ENZYMATIC MECHANISM OF STEROID RING A AROMATIZATION*

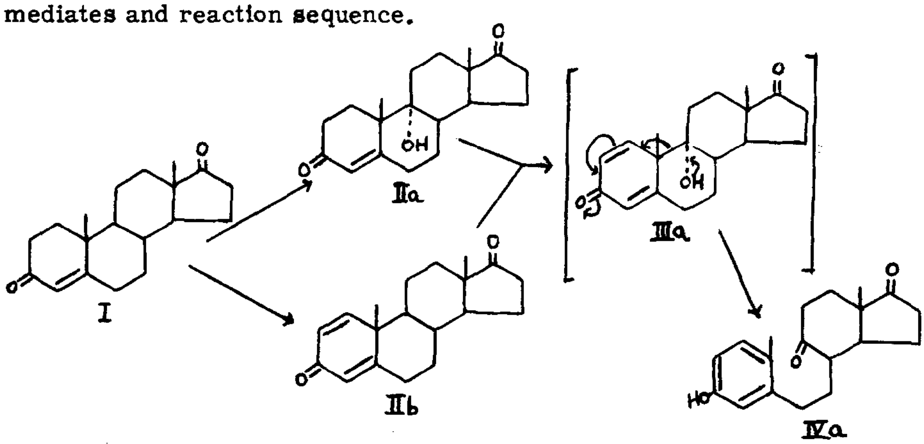
Charles J. Sih

School of Pharmacy, University of Wisconsin,

Madison, Wisconsin

Received January 12, 1962

One pathway of steroid degradation by microorganisms involves a 9α-hydroxylation, followed by a 1, 2-dehydrogenation (or vice versa) with the formation of a 9, 10-seco phenol. (Dodson and Muir 1958a, b). The degradation of Δ^4-androstene-3, 17-dione could thus be visualized as follows: Δ^4-androstene-3, 17-dione (I) $\longrightarrow$ 9α-hydroxy-4-androstene-3, 17-dione (IIa) or $\Delta^{1,4}$-androstadiene-3, 17-dione (IIb) $\longrightarrow$ 9, 10-seco-3-hydroxy-1, 3, 5(10)-androstatriene-9, 17-dione (IVa). This mechanism of steroid ring A aromatization bears close similarity to the formation of estrogens from androgenic steroids in mammals (Ryan 1959). Therefore the present investigation is undertaken in an attempt to define the intermediates and reaction sequence.



*This work has been supported in part by a grant from the National Institutes of Health (A-4874).

Nocardia restrictus (Dr. R. Gordon, Institute of Microbiology, Rutgers, The State University, New Brunswick, N. J.) is an organism capable of oxidizing a variety of steroids to CO_2 and H_2O via the 9, 10-seco phenol (Sih 1961). When IIa was incubated with partially purified steroid 1-dehydrogenase (10-fold purification) from this organism (Sih and Bennett 1961) in the presence of an electron acceptor, IVa was formed. In a typical experiment 200 mg of IIa mixed with 100 mg of phenazine methosulfate after 4-hour incubation at pH 7.0, afforded 94 mg of IVa, m.p. 122-124°; $[\alpha]_D^{25} = +98°$ (c, 0.9 in chloroform); λ_{max}^{alc} 280 mμ (ε 2700); λ_{max}^{Nujol} 2.95 μ, 5.75 μ, 5.86 μ, 6.23 μ and 6.66 μ, identical to an authentic specimen; (found: c, 76.23; H, 8.53).

This conversion could involve either 9α-hydroxy-1, 4-androstene-9, 17-dione (IIIa) or 9, 10-seco-4-androstene-3, 9, 17-trione (IIIb) as the intermediate (Fig 1). However, the steroid 1-dehydrogenase was inactive on IIIb whereas IIa was rapidly oxidized; this experiment ruled out IIIb as a possible intermediate in this aromatization reaction.

Phenazine methosulfate could be replaced by 2, 6-dichloro phenol indophenol or resazurin which have been also electron acceptors of steroid 1-dehydrogenase; inhibitors of steroid 1-dehydrogenase such as arsenite, Hg and Cu salts also inhibited this reaction; the pH optimum of this aromatization reaction (9.5) was the same as that of steroid 1-dehydrogenase (Sih and Bennett 1961). With the same enzyme system, 9α-fluorohydrocortisone was converted into 9α-fluoroprednisolone in 68% yield, m.p. 265-269° dec., $[\alpha]_D^{25} = +111°$ (c, 0.8 in ethanol), whose infrared spectrum was identical to an authentic sample of 9α-fluoroprednisolone. All these evidences suggest that the conversion of IIa to IVa proceeds through IIIa as the intermediate. Although attempts to demonstrate the presence of IIIa have been unsuccessful, it is well known that vinylogues of β-hydroxy-

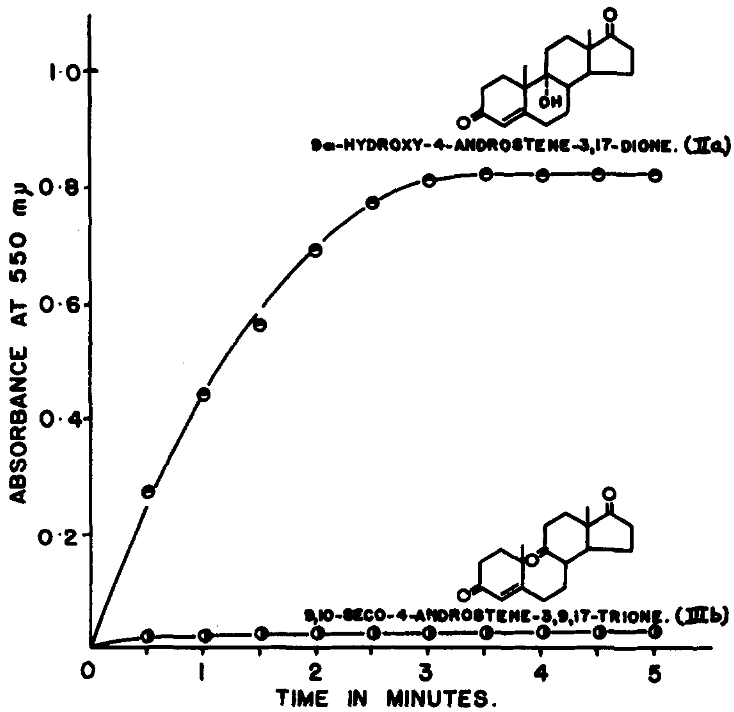
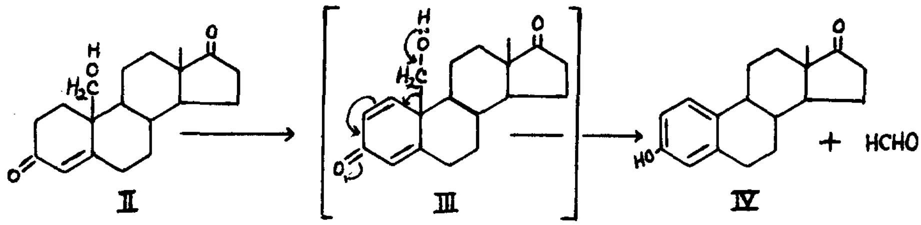


Fig. 1. Reaction of 9α-hydroxy-4-androstene-3, 17-dione and 9, 10-seco-4-androstene-3, 9, 17-trione with steroid 1-dehydrogenase. The assay system consisted of 2 mg of cytochrome c, 100 μg of steroid in 0.1 ml of methanol, and 25 units of enzyme in a total volume of 3.0 ml of 0.03 M phosphate buffer pH 7.0. The reaction was initiated by the addition of 0.1 ml of a 1% phenazine methosulfate solution.

ketones undergo rearrangement very easily even by ordinary chemical means (Talalay 1957, Ehrenstein and Otto 1959).

Similar conversion of 19-hydroxy-4-androstene-3, 17-dione (II) with the same enzyme system afforded estrone (IV), m.p. 256-260°; sulfuric acid spectrum (Axelrod 1953) showed peaks at 298 mμ and 448 mμ and the infrared spectrum was identical to an authentic sample of estrone. On paper chromatography the substance migrated as estrone and it gave the phenolic reaction with the diazotized sulfanilic acid reagent (Stowe and Thimann 1954). The distillates obtained after the enzymatic reaction gave positive reactions with chromotropic acid (Loke et al. 1955) indicating the presence of formaldehyde.



The aromatization of II and IIa appear to involve a direct 1,2-dehydrogenation to form vinylogues of β-hydroxyketones (III and IIIa) which undergo spontaneous non-enzymatic rearrangements (reverse aldolization) to give their respective phenols (IV and IVa).

REFERENCES

Axelrod, L. R. J. Biol. Chem. 201, 59 (1953).

Dodson, R. M. and Muir, R. D. J. Am. Chem Soc. 80, 5004 (1958).

Dodson, R. M. and Muir, R. D. J. Am. Chem. Soc. 80, 6148 (1958).

Ehrenstein, M. and Otto, K. J. Org. Chem. 24, 2006 (1959).

Loke, K. H., Marrian, G. J. and Watson, E. J. D. Biochem. J. 71, 43 (1955).

Ryan, K. J. J. Biol. Chem. 234, 268 (1959).

Sih, C. J. Am. Chem. Soc. Abstracts p, 61c Sept. (1961).

Sih, C. J. and Bennett, R. E. Biochim. et Biophys. Acta In press (1961).

Stowe, B. B. and Thimann, K. V. Arch. Biochem. Biophys. 51, 499 (1954).

Talalay, P. Physiol. Revs. 37, 362 (1957).