twice recrystallized from the same solvent, yellow needles which blackened in the range 247–257° and finally melted at 261°; ν_{max}^{Nujol} 3500, 3140 cm.$^{-1}$, transparent in the range 1750–1650 cm.$^{-1}$.

Anal. Calcd. for $C_{15}H_{20}N_4O_9$: C, 45.00; H, 5.04; N, 14.00. Found: C, 45.02; H, 4.99; N, 13.99.

URBANA, ILL.
DETROIT, MICH.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE RICE INSTITUTE]

Ouabagenin. III. Assignment of the Sixth Hydroxyl Group and a Structural Correlation with Strophanthidin

BY RICHARD B. TURNER AND JOSEPH A. MESCHINO[1]

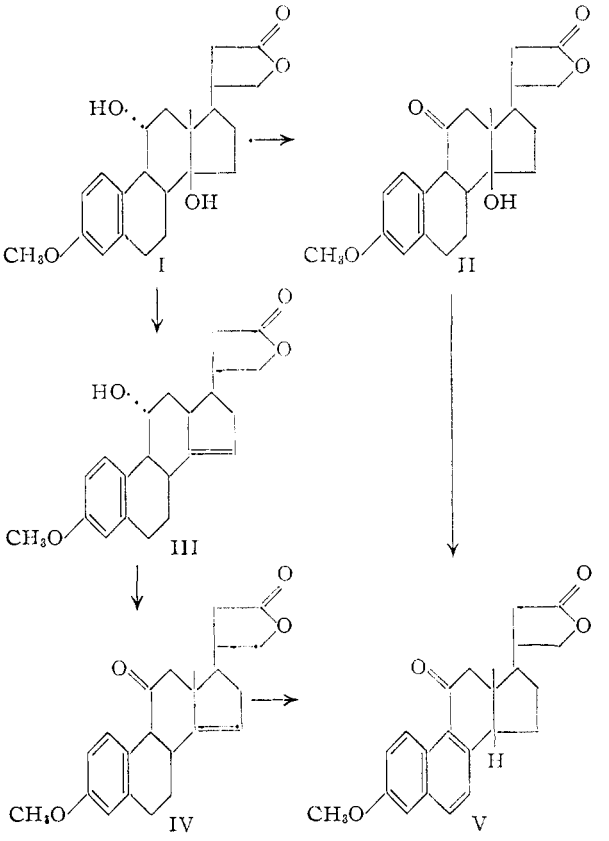
RECEIVED APRIL 21, 1958

Degradation of ouabagenin to a product (V) possessing a keto group conjugated with a naphthalene system (rings A and B) establishes the fact that ouabagenin contains a secondary hydroxyl group at C.11. Conversion of strophanthidin and of ouabagenin into a common derivative (Xb), still possessing the C.14 hydroxyl group and lactone side chain, provides additional evidence for the β-orientation of these functions in the latter genin and establishes a direct structural correlation between ouabagenin and other aglycones of the *Strophanthus* group.

In previous papers[2] evidence has been presented which permits assignment of four of the six hydroxyl groups of ouabagenin to the 1-, 3-, 5- and 19-positions, respectively. The further inference that a tertiary hydroxyl group is located at C.14 has been drawn from the early observation of Jacobs and Bigelow[3] that the parent rhamnoside, ouabain, is converted into isoöuabain by the action of base, and from the results of various dehydration experiments, notably those of Meyrat and Reichstein.[4] The position of the remaining (secondary) hydroxyl function has remained uncertain, although a tentative assignment of this group to C.11 was made by Fieser and Newman[5] as early as 1936. The Fieser–Newman suggestion, while based on arguments that are no longer tenable,[6] was incorporated in the 1942 formulation of Mannich and Siewert[7] and has received some support from work of Tschesche and Snatzke[8] in which epimerization of the hydroxyl group in question (by oxidation to the ketone and sodium borohydride reduction) furnished a derivative possessing a non-acylable secondary hydroxyl function. This behavior is characteristic of 11β-hydroxy steroids. Since all of the secondary hydroxyl groups of ouabagenin are readily acetylated, Tschesche and Snatzke were led to the conclusion that ouabagenin is an 11α-hydroxy compound. On the other hand, Djerassi and Ehrlich[9] have argued for an alternative assignment (presumably C.6) on the basis of the unsupported assumption that the very slow reaction of ouabagenin with lead tetraacetate represents the cleavage of a 1,2-glycol.

We have recently reported definitive evidence in support of the 11-hydroxy structure.[10] The present paper describes the details of this work as well as experiments that establish a direct structural correlation between ouabagenin and other aglycones of the *Strophanthus* group.

When the diol I[2a] is oxidized with the chromium trioxide–pyridine complex, a ketol II is obtained which shows only anisole absorption in the ultraviolet. The absence of a conjugated carbonyl system in this substance is further indicated by the position (5.80 μ) of the ketonic absorption band in the infrared. Compound II proved resistant to dehydration by hydrogen chloride in chloroform at 0°, and under more vigorous conditions (hydrogen chloride in refluxing methanol and *p*-toluenesulfonic acid in refluxing benzene) oily products were obtained that could not be induced to crystallize.



(1) Public Health Service Predoctoral Fellow, 1956–1958.

(2) (a) R. P. A. Sneeden and R. B. Turner, *Chemistry & Industry*, 1235 (1954); THIS JOURNAL, **77**, 130 (1955); (b) K. Florey and M. Ehrenstein, *J. Org. Chem.*, **19**, 1174 (1954); (c) Ch. Tamm, *Helv. Chim. Acta*, **38**, 147 (1955); (d) E. J. Becker and M. Ehrenstein, *Ann.*, **608**, 54 (1957).

(3) W. A. Jacobs and N. M. Bigelow, *J. Biol. Chem.*, **96**, 647 (1932); **101**, 15 (1933).

(4) A. Meyrat and T. Reichstein, *Helv. Chim. Acta*, **31**, 2104 (1948).

(5) L. F. Fieser and M. S. Newman, *J. Biol. Chem.*, **114**, 705 (1936).

(6) See the discussion of this point in ref. 2a.

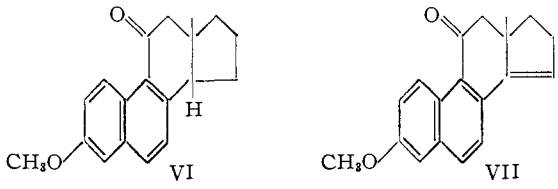
(7) C. Mannich and G. Siewert, *Ber.*, **75**, 737, 750 (1942).

(8) R. Tschesche and G. Snatzke, *ibid.*, **88**, 1558 (1955).

(9) C. Djerassi and R. Ehrlich, *J. Org. Chem.*, **19**, 1351 (1954).

(10) R. B. Turner and J. A. Meschino, THIS JOURNAL, **78**, 5130 (1956)

However, dehydrogenation of II over palladium black at 260° proceeded with concomitant loss of the C.14 hydroxyl group and furnished, although in poor yield, a naphthalenic ketone V, the ultraviolet absorption spectrum of which is virtually indistinguishable from that of *cis*-3-methoxy-11-keto-equilinane (VI), synthesized in an unambiguous manner by Eglinton, Nevenzel, Scott and Newman[11] (Fig. 1). The keto group of V must hence be located at C.11, and the position of the sixth hydroxyl group of ouabagenin is thereby established. The correspondence of the absorption of V and VI and the minor shifts observed in the



spectrum of *trans*-3-methoxy-11-ketoequilinane[11] (λ_{max} 220, 248, 316, 354 mμ) form the basis for tentative assignment of the *cis* configuration to the C/D ring fusion in V. In this connection it will be recalled that palladium dehydrogenation of estrone at 260° furnishes *d*-isoequilenin with inversion of the C/D junction from *trans* to *cis*.[12]

An alternate route to V is available in the transformation of I by acetylation and $POCl_3$–pyridine dehydration into the corresponding 11-acetoxy-Δ^{14}-derivative.[2a] Saponification of the latter substance to III, followed by chromium trioxide–pyridine oxidation affords the unsaturated ketone IV, which gives V on dehydrogenation, but with no significant improvement in yield over the previously described process. In the course of preliminary experiments on the palladium-catalyzed dehydrogenation of IV, in which *p*-benzoquinone was used as a hydrogen acceptor,[13] traces of material were encountered occasionally which showed ultraviolet absorption (λ_{max}^{MeOH} 238, 272, 315 mμ) corresponding to that of 3-methoxy-11-keto-14,15-dehydroequilinane (VII).[11] The substance could not be obtained consistently, however, and the matter was not pursued further.

Since at the time our investigation was undertaken correlation of ouabagenin with a steroid of known structure had not yet been accomplished,[14] attention was next directed toward transformations which might serve to establish such a connection. 20,22-Dihydrostrophanthidin (VIII)[15] provided an attractive starting point for this purpose, and this material was subjected to catalytic oxidation in the

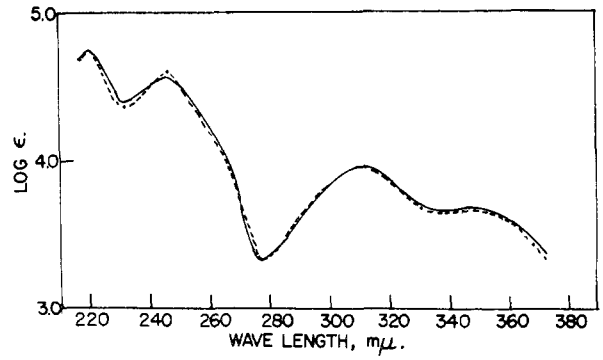
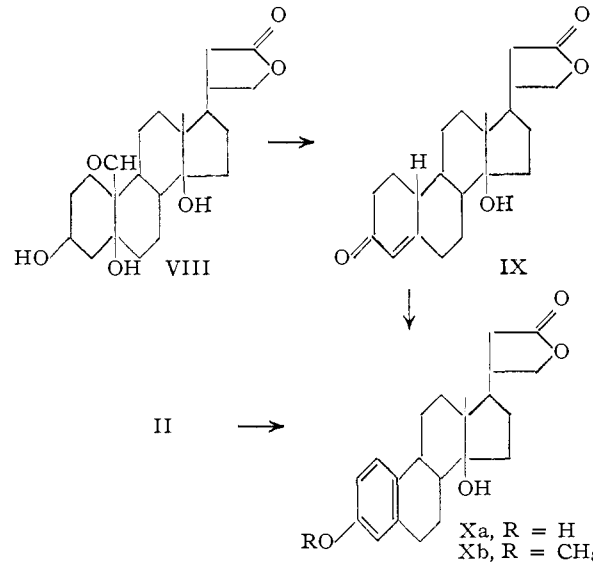


Fig. 1.—Compound V (solid line), λ_{max}^{MeOH} 220, 246, 312, 348 mμ, log ϵ 4.75, 4.56, 3.94, 3.63; *cis*-3-methoxy-11-keto-equilinane (VI) (broken line), λ_{max}^{MeOH} 220, 246, 313, 345 mμ, log ϵ 4.74, 4.60, 3.93, 3.64.

presence of platinum and oxygen.[16] Attempts to obtain a crystalline 3-keto derivative at this stage were unsuccessful, and the crude product was therefore submitted directly to the action of base. β-Elimination of the C.5 hydroxyl group and cleavage of the C.10 aldehyde function occurred under these conditions, and the α,β-unsaturated ketone IX was obtained in approximately 30% yield based on dihydrostrophanthidin. Dehydrogenation of IX over



palladium black in refluxing ethanol gave the phenol Xa, which was readily converted into the corresponding methyl ether Xb by treatment with dimethyl sulfate. Compound Xa also could be obtained directly from dihydrostrophanthidin by the action of Raney nickel and cyclohexanone in xylene,[17] but the yield by this route was only about 4%.

Initial attempts to convert ouabagenin into Xb *via* reductive elimination in the 11-*p*-toluenesulfonoxy derivative of diol I were unrewarding. In view of the considerable resistance toward dehy-

(11) G. Eglinton, J. C. Nevenzel, A. I. Scott and M. S. Newman, THIS JOURNAL, **78**, 2331 (1956). We are indebted to Professor Newman for supplying samples from his collection for direct spectral comparison.

(12) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 626.

(13) *Cf.* E. A. Braude, R. P. Linstead, P. W. D. Mitchell and K. R. H. Wooldridge, *J. Chem. Soc.*, 3595 (1954).

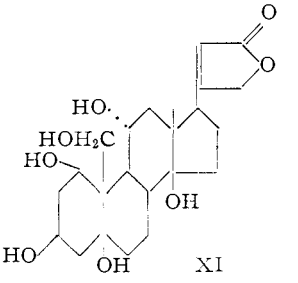
(14) Proof for the steroidal nature of ouabagenin recently has been provided by Ch. Tamm, G. Volpp and G. Baumgartner, *Helv. Chim. Acta*, **40**, 1469 (1957).

(15) W. A. Jacobs and M. Heidelberger, *J. Biol. Chem.*, **54**, 253 (1922).

(16) *Cf.* R. P. A. Sneeden and R. B. Turner, THIS JOURNAL, **77**, 190 (1955); A. Katz, *Helv. Chim. Acta*, **40**, 831 (1957), and reference 2a.

(17) *Cf.* E. C. Kleiderer and E. C. Kornfeld, *J. Org. Chem.*, **13**, 455 (1948); M. Ehrenstein, A. R. Johnson, P. C. Olmstead, V. I. Vivian and M. A. Wagner, *ibid.*, **15**, 264 (1950).

dration displayed by the ketol II in the presence of anhydrous hydrogen chloride at low temperature, the reaction of this substance with ethanedithiol and hydrogen chloride at 0° was next investigated.[18] The reaction proceeded smoothly in a mixture of alcohol-free chloroform and methylene chloride and furnished an amorphous product showing infrared absorption corresponding to hydroxyl and 5-ring lactone functions, but no bands attributable to a keto group. Hydrogenolysis of this material in the presence of Raney nickel afforded a sample of Xb identical according to all of the usual criteria with that obtained from strophanthidin *via* VIII. Since the structure and stereochemistry of strophanthidin are well established,[19] correlation of this substance with ouabagenin provides final proof for the orientation of the 14-hydroxyl group and lactone side chain in the latter molecule. Coupled with the recent establishment of the β-orientation of the 3-hydroxyl group[20] and with evidence to which references have already been given, the present work permits unambiguous assignment of structure XI to ouabagenin.



XI

Experimental[21]

Oxidation of I to II.—A solution of 500 mg. of I (m.p. 205–206°), prepared by the procedure of Sneeden and Turner,[2a] in 5 ml. of pyridine was added to a mixture of 500 mg. of chromium trioxide and 5 ml. of pyridine[22] at 20°. After standing for 3 hr. at room temperature, the reaction mixture was poured onto ice, and the product was extracted with ethyl acetate. After repeated washing with dilute hydrochloric acid, water and dilute sodium hydroxide solution, the organic layer was dried over anhydrous sodium sulfate, and the solvent was removed under reduced pressure. Crystallization of the residue from acetone–petroleum ether afforded 350 mg. of plates, m.p. 186–188.5°. The analytical sample melted at 189–191°, $[\alpha]_D$ +182° (*c* 1.34, acetone); λ_{max}^{MeOH} 276, 284 mμ, ε 1626, 1522; $\lambda_{max}^{CH_2Cl_2}$ 5.62 5.80 mμ.

Anal. Calcd. for $C_{23}H_{28}O_5$: C, 71.85; H, 7.34. Found: C, 71.60; H, 7.54.

Dehydrogenation of II.—A mixture of 100 mg. of II and 60 mg. of palladium black was sealed in an evacuated glass ampoule and heated to 260° in an oil-bath for 6 hr. The reaction product was taken up in acetone, filtered, and the solvent removed by evaporation. Chromatography of the residual oil on silicic acid furnished 20 mg. of crystalline material, which on recrystallization from acetone–petroleum ether gave the analytical sample, m.p. 260.5–262.5°, $[\alpha]_D$ +159° (*c* 0.97, chloroform); λ_{max}^{MeOH} 220, 246, 312, 348 mμ, log ε 4.75, 4.56, 3.94, 3.63.

Anal. Calcd. for $C_{23}H_{24}O_4$: C, 75.80; H, 6.64. Found: C, 75.91; H, 6.91.

Preparation of III.—Conversion of the diol into the corresponding 11-acetoxy-Δ^{14} derivative was carried out by acetylation and dehydration according to the previously described procedure.[2a] A solution of 240 mg. of the latter product in 20 ml. of 5% methanolic potassium hydroxide was heated under reflux for 1 hr. The basic solution was then neutralized with acetic acid, and the bulk of the methanol was removed under reduced pressure. The product was isolated by water dilution and extraction with ethyl acetate, and the organic extract was allowed to stand overnight at room temperature in the presence of a catalytic amount of *p*-toluenesulfonic acid in order to ensure relactonization. The solution finally was washed with water and dilute sodium hydroxide, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. Crystallization of the crude product from acetone–petroleum ether furnished 150 mg. of rectangular prisms melting at 217–219°. The analytical sample melted at 222–223°, $[\alpha]_D$ +30.3° (*c* 1.43, chloroform).

Anal. Calcd. for $C_{23}H_{28}O_4$: C, 74.97; H, 7.66. Found: C, 74.98; H, 7.87.

Preparation of IV.—A solution of 135 mg. of the product described in the preceding paragraph in 1 ml. of pyridine was added to a mixture of 100 mg. of chromium trioxide in 1 ml. of pyridine at 20°.[22] After standing for 3 hr. at room temperature the reaction mixture was poured over ice, and the product was extracted with ethyl acetate. The resulting solution was thoroughly washed, and, after removal of the solvent and chromatography on silicic acid, 65 mg. of rectangular prisms was obtained, m.p. 232–237° dec. Several recrystallizations from acetone–petroleum ether afforded the analytical sample, m.p. 239° dec., $[\alpha]_D$ +270° (*c* 1.01, chloroform); λ_{max}^{MeOH} 276, 284 mμ, ε 1626, 1522, $\lambda_{max}^{CH_2Cl_2}$ 5.66, 5.87 μ.

Anal. Calcd. for $C_{23}H_{26}O_4$: C, 75.38; H, 7.15. Found: C, 75.15; H, 7.24.

Dehydrogenation of IV.—Dehydrogenation of IV was carried out according to the procedure described for II. The crude product was chromatographed on silicic acid, and after recrystallization from acetone–petroleum ether, a pure sample of V was obtained, m.p. 260–262°. The infrared spectrum of this material was identical with that obtained in the dehydrogenation of II.

Conversion of 20,22-Dihydrostrophanthidin into IX.—A solution of 1.2 g. of 20,22-dihydrostrophanthidin (m.p. 189–190°, prepared by hydrogenation of a methanol solution of strophanthidin over a platinum catalyst) in 50 ml. of acetone and 150 ml. of distilled water was added to a suspension of platinum black (from 375 mg. of platinum oxide) in 45 ml. of water, and the mixture was stirred in an oxygen atmosphere until the absorption of oxygen ceased (about 48 hr.). The catalyst was removed by filtration, and the filtrate was concentrated to dryness at 40° under reduced pressure. The oily residue could not be induced to crystallize, and the total crude product was hence treated as follows.

The oil was stirred with 45 ml. of 4% sodium hydroxide solution at 0° under nitrogen. The material slowly dissolved, and after 4 hr. at 0° the reaction mixture was made acid to congo red and extracted with ethyl acetate. The organic phase was washed with water and allowed to stand overnight in the presence of a small amount of *p*-toluenesulfonic acid in order to ensure relactonization. The material was finally washed with base, dried and concentrated. Crystallization of the residue from acetone gave 125 mg. of IX. An additional 225 mg. was obtained from the mother liquors by chromatography on silicic acid. The two fractions were combined and recrystallized from acetone–petroleum ether, m.p. 197.5–199°, $[\alpha]_D$ +38.6° (*c* 1.0, chloroform); λ_{max}^{MeOH} 240 mμ, ε 17,700; $\lambda_{max}^{CH_2Cl_2}$ 2.82, 5.66, 6.01 μ.

Anal. Calcd. for $C_{22}H_{30}O_4$: C, 73.71; H, 8.44. Found: C, 74.06; H, 8.56.

Dehydrogenation of IX.—A solution of 100 mg. of IX in 5 ml. of absolute ethanol containing 60 mg. of suspended palladium black was heated under reflux in a nitrogen atmosphere for 10 hr. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. Crystallization of the resulting residue from acetone gave 27 mg. of Xa as fine prisms, m.p. 270–275° dec. The analytical sample melted at 271–272° dec., $[\alpha]_D$ +94.2° (*c* 1.0, pyridine); $\lambda_{max}^{CH_2Cl_2}$ 2.82, 5.66 μ; λ_{max}^{MeOH} 280 mμ, ε 1960; λ_{max}^{NaOH} 295 mμ, ε 2060.

(18) A similar procedure has been employed in an analogous case by Tamm (see footnote 14).

(19) *Cf.* R. B. Turner, *Chem. Revs.*, **43**, 1 (1948).

(20) G. Volpp and Ch. Tamm, *Helv. Chim. Acta*, **40**, 1860 (1957).

(21) Melting points were taken on a Fisher–Johns melting point stage. Analyses were carried out by S. M. Nagy, M.I.T., and by Midwest Microlab, Inc., Indianapolis.

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

Anal. Calcd. for $C_{22}H_{28}O_4$: C, 74.13; H, 7.92. Found: C, 73.96; H, 7.97.

Reaction of Dihydrostrophanthidin with Raney Nickel and Cyclohexanone.[23]—A solution of 1.0 g. of 20,22-dihydrostrophanthidin in 100 ml. of xylene and 50 ml. of freshly distilled cyclohexanone was refluxed in the presence of Raney nickel for 48 hr. The solvents and steam-volatile by-products were removed by steam distillation, which was continued until the distillate did not give a ferric chloride test for phenol. The residue was then extracted with methylene chloride, and, after chromatography on magnesium silicate, 40 mg. of Xa was obtained, m.p. 270–272° dec., λ_{max}^{EtOH} 280 mμ. The infrared spectrum of this material was identical with that of the sample of Xa obtained in the preceding experiment.

Preparation of Xb.—A solution of 53 mg. of the above phenol in 1 ml. of methanol was purged with nitrogen and treated with 5 ml. of deoxygenated water containing 2 pellets of sodium hydroxide. The mixture was stirred until solution was complete, and the flask and its contents were then cooled to 0°. Dimethyl sulfate (0.3 ml.) was added, and the reaction mixture was kept alkaline to alizarin yellow by the occasional addition of sodium hydroxide. The temperature then was allowed to rise to about 25° and stirring was continued for 2 hr., at the end of which time the solution was acidified to congo red. The product was extracted with ethyl acetate, and after remaining in contact with *p*-toluenesulfonic acid overnight, the organic phase was washed, dried and evaporated. Chromatography on silicic acid afforded crystalline material melting at 163–174°. The analytical sample was obtained by recrystallization from acetone–petroleum ether; m.p. 173.5–174.5°, $[\alpha]_D$ +82.1° (*c* 1.02, chloroform); $\lambda_{max}^{CH_2Cl_2}$ 2.75, 5.64 μ.

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 74.56; H, 8.16. Found: C, 74.75; H, 8.39.

Conversion of II into Xb.—A solution of 70 mg. of ketol II in 2 ml. of methylene chloride containing 10 drops of ethanedithiol was added at 0° to 2 ml. of alcohol-free chloroform saturated with hydrogen chloride. After standing at 0° for 24 hr., ice-water was added, and the product was extracted with ethyl acetate. Removal of the solvents furnished an amorphous residue which showed no ketonic absorption in the infrared. This material was dissolved in 10 ml. of freshly purified dioxane and refluxed with about 2.0 g. of W-5 Raney nickel[24] for 10 hr. Removal of the nickel and evaporation of the solvent gave 63 mg. of oil, which was chromatographed on silicic acid. Elution with benzene–chloroform mixtures yielded 25 mg. of Xb. Recrystallization from acetone–petroleum ether gave a sample melting at 173.0–174.5°, $[\alpha]_D$ +80.2° (*c* 0.98, chloroform); λ_{max}^{MeOH} 278, 286 mμ, ϵ 1620, 1520, $\lambda_{max}^{CH_2Cl_2}$ 2.75, 5.64 μ. A mixed melting point determination with the specimen of Xb prepared from dihydrostrophanthidin showed no depression, and the infrared spectra of the two samples were identical.

(23) We are indebted to Dr. R. P. A. Sneeden for this experiment.

(24) H. R. Billica and H. Adkins in "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 176, note 3.

HOUSTON, TEXAS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

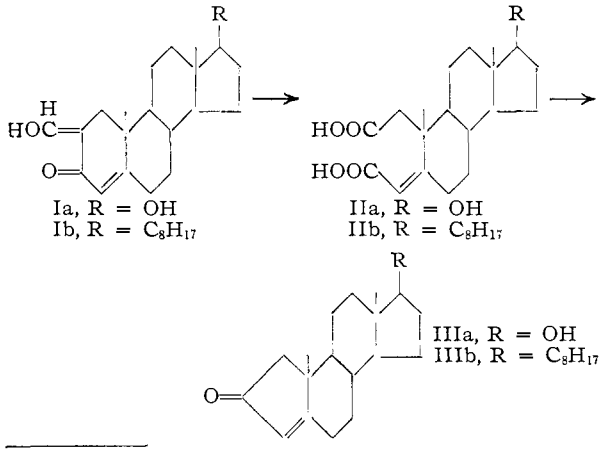
A-Nor-$\Delta^{3(5)}$-cholesten-2-one[1]

BY THOMAS L. JACOBS AND NOBUYOSHI TAKAHASHI

RECEIVED MARCH 17, 1958

A-Nor-$\Delta^{3(5)}$-cholestene-2-one was synthesized from A-norcholestane by dibromination, dehydrobromination of the dibromide to 3-bromo-A-norcholesten-2-one and debromination of the latter by catalytic hydrogenation under Rosenmund conditions. Attempted syntheses by cyclization of 2,3-*seco*-Δ^4-cholestene-2,3-dioic acid or its ester and by conventional methods from the enol lactone 3-oxa-$\Delta^{5,6}$-A-norcholesten-2-one were unsuccessful.

It was of interest to determine the effect of modification of the size of the A ring in various steroids on the physiological activity of these compounds. Acid IIa, a promising intermediate for the synthesis of A-nor-$\Delta^{3(5)}$-testosterone (IIIa), was obtained in connection with other work[2]



during the ozonization of hydroxymethylenetestosterone Ia. It was hoped that this acid was the geometrical isomer shown and that ring closure to IIIa could be accomplished easily. However, neither heating with acetic anhydride, the usual procedure for ring closure of similar saturated acids, nor the Dieckmann condensation of the dimethyl ester of IIa was successful for the preparation of IIIa.

The relative scarcity and cost of testosterone led to investigation of the cholesterol series at this point. Ozonization of Ib was reported earlier[2] to yield mainly a lactol, 5-hydroxy-3-oxa-A-norcholestan-2-one; no attempt was made to isolate IIb. We have obtained IIb from this ozonization, but the yield was never more than 4% and was often less. The yield of crude acidic material from the ozonization was considerably higher, but most of the substance was a glass that could not be obtained in crystalline form. The structure of IIb was shown by its ultraviolet spectrum and by hydrogenation to 2,3-*seco*-cholestan-2,3-dioic acid which readily was cyclized to the known A-norcholestan-2-one.[3] Cyclization of IIb or of its dimethyl ester was not successful.

It seems probable that the failure of attempts to cyclize IIa or b or their esters resulted because the

(1) Taken from a thesis submitted by Nobuyoshi Takahashi in partial fulfillment of the requirements for the Ph. D. degree, University of California, Los Angeles, February, 1958.

(2) F. L. Weisenborn, D. C. Remy and T. L. Jacobs, THIS JOURNAL, **76**, 552 (1954).

(3) A. Windaus and O. Dalmer, *Ber.*, **52**, 162 (1919).