sulfanilic acid of known concentration was prepared by diazotization and used as such. Previously the solid compound was isolated and weighed out for each run. The explosive character of the diazo salt made the use of a solution much more convenient. The reproducibility of the data was unaffected by the change and remained of the order ± 5%, with the greatest spread being 7%. In those cases where quenching of the reaction mixture in sulfuric acid resulted in a precipitate—(*e.g.*, calcium sulfate)—it was merely necessary to centrifuge the sample to obtain a clear supernatant liquid which could be used for the spectrophotometric estimation of the dye. The metals were added as their chloride or sulfate salts.

Rate Constants.—The rate constants are pseudo first-order rate constants obtained from the expression:

$$\text{Rate} = k_1 (\text{phenolate complex})(H^+)$$

The constant k_1 incorporates the product $k_2(RN_2^+)$, where (RN_2^+) is a constant excess of the diazonium salt and k_2 is the second order rate constant. This diazonium salt was present in fifty-fold excess. The concentration of the diazonium salt was 5.0×10^{-3} M while that of the phenol was 9.26×10^{-5} M in all of the runs. The second-order rate constants may be obtained from the relationship between the two rate constants and the concentration of diazonium salt.

Microbiological Transformations. III.[1] Reduction of the Steroid C-19 Aldehyde Group[2]

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Interconversions of ketone and hydroxyl groups were among the first described transformations of steroids by microörganisms, and many oxidations and reductions have been reported.[3] However, the conversion of 3-ketobisnor-4-cholen-22-al into 6β,11α,22-trihydroxybisnor-4-cholen-3-one has been the sole example of microbial reduction of a steroid aldehyde. We report herein the microbiological transformation of anhydrostrophanthidone (I) into 19-dihydroanhydrostrophanthidone (II). The conversion appears to constitute the first demonstration of reduction of a steroid *angular* aldehyde by a microörganism.

In preceding communications in this series,[1,4] we reported on the microbiological conversion of strophanthidin into anhydrostrophanthidone (I). For continuing studies, I was the substrate of choice, in view of the ease of detection of Δ^4-3-oxosteroid derivatives on paper chromatograms.

When I was incubated with *Penicillium thomii*, a more polar compound, m.p. 247–251°, was obtained in 55% yield. Analysis afforded figures in good agreement with the formula $C_{23}H_{30}O_5$. The ultraviolet spectrum showed λ_{max}^{MeOH} 219 mμ (Δ α,β-lactone), and the infrared spectrum showed bands at 2.92 μ (OH), 5.60 μ (Δ α,β-lactone), 6.06 and 6.20 μ (Δ α,β-ketone). Acetylation with acetic anhydride–pyridine afforded a monoacetate derivative. On the basis of the foregoing facts, the transformation product was characterized as II and the monoacetate as IV. The structural assignment was confirmed by synthesis of II by catalytic oxidation of strophanthidol (III) in the presence of platinum and oxygen. Direct comparison of the catalytic oxidation product and its acetate with the microbiological transformation product and its acetate indicated the identity of the respective compounds.

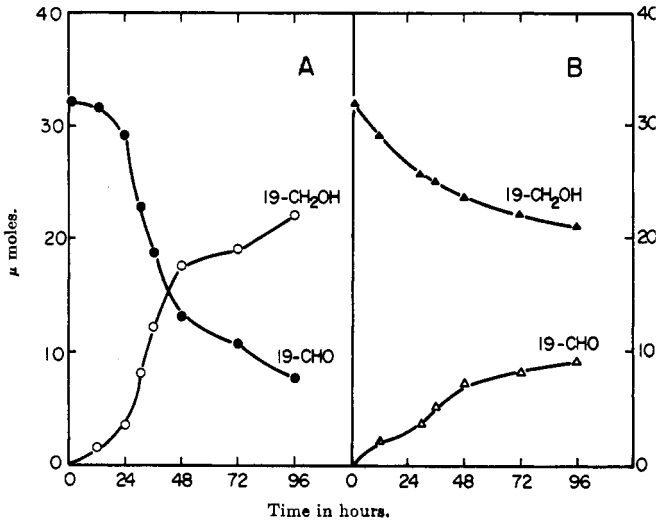
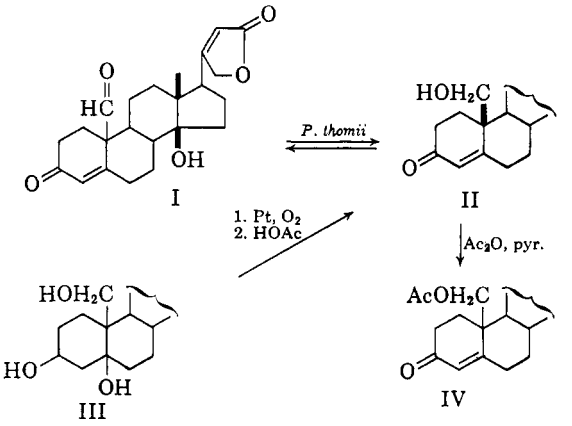


Fig. 1.—Interconversion of anhydrostrophanthidone (I) and 19-dihydroanhydrostrophanthidone (II) by *Penicillium thomii*. (A)—I as the substrate. (B)—II as the substrate.



The transformation is of biochemical interest because the reversal of the reaction parallels a possible biosynthetic step in the formation of estrogens from androgens. Recent studies with human placental microsomes support the sequence Δ^4-androstenedione → 19-hydroxyandrostenedione → 19-oxoandrostenedione ⇢ estrone for the steps in biological estrogen formation.[5] To demonstrate the reversibility of the microbial transformation, I and II were separately exposed to *P. thomii* under identical conditions. Fig. 1A and 1B show that approximately the same equilibrium

(1) Part II in the series: S. M. Kupchan, C. J. Sih, N. Katsui, and O. El Tayeb, *J. Am. Chem. Soc.*, **84**, 1752 (1962).

(2) This investigation was supported in part by research grants (H-2275, A-4069) from the National Institutes of Health.

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concentration of 19-hydroxy and 19-oxo compounds was obtained whether I or II was the substrate for the organism. The results indicate that the reaction is indeed a reversible one, similar to that of the interconversion of steroidal ketones and alcohols.[3,6]

Experimental[7]

Transformation of Anhydrostrophanthidone (I) into 19-Dihydroanhydrostrophanthidone (II) by *Penicillium thomii*.—The fermentation medium consisted of corn steep liquor, 0.6%; $NH_4H_2PO_4$, 0.3%; calcium carbonate, 0.25%; corn oil, 0.22%; yeast extract, 0.25%; and glucose, 1.0%. *P. thomii* was grown in 4.8 l. of this medium (twelve 2-l. erlenmeyer flasks); after 24 hr. of incubation at 27° on a rotary shaker, 1.0 g. of anhydrostrophanthidone in 12 ml. of dimethylformamide was distributed equally among the flasks. The fermentation was allowed to continue for 96 hr.; the culture broth then was filtered and the filtrate extracted with chloroform. The chloroform extract was dried with sodium sulfate and evaporated to dryness to yield 0.96 g. of residue. An aliquot of the chloroform extract was chromatographed on Whatman no. 1 paper and developed in a benzene–chloroform–propylene glycol system[8] for 6 hr. The paper chromatogram showed only two spots, one of which corresponded to anhydrostrophanthidone, when viewed under the ultraviolet scanner. The other compound showed an R_f of 0.10; anhydrostrophanthidone has an R_f of 0.70 in this system. Direct crystallization from acetone yielded 552 mg. of crystals, m.p. 247–251°, λ_{max}^{MeOH} 219 mμ (ε 22,000); λ_{max}^{chf} 2.92, 5.60, 6.06, and 6.20 μ.

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.54; H, 7.83.

The melting point was not depressed upon admixture of a sample of 19-dihydroanhydrostrophanthidone (II) prepared by catalytic oxidation (described below) and the paper chromatographic behavior and solution infrared and ultraviolet spectra were identical with those of the synthetic sample.

Catalytic Oxidation of Strophanthidol (III) to 19-Dihydroanhydrostrophanthidone (II).—A solution of strophanthidol[9] (700 mg.) in acetone (100 ml.) and water (100 ml.) was treated with platinum black (from 400 mg. of platinum oxide) under oxygen. After 24 hr. the consumption of oxygen had ceased, and the reaction mixture was treated as usual to yield 720 mg.of residue. The residue in acetic acid (35 ml.) was heated under reflux for 15 min. under nitrogen. Evaporation to dryness gave a residue which was dissolved in chloroform and chromatographed on Woelm neutral alumina (20 g.). Elution with chloroform–methanol (98:2) gave a crude crystalline product (250 mg.). Two recrystallizations from acetone–ether yielded II (75 mg.), m.p. 247–251°, $[\alpha]^{25}D$ +91° (*c* 1.46, methanol).

Anal. Calcd. for $C_{23}H_{30}O_5$: C, 71.48; H, 7.82. Found: C, 71.36; H, 7.71.

19-Dihydroanhydrostrophanthidone Acetate (IV).—A solution of III (108 mg.) in acetic anhydride (1.5 ml.) and pyridine (1.5 ml.) was allowed to stand at room temperature for 17 hr. The excess acetic anhydride was decomposed by cautious addition of methanol (2 ml.) and the solution was evaporated to dryness under reduced pressure. Crystallization from ethyl acetate yielded 87 mg. of crude crystalline product. Recrystallization from ethyl acetate–ether afforded colorless needles (41 mg.), m.p. 186–187°, $[\alpha]D$ +101° (*c* 1.28, methanol).

Anal. Calcd. for $C_{25}H_{32}O_6$: C, 70.07; H, 7.53. Found: C, 69.77; H, 7.51.

(7) Melting points are corrected for stem exposure. Values of $[\alpha]D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in methanol on a Cary recording spectrophotometer (Model 11 MS). Infrared spectra were recorded on a Beckman IR5-A double beam infrared recording spectrophotometer. Microanalyses by Dr. S. M. Nagy, Cambridge, Mass., and Mr. J. Alicino, Metuchen, N. J. Whatman no. 1 paper was washed twice with 95% ethanol prior to use for paper chromatography. For quantitative determinations the spots were eluted with 95% ethanol and the absorbancy at 240 mμ was used in measuring concentrations. The culture, *Penicillium thomii*, was kindly supplied by Professor K. B. Raper, Department of Bacteriology, University of Wisconsin.

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The Structure Proof of 16α-Carboxypregnenolone

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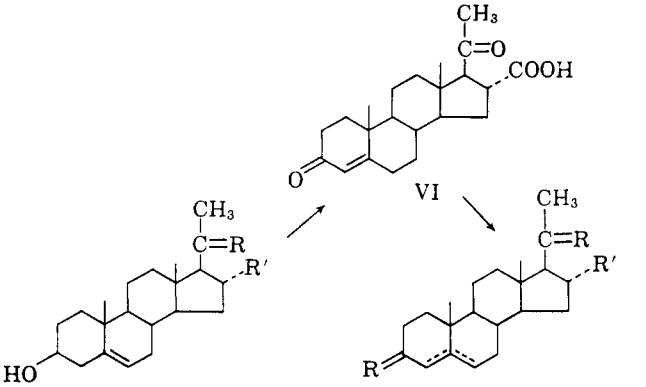
The recent publication of Heller, Stolar, and Bernstein[1] on the synthesis of 16α-hydroxymethylprogesterone prompts us to report some of our efforts in this area.

In the present work, we have converted 16α-carboxypregnenolone to the known 16α-methylprogesterone. Thus this sequence of reactions, for the first time, unequivocally establishes the configuration about carbons 16 and 17 in both the 16α-hydroxymethylprogesterone series of Heller, *et al.*,[1] and the 3β,20β-diacetoxypregn-5-ene-16α-carboxylic acid of Mazur and Cella.[2] This work also adds support to the evidence[2] that Romo[3] was working with 17α steroids substituted in the 16β position.

The synthesis of the acid IV and its conversion to 16α-methylprogesterone was accomplished in the following manner. The 20-cycloethylene ketal (II) of 16α-cyanopregnenolone was formed in the normal fashion. That no inversion had occurred at this step was confirmed by mild acid hydrolysis of the ketal to yield the starting cyano ketone I. When the ketal II was refluxed for 48 hours with ethanolic potassium hydroxide, there was obtained, in excellent yield after acid hydrolysis, the amide of 16α-carboxypregnenolone (III). When the hydrolysis of II was carried out in an



I. R = O, R′ = CN
II. R = 20-cycloethylene ketal, R′ = CN
III. R = O, R′ = $CONH_2$
IV. R = O, R′ = COOH
V. R = 20-cycloethylene ketal, R′ = COOH
VI. R = O, R′ = CH_2OH, Δ^5
VII. R = 20-cycloethylene ketal, R′ = $C(=O)-O-CH_2CH_2OH$, Δ^5
VIII. R = 20-cycloethylene ketal, R′ = CH_2OH, Δ^5
IX. R = O, R′ = CH_2OH, Δ^4
X. R = 20-cycloethylene ketal, R′ = CH_2—O—tosyl, Δ^5
XI. R = 20-cycloethylene ketal, R′ = CH_2I, Δ^5
XII. R = O, R′ = CH_3, Δ^4

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