[CONTRIBUTION FROM THE RESEARCH LABORATORIES, CHEMICAL DIVISION, MERCK & CO., INC., AND THE SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH]

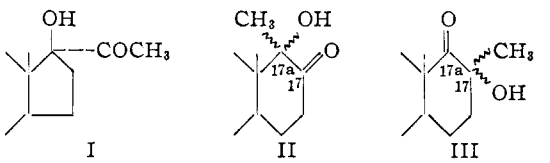
The Structure and Synthesis of D-Homosteroids[1]

BY N. L. WENDLER,[2] D. TAUB,[2] S. DOBRINER[3] AND D. K. FUKUSHIMA[3]

RECEIVED APRIL 2, 1956

The isolation and partial synthesis of the four possible D-homo structures derivable from 3α-acetoxy-17α-hydroxypregnane-11,20-dione permit unequivocal assignment of structure and configuration to the various D-homo systems.

The substances arising from D-homoannulation of 17β-hydroxy-20-keto steroids (I) were originally formulated as 17a-hydroxy-17a-methyl-17-keto systems (II) by Ruzicka and Meldahl[4] principally on the basis of transformations leading to 1-methylchrysene. Later Shoppee and Prins[5] demonstrated that the alkali- and acid-produced isomers were epimers by oxidation of the respective ketols to the same keto acid. More recently Turner,[6] in proposing a mechanism for the formation of the epimeric systems, extended conclusions from the earlier work to include the D-homo compounds arising from 17α-hydroxy-20-keto steroids as expressed by II.[7]



I II III

At the inception of the present work[1a] it was felt that the earlier structure proof was not entirely unambiguous since, among others, the transformations leading to 1-methylchrysene might possibly have involved rearrangement of the original ketol system.[8,9] In the meantime, independent proof for the structure and configuration of the products of D-homoannulation of 17β-hydroxy-20-keto steroids has been provided,[10] and they have been shown to conform to the 17-keto system (type II) as set forth by the original workers.[4,5] In the present investigation it was observed that in contrast to the 17β-hydroxy-20-keto steroids, the Lewis acid D-homoannulation of the epimeric 17α-hydroxy-20-keto compounds yields predominantly 17a-ketones of type III. This latter finding was also made independently in another series and reported recently.[11] The present account further describes the partial synthesis of the four possible D-homo systems arising from 3α-acetoxy-17α-hydroxypregnane-11,20-dione (IV) and corresponding to part structures II and III. This makes possible direct structural assignment to any individual in this particular compound series and by inference to any product of D-homoannulation of a 17-hydroxy-20-keto steroid.

D-Homoannulation of 3α-acetoxy-17α-hydroxypregnane-11,20-dione (IV) with boron trifluoride in acetic anhydride and acetic acid produced as the major isolable product the 17a-ketone V; there also was isolated a few per cent. of 3α,17α-diacetoxypregnane-11,20-dione.[12] Similarly, rearrangement of IV with aluminum *t*-butoxide in toluene followed by partition chromatography on a silica gel column afforded the 17a-ketone V as the major product together with a minor amount of the 17-ketone IX. The structure of the 17a-ketone V was assigned on the basis of its characteristic infrared absorption bands (see Experimental) and molecular rotation difference which paralleled those observed in the 11-desoxo series.[11] This substance further failed to give a Zimmermann reaction[13] or to form a benzylidene derivative[1b] characteristic of α-methylenic ketones.[13] Proof of structure of the 17a-ketone V was obtained by degradation and resynthesis in the following manner. Treatment of the 17a-ketone V with methanesulfonyl chloride in pyridine produced the corresponding non-crystalline methanesulfonic ester together with a crystalline isomeric substance.[14] The amorphous mesylate derivative was converted to the $\Delta^{\alpha\beta}$-ketone VI by refluxing with sodium iodide in acetone solution containing pyridine. The exact character of the $\Delta^{\alpha\beta}$-ketone was established

(1) Preliminary accounts of this work were communicated earlier: (a) N. L. Wendler and D. Taub, *Chem. & Ind.*, 505 (1955); (b) N. L. Wendler, D. Taub, D. K. Fukushima and S. Dobriner, *ibid.*, 1259 (1955).

(2) Merck & Co., Inc., Rahway, N. J.

(3) The Sloan-Kettering Institute for Cancer Research, New York, N. Y. That portion of the work done at the Sloan-Kettering Institute for Cancer Research was supported in part by an Institutional Grant from the American Cancer Society and Grant C-440 from the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

(4) L. Ruzicka and H. Meldahl, *Helv. Chim. Acta*, **23**, 364 (1940).

(5) C. Shoppee and D. A. Prins, *ibid.*, **26**, 201 (1943).

(6) R. B. Turner, THIS JOURNAL, **75**, 3484 (1953).

(7) It is now recognized that the D-homo system obtained by treatment of Reichstein's substance L with boron trifluoride was incorrectly formulated by Turner (reference 6) as a 17-ketone (type II). Recent developments (reference 1b, 11) including the present work make it apparent that this substance should be formulated as a 17a-ketone (type III). Dr. Turner (private communication) has acknowledged this point and indicated independent work supporting this conclusion.

(8) Compare for example: D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Sommers, *J. Chem. Soc.*, 2876 (1955).

(9) In the early communication on this subject (ref. 1a) the rearrangement II ⇄ III was believed actually to have been realized. The apparent demonstration of this interconversion was later shown to be at fault owing to the anomalous behavior of one of the compounds in question (*cf.* ref. 1b).

(10) R. B. Turner, R. Anliker, R. Helbling, J. Meier and H. Heusser, *Helv. Chim. Acta*, **38**, 411 (1955).

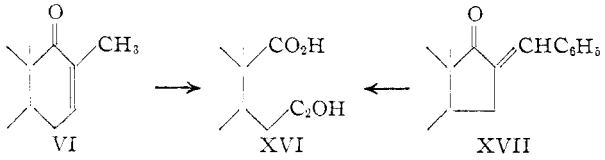
(11) D. K. Fukushima, S. Dobriner, M. S. Heffler, T. H. Kritchevsky, F. Herling and G. Roberts, *Federation Proc.*, **14**, 216 (1955); THIS JOURNAL, **77**, 6585 (1955).

(12) In his description of this reaction as applied to Reichstein's Substance L, Turner (ref. 6) excluded the possibility of acetylation at C_{17}.

(13) The somewhat hindered C_{12}-methylene group in the 11-keto systems under discussion does not respond to the Zimmermann reaction and does not form benzylidene derivatives.

(14) The nature of this isomeric individual and other anomalous reactions of V will be discussed elsewhere. Its appearance in 25% yield from the mesylation reaction together with other anomalous reactions of the 17a-ketone (V) served to confuse the initial interpretation of the formation of the $\Delta^{\alpha\beta}$-ketone (*cf.* ref. 1).

by permanganate oxidation to 3α-acetoxy-11-ketoetiobilianic acid XVI, identical with a sample obtained by oxidation of the benzylidene derivative XVII prepared from known 3α-acetoxyetiocholane-11,17-dione.[14a]

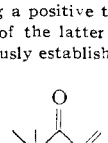


VI XVI XVII

The $\Delta^{\alpha\beta}$-ketone VI readily afforded the α-oxido ketone VII with either perbenzoic acid in benzene or, preferably, alkaline hydrogen peroxide. This oxido ketone VII was opened with hydrogen bromide to the bromohydrin VIII which could be reconverted with alkali to the oxido ketone. The bromohydrin VIII yielded the starting 17a-D-homo ketone V on reductive debromination in the presence of palladium on barium carbonate. This sequence of reactions provides a partial synthesis of V thereby confirming its structure. The α-orientation of the 17-hydroxyl function of V is inferred from the established steric course of reactions at similar double-bonded positions in the steroid nucleus.[15]

A significant feature of the above series of transformations is the directional opening of the oxido ketone VII with hydrogen bromide to give the 16β-bromo-17α-hydroxy ketone VIII having a diequatorial orientation of bromine and hydroxyl functions. Previous experience has indicated that isolated oxides generally open to give products having their functional substituents axially oriented.[16] The explanation for this directional course of oxide scission is presumably to be found in an interpretation of the transition state involved.[17] On the other hand, in the case of the α-oxido ketone VII the directional course of oxide opening may be restricted by the carbonyl function to β-scission. This presumably results from the fact that α-scission would lead to poorly stabilized transitional intermediates involving charge deficiency at an α-carbon adjacent to the positive end of a carbonyl dipole.[18]

(14a) NOTE ADDED IN PROOF.—Ozonolysis of VI (3α-OH) according to the procedure of D. H. R. Barton and P. de Mayo (*J. Chem. Soc.*, 142 (1956)) yielded acetic acid isolated as its *p*-bromophenacyl ester. Further, ozonolysis of VI afforded an amorphous acid soluble in potassium bicarbonate and giving a positive test with 2,4-dinitrophenylhydrazine reagent; oxidation of the latter with permanganate afforded XVI. These findings rigorously establish structure VI and exclude the exomethylenic possibility



(15) See, for example: (a) T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950); (b) N. L. Wendler and H. L. Slates, *Chem. & Ind.*, 167 (1955); also ref. 10.

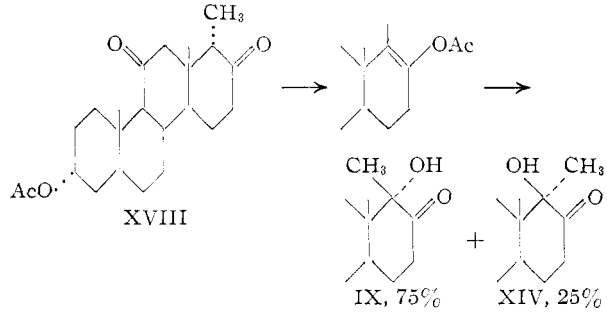
(16) A. Fürst and Pl. Plattner, *Helv. Chim. Acta*, **32**, 275 (1949); Abs. Papers, 12th Internat. Cong. Pure Appld. Chem., New York, 1951, p. 409.

(17) See, for example: E. J. Corey, THIS JOURNAL, **76**, 175 (1954).

(18) In this connection J. I. Shaw and R. Stevenson (*J. Chem. Soc.*, 3549 (1955)) have interpreted the conversion of 4,5β-oxido-coprostan-3-one to 4-bromocholestenone as proceeding *via* a *trans*-axial bromohydrin. This interpretation is at variance with our own experience as exemplified in the case of the oxido ketone VII.

The minor product from the Lewis acid D-homoannulation of I was the 17aα-hydroxy isomer IX. This substance, unlike V, gave a positive Zimmermann test for an α-methylenic ketone and afforded a benzylidene derivative X, m.p. 240–246°, (λ_{max} 288 mμ (16,600)).[19]

Partial synthesis of IX was achieved by epoxidation of the Δ^{17}-enol acetate derivative of 3α-acetoxy-17aα-methyl-D-homoetiocholane-11,17-dione (XVIII). By this route there was obtained a 3:1 mixture of the 17aα-hydroxy isomer IX and the 17aβ-hydroxy isomer XIV which were separated by paper partition chromatography into the pure compounds. The preponderance of the 17aα-hydroxy isomer IX is consistent with this configurational assignment by virtue of previous experience with epoxidation of steroid double bonds.[15]



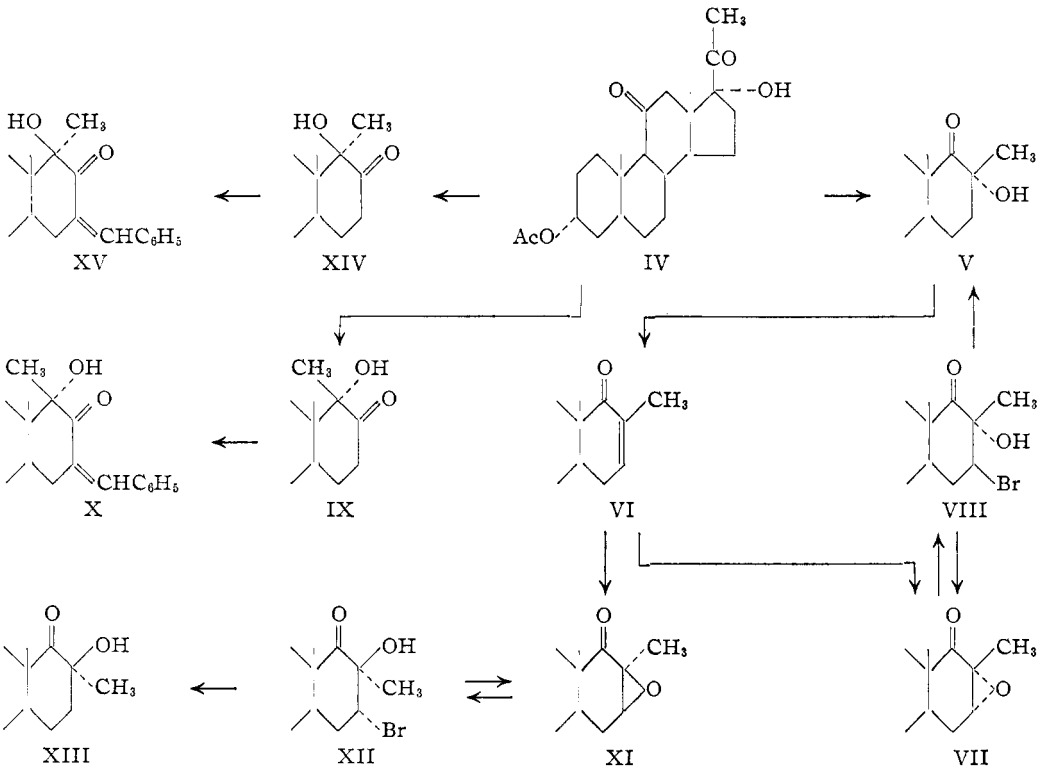
XVIII IX, 75% XIV, 25%

The addition of hypobromous acid to the $\Delta^{\alpha,\beta}$-ketone VI afforded an oily bromohydrin (16β-OH/17α-Br) which was converted smoothly by alkali to the crystalline β-oxide XI.[15b] The latter in turn gave a crystalline isomeric bromohydrin XII on treatment with hydrogen bromide in acetic acid. Reductive debromination of the latter bromohydrin XII afforded the 17β-hydroxy-17a-ketone XIII epimeric with V and an isomer as yet never isolated by direct D-homoannulation of a 17-hydroxy-20-keto steroid. This isomer, XIII, like its 17α-hydroxy epimer V did not give a Zimmermann reaction nor form a benzylidene derivative.

D-Homoannulation of 3α-acetoxy-17α-hydroxypregnane-11,20-dione IV with alkali followed by acetylation afforded as the main product the 17aβ-hydroxy ketone XIV together with a lesser amount of the 17aα-hydroxy epimer IX. The 17aβ-hydroxy ketone XIV gave a positive Zimmermann reaction and formed a benzylidene derivative, m.p. 220–222° (λ_{max} 285 mμ (23,300)); the structure of XIV was established, as already described, by partial synthesis from XVIII thereby completing the synthesis and correlation of the four D-homo ketols conforming to part-structures II and III.

The infrared spectra of the four D-homo ketols (see Experimental) reveal the interesting relationship that the two ketols, V and XIV, with equa-

(19) In comparison with the benzylidene derivative of the 17aβ-hydroxy epimer XV (see below), the derivative X exhibits a somewhat lower intensity. This spectral difference is not inconsistent with a factor of steric hindrance operative in X. Examination of models, in fact, shows interaction between the C_{13}- and C_{17a}β-methyl groups tending to force the Δαβ-chromophore out of the plane of the ring system.



torial hydroxy groups, exhibit a single band in the OH region at *ca.* 3500 cm.$^{-1}$ (2.85 μ), whereas the two ketols IX and XIII with axial hydroxyl functions show hydrogen bonded absorption in the OH region at 3600 cm.$^{-1}$ (2.78 μ) and association at *ca.* 3500–3340 cm.$^{-1}$ (2.85–2.9 μ).[20,21] This infrared spectral relationship may prove to be quite useful for configurational assignment in ketol systems.[22]

Acknowledgments.—The authors (N. L. W. and D. T.) express their appreciation to R. W. Walker for infrared spectral determinations and to R. N. Boos for analytical results. The authors (D. K. F. and S. D.) are grateful to Dr. T. F. Gallagher for his interest and support of their work and to Friederike Herling for the determination of infrared spectra.

(20) Dr. Ch. Tamm of the University of Basel, Switzerland, has informed us that he, too, has observed a similar relationship in the infrared for 11,12-ketols in the cholanic acid series.

(21) The significant difference in the position of the absorption maxima of the 17a C=O at 1700 cm.$^{-1}$ (5.88 μ) and the 17 C=O at 1720 cm.$^{-1}$ (5.82 μ) clearly evident in the 11-desoxo series[11] is largely masked in the present series because of absorption of the 11 C=O.

(22) In the light of the present findings the rearrangement of Reichstein's Substance "S" acetate, hydrocortisone acetate and cortisone acetate with boron trifluoride and aluminum isopropoxide [see V. Georgian and N. Kundu, *Chem. and Ind.*, 431 (1954); E. Batres, G. Rosenkranz and F. Sondheimer, THIS JOURNAL, **76**, 5171 (1954)] would also appear to have resulted in the formation of the 17a-keto-D-homo system. Thus, the products obtained from Substance "S" and hydrocortisone exhibit the characteristic absorption band for the 17a-ketone at 1700 cm.$^{-1}$ (5.88 μ). Although this band is obscured in the product from cortisone due to the absorption band for the 11-ketone, this substance, nonetheless, has been interrelated with the D-homo steroid derived from hydrocortisone. Further, it has been independently observed (unpublished observation by N. L. Wendler and R. P. Graber) that the corresponding product from the D-homoannulation of 3α,21-diacetoxy-17α-hydroxypregnane-11,20-dione with aluminum isopropoxide not only fails to give a 2,4-dinitrophenylhydrazone derivative but also does not give a Zimmermann reaction in keeping with a 17a-keto-D-homo system; furthermore, this same substance exhibits an OH band at 3490 cm.$^{-1}$ (2.86 μ) in conformity with an equatorial 17α-hydroxyl group (see above). Therefore the products of D-homoannulation of Substance "S" acetate, hydrocortisone acetate and cortisone acetate are quite probably 17α-hydroxy-17a-keto-D-homo structures instead of the 17-keto-D-homo systems as previously reported.

Experimental[23]

D-Homoannulation of 3α-Acetoxy-17α-hydroxypregnane-11,20-dione (IV). (A) Rearrangement with Boron Trifluoride.—A solution of 1.6 g. of IV in 160 cc. of acetic acid was treated with 6.4 cc. of acetic anhydride and 6.4 cc. of boron trifluoride etherate and allowed to stand at room temperature for 15–18 hr. At the end of this time 160 cc. of water was added and the reaction mixture concentrated *in vacuo* to a low volume. The residue was extracted with ether and the ether extract washed with aqueous 10% potassium bicarbonate solution, dried over magnesium sulfate and concentrated *in vacuo* to an oil, wt. 2 g. This oil was chromatographed on 100 g. of acid washed alumina. Material eluted with benzene through 5% ether in benzene afforded 1.3 g. of **3α,17α-diacetoxy-17β-methyl-D-homoetiocholane-11,17a-dione** as plates from acetone–hexane; m.p. 167–168.5°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.44; H, 8.39. Found: C, 69.22; H, 8.57.

Fractions eluted with 5% ether in benzene afforded 190 mg. of 3α,17α-diacetoxypregnane-11,20-dione, m.p. 203–206° not depressed on admixture with authentic material.[24] The infrared spectra of the two samples were the same.

The above D-homo ketol diacetate (3 g.) was saponified by refluxing for 4 hr. with 100 cc. of 20% potassium hydroxide in methanol. The product was crystallized from acetone–hexane to give 2.6 g. of 3α,17α-dihydroxy-17β-methyl-D-homoetiocholane-11,17a-dione, m.p. 177–178°, $[\alpha]_D$ +54. This substance was also obtained by room tem-

(23) All melting points unless otherwise specified were taken on a micro hot-stage apparatus and are corrected. The infrared spectra were determined on a Perkin-Elmer Model 21 Spectrophotometer in carbon disulfide and carbon tetrachloride and are reported in cm.$^{-1}$ (μ), respectively. Optical rotations were determined in chloroform.

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

perature saponification as lower melting polymorphs, m.p. 120–122° and 156–158.5°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.66; H, 9.30.

Acetylation of the above diol (2.6 g.) in 5 cc. of pyridine and 2 cc. of acetic anhydride at room temperature overnight gave **3α-acetoxy-17α-hydroxy-17β-methyl-D-homoetiocholane-11,17a-dione (V)** directly from ether, recrystallized from acetone–hexane; m.p. 169–170°, $[\alpha]_D$ +80°, λ_{max} 3505 (2.86) –OH; 1738 (5.75) –OAc; 1712, 1702 (5.85; 5.87) >CO; 1432 (6.98) $-\alpha CH_2$.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.71. Found: C, 70.88; H, 8.48.

This ketol monoacetate V is dimorphic as revealed from chromatography of the total acetylation product which yielded V in 75% yield as needles from ether; m.p. 155°; these showed a phase change at the m.p. or when seeded gave prisms, m.p. 169–170°. The remainder of the acetylation product was 3,17-diacetate (25%) which became the only product when the duration of acetylation was extended to 3–4 days or when carried out at steam-bath temperatures.

(B) **Rearrangement with Aluminum *t*-Butylate.**—A solution of 1 g. of IV in 30 cc. of toluene was treated with 1 g. of commercial aluminum *t*-butylate[25] and refluxed for 2 hr. The reaction product was cooled and treated with ice and enough 5% hydrochloric acid to dissolve the aluminum hydroxide formed. The organic material was extracted with ether and the ether layer washed with water, dried over magnesium sulfate and concentrated to an oil. Chromatography of this oil on 25 g. of acid-washed alumina afforded 350 mg. of V identical in m.p., mixed m.p. and infrared spectrum with material obtained in Part A. In addition to V there was also isolated from the chromatogram 450 mg. of a by-product triol monoacetate formed by the reduction of IV (*cf.* ref. 1b).[26]

In another run 200 mg. of IV was refluxed with 1 g. of aluminum *t*-butylate in 40 cc. of benzene for 30 hr. The reaction product was chromatographed on 100 g. of silica gel containing 40 cc. of ethanol. Elution with 1% ethanol in methylene chloride–petroleum ether (1:1) afforded 133 mg. of the 17a-ketone V followed by 36 mg. of **3α-acetoxy-17aα-hydroxy-17aβ-methyl-D-homoetiocholane-11,17-dione (IX)**, recrystallized from benzene–cyclohexane; m.p. 183.5–185.5°, $[\alpha]_D$ +37.2°, λ_{max} 3600, 3510–3450 (2.78, 2.85–2.90) –OH; 1738 (5.75) –OAc; 1724, 1713 (5.80, 5.84) >CO; 1435, 1422 (6.97, 7.04) $-\alpha CH_2$.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.71. Found: C, 70.94; H, 8.94.

The **benzylidene derivative X** prepared from IX in the manner described for XV (see below) was crystallized from acetone m.p. 240–246° $\lambda\lambda_{max}^{CH_3OH}$ 288 mμ (16,600), 233 mμ (7,200).

Anal. Calcd. for $C_{30}H_{38}O_5$: C, 75.28; H, 8.00. Found: C, 75.25; H, 8.00.

(C) **Thermal Rearrangement.**—3α-Acetoxy-17α-hydroxypregnane-11,20-dione IV (200 mg.) was melted in a quartz tube and heated at 240° for 30 minutes. Chromatography of the product on silica gel employing 10% ethanol in petroleum ether–methylene chloride (3:1) for elution afforded 76 mg. of crude V and 54 mg. of crude IX. The pure compounds were obtained by recrystallization.

3α-Acetoxy-17-methyl-Δ[16]-D-homoetiocholene-11,17a-dione (VI).—A sample of monoacetate V (1.4 g.) in 5 cc. of pyridine was treated at 0–5° with 1.5 cc. of methanesulfonyl chloride and allowed to react for 15–18 hr. The reaction product was poured onto ice and the organic material extracted with ether. The ether extract was washed free of pyridine with dilute hydrochloric acid and was washed finally with dilute potassium bicarbonate solution. The ether extract was dried over magnesium sulfate, filtered and evaporated to a point where turbidity could be just clarified by addition of a few drops of ether. At this point very slow crystallization occurred depositing in all *ca.* 350 mg. of a crystalline by-product, m.p. 142–144°.[14] From the mother liquors there was obtained *ca.* 1 g. of the amorphous mesylate ester of V. The latter on chromatography gave no crystalline material.

The above amorphous mesylate derivative of V (1 g.) was refluxed for 1.5 hr. in 30 cc. of acetone containing 1 g. of sodium iodide and 0.5 cc. of pyridine. The reaction product was evaporated to dryness *in vacuo* and the residue treated with water and ether. The ether extract was washed with dilute hydrochloric acid and potassium bicarbonate solution, dried over magnesium sulfate and concentrated to dryness. The residue crystallized from ether as needle-like prisms, wt. 600 mg. Recrystallization was effected from acetone–hexane, m.p. 213–215°, $\lambda_{max}^{CH_3OH}$ 235 mμ (8,300).

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.19; H, 8.59. Found: C, 73.95; H, 8.30.

3a-Acetoxy-16,17α-oxido-17β-methyletiocholane-11,17a-dione (VII). (A).—A solution of 1.2 g. of the Δαβ-ketone VI in 90 cc. of methanol was cooled to *ca.* 10° and treated with 2.4 cc. of 4 *N* aqueous sodium hydroxide followed by 4.8 cc. of 30% hydrogen peroxide. The reaction mixture was stored at 5–10° for 20 hr., concentrated *in vacuo* to 50 cc. and quenched with 350 cc. of water. The crystalline reaction product was filtered, washed with water and dried. The dry product was acetylated in 10 cc. of pyridine with 15 cc. of acetic anhydride to give 880 mg. (1st crop) of α-oxido ketone VII as felted needles from ether, m.p. 185–186°, $[\alpha]_D$ +98°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.80; H, 7.97.

(B).—A solution of 540 mg. of the Δαβ-ketone VI in 5 cc. of benzene was treated with 9.5 cc. of a 0.315 *M* solution of perbenzoic acid in benzene and allowed to stand for 4 days at room temperature. The reaction product was worked up to afford 225 mg. of α-oxido ketone VII as felted needles from ether identical in m.p. and m.m.p. with material prepared in Part A.

3α-Acetoxy-17α-hydroxy-16β-bromo-17β-methyl-D-homoetiocholane-11,17a-dione (VIII).—A solution of 780 mg. of α-oxide VII in 32 cc. of acetic acid was treated with 4 cc. of a solution of 24% hydrogen bromide in acetic acid at 10–15°. The reaction mixture was allowed to stand at this temperature for 45 minutes, then concentrated *in vacuo* to dryness. The residue was dissolved in benzene and again concentrated to dryness and finally crystallized from ether to give 795 mg. of bromohydrin VIII, m.p. 226–228° dec.

Anal. Calcd. for $C_{23}H_{33}O_5Br$: C, 58.85; H, 7.04; Br, 17.06. Found: C, 58.95; H, 6.94; Br, 17.40.

A solution of the bromohydrin VIII (75 mg.) in 5 cc. of methanol was treated with 70 mg. of potassium hydroxide dissolved in 1 drop of water and 5 cc. of methanol. After 45 minutes at room temperature the reaction product was isolated and acetylated to yield starting α-oxido ketone VII, m.p. 185.5–186.5° not depressed on mixture with authentic VII.

Conversion of Bromohydrin VIII to Ketol V.—A solution of 63 mg. of VIII in 7 cc. of 90% methanol was hydrogenated in the presence of 130 mg. of 25% palladium-on-calcium carbonate. The product was chromatographed to afford 44 mg. of V, m.p. 155–157° with phase change melting again at 167–169°; mixed m.p. with V obtained by D-homoannulation of IV was not depressed. Infrared spectra of the two samples were identical.

Reductive debromination of VIII with Raney nickel according to the method of Julian, *et al.*,[27] produced, instead of IV, a 17,17a-diol,[1b] the detailed chemistry of which will be discussed elsewhere (see ref. 14).

3α-Acetoxy-16,17β-oxido-17α-methyl-D-homoetiocholane-11,17a-dione (XI).—A solution of 300 mg. of Δαβ-ketone VI in 10 cc. of dioxane was treated with 1.6 g. of N-bromosuccinimide and 2 cc. of water. To this solution, cooled in an ice-bath, was added dropwise with stirring 7.5 cc. of 1 *N* perchloric acid.[28] The reaction mixture was allowed to stir at room temperature for 3 hr. At the end of this time the excess N-bromosuccinimide was decomposed with 5% aqueous sodium bisulfite at the same time discharging the color of the solution. The product was precipitated by addition of water and extracted with ethyl acetate. The ethyl acetate extract was washed with water and a saturated solution of sodium chloride, dried over magnesium sulfate and concentrated *in vacuo* to give the 16β-hydroxy-17α-bromo derivative as an oil. The latter was dissolved in 10 cc. of methanol and treated with 250–300 mg. of potassium

(25) Obtained from the Matheson Co., Inc., East Rutherford, N. J.

(26) The chemistry of this compound will be discussed elsewhere.

(27) P. L. Julian, E. W. Meyer, W. J. Karpel and I. R. Walker, THIS JOURNAL, **72**, 5145 (1950).

(28) Method of J. Fried and E. Sabo, *ibid.*, **76**, 1455 (1954).

hydroxide dissolved in 0.5 cc. of water and 2 cc. of methanol. Within a period of 10–15 seconds after addition of the alkali to the bromohydrin a copious crystallization of needles ensued (probably oxide acetate) that gradually redissolved. At the end of 30 minutes the reaction mixture was evaporated to dryness *in vacuo* and the residue extracted with ethyl acetate. The ethyl acetate solution was washed with saturated sodium chloride solution, dried over magnesium sulfate and evaporated *in vacuo* to dryness. The residue was acetylated at room temperature with 2 cc. of acetic anhydride in 5 cc. of pyridine. The product was extracted with ethyl acetate and washed with dilute hydrochloric acid and bicarbonate solution to afford 200–250 mg. of the **β-oxido ketone XI** as needle-like prisms from acetone–ether; m.p. 230–232°, $[\alpha]_D$ +21°.

Anal. Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.88; H, 8.01.

3α-Acetoxy-17β-hydroxy-16α-bromo-17α-methyl-D-homoetiocholane-11,17a-dione (XI).—A solution of 60 mg. of β-oxido ketone XI in 4 cc. of acetic acid was treated at 10–15° with 0.5 cc. of a solution of hydrogen bromide in acetic acid. The reaction was allowed to proceed for 45 minutes and then concentrated to dryness *in vacuo*. The residue was dissolved in benzene and reconcentrated to dryness *in vacuo* to give 70 mg. of crystalline bromohydrin on addition of ether. Recrystallized from acetone–hexane; m.p. 192–195°.

Anal. Calcd. for $C_{23}H_{33}O_5Br$: C, 58.85; H, 7.04; Br, 17.06. Found: C, 59.18; H, 6.94; Br, 17.0.

3α-Acetoxy-17β-hydroxy-17α-methyl-D-homoetiocholane-11,17a-dione (XIII).—A solution of 70 mg. of bromohydrin XII in 7 cc. of methanol and 0.7 cc. of water was hydrogenated in the presence of 125 mg. of 25% palladium-on-calcium carbonate. The reaction product was filtered and crystallized from acetone–hexane to give 40 mg. of XIII, m.p. 212–215°, $[\alpha]_D$ +59°, λ_{max} 3600 (2.78)[29] –OH; 1740 (5.75) –OAc; 1715 (5.83) >CO; 1432 (6.98) $-\alpha CH_2$.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.77; H, 8.72. Found: C, 70.72; H, 8.48.

This ketol XIII was also obtained from the bromohydrin XII with Raney nickel.

D-Homoannulation of 3α-Acetoxy-17α-hydroxypregnane-11,20-dione (IV) with Base.—A solution of 2 g. of IV in 100 cc. of 5% methanolic potassium hydroxide was refluxed for 5–6 hr. At the end of this period the reaction mixture was evaporated to dryness *in vacuo* and the residue extracted with ethyl acetate. The residue from evaporation of the ethyl acetate solution was acetylated with 2 cc. of acetic anhydride in 5 cc. of pyridine at room temperature for 18 hr. Chromatography of the acetylated material on acid washed alumina afforded 1.2 g. of XIV as plates from acetone–hexane; m.p. 223–227°, $[\alpha]_D$ +37°, λ_{max} 3480 (2.87) –OH; 1742 (5.74) –OAc; 1720–1716 (5.81–5.83) >CO; 1435, 1428 (6.97, 7.01) $-\alpha CH_2$.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.77; H, 8.72. Found: C, 71.05; H, 8.55.

Acetylation of XIV with boron trifluoride in acetic acid and acetic anhydride provided the corresponding 3,17-diacetate as flat prisms from acetone–hexane; m.p. 227–228.5°.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.44; H, 8.39. Found: C, 70.01; H, 8.40.

Treatment of 150 mg. of XIV in 2 cc. of ethanol with 0.1 cc. of benzaldehyde and 1 cc. of 15% aqueous potassium hydroxide for 18 hr. at room temperature afforded the **benzylidene derivative XV** as needle-like prisms from acetone–hexane; m.p. 220–222°, $\lambda\lambda_{max}^{CH_3OH}$ 285 mμ (23,000), 220 mμ (8,000).

Anal. Calcd. for $C_{30}H_{38}O_5$: C, 75.28; H, 8.00. Found: C, 75.72; H, 7.81.

In another run 500 mg. of IV was refluxed for 4 hr. in a mixture of 125 cc. of ethanol and 125 cc. of 10% aqueous potassium hydroxide. The reaction product was chromatographed on a 250-g. silica gel column. Elution with 5% ethanol in methylene chloride afforded 224 mg. of **3α,17aβ-dihydroxy-17aα-methyl-D-homoetiocholane-11,17-dione (XIV)** recrystallized from acetone; m.p. 228–229.5°, $[\alpha]_D$ +12.5°.

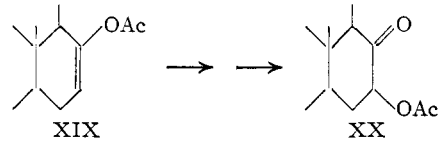
(29) In chloroform the additional broad maximum due to association is present at 3535–3370 cm.$^{-1}$ (2.83–2.97 μ).

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 72.49; H, 9.13.

Further elution of the column with 5% ethanol in methylene chloride afforded 105 mg. of 3α,17aα-dihydroxy-17aβ-methyl-D-homoetiocholane-11,17-dione (IX), recrystallized from acetone–petroleum ether; m.p. 187–188°, $[\alpha]_D$ +11.6°.

Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.38; H, 9.26. Found: C, 71.93; H, 9.39.

Partial Synthesis of IX and XIV.—A solution of 1.00 g. of 3α-acetoxy-17aα-methyl-D-homoetiocholane-11,17-dione[26] in 20 cc. of carbon tetrachloride was enol acetylated by the method of Barton, *et al.*[30] The total product from the enol acetylation (1.04 g.) was dissolved in 10 cc. of benzene and treated with 10.9 cc. of 0.49 *M* perbenzoic acid in benzene at 20° for 18 hr. The reaction product (1.01 g.) was dissolved in 40 cc. of ethanol and treated with 1.00 g. of sodium hydroxide in 30 cc. of water for 2 hr. at room temperature. At the end of this time the product was precipitated by addition of water and extracted with chloroform to give 400 mg. of neutral material giving a positive Zimmermann test for α-methylenic ketones.[31] The remainder of the product was acidic. The neutral product was acetylated and chromatographed[32] on acid washed alumina to afford 64 mg. of



XIX XX

a crystalline mixture which by infrared analysis was found to consist of 75% of IX and 25% of XIV.[33] Complete resolution of the two ketols IX and XIV was achieved by partition chromatography on paper employing dimethylformamide as the stationary phase and a 3:1 mixture of cyclohexane in benzene as the mobile phase; the Zimmermann test[34] was employed in following the resolution. In this way pure IX, as the more polar component, was obtained, m.p. 181–183°; this material showed no depression on mixed m.p. with 17aα-hydroxy ketone IX obtained from the Lewis acid-D-homoannulation of IV (see above), and the infrared spectra of the two samples were identical. The less polar component from the chromatography represented pure 17aβ-hydroxy ketone XIV, m.p. 223–226°. The mixed m.p. of this material with XIV obtained from the alkaline D-homoannulation of IV showed no depression. The infrared spectra of the two samples were identical.

3α-Acetoxy-11-ketoetiobilianic Acid (XVI).—A solution of 335 mg. of Δαβ-ketone VI in 35 cc. of acetone was treated at 0–5° with 450 mg. of potassium permanganate and stirred at 0–5° for 1 hr. and at room temperature for 1 hr. At the end of this time the acetone was blown off in a stream of nitrogen and replaced with water. The aqueous mixture was acidified with 1.5 cc. of 50% sulfuric acid and with stirring treated with saturated sodium bisulfite solution until the color had been discharged. The organic material was taken up in ether and the organic acid extracted with aqueous potassium bicarbonate solution. Acidification of the bicarbonate extract precipitated the acid XVI. The latter was taken up in ether and crystallized from this solvent; m.p. 225–230°.

Anal. Calcd. for $C_{21}H_{30}O_7$: C, 63.96; H, 7.62. Found: C, 63.89; H, 7.50.

(30) D. H. R. Barton, R. M. Evans, J. C. Hamlett, P. G. Jones and T. Walker, *J. Chem. Soc.*, 747 (1954).

(31) W. Zimmermann, *Z. physiol. Chem.*, **245**, 47 (1936); N. H. Callow, R. K. Callow and C. W. Emmens, *Biochem. J.*, **32**, 1312 (1938).

(32) The bulk of the chromatographed material was eluted by benzene–10% ether in benzene and did not give a positive Zimmermann test. It probably consisted largely of the 16-acetoxy compound XX derived from the Δ-16 enol acetate XIX present in the total product of enol acetylation. The desired ketols IX and XIV (strong positive Zimmermann test) derived from the Δ-17 enol acetate were eluted from the column by 30–50% ether in benzene and were therefore readily separable from XX.

(33) An accurate analysis of mixtures of IX and XIV was made possible by the intense band at 1159 cm.$^{-1}$ (8.6 μ) present in XIV but absent in IX.

(34) Procedure of K. Savard, *J. Biol. Chem.*, **202**, 457 (1953).

The mixed m.p. of this acid with material prepared from the oxidation of XVII (see below) was not depressed. The infrared spectra of the two samples were the same.

Benzylidene Derivative of 3α-Acetoxyetiocholane-11,17-dione (XVII).—A 0.68-g. sample of 3α-acetoxyetiocholane-11,17-dione was converted to its 16-benzylidene derivative by the method already described for the preparation of XV. The product was acetylated at room temperature with pyridine and acetic anhydride to give XVII as prisms from acetone–hexane; m.p. 245–247°, $\lambda\lambda_{max}^{CH_3OH}$ 295 mμ (25,800), 229 mμ (8,300).

Anal. Calcd. for $C_{28}H_{34}O_4$: C, 77.42; H, 7.83. Found: C, 77.20; H, 7.56.

Oxidation of XVII with potassium permanganate gave the acid XVI, m.p. 226–231°, identical with material prepared from the $\Delta^{\alpha\beta}$-ketone VI (*cf.* above).

3α-Acetoxy-11-ketoetiobilianic Acid Anhydride.—Treatment of the acid XVI with acetic anhydride in pyridine at room temperature produced the corresponding anhydride, crystallized from acetone–hexane; m.p. 213–215°, λ_{max} 1800 (5.55), 1765 (5.67), 1710 (5.85) and 1250 cm.$^{-1}$ (8 μ).

Anal. Calcd. for $C_{21}H_{28}O_6$: C, 67.02; H, 7.45. Found: C, 67.28; H, 7.40.

RAHWAY, N. J.
NEW YORK, N. Y.

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

The Polarographic Reduction of Prednisone (17α,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione), Prednisolone (11β,17α,21-Trihydroxy-1,4-pregnadiene-3,20-dione) and Their Precursors[1]

BY PETER KABASAKALIAN AND JAMES MCGLOTTEN

RECEIVED MARCH 22, 1956

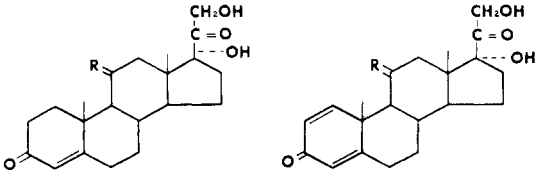
Polarographic data are compiled for prednisone (17α,21-dihydroxy-1,4-pregnadiene-3,11,20-trione), prednisolone (11β,-17α,21-trihydroxy-1,4-pregnadiene-3,20-dione), cortisone and hydrocortisone (17α-hydroxycorticosterone) in well-buffered 50% ethanol solutions. The effects of *p*H, temperature, concentration and mercury pressure on the polarographic waves obtained are discussed. A possible electrode reaction mechanism has been proposed on the basis of the results obtained from wave analyses and diffusion coefficient determinations. Preliminary studies of controlled potential electrolysis products support the proposed mechanism.

Introduction

Although it is now well known that the polarographic reduction characteristics of carbonyl compounds depend greatly on many external factors such as *p*H, buffer type, buffer concentration and type of solvent, much of the early work done on the α,β-unsaturated ketosteroids was performed in unbuffered or poorly buffered systems. Adkins and Cox[2] obtained reduction waves for cholestenone in 0.1 *N* ammonium chloride solutions. Later Eisenbrand and Picher[3] observed the behavior of several Δ^4-3-ketosteroids in 90% ethanol 0.1 *N* in lithium chloride. Wolfe, Hershberg and Fieser[4] studied the polarographic reduction of cholestenone, corticosterone, cortisone, desoxycorticosterone, progesterone and testosterone in 35% isopropyl alcohol solutions 0.1 *N* with respect to tetraethylammonium hydroxide.

Sartori and Bianchi[5] reported the half-wave dependence on *p*H for 17-ethinyltestosterone and methyltestosterone, but did not describe any changes in wave form or currents with *p*H. More recently Zuman, Tenygl and Brezina[6] made a fairly exhaustive study on the behavior of certain Δ^4-3-ketosteroids in 30–60% ethanol solutions buffered with Britton and Robinson buffer mixtures.

The present work is concerned with the polarographic behavior of two Δ^4-3-ketosteroids (cortisone and hydrocortisone) and two of the recent $\Delta^{1,4}$-3-ketosteroids (prednisone and prednisolone) in well-buffered 50% ethanol solutions. Some earlier work carried out in 50% methanol is also included.



CORTISONE R O PREDNISONE R O

HYDROCORTISONE R = HO / H PREDNISOLONE R HO / H

Experimental

Apparatus.—The curves for the analysis of the wave were obtained with the Fisher Elecdropode. The remainder of the polarographic work was carried out with the Sargent model XXI recording polarograph. The cells used were small H-type cells (3-ml. sample volume) containing a normal calomel electrode separated from the sample compartment by an agar plug and fritted glass diaphragm. The chloride ion in the calomel cell was furnished by a normal tetramethylammonium chloride solution. The electrode capillary delivered 1.698 mg. of mercury per second at a column height of 41 cm. The drop time was 4.12 seconds and the capillary constant, $m^{2/3}t^{1/6}$, was 1.80 mg.$^{2/3}$ sec.$^{-1/2}$. The constants were determined at an open circuit with the mercury dropping into a 0.1 *N* potassium chloride solution.

All *p*H measurements were made with the Beckman model G *p*H meter using the glass electrode.

The magnetic stirring device and fritted glass diaphragm cell in the diffusion coefficient experiments were similar to those used by Stokes[7] in his diffusion work.

The electrolysis cell in the controlled-potential electrolysis work was similar in design to that used by Pasternak.[8]

(1) Presented at the Seventh Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., February 28, 1956.

(2) H. Adkins and F. W. Cox, THIS JOURNAL, **60**, 1151 (1938).

(3) J. Eisenbrand and H. Picher, *Z. physiol. Chem.*, **260**, 83 (1939).

(4) J. K. Wolfe, E. B. Hershberg and L. F. Fieser, *J. Biol. Chem.*, **136**, 653 (1940).

(5) G. Sartori and E. Bianchi, *Gazz. chim. ital.*, **74**, 8 (1944).

(6) P. Zuman, J. Tenygl and M. Brezina, *Collection Czechoslov. Chem. Communs.*, **19**, 46 (1954).

(7) R. H. Stokes, THIS JOURNAL, **72**, 763 (1950).

(8) R. Pasternak, *Helv. Chim. Acta*, **31**, 753 (1948).