at 0°. After 15 hr. the supernatant ether solution was decanted, washed once with sodium bicarbonate solution, then with water and dried over anhydrous potassium carbonate. Removal of the ether in a stream of dry air left the bromide which is very sensitive to heat and moisture. It was used directly for the next step.

2-[β-(6-Methoxy-3-indolyl)ethyl]-5-carbomethoxyisoquinolinium bromide (XX). Condensation of XIX with 5-carbomethoxyisoquinoline as in the preparation of IV gave XX as clusters of orange needles, m.p. 270°, with a color change about 220°. The yield was 61% from XIX.

Anal. Calcd. for $C_{22}H_{21}BrN_2O_3$: C, 59.85; H, 4.77; N, 6.35. Found: C, 59.57; H, 4.78; N, 6.35.

Tetrahydroalstonilinol (XXI). To a solution of 441 mg. of XX in 20 ml. of anhydrous tetrahydrofuran and 20 ml. of anhydrous ether was added 1 g. of lithium aluminum hydride. After standing overnight at room temperature with occasional shaking the excess hydride was carefully decomposed with a few drops of water. After filtering through Celite and thorough washing of the filter cake, the solvent was removed under reduced pressure and the residue was recrystallized from chloroform–petroleum ether to yield 210 mg. (64%) of XXI as fine white needles, m.p. 220–224°.

Anal. Calcd. for $C_{21}H_{22}N_2O_2$: C, 75.42; H, 6.63; N, 8.38. Found: C, 75.28; H, 6.77; N, 8.09.

The *hydrochloride* of XXI, prepared in and recrystallized from absolute ethanol, formed white needles, m.p. 278° (dec.) with previous darkening about 250°.

Anal. Calcd. for $C_{21}H_{23}ClN_2O_2$: C, 68.03; H, 6.29; N, 7.51. Found: C, 67.97; H, 6.01; N, 7.29.

The infrared spectra of tetrahydroalstonilinol and its hydrochloride were identical with those of the substances prepared from natural alstoniline (XXIII) according to Elderfield and Wythe.[8]

Alstonilinol (XXII). Dehydrogenation of XXI with iodine and potassium acetate as in the case of XIII gave alstonilinol iodide as orange needles, m.p. 310° (dec.), from methanol. The yield was 90%.

Anal. Calcd. for $C_{21}H_{19}IN_2O_2$: C, 54.90; H, 4.17; N, 6.10; I, 27.86. Found: C, 55.02; H, 4.20; N, 6.07; I, 27.52.

Reaction of β-(3-indole)glyoxalyl chloride (XVI) *with 5-carbomethoxyisoquinoline* (XXIV). To a solution of 2.08 g. of XVI[16] in 15 ml. of tetrahydrofuran was added a solution of 1.87 g. of 5-carbomethoxyisoquinoline in 10 ml. of tetrahydrofuran. When recrystallization of the yellow precipitate from methanol-ether was attempted solvolysis occurred. The crystalline material which separated first as white needles, m.p. 200° (dec.), was identified as the hydrochloride of 5-carbomethoxyisoquinoline by infrared comparison with a known sample.

Anal. Calcd. for $C_{11}H_9NO_2$: C, 59.10; H, 4.48; N, 6.28; Cl, 15.86. Found: C, 59.47; H, 4.49; N, 6.22; Cl, 15.51.

The mother liquors from the above hydrochloride were concentrated and the residue was recrystallized from methanol-benzene to give 1.45 g. (72%) of white prisms, m.p. 226°.

Anal. Calcd. for $C_{11}H_9NO_3$: C, 65.02; H, 4.43; N, 6.85. Found: C, 64.71; H, 4.21; N, 6.64.

Hydrolysis of the above ester with 0.2*N* sodium hydroxide gave an acid, m.p. 214°, after recrystallization from methanol. Indole-3-glyoxylic acid is reported as melting at 216° and its methyl ester at 225°.[17]

Attempted Japp-Klingemann Reaction with m-methoxybenzenediazonium chloride and diethyl α-acetogluturate. To a solution of 24 g. of diethyl α-acetylgluturate[18] in 200 ml. of ethanol and 200 ml. of 20% sodium hydroxide solution at 5° a solution of *m*-methoxybenzenediazonium chloride prepared from 13 g. of *m*-anisidine,[19] 7 g. of sodium nitrite, and 61 ml. of 18% hydrochloric acid was added. The mixture was kept in an ice-salt bath for 3 hr. and allowed to come to room temperature. Green needles, m.p. 130° (14.2 g., 79%), were collected and recrystallized from ethanol.

Anal. Calcd. for $C_9H_{12}N_2O_2$: C, 59.98; H, 6.71; N, 15.55. Found: C, 59.64; H, 6.83; N, 15.66.

The *acid sulfate*, prepared in and recrystallized from 90% ethanol, formed long yellow needles, m.p. 180° (dec.).

Anal. Calcd. for $C_9H_{12}N_2O_2 \cdot H_2SO_4$: C, 38.85; H, 5.07; N, 10.07. Found: C, 38.55; H, 5.10; N, 9.83.

The structure of this green compound is under investigation.

ANN ARBOR, MICH.

(16) M. S. Kharasch, S. S. Kane, and H. C. Brown, *J. Am. Chem. Soc.*, **62**, 2243 (1940).

(17) J. N. Baker, *J. Chem. Soc.*, 459 (1940).

(18) The ester, b.p. 134–138° (2 mm.), was prepared in 69% yield by refluxing the product of the condensation of pyrrolidine and ethyl acetoacetate with ethyl acrylate. (Private communication from Dr. R. E. Ireland of these Laboratories.)

(19) P. K. Kadaba and S. P. Massie, *J. Org. Chem.*, **22**, 333 (1957).

[CONTRIBUTION FROM THE MERCK SHARP & DOHME RESEARCH LABORATORIES, MERCK & CO., INC.]

Transformations in the D-Homosteroid Series. The Isomeric 17α,17a-Glycols[1]

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Received December 30, 1957

The formation and various transformations of the isomeric 17α,17a-dihydroxy-3α-acetoxy-17β-methyl-D-homoetiocholan-11-ones are described.

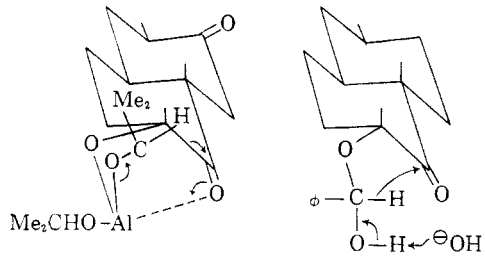
The formation and structure elucidation of two 3α-acetoxy-17α-17a-diols isomeric at position 17a arising from reduction of 3α-acetoxy-17α-hydroxy-17β-methyl-D-homoetiocholane-11,17a-dione (I) were reported recently.[1,2a] Reduction of I with aluminum isopropoxide affords in good yield a glycol, m.p. 213–15° which reverted to I on chromic acid oxidation. When I was refluxed in ethanol solu-

(1) Presented in part at the Symposium on Steroids and Related Natural Products, The Gordon Research Conferences, New Hampton, N. H., July 30–August 3, 1956.

(2) (a) N. L. Wendler, D. Taub, S. Dobriner, and D. K. Fukushima, *Chem. & Ind. (London)*, 1259 (1955); (b) *J. Am. Chem. Soc.*, **78**, 5027 (1956).

tion with Raney nickel, there was produced in minor amount (25%) the same glycol, m.p. 213–215° and to a major extent (70%) a new glycol, m.p. 244–246°. The latter on chromic acid oxidation also reverted to I thereby establishing the 17a epimeric character of the two compounds. Saponification of the higher melting (244–246°) isomer produced a triolone, m.p. 250–252° which was identical with material obtained from I by treatment with benzaldehyde and alkali. The formation of the triolone by the latter procedure presumably occurs, as has been pointed out previously,[2a] by way of an unusual crossed Cannizzaro reaction.[3]

Configurational assignment at C-17a was made on the basis of the relative rates of cleavage with periodic acid[4] as well as evidence based on the Wagner rearrangement of their sulfonic ester derivatives (see later). The lower melting glycol reacted within 5 minutes with 1 mole of periodic acid and is assigned the *cis*-configuration (17aα-OH, axial) II; the higher melting glycol, in contrast, required 2 hours for cleavage and is consequently assigned the *trans*-configuration (17aβ-OH, equatorial) III. The transition state for the aluminum isopropoxide reduction of I probably involves coordination of the aluminum with the 17α-hydroxyl and 17a keto groups necessarily from below the ring system as in A.[5] Transfer of hydride ion would



A B

then occur from above (β-face) leading to II. In general aluminum isopropoxide reductions of unhindered ketones lead predominantly to the axial isomers.[6]

The formation of the 17aβ-epimer from the crossed Cannizzaro reaction with benzaldehyde and alkali may be depicted as in B. The C-17 epimer of I[2b] did not react in the Cannizzaro reduction, a result explicable on the basis of steric hindrance to the pertinent transition state on the β-face of ring-D. The crossed Cannizzaro reaction as demonstrated here may prove to have utility as a means for effecting differential reduction of ketolic systems. In the Raney nickel reduction there is a precedent for the predominant formation of the thermodynamically favored 17aβ-equatorial hydroxyl group.[7]

Reaction of either glycol[8] II or III with periodic acid produced a noncrystalline keto aldehyde intermediate (i, footnote 9) which was cyclized under alkaline conditions to the $\Delta^{\alpha\beta}$-unsaturated ketone IV, m.p. 208–210°, $\lambda_{max}^{CH_3OH}$ 237 mμ (12,300).[9] The structure of the $\Delta^{\alpha\beta}$-ketone IV was established by oxidation of its 3 acetate derivative to 3α-acetoxy-11-keto etiobilianic acid[2b] as well as its transformation *inter alia* to the 16 *iso*-analogs of cortisone.[10]

(3) Recently R. B. Turner, M. Perelman, and K. T. Park, Jr., [*J. Am. Chem. Soc.*, **79**, 1108 (1957)] have prepared the corresponding 17a epimeric glycol systems in another series.

(4) See for example: P. F. Fleury, J. E. Courtois, and A. Breder, *Bull. soc. chim. France*, **118**, (1952).

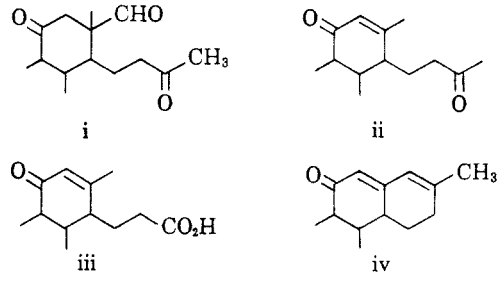
(5) H. Felkin [*Bull. soc. chim. France*, 1050 (1956)] has recently formulated similar transitional intermediates to explain the directional course of reduction of acylic ketols with aluminum alkoxides.

(6) W. Klyne, *Progress in Stereochemistry*, **1**, Butterworth Scientific Publications, London, 1954, p. 74.

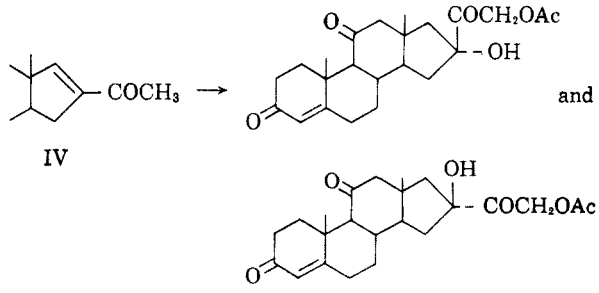
(7) H. Heusser, P. Th. Herzig, A. Fürst, and Pl. A. Plattner, *Helv. Chim. Acta*, **33**, 1093 (1950).

(8) For practical purposes, it proved preferable to utilize the triol IIa which was prepared in good yield by one-step reductive D-homoannulation of 3α,17α-dihydroxypregnane 11,20-dione with aluminum isopropoxide–isopropyl alcohol in toluene (see Experimental).

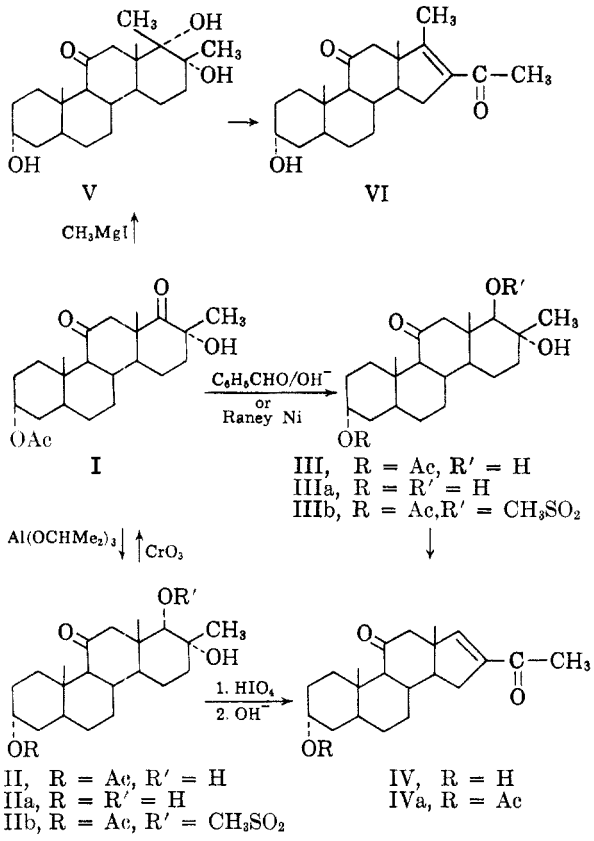
(9) β-Elimination of the formyl group from the intermediate aldehyde i obtained from II and III with periodic acid would give ii which would be expected to have ultraviolet absorption similar to that of IV. However, structure IV can be differentiated from ii on the basis of its empirical formula and the ultraviolet of the semicarbazone derivative λ_{max} 265 mμ (16,800). Semicarbazide would be expected to react with ii at the saturated carbonyl group only to give a semicarbazone with λ_{max} near 230 mμ. In this connection the Δ^{12}-11-keto acid iii [N. L. Wendler, D. Taub, and H. L. Slates, *J. Am. Chem. Soc.*, **77**, 3559 (1955)] did not react with carbonyl reagents under the usual conditions. The ultraviolet of the cyclization mother liquors had a shoulder in the 280–290 mμ region possibly indicating the presence of a small amount of iv formed by cyclization of ii.



(10) The transformation series:



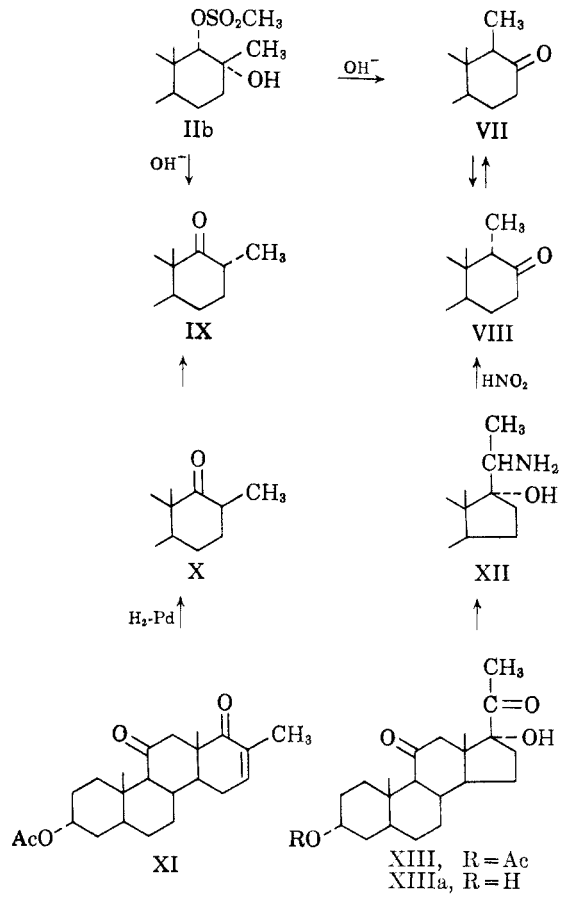
was described on the occasion cited in ref. 1 and will be published in detail at a later date. More recently J. Fajkoš and F. Šorm [*Coll. Czech. Chem. Comm.*, **21**, 1013 (1956); *Chem. listy*, **51**, 579 (1957)] have prepared systems of the class IV in the 11-desoxy series by another route.



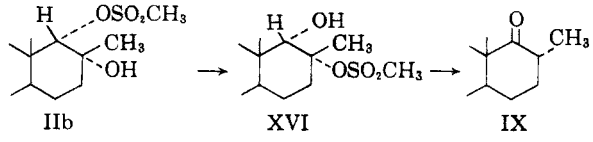
In a like manner reaction of I with methyl Grignard reagent afforded a triol m.p. 197–200° formulated as the *cis* diol V for mechanistic reasons considered to be similarly applicable as in the case of the aluminum isopropoxide reduction (see earlier). Thus a complex formed from the Grignard reagent and I, similar to B, may be envisaged. Cleavage of this glycol with periodic acid followed by alkaline catalyzed ring closure produced the $\Delta^{\alpha\beta}$-ketone VI, m.p. 197–200°, $\lambda_{max}^{CH_3OH}$ 251 mμ (10,300).

Acylation of the *cis*-glycol, II at room temperature or below in pyridine occurs at the 17α-OH. This latter fact was established by acetylation which gave a noncrystalline derivative having OH absorption in the infrared which remained essentially unchanged after chromic acid oxidation. Mesylation of II likewise afforded an amorphous mesylate IIb. Treatment of the latter with methanolic potassium hydroxide caused Wagner rearrangement with production of a mixture of the epimeric 17a methyl ketones VII and VIII as the major product. A sample of the pure 17α-methyl epimer VIII[2b] was prepared by nitrous acid-amine ring-expansion of XII obtained in turn from the oxime of XIII by hydrogenation. Ramirez and Stafiej[11] have shown that this sequence in the 17α-OH series leads to 17aα-methyl 17-ketones. When the 17aα epimer VIII was submitted to alkaline treatment it afforded the same inseparable mixture of VII and VIII as determined by melting point behavior and infrared comparison.[12]

Also formed in small amounts from the alkaline treatment of IIb was the 17α-methyl-17a-ketone IX. The latter proved to be identical with a sample prepared from the $\Delta^{\alpha\beta}$-ketone XI[2b] by hydrogenation followed by alkaline isomerization at C-17. In the latter sequence the hydrogenation of XI is



presumed to proceed from the rear to give the unstable axial 17β-methyl ketone X, m.p. 156–159° which isomerizes with base to the stable equatorial epimer IX, m.p. 165–166° (mixed melting point depressed). The formation of IX from the mesylate derivative IIb is an interesting and perhaps novel change mechanistically. This transformation would appear to be predicated on an initial sulfonyl group transfer 17a-O→17-O with ensuing hydrogen



(11) F. Ramirez and S. Stafiej, *J. Am. Chem. Soc.*, **78**, 644 (1956).

(12) Ramirez and Stafiej (ref. 11) working in another series were quite fortunately able to separate their mixture of 17aα- and 17aβ-methyl epimers by fractional crystallization. They determined the ratio of isomers to be *ca.* 30% 17aα- and 70% 17aβ.

migration C-17a→C-17. The latter phase is by no means an ideal one from the point of view of steric considerations[13] but has, however, some precedent in the formation of hecogenin XV to a minor extent from the alkaline decomposition of XIV.[14] In the latter case a migration of axial hydrogen accompanies the departure of an equatorial mesyloxy function. In analogy it is presumed that the mesylate derivative IIb may rearrange secondarily in a like manner after initial acyl transfer. (IIb → XVI → IX).[15]

The formation of the 17a methyl ketones VII and VIII as the major product from the rearrangement of the mesylate derivative IIb provides additional substantiation for the *cis*-orientation of the functions at 17,17a as ascertained by rate of reaction with periodic acid.

EXPERIMENTAL

3α,17α,17aα-Trihydroxy-17β-methyl-D-homoetiocholane-11-one (IIa). Fifty grams of aluminum isopropoxide was added to a stirred hot solution of 50 g. of pregnane-3α,17α-diol-11,20-dione (XIIIa) in 750 ml. of toluene, 250 ml. of dioxane, and 250 ml. of isopropyl alcohol and the mixture refluxed for 2 hr. At the end of this time the reaction mixture was cooled and 1500 ml. of cold 2*N* HCl was added. The layers were separated and the aqueous phase was extracted three times with ethyl acetate. The combined organic layers were washed with water and saturated salt solution and dried over magnesium sulfate. On concentration *in vacuo* to half the original volume (*ca.* 600 ml.) the triol (IIa) precipitated. The chilled precipitate was filtered, washed with hexane, and dried to give 34.9 g., m.p. 216–220°. Further concentration gave an additional 7 g., m.p. 206–214° (total yield 84%). Recrystallization from acetone-hexane raised the m.p. to 220–225°; λ_{max}^{Nj} 3.00, 5.90 μ.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.77. Found: C, 72.05; H, 9.51.

Aluminum isopropoxide treatment of 3α-acetoxypregnane-17α-ol-11,20-dione (XIII) in refluxing toluene produced the triol monoacetate II (50%) (see below) as well as considerable (30%) triol (IIa) readily separable on alumina. The formation of the triol (IIa) must be a consequence of ester interchange of the 3α-acetate function with isopropyl alcohol released from the aluminum isopropoxide.

Aluminum isopropoxide reduction of 3α-acetoxy-17α-hydroxy-17β-methyl-D-homoetiocholane-11,17a-dione (I). A solution of 500 mg. of I in 15 cc. of toluene was refluxed for 2 hr. with 500 mg. of aluminum isopropoxide and worked up as described above. Chromatography of the reaction product on alumina afforded 300 mg. of II m.p. 213–215° from acetone-hexane[16] $[\alpha]_D^{Chf}$ +71.9°.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.34; H, 9.24.

Raney nickel reduction of 3α-acetoxy-17α-hydroxy-17β-methyl-D-homoetiocholane-11,17a-dione (I). A stirred solution of 3.0 g. of the D-homoketol monoacetate (I) in 150 ml. of absolute ethanol was refluxed 5 hr. with 30 g. of W-4 Raney nickel, which had been partly deactivated by washing with ethyl acetate shortly before use. The reaction mixture was cooled, filtered through celite, and the filtrate taken to dryness *in vacuo*. The crystalline residue (3.0 g.; m.p. 215–230°) was chromatographed on 105 g. of acid-washed alumina. The 20–30% chloroform-benzene eluates afforded 704 mg. (23%) of *3α-acetoxy-17α,17aα-dihydroxy-17β-methyl-D-homoetiocholane-11-one* (II) recrystallized from acetone-ether, m.p. 213–215°; this material was identical with that obtained from the reduction of I with aluminum isopropoxide (see above).

Fractions eluted with chloroform through 5% methanol-chloroform gave 2.0 g. of *3α-acetoxy-17α,17aβ-dihydroxy-17β-methyl-D-homoetiocholane-11-one* (III), which crystallized as needles from acetone-hexane, m.p. 244–246°; $[\alpha]_D^{Chf}$ +75°.

Anal. Calcd. for $C_{23}H_{36}O_5$: C, 70.38; H, 9.24. Found: C, 70.17; H, 9.25.

Saponification of III with sodium hydroxide in aqueous methanol gave the corresponding triol (IIIa) identical with material obtained by Cannizzaro-reduction of I (see below).

Cannizzaro reduction of 3α-acetoxy-17α-hydroxy-17β-methyl-D-homoetiocholane-11,17a dione (I) *with benzaldehyde and alkali.* A solution of 200 mg. of I in 20 cc. of ethanol was treated with 2 cc. of benzaldehyde and 10 cc. of 15% aqueous potassium hydroxide and allowed to stand at room temperature for 18 hr. The product was watered out and crystallized from ethyl acetate to give 100 mg. of IIIa, m.p. 250–252°; $[\alpha]_D^{Me_2CO}$ +55.3°.

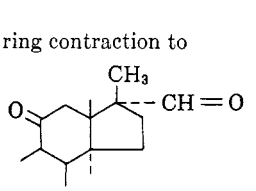
Anal. Calcd. for $C_{21}H_{32}O_4$: C, 72.41; H, 9.20. Found: C, 72.14; H, 9.67.

This material was identical with that obtained by saponification of the 244–246° melting monoacetate obtained from the Raney nickel reduction (see above). Conversely acetylation of this triol followed by chromatography yielded some of the 3-monoacetate, m.p. 244–246°.

Chromium trioxide oxidation of 3α-acetoxy-17α,17aβ-dihydroxy-17β-methyl-D-homoetiocholane-11-one (III). To a solution of 198 mg. of the triol monoacetate (III) in 2.0 ml. of acetic acid was added 37 mg. (10% excess) of chromium trioxide in 1 drop of water and 2 ml. of acetic acid. After 17 hr. at 25°, water and chloroform were added and the chloroform extracts were washed with potassium bicarbonate, water, and dried over magnesium sulfate. The crude crystalline neutral product (140 mg.) was purified by chromatography on acid-washed alumina to give prismatic needles with m.p. 155° phase change to hexagonal prisms, m.p. 169–170°, identical with the D-homo ketol (I) by mixed melting point and infrared spectral comparisons.

Chromium trioxide oxidation of 3α-acetoxy-17α,17aα-dihydroxy-17β-methyl-D-homoetiocholane-11-one (II). The isomeric triol monoacetate II, when treated with a slight excess

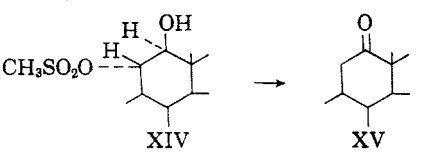
(13) Sterically, ring contraction to



would have been anticipated to follow migration of the sulfonyl group.

(14) N. L. Wendler, R. F. Hirschmann, H. L. Slates, and R. W. Walker, *J. Am. Chem. Soc.*, **77**, 1632 (1955).

(15) Inasmuch as IIb was not obtained crystalline the possibility remains that IX arises from a small amount of a dimesylate species present in IIb. In this event the formation of IX would be comparable to the formation of XV from XIV.



(16) The D-homoannulation of XIII with "aluminum-*t*-butoxide" in toluene and cyclohexanone, was found to give in addition to I (see ref. 2a) as much as 40% of II. The aluminum *t*-butoxide was commercial reagent obtained from Matheson Co., East Rutherford, N. J. It is believed that this reagent was probably contaminated with an appreciable amount of aluminum isopropoxide which was responsible for reduction of the initially formed I.

over one equivalent of chromium trioxide, as described above, also produced material identical with the D-homoketol I.

Periodic acid cleavage experiments. (A) Cleavage of the cis-glycol 3α-acetate (II). To a solution of the *cis*-glycol 3α-acetate (784 mg.; 2.00 millimoles) in 10 ml. of methanol and 3 ml. of dioxane was added 684 mg. (3.00 millimoles) of periodic acid dihydrate in 10 ml. of water. Iodimetric titration of aliquots[17] showed the reaction to be complete within 5 min. Concentration on the water pump followed by chloroform extraction gave the noncrystalline keto-aldehyde (i).

(B) Cleavage of the cis-glycol 3α-ol (IIa). Similar treatment of the *cis*-glycol 3α-ol (14.00 g.) indicated consumption of an equivalent of periodic acid within 1 min. to give the amorphous keto-aldehyde (i); $\lambda_{max}^{CHCl_3}$ 3.00, 3.70, 5.80, 5.82 μ.

(C) Cleavage of the trans-glycol 3α-acetate (III). Under similar conditions the *trans*-glycol 3α-acetate (589 mg.) required *ca.* 2 hr. to react with an equivalent of periodic acid.

16-Acetyl-Δ[16]-etiocholene-3α-ol-11-one (IV). The keto aldehyde (i) (28 g.) was dissolved in 700 ml. of *t*-butyl alcohol and the air displaced by nitrogen. A solution of 1.30 g. of potassium in 43 ml. of *t*-butyl alcohol was added rapidly to the stirred steroid solution maintained at 20°. After 20 min. the mixture was neutralized with 13.5 ml. of 2.5*N* HCl and the solvent removed *in vacuo*. Saturated salt solution and chloroform were added and the mixture extracted thoroughly with chloroform. The partly crystalline residue was chromatographed on acid-washed alumina (15:1). The eluates from 10% benzene-chloroform to 100% chloroform which contained crystalline material melting over 190° were combined (15.7 g.). Crystallization from acetone-ether gave clusters of needles, m.p. 204–206° with partial softening and phase change to individual needles at 170–180°; $\lambda_{max}^{CH_3OH}$ 237 mμ (12,300); $\lambda_{max}^{CHCl_3}$ 2.79, 2.90–2.95, 5.86, 6.00, 6.24 μ.

Anal. Calcd. for $C_{21}H_{30}O_3$: C, 76.33; H, 9.15. Found: C, 76.21; H, 9.33.

The mother liquors possessed weak absorption in the 280-mμ region indicating the presence of small amounts of a dienone, the quantity of which was increased at the expense of the enone IV by extending the reaction time. This substance was not investigated further.

The periodic acid cleavage product of the *trans*-glycol 3α-acetate (III) when treated with potassium *t*-butoxide as above also gave (IV).

Room temperature acetylation of IV with acetic anhydride in pyridine produced 3α-acetoxy-16-acetyl-Δ[16]-etiocholene-11-one (IVa) which was obtained as a colorless oil; $\lambda_{max}^{CH_3OH}$ 237.5 mμ (10,500); $\lambda_{max}^{CHCl_3}$ 5.82, 5.88, 6.02, 6.27, 8.01 μ.

Oxidation of IVa. The 3-acetate of IV was treated with potassium permanganate as described in reference 2b. The acidic product, m.p. 225–230°, was produced in good yield and was identical with 3α-acetoxy-11-keto-etiobilianic acid by mixed melting point and infrared comparisons.

3α,17α,17aα-Trihydroxy-17β,17aβ-dimethyl-D-homoetiocholane-11-one (V). Methylmagnesium iodide was prepared in ether solution from 2.4 g. of magnesium. After complete reaction the ether was distilled off and replaced with benzene until the distillation temperature reached 65°. To the benzene-ether solution of the Grignard reagent was added dropwise with stirring at room temperature 1.17 g. of I in 20 cc. of benzene. The reaction mixture was refluxed for 1 hr., cooled, and hydrolyzed with water and ammonium chloride. Product crystallized from acetone-hexane, m.p. 208–213°.

Anal. Calcd. for $C_{22}H_{36}O_4$: C, 72.53; H, 9.90. Found: C, 72.66; H, 9.98.

3α-Hydroxy-17-methyl-16-acetyl-Δ[16]-etiocholene-11-one (VI). A solution of 300 mg. of the triolone (V) in 10 cc. of dioxane was treated with 1 g. of periodic acid in 5 cc. of water for 18 hr. The amorphous product in 10 cc. of methanol was treated with 5 cc. of 15% aqueous potassium hydroxide and refluxed for 2 hr. Concentration of the methanol afforded a product which was crystallized several times from acetone-hexane to give VI m.p. 196.5–200°. $\lambda_{max.}^{CH_3OH}$ 251 mμ (10,320).

Anal. Calcd. for $C_{22}H_{32}O_3$: C, 76.74; H, 9.30. Found: C, 76.84; H, 9.03.

Acylation of 3α-acetoxy-17α,17aα-dihydroxy-17β-methyl-D-homoetiocholane-11-one (II). Acetylation of II with acetic anhydride in pyridine at room temperature afforded a noncrystalline diacetate exhibiting OH in the infrared. Oxidation of the latter with CrO_3 in acetic acid was essentially without effect as judged by infrared spectral comparison. These observations confirm the structure of a 3α,17aα-diacetate. Similarly mesylation of II (400 mg.) in pyridine (5 cc.) with mesyl chloride (1.5 cc.) at 0–5° for 16 hr. afforded an amorphous mesylate showing OH in its infrared spectrum $\lambda_{max.}^{Nj}$ 2.85–3.02 (OH), 5.8, 8.0 (OAc); 5.85 (C=O), 7.4, 8.5 μ (OSO_2CH_3). This substance is consequently assigned structure IIb.

Alkaline decomposition of 3α-acetoxy-17α-hydroxy-17aα-methanesulfonyloxy-17β-methyl-D-homoetiocholane-11-one (IIb). The product from mesylation of 1 g. of II in 10 cc. of tetrahydrofuran was added to a refluxing solution of 10% sodium methoxide (50 cc.) and refluxed for 2 hr. The reaction mixture was concentrated, the residue extracted and acetylated with acetic anhydride in pyridine. The acetylated product was chromatographed on acid-washed alumina. The eluates consisting of benzene and 1% ether in benzene afforded *3α-acetoxy-17α-methyl-D-homoetiocholane-11,17a-dione* (IX), m.p. 162–163°, not depressed on admixture with an authentic sample prepared from XI → X → IX (see below). The eluates consisting of 5% ether in benzene afforded a mixture of the 17aα and 17aβ-methyl-17 ketones VII and VIII, m.p. 190–210°. This product mixture was identical in the infrared with the product of base isomerization of pure VIII prepared below.

3α-Acetoxy-17β-methyl-D-homoetiocholane-11,17a-dione (X). 3α-Acetoxy-17-methyl-Δ[16]-D-homoetiocholene 11,17a-dione (186 mg.; 0.500 millimole) in 15 ml. of methanol was hydrogenated at atmospheric pressure and 25° over 290 mg. of 25% Pd-on-$CaCO_3$ catalyst. After uptake of one mole of hydrogen (20 min.) the catalyst was removed by filtration and the solvent removed on the water pump. The crystalline residue on two crystallizations from ether–petroleum ether gave 168 mg. of rectangular prisms, m.p. 156–159°, $\lambda_{max.}^{CCl_4}$ 5.76, 5.82, 8.0 μ.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.75; H, 9.15. Found: C, 73.60; H, 8.85.

3α-Acetoxy-17α-methyl-D-homoetiocholane-11,17a-dione (IX). To 120 mg. of the 17β-methyl-D-homoetiocholane (X) in 2.0 ml. of methanol was added a solution of 250 mg. of potassium hydroxide in 3.0 ml. of methanol. The mixture was refluxed for 90 min. under nitrogen. The reaction mixture was cooled, water added, and the crystalline 3α-ol filtered and washed with water. Room temperature acetylation in 1 ml. of acetic anhydride and 1 ml. of pyridine for 18 hr. gave the 17α-methyl compound (IX) as needles from ether–petroleum ether, m.p. 164–166°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.75; H, 9.15. Found: C, 73.85; H, 8.97.

A mixture melting point of (IX) and (X) depressed to 133–149° and the respective infrared spectra differed in the fingerprint region.

3α-Acetoxy-17aα-methyl-D-homoetiocholane-11,17-dione (VIII). A solution of 3.75 g. of 3α-acetoxy-17α-hydroxy pregnane-11,20-dione (XIII) in 50 cc. of hot methanol was treated with a solution of 3.75 g. of hydroxylamine hydrochloride and 5 g. of sodium acetate in 15–20 cc. of water. Sufficient water was added to maintain homogeneity and

(17) *Scott's Standard Methods of Chemical Analysis*, Fifth Edition; D. Van Nostrand Company, Inc., New York, N. Y., p. 1208.

the reaction mixture was refluxed for 1 hr. on a steam bath and allowed to stand at room temperature overnight. The solvents were evaporated and product dissolved in ether and the ether solution washed with water, dried, and concentrated to give the C-20 oxime as a solid, m.p. 216–220°.[18]

Anal. Calcd. for $C_{23}H_{35}O_5N$: C, 68.21; H, 8.69; N, 3.45. Found: C, 68.38; H, 8.91; N, 3.18.

The above oxime (3 g.) was hydrogenated in 30 cc. of acetic acid with 600 mg. of platinum oxide catalyst. The hydrogenation product was filtered, evaporated *in vacuo*, and redissolved in 15 cc. of acetic acid and 45 cc. of water. To the acetic acid solution was slowly added at 0° 5 g. of sodium nitrite dissolved in 5 cc. of water. A gummy oil slowly separated which solidified overnight at room temperature. The solid was extracted with ethyl acetate and the ethyl acetate extract washed free of acid with aqueous potassium bicarbonate solution and dried over magnesium sulfate. The product obtained after evaporation of the solvent was acetylated with pyridine and acetic anhydride and the acetylated product chromatographed on acid-washed alumina. The initial eluate afforded a small amount of the 17a ketone IX. Eluates consisting of 10% to 20% ether in benzene gave an appreciable amount (500 mg.) of a nitrogenous individual (see ref. 9), m.p. 180–183°. Found: C, 65.91, 65.70; H, 8.06 which was not further investigated. The eluates consisting of 5–10% ether in benzene afforded 500–600 mg. of VIII, m.p. 237–240°.

Anal. Calcd. for $C_{23}H_{34}O_4$: C, 73.75; H, 9.15. Found: C, 73.76; H, 9.02.

Treatment of 150 mg. of VIII in 15 cc. of 10% sodium methoxide in methanol and refluxing for 2 hr. afforded, on working up, a crystalline mixture of VII and VIII from ether, m.p. 193–209°. Mixed melting point with material obtained from alkaline decomposition of IIb 190–212°, the infrared spectra of the two samples were identical.

Acknowledgment. The authors express their appreciation to R. D. Hoffsommer for valuable assistance in the preparation of certain key intermediates.

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(18) Prepared by H. Kuo of these Laboratories.

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Microbially Produced 7α- and 7β-Hydroxy-Δ⁴-3-keto Steroids

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Received December 30, 1957

Microbial methods for producing 7α- and 7β-hydroxy-Δ⁴-3-keto steroids are described. Progesterone and desoxycorticosterone were converted to their 7α-hydroxylated analogs with a *Helminthosporium* culture and to their 7β-hydroxylated analogs with a *Cladiosporium* culture. Characterization of the 7-hydroxy progesterones was effected by conversion to the common intermediate, Δ⁴,⁶-pregnadiene-3,20-dione. Assignment of configuration is based on the differential rates of oxidation with chromium trioxide.

In examining a wide variety of microbially produced hydroxyprogesterones we have recently encountered two products which upon treatment with methanolic sodium hydroxide showed the shift in ultraviolet absorption maximum from 240 mμ to 285 mμ characteristic of 7-hydroxy-Δ⁴-3-keto steroids.

Characterization of these two isolates as 7-hydroxyprogesterones was accomplished by converting both to Δ⁶-progesterone (Δ⁴,⁶-pregnadiene-3,20-dione) by dehydrating in methanolic sodium hydroxide. Differentiation was established by the different melting points (7α-, m.p. 227–231°, 7β-, m.p. 188–191°) and the nonidentity of the infrared spectra at the longer wave lengths, particularly bands at 9.78 μ and 11.24 μ present in the 7α-hydroxy spectrum and absent in the 7β-hydroxyprogesterone spectrum.

Evidence for the assignment of configuration for the epimeric 7-hydroxyprogesterone was obtained from a study of their relative rates of oxidation with chromium trioxide. Employing the excellent micromethod recently described by Grimmer[1] we found that the epimer produced by the *Helminthosporium* culture (m.p. 227–231°) oxidized much more rapidly than the epimer produced by the *Cladiosporium* culture (m.p. 188–191°). Grimmer reported that under the conditions used axial hydroxyls react more rapidly than the corresponding equatorial epimers. On this basis we have assigned the 7α-hydroxy configuration to the higher melting epimer and conversely the 7β-hydroxy configuration to the lower melting epimer.

No conclusions as to the configuration of the two epimeric 7-hydroxy progesterones could be derived from their optical rotations since both compounds showed nearly identical rotations.

Careful paper strip chromatography, using the system benzene:cyclohexane/formamide:methanol, was successful in differentiating these two compounds. Two points of interest were noted in this chromatogram: (*a*) The 7β (equatorial) epimer was found to be more mobile (17.0 cm. in 30 hr.) than the 7α (axial) epimer (14.0 cm. in 30 hr.). This is the first example, in the hydroxyprogesterone series, of a violation of Savard's rule[2] which proposes that equatorially hydroxylated steroids are more polar than the axial epimers on paper strip chromatograms with Zaffaroni systems.[3]

(1) G. Grimmer, *Angew. Chem.*, 69, 400 (1957).

(2) K. Savard, *J. Biol. Chem.*, 202, 457 (1953).

(3) A. Zaffaroni, R. B. Burton, and E. H. Keutmann, *Science*, 111, 6 (1950).