloride solution. Drying over magnesium sulfate and removal of the solvent

(15) Melting points were taken on a micro hot-stage apparatus and are corrected. Ultraviolet spectra were determined in methanol, infrared spectra in chloroform or as Nujol mulls and optical rotations in chloroform (*c* = 0.5–1.0). Paper chromatograms were run on strips of Whatman No. 1 filter paper using the benzene–formamide system [A. Zaffaroni, R. B. Burton and E. H. Keutmann, *Science*, **111**, 6 (1950)]. We are indebted to R. N. Boos and associates for the microanalyses, F. W. Bacher and associates for the ultraviolet spectra and R. W. Walker for the infrared spectra.

(16) The procedure is essentially that of R. E. Jones and S. A. Robinson, *J. Org. Chem.*, **21**, 586 (1956).

gave an amorphous residue which crystallized when triturated with methanol to give 13.6 g. (70%) of 12α-bromocorticosterone (IVa). Recrystallization from acetone–hexane gave rectangular plates, m.p. 215–219° dec., $[\alpha]_D$ + 126°, λ_{max} 240 mμ (16,300); λ_{max}^{chf} 2.90, 5.87, 6.02 and 6.18 μ.

Anal. Calcd. for $C_{21}H_{29}O_4Br$: C, 59.32; H, 6.87. Found: C, 59.75; H, 6.59.

The non-crystalline mother liquors (4.77 g., 24.5%) were treated as described below.

Δ⁴-Pregnene-11β,12β-oxido-21-ol-3,20-dione Acetate (Vb).—Crude crystalline 12α-bromocorticosterone (IVa, 13.0 g.) was dissolved in 190 ml. of methanol and the air displaced by nitrogen. A solution of 5.2 g. of potassium hydroxide in 2.5 ml. of water and 50 ml. of methanol was added under nitrogen with stirring with the temperature maintained at 25°. After 30 minutes the reaction mixture was cooled to 15°, and 5.2 ml. of acetic acid in 30 ml. of water was added. Most of the methanol was removed on the water-pump and the mixture partitioned between ethyl acetate and water. Ethyl acetate extraction gave Δ⁴-pregnene-11β,12β-oxido-21-ol-3,20-dione (Va) as a non-crystalline bromine-free residue, which was acetylated in 20 ml. of pyridine and 15 ml. of acetic anhydride at 25° for 16 hr. The reaction mixture was chilled and cold water was added slowly followed by chloroform extraction. The chloroform extract was washed with dilute hydrochloric acid, aqueous potassium bicarbonate and water. The residue (12.3 g.) crystallized from ether to give 6.32 g. (54%) of 11β,12β-oxide acetate Vb, m.p. 165–171°. Recrystallization from ether–acetone gave rectangular prisms, m.p. 172–173°, $[\alpha]_D$ + 192°, λ_{max} 238.5 mμ (17,400); λ_{max}^{Nujol} 5.72, 5.80, 5.99 and 6.17 μ.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.83. Found: C, 71.68; H, 7.70.

Chromatography of the non-crystalline mother liquors (6 g.) on neutral alumina afforded an additional 0.60 g. of oxide acetate Vb, m.p. 170–172°.

Treatment of the 12α-bromocorticosterone (IVa) mother liquors (4.77 g.) with methanolic potassium hydroxide followed by acetylation and chromatography gave an additional 0.50 g. of 11β,12β-oxide Vb, m.p. 170–172°. The over-all yield of Vb from I was 39%.

12α-Bromocorticosterone 21-Acetate (IVb).—To 75 mg. of the 11β,12β-oxide Vb in 1.5 ml. of acetic acid at 15° was added dropwise with stirring 0.3 ml. of 24% anhydrous hydrogen bromide in acetic acid. After 45 minutes at 15°, the reaction mixture was pumped to dryness *in vacuo*. The solid residue was crystallized from acetone–petroleum ether to give 77 mg. of 12α-bromocorticosterone-21-acetate (IVb), m.p. 210–215° dec., $[\alpha]_D$ +145°; λ_{max} 240 mμ (15,600); λ_{max}^{Nujol} 2.90, 5.73 and 5.82 μ.

Anal. Calcd. for $C_{23}H_{31}O_5Br$: C, 59.24; H, 6.68; Br, 17.12. Found: C, 58.61; H, 6.99; Br, 17.26.

Acetylation (acetic anhydride–pyridine 25° for 16 hr.) of 12α-bromocorticosterone (IVa) gave material of identical melting point, mixed melting point and infrared spectrum as IVb prepared from the 11β,12β-oxide Vb.

12α-Bromo-11-dehydrocorticosterone Acetate (I).—To 35 mg. of 12α-bromocorticosterone 21-acetate (IVb) in 1 ml. of acetic acid was added 10 mg. of sodium dichromate dihydrate in 1 ml. of acetic acid. After 2.5 hr. at 25°, water was added, and the mixture was extracted with chloroform. Crystallization of the solid product from acetone–ether gave I as prismatic needles, m.p. 205–208 dec., identical with authentic I by mixed melting point and comparison of infrared spectra.

12α-Chlorocorticosterone (VIa) and 12α-Chlorocorticosterone 21-Acetate (VIb).—To a solution of 100 mg. of the 11β,12β-oxide Vb in 5 ml. of chloroform was added 5 ml. of concentrated hydrochloric acid. The two-phase mixture was stirred at 25° 1 hr. Addition of water and chloroform extraction gave a crude crystalline product (105 mg.) which was found to contain two components by paper chromatography and which was resolved by chromatography on neutral alumina. The benzene and benzene–chloroform eluates gave 12α-chlorocorticosterone acetate (VIb) (63 mg.) as needles, m.p. 166–170°, converted into prisms, m.p. 228–233°, on crystallization from acetone–ether; $[\alpha]_D$ +179°, λ_{max} 240 mμ (15,600); λ_{max}^{Nujol} 2.87, 5.70, 5.80, 6.01 and 6.15 μ.

Anal. Calcd. for $C_{23}H_{31}O_5Cl$: C, 65.35; H, 7.39; Cl, 8.38. Found: C, 65.28; H, 7.62; Cl, 8.61.

The paper chromatographic mobility in the benzene–formamide system of 12α-chlorocorticosterone 21-acetate (VIb) was somewhat surprisingly equal to that of 11-dehydrocorticosterone acetate.

From the chloroform and chloroform–2% methanol eluates, there was isolated 28 mg. of 12α-chlorocorticosterone (VIa), prisms from acetone–ether, m.p. 200–205°, $[\alpha]_D$ +163°; λ_{max} 240 mμ (16,400); λ_{max}^{Nujol} 3.00, 5.85, 6.04 and 6.18 μ.

Anal. Calcd. for $C_{21}H_{29}O_4Cl$: C, 66.22; H, 7.67; Cl, 9.31. Found: C, 66.43; H, 7.39; Cl, 9.36.

Acetylation of VIa (acetic anhydride–pyridine 25° for 16 hr.) gave the 21-acetate VIb.

12α-Fluorocorticosterone 21-Acetate (VIIb).—The 11β,12β-oxide Vb (1.200 g.) was dissolved in 12 ml. of chloroform and 13 ml. of dry tetrahydrofuran and the solution cooled to −60° (acetone–Dry Ice-bath). Over a period of 5 minutes this solution was added to 12 ml. of a 2:1 (by weight) mixture of anhydrous hydrogen fluoride and tetrahydrofuran in a polyethylene bottle maintained at −60° (see ref. 11). When addition was complete the bath temperature was raised to −10° for 30 minutes and maintained at −5° for 4 hr.[17] The reaction mixture was then cooled to −60° and by means of a polyethylene pipet cautiously added to a stirred mixture of 200 ml. of 25% aqueous potassium carbonate and 75 ml. of chloroform kept at −5°. The aqueous phase was further extracted with chloroform, the extracts washed with water and saturated sodium chloride solution and dried over magnesium sulfate. The residue crystallized from acetone–ether to give 12α-fluorocorticosterone acetate (VIIb) (640 mg., 51%) as massive rhombs, m.p. 195–200°, raised to 197–200° on recrystallization; $[\alpha]_D$ +200°; λ_{max} 241 mμ (16,600); λ_{max}^{Nujol} 3.02, 5.70, 5.78, 6.06 and 6.15 μ.

Anal. Calcd. for $C_{23}H_{31}O_5F$: C, 67.94; H, 7.71; F, 4.68. Found: C, 68.21; H, 7.67; F, 4.72.

Chromatography of the mother liquors gave an additional 40 mg. (3%) of VIIb.

On paper VIIb was slightly more mobile than 9α-fluorocorticosterone-21-acetate.

12α-Fluorocorticosterone (VIIa) was obtained from VIIb by mild treatment with sodium methoxide in methanol[18]; prisms from ether, m.p. 189–192, λ_{max} 241 mμ (15,500); λ_{max}^{Nujol} 2.92, 3.00, 5.86, 6.05 and 6.17 μ.

12α-Fluoro-11-dehydrocorticosterone Acetate (VIII).—To a stirred solution of 72 mg. of 12α-fluorocorticosterone acetate (VIIb) in 5 ml. of acetic acid at 25° was added a solution of 23 mg. of sodium dichromate in 3 ml. of acetic acid. After 2.5 hr. water was added and the mixture extracted with chloroform. The extract was washed with aqueous potassium bicarbonate, water and saturated sodium chloride and dried over magnesium sulfate. Crystallization of the solid residue from acetone–hexane gave 12α-fluoro-11-dehydrocorticosterone acetate (VIII) as hexagonal prisms (51 mg., 1st crop), m.p. 177–180°, λ_{max} 237 mμ (15,800); λ_{max}^{Nujol} 5.75, 5.81, 5.88, 6.01 and 6.20 μ.

Anal. Calcd. for $C_{23}H_{29}O_5F$: C, 68.30; H, 7.23. Found: C, 68.35; H, 7.45.

Δ¹-12α-Fluorocorticosterone 21-Acetate (IXb).—12α-Fluorocorticosterone acetate (VIIb) (200 mg.) was incubated with *bacillus sphaericus* for 24 hr. at 28° as described in reference 12. A sample of the crude non-crystalline product chromatographed on paper indicated the presence of Δ¹-12α-fluorocorticosterone (IXa) as the major steroid component on the basis of its polarity. The residue was acetylated (acetic anhydride–pyridine, 25°, overnight) and chromatographed on neutral alumina. The benzene–chloroform eluates gave Δ¹-12α-fluorocorticosterone-21 acetate (IXb) as prismatic needles from acetone–hexane, m.p. 218–222°, λ_{max} 242 mμ (15,700); λ_{max}^{Nujol} 3.08, 5.72, 5.78, 6.03, 6.18, 6.23 and 11.10 μ.

Anal. Calcd. for $C_{23}H_{29}O_5F$: C, 68.30; H, 7.23. Found: C, 68.14; H, 7.11.

12α-Hydroxycorticosterone (Xa).—To 200 mg. of 11β,12β-oxide Vb in 3.5 ml. of dioxane was added 1.75 ml. of 2 *M*

(17) The optimum temperature for hydrogen fluoride reaction with Δ⁴-pregnene-9β,11β-oxido-17α,21-diol-3,20-dione 21-acetate was −30°.[11]

(18) Huang-Minlon, E. Wilson, N. L. Wendler and M. Tishler, THIS JOURNAL, 74, 5394 (1952).

perchloric acid. After 3 days at 25° saturated sodium chloride was added and the mixture extracted with chloroform. Paper chromatography of the crude crystalline product showed it to consist of 2 substances both considerably more polar than Vb. The major and more polar component (isolated by fractional crystallization from acetone–ether) was 12α-hydroxycorticosterone (Xa), m.p. 208–212°, $[\alpha]_D$ +194°, λ_{max} 241 mμ (15,900); λ_{max}^{Nujol} 2.90, 3.02, 5.84, 6.08 and 6.18 μ.

Anal. Calcd. for $C_{21}H_{30}O_5$: C, 69.58; H, 8.34. Found: C, 69.97; H, 8.47.

The minor component, m.p. 175–177°, was assigned the structure 12α-hydroxycorticosterone 21-acetate (Xb) on the basis of its polarity.

12α-Hydroxycorticosterone 12α,21-Diacetate (Xc).—Acetylation of 120 mg. of the perchloric acid product (Xa and a minor amount of Xb) in 3 ml. of pyridine and 2 ml. of acetic anhydride for 16 hr. at 25° followed by chromatography on neutral alumina gave 95 mg. of the 12α,21-diacetate Xc, m.p. 221–223°; λ_{max}^{Nujol} 2.90, 5.70, 5.79, 5.98 and 6.16 μ as well as 16 mg. of Xb. Thus the 12α-hydroxyl group appears to be partly resistant to acetylation under these conditions.

12α-Hydroxy-11-dehydrocorticosterone 12α,21-Diacetate (XI).—To 90 mg. of the 11β-hydroxy-12α,21-diacetate Xc in 5 ml. of acetic acid was added 17 mg. of chromic oxide in 0.1 ml. of water and 0.5 ml. of acetic acid. After 16 hr. at 20°, 5% sodium sulfate solution was added and the mixture extracted with chloroform. The solid residue on crystallization from acetone–ether gave the 11-keto-12α,21-diacetate XI as prismatic plates (70 mg., 1st crop), m.p. 171–172°, $[\alpha]_D$ +241°; λ_{max} 238 mμ (16,000); λ_{max}^{Nujol} 5.71, 5.80, 5.97 and 6.12 μ.

Anal. Calcd. for $C_{25}H_{32}O_7$: C, 67.54; H, 7.26. Found: C, 67.29; H, 7.06.

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[CONTRIBUTION FROM THE MERCK-SHARP & DOHME RESEARCH LABORATORIES]

The Transformation of Stigmasterol to 17α-Hydroxypregnenolone and 17α-Hydroxyprogesterone

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The transformation of stigmasterol to 17α-hydroxypregnenolone and 17α-hydroxyprogesterone is described. Stigmasterol acetate is hydrochlorinated to 5α-chlorostigmasterol acetate which on ozonization gives 3β-acetoxy-5α-chlorobisnorcholan-22-al. This aldehyde on bromination and dehydrobromination at C-20 gives 3β-acetoxy-5α-chloro-17(20)-bisnorcholen-22-al. The α,β-unsaturated aldehyde on treatment with excess peracid gives 3β-acetoxy-5α-chloro-17,20-epoxypregnan-20-ol 20-formate which compound on alkaline hydrolysis gives 17α-hydroxypregnenolone. Acid hydrolysis of the epoxy formate followed by oxidation with chromic acid–pyridine complex and dehydrohalogenation with base gives 17α-hydoxyprogesterone.

The importance of bio-oxidative methods for the introduction of oxygen into ring C of steroids[1] has focused attention on the synthesis of substrates (*e.g.*, progesterone, Compound S) for this oxidation. The most abundant raw materials for this purpose are stigmasterol, ergosterol and diosgenin. Several simplified and improved methods of preparation of progesterone and pregnane-3,20-dione from the first two sterols have been reported.[2] A more valuable substrate than progesterone or pregnane-3,20-dione would result from a degradation procedure which resulted in the introduction of the 17α-hydroxy group in the final product without materially increasing the number of steps in the process. This paper describes such a procedure utilizing stigmasterol as a raw material. As a final product either 17α-hydroxypregnenolone or 17α-hydroxyprogesterone may be obtained. The reaction scheme is formulated in the flow sheet.

This scheme required the C-22 aldehyde from stigmasterol with the 5,6-double bond protected. In the usual ozonization of stigmasterol to obtain the C-22 aldehyde the 5,6-double bond is protected as the 5,6-dibromide. Since subsequent decomposition of the ozonide by zinc dust removes the bromine, this practice was not useful for our purpose. Although the 5,6-dichloro derivative of stigmasterol acetate offered a more attractive prospect, it proved impracticable to test since partial chlorination could not be achieved. It was found that the 5α-chloro compound I obtained by the addition of hydrogen chloride to stigmasterol acetate was stable to the zinc dust treatment for the decomposition of the ozonide and this compound I represents the key intermediate in the degradation.

5α-Chlorostigmasterol acetate (I) had been prepared previously by Ruzicka, Fischer and Meyer[3] by hydrochlorination in ether. The low solubility of stigmasterol acetate in ether is disadvantageous. Hydrochlorination proceeds slowly in chloroform because of the low solubility of hydrogen chloride. Finally a mixture of chloroform–ethanol saturated with hydrogen chloride at 5–10° was found to be an excellent medium for the hydrochlorination of stigmasterol acetate in good, reproducible yields. During the 70-hour period at room temperature, partial deacetylation is effected by ester interchange and a reacetylation is required. The steric orientation of the chlorine atom to the 5α (axial) position was assigned by analogy with Barton's[4] results on the stereochemistry of the ionic addition of chlorine to cholesterol acetate. Fur-

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