The residue was dissolved in 50 ml. of hexane and chromatographed on 20 g. of silica gel. Elution with 5% ether–95% hexane yielded 226 mg. of β-ol (m.p. 153–155°), 10% ether–90% hexane yielded 140 mg. of a mixture of epimers and 20% ether–80% hexane yielded 120 mg. of α-ol (m.p. 177–179°). Rechromatography of the center fraction yielded 33 mg. of β-ol (m.p. 153–155°), 39 mg. of α + β-ols and 58 mg. of α-ol (m.p. 177–179°). The total recovery was 259 mg. (52%) of β-ol and 178 mg. (37%) of α-ol. Recrystallization of the β-ol from methanol gave material melting 153.3–155.1° (lit.[13] 154–155°) and recrystallization of the α-ol from ether gave material melting 177.1–178.9° (lit.[13] 178–180°).

(b) $NaBH_4$: A solution of 0.2 g. (5.3 mmoles) of $NaBH_4$, prepared by first dissolving the hydride in 5 ml. of H_2O and then diluting with 20 ml. of MeOH, was added over a period of 20 minutes to a refluxing solution of 500 mg. of the ketone in 20 ml. of MeOH and 5 ml. of H_2O. After heating for 3 hours, the reaction mixture was decomposed with 3 ml. of concd. HCl and then heated for an additional hour. The mixture was cooled, diluted with H_2O and processed as above; yield 488 mg. The crude product was chromatographed as before. The original hexane eluate gave 22 mg. of unreacted ketone and the yield of pure β-ol was 342 mg. (71%), m.p. 153–155°, and of pure α-ol was 76 mg. (16%), m.p. 177–179°.

Cholestan-3β-ol-7-one.—In our hands, the preparation of this compound by direct hydrogenation of 7-ketocholesteryl acetate followed by alkaline hydrolysis, as described by Buser,[19] was found to yield material containing some unsaturated ketone (ϵ^{235} 3000). The following indirect method was employed in the present work.

7-Ketocholesteryl acetate (15.0 g., 0.034 mole), prepared in 65% yield from cholesteryl acetate by the procedure of Oppenauer and Oberrauch[23] using *t*-butyl chromate, was dissolved in 200 ml. of ethyl acetate and hydrogenated using 1.0 g. of PtO_2. After the uptake of hydrogen had ceased (1.2 moles), the catalyst was filtered and the solvent evaporated. The crude product had a m.p. of 134–140°.

The crude material was then dissolved in 200 ml. of acetic acid and hydrogenated using 1.0 g. of PtO_2 until no further uptake of hydrogen occurred. The catalyst was filtered, the solvent evaporated and the residue recrystallized from MeOH to yield 7-hydroxycholestanyl acetate, m.p. 95–105°, yield 9.20 g. (61%). The molar extinction at 235 mμ was 30.

The above solid (9.20 g., 0.021 mole) was dissolved in 125 ml. of acetic acid and oxidized with a solution of CrO_3 (2.28 g., 0.029 mole) in 4.0 ml. of H_2O and 150 ml. of acetic acid. The reaction was allowed to proceed for 16 hours at 20°. The solvents were removed under reduced pressure at 20–30°. The residue was diluted with H_2O, extracted 3 times with ether and the ethereal extracts washed with dilute H_2SO_4, $NaHCO_3$ solution and H_2O and then dried. After evaporation of the solvent, the material was recrystallized to yield 7-ketocholestanyl acetate, m.p. 146.3–147.4° (lit.[24] 148–149°), yield 8.08 g. The over-all yield based on 7-ketocholesteryl acetate was 54%.

The acetate (4.55 g., 0.01 mole) was saponified by refluxing for one hour with 125 ml. of EtOH containing 6.0 g. of KOH. The cooled reaction mixture was diluted with H_2O, filtered and recrystallized from aqueous EtOH to give 7-ketocholestanol, m.p. 156.3–158° (lit. 156–157°,[25] 164–165°,[24] yield 3.89 g. (94%).

Reduction of Cholestan-3β-ol-7-one Acetate with $LiAlH_4$.

(a) Diethyl Ether as Solvent.—A solution of 2.24 g. (5.03 mmoles) of the acetate in 50 ml. of dry ether was reduced in the usual manner with 100 ml. of an ethereal solution of $LiAlH_4$ containing 0.056 mmole per ml. The yield of epimeric 7-hydroxy compounds was 2.05 g. (100%), $[\alpha]^{25}D$ +28.1° (α + 0.285, *c* 1.012). The infrared spectrum of this material showed no band in the carbonyl region.

A sample (1.080 g.) of the crude mixture was absorbed on 30 g. of Woelm neutral alumina. Elution with benzene gave 3 mg. of non-crystalline material. Elution with redistilled ether gave 1.01 g. (93.5%) of the isomeric 7-hydroxy compounds, $[\alpha]^{26}D$ +26.9°, (α +0.286, *c* 1.062). This rotation corresponds to a mixture of 57% α and 43% β.[26]

(b) Tetrahydrofuran as Solvent.—A mixture of 0.500 g. (1.13 mmoles) of the acetate and 0.171 g. (4.55 mmoles) of $LiAlH_4$ in 60 ml. of tetrahydrofuran was heated under reflux for 1 hour and processed as above. A quantitative yield of crude product was obtained. Chromatography yielded 2 mg. of material with hexane. The mixture of epimeric 7-hydroxy compounds showed an $[\alpha]^{25}D$ +23.2° (α +0.252, *c* 1.086) which corresponds to 65% α and 35% β.

Reduction of Cholestan-3β-ol-7-one with $NaBH_4$.—To a solution of 0.822 g. (2.04 mmoles) of the compound in 40 ml. of MeOH and 10 ml. of ether was added a solution of 0.679 g. (18.4 mmoles) of $NaBH_4$ in 5 ml. of H_2O and 25 ml. of MeOH. The reaction mixture was stirred at room temperature for 22 hours and then heated under reflux for 2 hours. After cooling, ether was added and the solution acidified with 5% HCl until acid to congo red. The layers were separated and the aqueous layer extracted 3 times with ether. The combined etheral solution was washed with 5% HCl, H_2O and dried. Evaporation of the solvent gave 0.792 g. (96%) of crude product. Chromatography of 0.700 g. as described above yielded only 2 mg. of material with benzene. The mixture of isomeric 7-hydroxy compound had $[\alpha]^{22}D$ +19.8° (α +0.252, *c* 1.282) which corresponds to 73% α and 27% β.

(23) R. V. Oppenauer and H. Oberrauch, *Anales asoc. quim. argentina*, **37**, 246 (1949).

(24) O. Wintersteiner and M. Moore, THIS JOURNAL, **65**, 1503 (1943).

(25) A. Windaus and E. Kirchner, *Ber.*, **53**, 614 (120).

(26) The $[\alpha]D$ used for the pure epimers was +51.0° for 7β and +8.5° for 7α.

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[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, WESTERN RESERVE UNIVERSITY, AND THE LAKESIDE HOSPITAL]

The Preparation of 16-Oxygenated Etianates and their Relation to Gitoxigenin[1]

BY H. HIRSCHMANN AND FRIEDA B. HIRSCHMANN

RECEIVED FEBRUARY 13, 1956

The preparation and reduction of both 17-epimers of methyl 3β-acetoxy-16-oxoetianate is described. The principal reduction product of the 17β-isomer is a *cis*-hydroxy ester which is shown to be methyl 3β-acetoxy-16β-hydroxyetianate. It gave a diacetate identical with a degradation product of gitoxigenin. The results strongly indicate that gitoxigenin is 16β-hydroxydigitoxigenin.

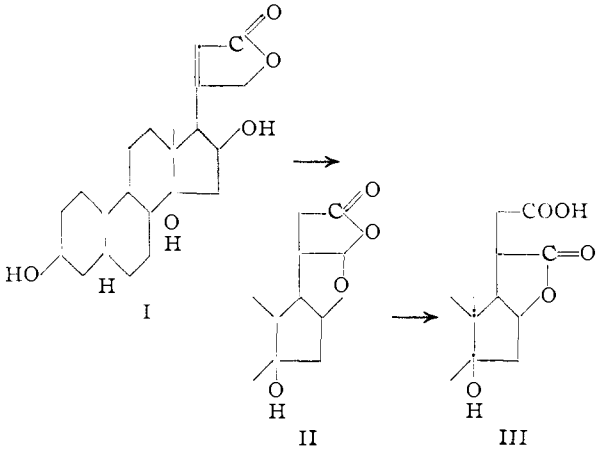
Gitoxigenin (I) has been obtained as the steroid moiety from several cardiac glucosides. It has been intensively studied in many laboratories, notably by Jacobs and his collaborators[2] and by Meyer,[3] who found it to differ from digitoxigenin by an additional hydroxyl group at C-16. The sole structural features not yet fully established concern configura-

(1) This investigation was supported by grant C-1679 of the National Institutes of Health, U. S. Public Health Service.

(2) (a) W. A. Jacobs and E. L. Gustus, *J. Biol. Chem.*, **79**, 553 (1928); (b) **82**, 403 (1929); (c) **86**, 199 (1930); (d) **88**, 531 (1930); (e) W. A. Jacobs and R. C. Elderfield, *ibid.*, **108**, 497 (1935).

(3) (a) K. Meyer, *Helv. Chim. Acta*, **29**, 718 (1946); (b) **29**, 1580 (1946); (c) **29**, 1908 (1946).

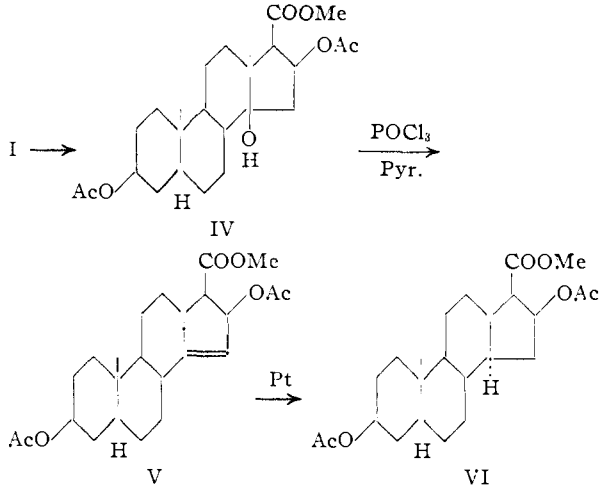
tions of ring D. The substituents at C-14 and C-17 were shown to be *cis* to each other[4] and their attachment, while not rigorously proven, is generally believed to be beta as it is in other cardiac aglycones. No such agreement exists with respect to C-16. Many years ago Shoppee[6] proposed the *cis*-relationship of all 3-substituents, since gitoxigenin forms a 16-21 oxide II on treatment with alkali,[2d] and was thought to form a 14-21 oxide upon oxidation of the 16-hydroxyl group.[7] Hydrolysis of II followed by oxidation gave a lactone III which



showed extraordinary stability toward alkali.[2a,b] This compound therefore must be a *cis*-lactone since great strain can be deduced for the epimeric structure from the repeated failures to obtain *trans*-2-hydroxycyclopentaneacetic lactone from its parent acid.[8] *cis*-Lactones of this type show normal reactivity toward alkali.[8,9] The inertness of III can be explained, however, by steric hindrance if the lactone ring is *cis* also to C-18 and to the 14-hydroxyl but seems quite inexplicable if the ring were attached to the opposite (α) side of ring D. If the 14-hydroxyl group is removed, the lactone ring hydrolyzes at a faster rate.[2b] Moreover, as pointed out by Turner,[10] the conversion[2c,e] of the lactone to etianic acid demonstrates that III has the β-configuration at C-17. The same assignment, therefore, appears justified for the oxygen function at C-16 in III and II and probably also in I.

The 16α-configuration was first proposed for gitoxigenin by Reichstein[11] and was adopted by Cardwell and Smith.[12] The main point in its favor is the facile elimination of the 16-acetoxy group and the 17-hydrogen under conditions which normally favor *cis*-elimination.[13]

Ten years ago Meyer[3a] reported a degradation of gitoxigenin to a methyl 3β,16-diacetoxyetianate (VI) and thus obtained the only degradation product which retained the oxygen function at C-16 and possessed a hydrogen with the normal orientation[3b] at C-14. Although his sequence (I to VI) of reactions involved an allylic intermediate V, the conditions used seemed mild enough[15] to assure the



retention of configuration at C-16. Our development of a procedure for the introduction of a 16α-hydroxyl group into the steroid molecule[16] prompted us to attempt the synthesis of compound VI with the aim of determining its configuration. While this work was in progress, Moore[5] reported a synthesis of the crystalline methyl 3β,16α-dihydroxyetianate and of its amorphous diacetate. Since the degradation product from gitoxigenin failed to induce crystallization and possessed a different rotation, it was concluded that the two diacetates were not identical but most likely epimeric at C-16. As Dr. Moore was unable to continue with this work, we have proceeded with our studies and have obtained the isomer of methyl 3β,16-diacetoxyetianate that is identical with the degradation product from gitoxigenin.

The procedure used by Moore for the preparation of 16α-hydroxyetianates consisted in the ozonization of the 21-benzylidene derivative of VIII, followed by hydrolysis, hydrogenolysis and methylation of the resulting acids. Our reaction sequence is longer but gave better yields. The first steps were patterned after our preparation of 16α,21-diace-

(4) K. Meyer, *Helv. Chim. Acta*, **32**, 1993 (1949); see also the discussion by Moore.[5]

(5) J. A. Moore, *ibid.*, **37**, 659 (1954).

(6) C. W. Shoppee, *Ann. Rev. Biochem.*, **11**, 125 (1942).

(7) This interpretation failed to account for the facile isomerization of the product and appears to be incorrect (*cf.* C. W. Shoppee and E. Shoppee in E. H. Rodd, "Chemistry of Carbon Compounds," Vol. IIB, Elsevier Publishing Company, Houston, Texas, 1953, p. 1012).

(8) W. Hueckel and W. Gelmroth, *Ann.*, **514**, 233 (1934); W. E. Grigsby, J. Hind, J. Chanley and F. H. Westheimer, THIS JOURNAL, **64**, 2606 (1942).

(9) J. W. Corcoran and H. Hirschmann, *ibid.*, **78**, 2325 (1956).

(10) R. B. Turner, *Chem. Revs.*, **43**, 7 (1948).

(11) T. Reichstein, *Angew. Chem.*, **63**, 418 (1951).

(12) H. M. E. Cardwell and S. Smith, *J. Chem. Soc.*, 2012 (1954).

(13) For references and discussion see Moore[5] and Cardwell and Smith.[12] A second argument presented by the British workers for the 16α-configuration is much more tenuous as it is based not on observations of gitoxigenin but a companion substance, adynerigenin, which has been obtained from the same plant. Since the hydroxyl group which this compound is alleged to have in the 16α-position could not be acetylated,[14] it is improbable that the reactions of adynerigenin proceed as formulated by Cardwell and Smith.

(14) R. Tschesche and G. Grimmer, *Chem. Ber.*, **87**, 418 (1954); R. Tschesche and G. Snatzke, *ibid.*, **88**, 511 (1955).

(15) See, *e.g.*, the retention of configuration of an unstable (axial) 6β-acetoxy group under similar conditions of dehydration and hydrogenation (D. N. Jones, J. R. Lewis, C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 2876 (1955); V. A. Petrow, O. Rosenheim and W. W. Starling, *ibid.*, 677 (1938)). In compound V the α-site is less crowded and if isomerization to the more stable form occurs, one would expect it to be from β to α. Hence if VI, as will be shown, is 16β, this configuration most likely was present already in gitoxigenin.

(16) (a) H. Hirschmann, F. B. Hirschmann and M. A. Daus, THIS JOURNAL, **74**, 539 (1952); (b) H. Hirschmann, F. B. Hirschmann and J. W. Corcoran, *J. Org. Chem.*, **20**, 572 (1955).

toxyprogesterone.[17] 3β-Acetoxy-Δ16-pregnen-20-one was hydroxylated at C-16 (IX) by the benzyl alcohol method[16b] and after acetylation (X) acetoxylated at C-21 with lead tetraacetate.[18] The 21-acetoxy group in XI was hydrolyzed with bicarbonate to the ketol which was degraded with *m*-periodate.[19] The removal of unchanged compound X is carried out most readily at this stage. Methylation of the acidic material gave chiefly methyl 3β,16α-diacetoxyetianate (XII). This product could be hydrolyzed without appreciable elimination at C-16 to yield methyl 3β-acetoxy-16α-hydroxyetianate (XIIIb) and the corresponding dihydroxy ester XIIIa. Their over-all yields from VII were 13.6 and 3%, respectively. When the pure dihydroxy ester was reacetylated, the product XII, like that of Moore, failed to crystallize. Non-identity with the degradation product VI from gitoxigenin[3a] was confirmed by infrared comparison.

The oxidation of the β-hydroxy ester XIIIb with the chromium trioxide–pyridine complex[20] gave very little acidic material. Chromatography of the neutral fraction removed some unchanged starting compound but otherwise caused little fractionation. Nevertheless the main product could be separated by crystallization from non-polar solvents into two products with the composition of a methyl acetoxyoxoetianate. They were recognized as distinct compounds by their rotations and their infrared spectra which differed widely in the fingerprint region. The carbonyl peaks indicated that both were 16-ketoetianates.[21] Since only epimerism at C-17 is consistent with the mode of formation and the spectra of these isomers, they have been assigned structures XIV and XV.[22] Although vicinal interaction effects were expected, the observed rotations agreed quite well with the calculated values.[23] Accordingly, the less levorotatory isomer has been assigned the 17-normal configuration.

Since two β-keto esters epimeric at the α-carbon atom possess a common enol, their ready interconversion was to be anticipated. Partial epimerization of either isomer could in fact be observed spectrographically on several occasions.[25] Nevertheless the process must be considerably slower than the enolization of simple β-ketoesters[26] to have permitted the fractionation achieved. Since the melting ranges of the final products were rather wide it is probable that epimerization interfered to some extent with purification. Nevertheless this mutual contamination could not have been very serious judging from the very good reproducibility of the infrared curves and the results of the hydrogenations to be reported. The enol content of compound XIV, in 95% ethanol, was estimated spectrographically as less than 1% and hence as substantially lower than that of ethyl 2-oxocyclopentanecarboxylate.[27] This difference probably can be ascribed to the attachment of a chair form of a cyclohexane ring which should interfere with the coplanarity of the ethenoid carbons and their substituents, particularly if the fusion is *trans*.[28]

The conversion of 16-ketosteroids to 16β-hydroxy compounds has been realized by a variety of procedures including hydrogenation with platinum in acetic acid[24] or with nickel in alcohol[24,30] or reduction with sodium borohydride.[9] When these methods were applied to compound XIV, they all gave the same hydroxy ester XVI although in greatly varying yields. When nickel was used in large amounts, compound XVI was the sole product that could be detected. In contrast to the isomeric structure XIIIb which possessed a hydroxyl peak at 2.78 and a single carbonyl peak at 5.77 μ, the new hydroxy ester showed a hydroxyl peak at 2.86 and two clearly separated maxima at 5.76 and 5.83 μ. Since this spectral pattern persisted on dilution, it was concluded that the hydroxyl group is linked by hydrogen bonding to the ester carbonyl of the same molecule.[31] This signified a *cis* relationship between these groups.[32] When the nickel reduction was applied to the 17-iso ester XV, the reaction product, which failed to crystallize, possessed a spectrum very different from XVI. The main constituent XVIIIa again showed hydrogen

(17) H. Hirschmann, F. B. Hirschmann and G. L. Farrell, THIS JOURNAL, **75**, 4862, 6363 (1953).

(18) T. Reichstein and C. Montigel, *Helv. Chim. Acta*, **22**, 1212 (1939).

(19) S. A. Simpson, J. F. Tait, A. Wettstein, R. Neher, J. v. Euw, O. Schindler and T. Reichstein, *ibid.*, **37**, 1200 (1954).

(20) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953).

(21) N. J. Leonard, H. S. Gutowsky, W. J. Middleton and E. M. Petersen (*ibid.*, **74**, 4070 (1952)) observed a frequency shift of both carbonyl peaks, if a keto group is in a 5-membered ring and β to the carboxylate. Ethyl 2-oxocyclopentanecarboxylate (pure liquid) had maxima at 5.69 and 5.80 μ. If the molecule contained a second more distant ester group, the 5.8 μ maximum and the normal ester peak were not resolved (λ_{max} 5.76 or 5.77 μ). Compound XIV showed maxima at 5.66 and 5.76 μ, compound XV at 5.69 and 5.78 μ in carbon disulfide.

(22) Recently a lecture was published in which Fried reported the conversion of biosynthetic 16α,21-dihydroxyprogesterone to methyl 3,16-dioxo-Δ4-etienate (J. Fried, R. W. Thoma, D. Perlman, J. E. Herz and A. Borman, *Recent Progr. Hormone Research*, **11**, 149 (1955)). Isomerism of the keto ester was not mentioned.

(23) [*M*]D calculated for XIV −334°, for XV −606°; found −320° and −537°. The calculations are based on [*M*]D for 3β,20β-diacetoxyallopregnan-16-one[24] −406°, 3β,20β-diacetoxyallopregnane +125° (H. Hirschmann, M. A. Daus and F. B. Hirschmann, *J. Biol. Chem.*, **192**, 115 (1951)), methyl 3β-acetoxyetianate (XVII) +197° (all in alcohol), methyl 3β-acetoxy-17α-etianate −105° (chloroform, M. Sorkin and T. Reichstein, *Helv. Chim. Acta*, **29**, 1209 (1946)), approximate solvent correction Δ[*M*]D EtOH − Chl. +30° (D. H. R. Barton and W. Klyne, *Chemistry & Industry*, 755 (1948)).

(24) H. Hirschmann, F. B. Hirschmann and M. A. Daus, *J. Biol. Chem.*, **178**, 751 (1949).

(25) In view of this it was most startling to find that XIV gave a transitory purple color with ferric chloride, while XV did not unless it had first been partially isomerized by exposure to pyridine.

(26) Ethyl acetoacetate is quite stable in either one of its tautomeric forms only if the preparation is highly purified. Polar solvents like alcohol which favor the keto form greatly accelerate equilibration. Non-polar solvents shift the equilibrium toward the enol but do not catalyze equilibration. However, trace impurities act as very potent accelerators (P. Grossmann, *Z. physik. Chem.*, **109**, 305 (1924); F. O. Rice and J. J. Sullivan, THIS JOURNAL, **50**, 3048 (1928)).

(27) W. Dieckmann, *Ber.*, **55**, 2470 (1922); Buu-Hoi and P. Cagniant, *Bull. soc. chim. France*, [5] **10**, 251 (1943).

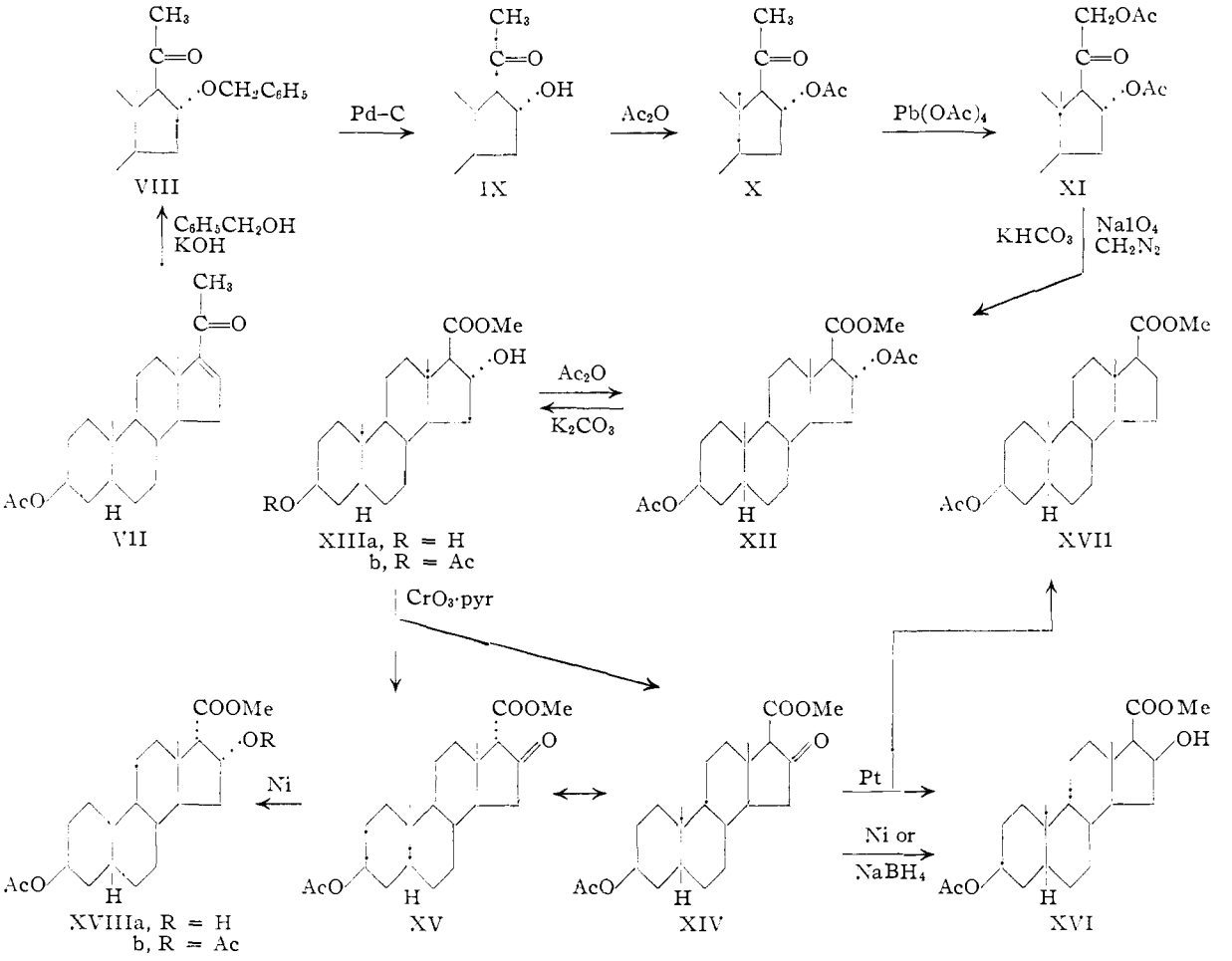
(28) In keeping with the usual behavior of β-diketones, 16,20-dioxosteroids enolize much more extensively. Nevertheless only a single crystalline species has been described in every case (G. E. Arth, G. I. Poos and L. H. Sarett, THIS JOURNAL, **77**, 3834 (1955); S. Bernstein, M. Heller and S. M. Stolar, *ibid.*, **77**, 5327 (1955)). This, however, appears not to be a diketone but a chelated enol.[21,29]

(29) R. S. Rasmussen, D. D. Tunnicliff and R. R. Brattain, *ibid.*, **71**, 1068 (1949).

(30) S. Kaufmann and G. Rosenkranz, *ibid.*, **70**, 3502 (1948).

(31) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *ibid.* **74**, 2820 (1952).

(32) P. Hirsjaervi, *Acta Chem. Scand.*, **8**, 12 (1954).



bonding between the hydroxy group and the ester carbonyl.[33] The formation of two different *cis*-hydroxy esters from the isomeric keto esters XIV and XV demonstrated that the hydrogenation could not have been preceded by an equilibration of the two keto esters. Moreover, the substrate must have been adsorbed on the catalyst as the ketone rather than the enol. If the reaction represents the hydrogenation of the carbonyl group, the hydroxy esters have the same configurations at C-17 as the keto esters from which they were derived. However an alternative mechanism seems at least conceivable, namely, that the keto ester enolized while attached to the catalytic surface and was reduced as such. In this case the configuration that results at C-17 would depend entirely on the direction of the attachment of the catalyst to the steroid. If the catalyst were attached to the β-side of compound XIV, inversion would result from this mechanism and the reduction product would then possess structure XVIIIa.

The results of the hydrogenation of the 17-normal keto ester XIV with platinum, however, render this alternative formulation very unlikely. In this case the hydroxy ester XVI was accompanied by a hydrogenolysis product[34] XVII which was unequivocally identified as methyl 3β-acetoxyetianate. Since the hydrogenolysis of enol acetates is a common occurrence with this catalyst[35] we should expect that the postulated reduction through the enol would also be accompanied by hydrogenolysis. If this is the mode of formation of XVII, the α-attachment of the catalyst can be deduced from the configuration of the hydrogenolysis product at C-17. This, however, is the opposite of that postulated for the alternative formulation of the hydroxy ester.

The results obtained with sodium borohydride fully confirm that the reduction of XIV to XVI involved no inversion at C-17. If it did, the epimerization would have had to occur prior to reduction, and the borohydride reduction of XV which already possesses the 17-iso structure should then have given at least the same amount of XVI as was obtained from the normal ester XIV. Examination

(33) The wave length of the hydroxyl peak (2.83 μ) was significantly shorter than in XVI, but the maximum at 5.83 μ provided full confirmation of hydrogen bonding. (In order not to mistake an α,β-unsaturated for a hydrogen bonded carboxylate, the sample was acetylated. The peak at 5.83 μ disappeared.) The shorter wave length of the hydroxyl peak indicates a weaker (longer) hydrogen bond (L. P. Kuhn, THIS JOURNAL, **74**, 2492 (1952)). The reason for this difference between the two isomers is not clear.

(34) Carbon-oxygen hydrogenolysis has been reported also for acetoacetate when a platinum, but not when a nickel catalyst was used: (a) M. Faillebin, *Ann. chim.* (*Paris*), [10], **4**, 156 (1925); (b) H. Adkins, "Reactions of Hydrogen with Organic Compounds over Copper-Chromium Oxide and Nickel Catalysts," The University of Wisconsin Press, Madison, Wis., 1944, p. 51; (c) H. Adkins and H. R. Billica, THIS JOURNAL, **70**, 695 (1948).

(35) H. H. Inhoffen, G. Stoeck, G. Koelling and U. Stoeck, *Ann.*, **568**, 52 (1950), and references there cited; R. Hirschmann, M. Brown and N. L. Wendler, THIS JOURNAL, **73**, 5373 (1951).

of the crude reaction product, however, failed to give any indication of the formation of the hydroxy ester XVI from the 17-isoester XV. We conclude, therefore, that the hydroxy ester XVI has the same β-configuration at C-17 as compound XIV. Since the carboxylate was shown to be *cis* to the hydroxyl. compound XVI is methyl 3β-acetoxy-16β-hydroxyetianate.

Comparison of the rotations of the two 16,17-*cis* methyl 3β,16-diacetoxyetianates lends support to this assignment. Inversions of a carboxylate at C-17 from β to α and of an acetoxy group at C-16 in the same direction generally cause large negative shifts. The 16-acetate of XVI, therefore, should be more dextrorotatory than XVIIIb. Although the rotational difference was much smaller than estimated, assuming no vicinal effect, it has the correct sign.

According to our formulations compounds XVI and XIIIb are epimeric at C-16. The relative orientations of the substituents at C-16 and C-17 in these compounds are consistent with the following observations: Whereas the *trans*-ester XIII acetylates readily with pyridine and acetic anhydride at room temperature under our standard conditions, a complete reaction with XVI required much longer periods. Furthermore, treatment of XII with carbonate caused little elimination at C-16, whereas the acetate of XVI under these conditions gave large amounts of the α,β-unsaturated ester. Since the 16-acetoxy group in XVI is *trans* to the hydrogen at C-17, the more facile elimination in an ionic reaction is to be anticipated.

The acetate of methyl 3β-acetoxy-16β-hydroxyetianate (XVI) was identical with the degradation product VI from gitoxigenin.[3a] Since the reactions reported by Meyer could not have altered the configuration at C-17, it follows that the side chain at C-17 and, therefore,[4] the hydroxyl group at C-14 of gitoxigenin likewise have the β-orientation. As pointed out above, the degradations most probably also preserved the configuration at C-16. The identification of this degradation product as a 16β-acetoxy steroid, therefore, lends strong support to the view that gitoxigenin likewise possesses a β-oriented hydroxyl at C-16. If the new evidence is considered jointly with the observations of Jacobs, *et al.*,[2] there remains little ground to question the correctness of this assignment.

Experimental

General Procedures.—All m.p.'s are corrected. Unless when noted otherwise, rotations, ultraviolet spectra were measured in 95% ethanol, infrared spectra in carbon disulfide with a Perkin–Elmer single beam spectrometer with double pass. The infrared bands given in parentheses are those particularly useful for the differentiation from the other stereoisomers described in this paper, the others serve to characterize functional groups. These include the complex ester peak near 8 μ (axial acetate)[36] and the maxima near 8.63 (acyl–oxygen stretching of methyl etianates)[36] and 9.80 μ (alkyl–oxygen stretching of 3β-acetoxy-5β-steroids).

Reaction products were isolated by ether extraction, washed free of basic or acidic reagents with hydrochloric acid or bicarbonate in the cold and then with water. In chromatography, a 2:1 mixture of silica gel and Celite (50 times the weight of the crude steroid) was used and prewashed as described.[9] The eluent generally was benzene containing increasing amounts of ether. The ligroin used had b.p. 90–96°, the petroleum ether 60–70°.

(36) R. N. Jones and F. Herling, *J. Org. Chem.*, **19**, 1252 (1954).

3β-Acetoxy-16α-benzyloxypregnan-20-one (VIII).—The treatment of 1.33 g. of 3β-acetoxy-Δ^{16}-pregnen-20-one (VII) with 40 ml. of 3% benzyl alcoholic potassium hydroxide was carried out for 3.5 hr. at 28° as described previously.[16] The crude reaction product was reacetylated with 8 ml. of pyridine and 4 ml. of acetic anhydride at room temperature for 17 hr. The mixture of acetates (1.6 g.) was chromatographed. Most of the earlier eluates gave unreacted starting material, the later fractions furnished 3β-acetoxy-16α-benzyloxypregnan-20-one which crystallized from methanol in needles (798 mg., m.p. 133–136.5°). The analytical sample gave m.p. 135–137°, $[\alpha]^{26}D$ +24° (*c* 0.8, chloroform) λ_{max} 5.76, 5.86, 13.66 and 14.36 μ (last two, benzyloxy).[16] *Anal.* Calcd. for $C_{30}H_{42}O_4$: C, 77.21; H, 9.07. Found: C, 77.05; H, 9.23.

Methyl 3β-Acetoxy-16α-hydroxyetianate (XIIIb).—The procedure finally adopted was carried out without purification of intermediates. Infrared spectroscopy and, in the case of step c, the tetrazolium reaction[37] were used to check on the success of the reactions.

(a) **Reduction.**—A mixture of catalyst (303 mg. of 5% palladium chloride–charcoal, pre-reduced and washed as described before),[16b] of 798 mg. of 3β-acetoxy-16α-benzyloxypregnan-20-one (VIII) and of 180 ml. of 95% alcohol was stirred magnetically in an atmosphere of hydrogen. The gas uptake ceased after 37 minutes. After removal of the catalyst, the solution gave 646 mg. of crystalline residue. Recrystallization of such material from acetone gave 3β-acetoxy-16α-hydroxypregnan-20-one (IX) (m.p. 165–166.5°, $[\alpha]^{28}D$ +49° (*c* 0.45)). *Anal.* Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.08; H, 9.65.

(b) **Acetylation.**—Crude compound IX (646 mg.) in 5 ml. of pyridine and 3 ml. of acetic anhydride was kept at room temperature for 14.5 hr. The reaction product (744 mg.) failed to crystallize. Its infrared spectrum showed no hydroxyl peak nor any of the maxima assigned to the benzyloxy group.

(c) **Acetoxylation.**—The crude acetate (744 mg.) was distributed over 12 tubes. (This may not be necessary. It was done because in the Δ^5-series[17] yields were much better with small batches.) To each was added 5.5 ml. of a warm solution of lead tetraacetate (6.72 g. in 92 ml. of glacial acetic acid and 7.75 ml. of acetic anhydride). The sealed tubes were kept at 68° for 46 hr. The excess anhydride was hydrolyzed with water after the addition of pyridine (1 ml. per tube). The combined reaction mixture gave 785 mg. of neutral product.

(d) **Hydrolysis and Ketol Cleavage.**—A mixture of this material in 340 ml. of methanol and 8.25 ml. of 1 *N* aqueous potassium bicarbonate was kept under nitrogen for 4.5 hr. at 24°, acidified with 0.59 ml. of glacial acetic acid and concentrated *in vacuo*. Since the infrared spectrum of the neutral reaction product showed incomplete hydrolysis of the 21-acetoxy group, the hydrolysis was repeated (7 hr.). (The shorter reaction period is adequate if the 16-hydroxy group is formylated rather than acetylated.) A mixture of the neutral fraction (713 mg.) in 100 ml. of methanol and of 60 ml. of 2% sodium *m*-periodate in water was kept in the dark for 135 minutes and then concentrated *in vacuo*. The product was extracted from the acidified solution with ether–chloroform and separated with sodium carbonate into an acidic and neutral (363 mg.) fraction. The latter can be reworked by repeating steps b, c and d. (Sodium bicarbonate proved to be quite ineffective in extracting etianic acids.)

(e) **Methylation and Carbonate Hydrolysis.**—The acidic fraction in 2 ml. of methanol was treated with an excess of diazomethane in ether (22 ml.). The product (341 mg.) was mostly the diacetate XII of methyl 3β,16α-dihydroxyetianate, but contained some 3-monoacetate XIIIb owing to partial hydrolysis of a nuclear acetate group during the prolonged bicarbonate treatment in step d. To complete the hydrolysis, a mixture of this product in 25 ml. of methanol and 5 ml. of 0.5 *N* aqueous potassium carbonate was kept at 20° for 15 hr. The neutral reaction product (309 mg.) was chromatographed. The early eluates failed to crystallize. The middle fractions (benzene + 10% ether) were freed of a sparingly soluble brown pigment by repeated extractions with ligroin–methanol (20:1). Recrystallization from ligroin gave 198 mg. of methyl 3β-acetoxy-16α-hydroxyetianate (XIIIb) (m.p. 122.5–124.5°). One specimen on reheating of the solidified melt gave m.p. 129.5°. The analytical

(37) W. J. Mader and R. R. Buck, *Anal. Chem.*, **24**, 666 (1952).

sample (m.p. 123.5–124.5°) had $[\alpha]^{27}D$ +20° (*c* 0.6), λ_{max} 2.78, 5.77, 8.00, 8.11, 8.63, 9.79 (9.50 and 13.15) μ. *Anal.* Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.25. Found: C, 70.21; H, 9.36.

The late eluates upon recrystallization from acetone gave 40 mg. of **methyl 3β,16α-dihydroxyetianate (XIIIa)**. One preparation showed m.p. 204–205°, another 190–192° and 204° on reheating of the solidified melt, $[\alpha]^{27}D$ +22° (*c* 0.5, methanol). Moore[5] reported m.p. 190–192° and $[\alpha]D$ +18.6°.

Alternative Preparation of Compound XIII.—At the outset of the experiments it was unknown whether the carbonate hydrolysis of step e would cause elimination. Therefore, compound IX was formylated at C-16 by the procedure described.[16b] Although the formate group was quantitatively hydrolyzed by the short hydrolysis of step d, ketol cleavage and methylation gave a mixture of XIIIb and XII which were separated chromatographically. Evidently step c had caused some ester exchange. This modification, therefore, does not eliminate step e (hydrolysis).

Methyl 3β,16α-Diacetoxyetianate (XII).—A solution of 10.3 mg. of methyl 3β,16α-dihydroxyetianate (XIIIa) in 1 ml. of pyridine and 0.5 ml. of acetic anhydride was kept at room temperature for 15.5 hr. The neutral product (12.6 mg.), which failed to crystallize, showed an infrared spectrum identical with that of compound XII obtained in the preparation of XIIIb by the alternative procedure. There was no hydroxyl peak, λ_{max} 5.75, 8.62, 9.78 (7.73, 8.34 and 13.15) μ.

Methyl 3β-Acetoxy-16-oxoetianate (XIV).—A solution of 195 mg. of chromium trioxide in 13 ml. of pyridine (for precautions see Poos, *et al.*[20]) was added to 194 mg. of methyl 3β-acetoxy-16α-hydroxyetianate and kept in the dark at 27° for 28 hr. The neutral reaction product (183 mg.) was freed by chromatography from unchanged starting material (eluted with benzene and 15% ether). The earlier eluates (153 mg. with benzene and 5% ether) were recrystallized from ligroin and gave 71.3 mg. of methyl 3β-acetoxy-16-oxoetianate (XIV) as needles melting at 137–144°. Continued recrystallization failed to sharpen the m.p.; $[\alpha]^{26}D$ −82° (*c* 0.6), ultraviolet λ_{max} 259 (ϵ 78, enol), shoulder ~293 mμ (ϵ 34, ketone) (*c* 0.09); infrared λ_{max} 5.66, 5.76, 8.00, 8.11, 8.64, 9.80 (7.44, 8.81, 10.42 and 12.41) μ. *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.82; H, 9.04. The sample recovered from the rotation had practically the original spectrum, but others kept for similar periods in neutral polar solvents showed partial isomerization to compound XV.

Reference data for estimating % enol[27]: ethyl 2-oxocyclopentanecarboxylate in absolute ethanol showed λ_{max} ~258 mμ, ϵ ~ 640 and bromometric enol content of 6.4%; in 95% alcohol ϵ ~ 350.

Methyl 3β-Acetoxy-16-oxo-17α-etianate (XV).—The first mother liquor (70.7 mg.) of XIV was allowed to evaporate slowly. Heavy blocks separated which were removed mechanically (43.7 mg.) and recrystallized from dilute acetone and from ligroin to yield 23.1 mg. of XV which had m.p. 150–159°, $[\alpha]^{25}D$ −137° (*c* 0.4); λ_{max} 5.69, 5.78, 8.00, 8.11, 8.62, 9.79 (7.63, 8.71, 10.15 and 10.29) μ. *Anal.* Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78. Found: C, 70.98; H, 9.04. Although there were no manifestations of mutarotation, the sample recovered from this measurement on recrystallization gave m.p. 125–134° and infrared spectrum showing extensive isomerization to XIV.

Isomerization of Keto Esters with Pyridine.—Compounds XIV (2.5 mg.) and XV (2.5 mg.) were each kept in 1 ml. of pyridine for 28 hr., diluted with ether and washed with acid. The spectra of the neutral residues were nearly identical, the minor differences indicating incomplete equilibration. The equilibrium appears to be in favor of the 17-normal epimer XIV.

Reductions of Methyl 3β-Acetoxy-16-oxoetianate (XIV).
A. With Nickel.—The catalyst for all experiments was W-5 of Adkins and Billica[34c] prepared on 0.1 scale and washed with more water (10 l.) than was needed to obtain a neutral supernatant. It was used within 3 days of preparation. This catalyst (300 mg.), 24.8 mg. of XIV and 6 ml. of 95% ethanol were shaken in an atmosphere of hydrogen for 30 minutes. The gas uptake stopped after 5 minutes. The product was recrystallized from dilute acetone and from ligroin to give **methyl 3β-acetoxy-16β-hydroxyetianate (XVI)** with m.p. 108.5–110°, $[\alpha]^{28}D$ +42° (*c* 0.6); λ_{max} 2.86, 5.76, 5.83, 7.99, 8.11, 8.60, 9.80 (9.24, 9.40, 12.35 and 13.02) μ. *Anal.* Calcd. for $C_{23}H_{36}O_5$: C, 70.37; H, 9.25. Found: C, 70.18; H, 9.38. The infrared spectrum of the crude reduction product agreed closely with that of pure XVI and showed none of the bands characteristic of XIIIb or XVIIIa. A non-crystalline preparation of this compound was described by Meyer.[35b] It probably contained methyl 3β-acetoxy-Δ[16]-etienate. Reductions of XIV using only 30 mg. of catalyst came to a standstill before completion.

Compound XVI (9.0 mg.) and its mother liquors were each kept in 1 ml. of pyridine and 1 ml. of acetic anhydride for 44 hr. The reaction products had virtually identical infrared spectra and both gave on recrystallization from dilute acetone and from petroleum ether **methyl 3β,16β-diacetoxyetianate (VI)** with m.p. 152.5–153.5°, $[\alpha]^{25}D$ +24° (*c* 0.6, 95% alcohol), +22° (*c* 0.5, chloroform); λ_{max} 5.75, 8.64, 9.80 (8.23, 8.40 and 13.01) μ. *Anal.* Calcd. for $C_{25}H_{38}O_6$: C, 69.09; H, 8.81. Found: C, 68.99; H, 8.91. The infrared spectrum was identical in every respect with that of a specimen (m.p. 152.5–153.5°) prepared by Prof. K. Meyer from gitoxin. There was no change in m.p. on admixture. The reported $[\alpha]D$ is +30.9° (chloroform).[35a] When XVI was acetylated as described for XIIIa, the hydroxyl and carbonyl peaks of the starting compound were still very distinct.

B. With Platinum.—A mixture of 14 mg. of compound XIV, of 15 mg. of platinum oxide (according to Adams and Shriner) and of 6 ml. of prereduced glacial acetic acid were shaken with hydrogen for 60 minutes. The product was acetylated and chromatographed. The early eluates (benzene + 1% ether) yielded **methyl 3β-acetoxyetianate (XVII)**. Its m.p. (127.5–128.5°) was not depressed by admixture of a reference sample, supplied by Prof. Reichstein, $[\alpha]^{25}D$ +52° (*c* 0.3); λ_{max} 5.77, 7.99, 8.13, 8.64 and 9.80 μ; lit. m.p. 126–128°, $[\alpha]D$ +54° (acetone).[38] The infrared spectrum was identical with that of the reference sample. *Anal.* Calcd. for $C_{23}H_{36}O_4$: C, 73.36; H, 9.64. Found: C, 73.71; H, 9.75.

The later eluates gave methyl 3β,16β-diacetoxyetianate (m.p. 152.5–153.5°) identified by mixture m.p. and infrared comparison. All maxima of the crude unacetylated reduction product were present in the spectra of either XVI or XVII.

C. With Sodium Borohydride.—A mixture of 8.4 mg. of compound XIV in 0.5 ml. of 80% dioxane and of 19 mg. of sodium borohydride in 0.95 ml. of the same solvent was kept at 22° for 6 hr. and then acidified with hydrochloric acid. The product showed hydroxyl peaks at 2.78 and 2.87 μ. The twin maxima of XVI at 9.24 and 9.40 μ were easily seen, but the very strong peak of XVIIIa at 9.34 μ was absent. The spectrum indicated the presence of XIIIb in this mixture, but only XVI was definitely identified by the isolation of VI after acetylation, chromatography and recrystallization.

Reductions of Methyl 3β-Acetoxy-16-oxo-17α-etianate (XV). A. With Nickel.—Ten mg. of compound XV was reduced with 350 mg. of nickel as described above. The product which failed to crystallize had λ_{max} 2.83, 5.76, 5.83, 7.99, 8.09, 8.14, 8.57 (etianate?), 9.79 and (9.34) μ and a shoulder or weak maximum near 2.78 μ. The maxima characteristic of XVI were absent. Acetylation for 45 hr. gave a product with λ_{max} 5.75, 8.65, 9.79 and (10.30) μ clearly different from VI. It did not crystallize and was chromatographed. The middle fractions had $[\alpha]^{25}D$ −12° (*c* 0.4).

B. With Sodium Borohydride.—Compound XV (2.2 mg.) was reduced as described for XIV. The product showed two hydroxyl peaks (2.77, 2.83 μ). Although evidently less pure, it was spectrographically similar to the one obtained with nickel. The twin peaks at 9.24 and 9.40 μ and other maxima characteristic of XVI were absent.

Elimination Reactions.—Compound XII (2.5 mg.) and VI (2.5 mg.) after examination of their ultraviolet spectra were each dissolved in 0.4 ml. of methanol and treated with 0.08 ml. of 0.54 *N* potassium carbonate at 18° for 17 hr. The product derived from XII showed a shoulder near 225 mμ with an increment in ϵ of 500, the one from VI had a maximum at 225.5 mμ with $\Delta\epsilon$ of 5,600; reported[35b] for methyl 3β-acetoxy-Δ[16]-etienate, λ_{max} ~ 225 mμ (log ϵ 4.1).

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(38) R. Casanova and T. Reichstein, *Helv. Chim. Acta*, **32**, 647 (1949); T. Reichstein and H. G. Fuchs, *ibid.*, **23**, 658 (1940).

Basel, for preparing a fresh sample of methyl 3β,16β-diacetoxyetianate from gitoxin to permit direct comparison; to Professor T. Reichstein for a sample of methyl 3β-acetoxyetianate; to Dr. J. J. Pfiffner of Parke, Davis and Company for supplying the starting material, 3β-acetoxy-Δ^{16}-pregnen-20-one; and to Dr. E. W. D. Huffman for the microanalyses reported in this paper.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Optical Rotatory Dispersion Studies. VI.[1] The Bile Acid Series. Polycarbonyl Compounds and Stereochemical Differentiations[2]

BY CARL DJERASSI AND W. CLOSSON

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Rotatory dispersion curves for various bile acid derivatives with carbonyl groups at carbon atoms 3, 6, 7, 11 and 12 are presented, thus illustrating further the utility of the rotatory dispersion method for the localization of isolated ketonic functions. More subtle differentiations are also possible and in the case of 3-, 6- or 7-ketosteroids the stereochemistry of the A/B ring juncture can be determined readily by this procedure. The additive nature of individual rotatory contributions of carbonyl groups in steroidal diketones has been investigated by examining the degree of coincidence between observed and calculated rotatory dispersion curves.

Our earlier investigations in the androstane,[3] cholestane[1,4] and sapogenin[5] series have clearly demonstrated that isolated ketone functions are strongly optically active and that the resulting rotatory dispersion curves are typical of that particular carbonyl group and its stereochemical environment and rather independent of additional weak chromophores such as hydroxyl and acyloxyl substituents or isolated double bonds. As pointed out in our last paper,[1] the characteristic shapes of certain dispersion curves permit in many instances the precise localization of isolated carbonyl groups which is often not possible by infrared or ultraviolet spectroscopic procedures. The scope and precision of the rotatory dispersion method—for identification purposes,[1] analytical applications[5,6] and for the assignment of absolute configurations to various alicyclic ketones[7]—requires the accumulation of dispersion curves for a variety of structural systems and the present paper is concerned with bile acids. This series is particularly useful since it affords a great number of representatives with the 5β (A/B *cis*) stereochemistry (most of the earlier examples[1,3,5] possessed the 5α (A/B *trans*) configuration) and, furthermore, there are available various diketones which permit an evaluation of the additive effects of individual optically active chromophores.

As in the earlier studies,[3,5] it was first necessary

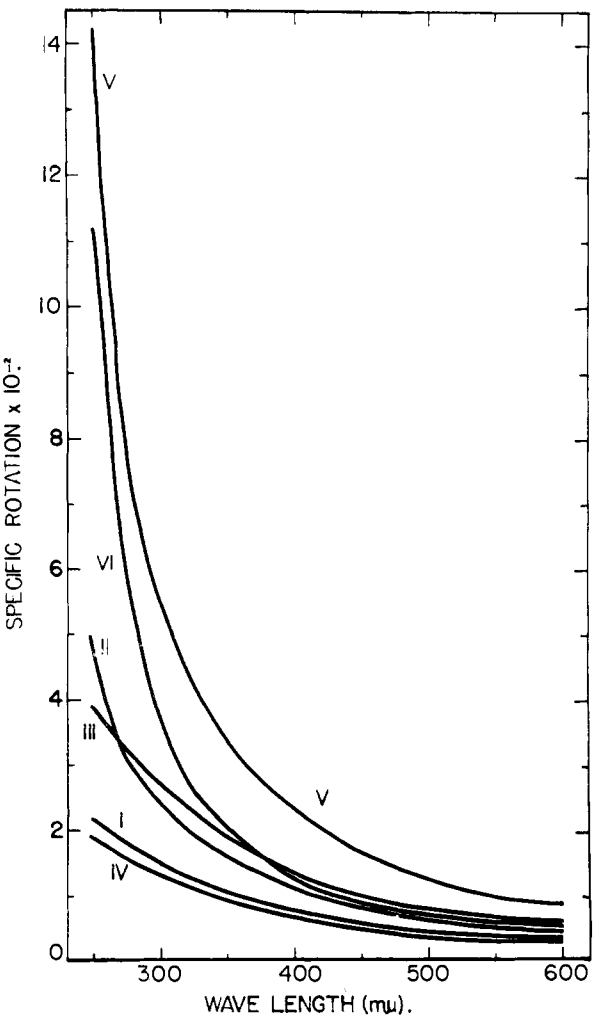

Fig. 1.—Rotatory dispersion curves of: methyl cholanate (I), desoxycholic acid (II), 3α,12α-dihydroxynorcholanic acid (III), 3α,12α-dihydroxybisnorcholanic acid (IV), 3α,12α-dihydroxyetianic acid (V) and methyl 3β-hydroxy-5α-etianate (VI).

(1) Paper V, C. Djerassi, W. Closson and A. E. Lippman, THIS JOURNAL, **78**, 3163 (1956).

(2) Supported by a research grant from the Damon Runyon Memorial Fund for Cancer Research.

(3) C. Djerassi, E. W. Foltz and A. E. Lippman, THIS JOURNAL, **77**, 4350 (1955).

(4) A. E. Lippman, E. W. Foltz and C. Djerassi, *ibid.*, **77**, 4364 (1955).

(5) C. Djerassi and R. Ehrlich, *ibid.*, **78**, 440 (1956).

(6) As illustrated in this and earlier papers, the characteristic features of the rotatory dispersion curves of carbonyl-containing steroids and related substances (ref. 7) become noticeable only below 400 mμ. The magnitude of the rotations in those regions often permits the determination of dispersion curves with 0.1–0.2 mg. of material (1–2 cc. of solvent), thus making it possible to utilize this procedure on a micro scale.

(7) This application in the field of terpenes, alkaloids and certain miscellaneous ketones will be reported in a forthcoming paper of this series (part VII).