THE STEREOCHEMICAL COURSE OF ENZYMATIC STEROID 1,2-DEHYDROGENATION[1]

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The enzymatic unsaturation at positions 1,2 of various steroid substrates by the steroid Δ^1-dehydrogenase has been examined in the respiring microorganism (cf. Peterson, 1958) and in cell-free preparations of certain strains (cf. Levy and Talalay, 1959). It is now generally concluded that the mechanism of this reaction is one of direct dehydrogenation, rather than that of a hydroxylation followed by dehydration, as observed for example in the formation of the isolated double bond in the long chain fatty acid (Bloomfield and Bloch, 1960; Lennarz and Bloch, 1960). In our laboratory, examination of the stereochemical course of this reaction has been undertaken with the use of various steroid substrates with substituents at C-1α, 1β, 2α, and 2β. The compounds employed were 1α-methyl-androstan-17β-ol-3-one, 1β-methyl-androstan-17β-ol-3-one, 1α-methyl-19-nor-Δ^4-androsten-17β-ol-3-one, 1α-hydroxy-Δ^4-androstene-3,17-dione, 2α-methyl-testosterone, 2α-hydroxy-testosterone and 2β-hydroxy-Δ^4-androstene-3,17-dione (Fig. 1). Controls of Δ^4-androstene-3,17-dione, testosterone, androstane-3,17-dione and androstan-17β-ol-3-one were run in parallel.

Five mg. of each of the above steroids were fermented with a 24 hour culture of Bacillus sphaericus (ATCC 7055) (Stoudt et al., 1955) in 250 ml. Erlenmeyer flasks at 30° for 72 hours. The incubations and work-up were carried out as described by Gual et al., (1959). The residues from the ethyl acetate extracts of the incubated media were resolved by paper chromatography

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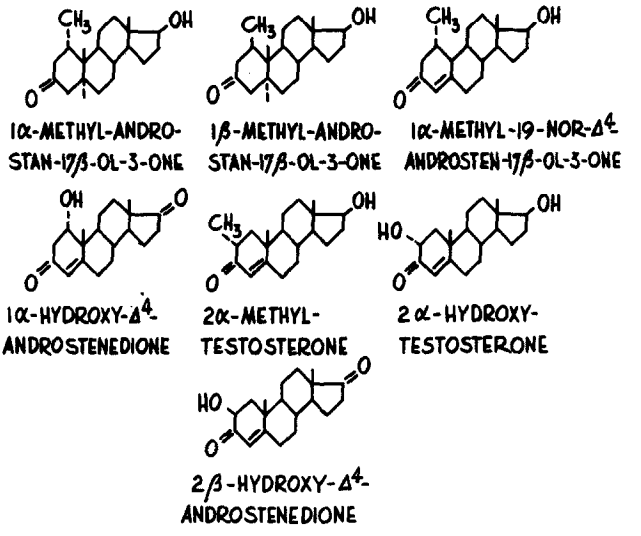

Figure 1.

using the ligroin-propylene glycol system (Fig. 2). The following means were employed for the detection of steroids on paper: Scanning with ultraviolet light for conjugated ketones; the isonicotinic acid hydrazide test (Smith and Foell, 1959) for mono conjugated 3-ketones; the Zimmermann test for 17-ketosteroids; the dinitrophenylhydrazine reaction for 3-keto structures; and the Turnbulls blue color (ferric chloride and potassium ferricyanide) for enols and phenols.

Δ^1-Dehydrogenated products were formed from each of the steroids run as controls (Table I, strips 1-4). In addition 17β-hydroxyl groups present in the substrates were oxidized to 17-keto functions. Out of this group of test compounds only 1β-methyl-androstan-17β-ol-3-one (strip 6), 2α-methyl-testosterone (strip 9) and 2α-hydroxy-testosterone (strip 10) gave single products in high yield with characteristics of newly introduced unsaturation. No indication of 1,2-dehydrogenated products was noted in fermentations of 1α-substituted structures. A small amount (± 10%) of dienone was observed from the incubation of 2β-hydroxy-androstenedione (strip 11).

These products and those from the control incubations were eluted from

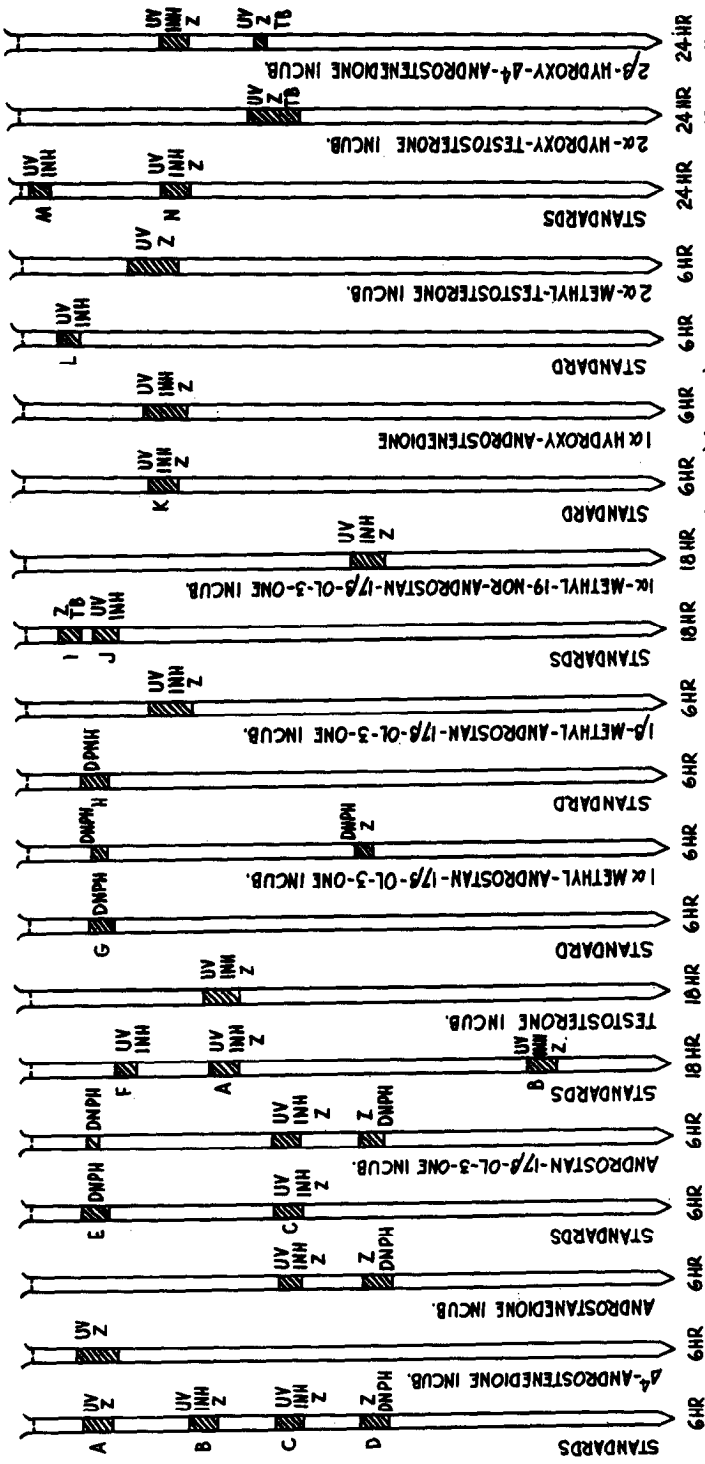

Figure 2. Paper Chromatograms of Steroid Incubations with B. sphaericus. Strips: 1 cm. x 45 cm. (starting line to tip). Steroid standards: A = Δ^{1},4-androstadiene-3,17-dione; B = Δ^{4}-androstene-3,17-dione; C = Δ^{1}-androstene-3,17-dione; D = androstane-3,17-dione; E = androstan-17β-ol-3-one; F = testosterone; G = 1α-methyl-androstan-17β-ol-3-one; H = 1β-methyl-androstan-17β-ol-3-one; I = estrone; J = 1α-methyl-19-nor-androstan-17β-ol-3-one; K = 1α-hydroxy-Δ^{4}-androstene-3,17-dione; L = 2α-methyl-testosterone; M = 2α-hydroxy-testosterone; N = 2β-hydroxy-Δ^{4}-androstene-3,17-dione. Ligroin-propylene glycol system 6, 18 and 24 hours, except for incubation 8, toluene-propylene glycol system 6 hours. Positive color reactions and ultraviolet (UV) absorption are noted on the right of each spot; INH = isonicotinic acid hydrazide test, Z = Zimmermann test, DNPH = dinitrophenylhydrazine test, TB = Turnbulls blue test.

Table I

UV Absorption Maxima of Products from B. sphaericus incubations

Compound Incubated		Product from Incubation		Steroid Standard
Δ^4-Androstenedione	242 mμ*	$\Delta^{1,4}$-Androstadienedione	243 mμ	244 mμ
Androstanedione		Δ^1-Androstenedione	230	231
Androstan-17β-ol-3-one		Δ^1-Androstenedione	230	231
Testosterone	242	$\Delta^{1,4}$-Androstadienedione	243	244
1β-Methyl-androstan-17β-ol-3-one		Δ^1-1-Methyl-androstenedione	242	243**
2α-Methyl-testosterone	242	$\Delta^{1,4}$-2-Methyl-androstadienedione	248	248#
2α-Hydroxy-testosterone	244	$\Delta^{1,4}$-2-Hydroxy-androstadienedione	253 (284)	253 (284)##

*All absorbtion maxima were determined in methanol.
**Δ^1-1-Methyl-androsten-17β-ol-3-one
#Triarte, J. and Ringold, H. J., Tetrahedron 3, 28 (1958).
##Gual, C., Stitch, S. R., Gut, M., and Dorfman, R. I., J. Org. Chem. 24, 418 (1959).

the paper strips with methanol and their ultraviolet absorption maxima determined[2]. These data are presented in Table I together with absorption values determined of steroid standards where these were available to us. References to values in the literature are listed for others.

It is clear from the above findings that the bacterial dehydrogenation involves the diaxial loss of the 1α- and 2β-hydrogen atoms. The low yield of dienone from the 2β-hydroxy compound is probably due to partial inversion of the thermodynamically unstable 2β-hydroxy to the 2α-hydroxy isomer subsequent to dehydrogenation.

A simple two step mechanism depicted here for a Δ^4-3-ketosteroid (Fig. 3A) may be written for the bacterial dehydrogenation. Coordination of the enzyme with the 3-keto group of the steroid would facilitate enolization by loss of the 2β-hydrogen atom, axial rather than equatorial hydrogen removal being favored by more efficient bonding in the transition state. Once the

[2]Cary Recording Spectrophotometer, Model 11 MS.

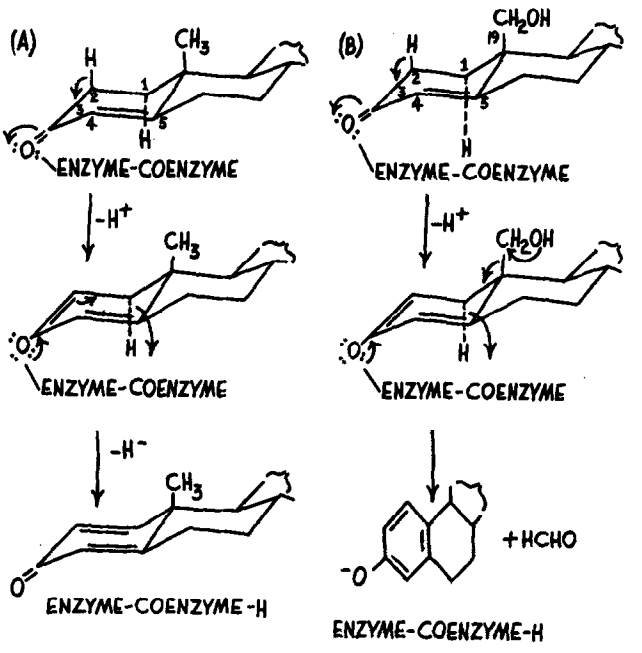

Figure 3. A mechanism for dehydrogenation (see text).

$\Delta^{2(3)}$-enol has been formed the hydrogen atoms attached to the 1-carbon atom are activated and may be readily abstracted as hydride ion by a suitable, in this case as yet unidentified, co-enzymatic acceptor. Again, loss of the axial (1α) hydride would be favored. Formation of the 1(2)-double bond proceeds concomitant with the loss of hydride ion from the enol.

Work is in progress to establish by means of suitable deuterium labeled substrates whether the enolization step or the loss of the 1α-hydride ion is the rate-determining step in these transformations.

It is pertinent to note that the placental aromatization of 19-hydroxy-Δ^{4}-androstene-3,17-dione to estrone (Fig. 3B) may be accommodated by a similar mechanism of enolization followed by co-enzyme abstraction of the 1α-hydride ion with a concomitant loss of formaldehyde, aromatization being the driving force in this case.

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