Enzymic mechanism of the 12β-hydroxylation of steroids

Recently the first series of experiments on the mechanism of enzymic hydroxylation at carbons 11α and 11β of steroid substrates by *Rhizopus nigricans* and bovine adrenal glands respectively was reported[1]. In both instances a stereospecific replacement of the hydrogen (or its isotope) of the position subsequently oxygenated was noted. [11α,12α-$^3H_2$]pregnanedione was found transformed to 11α-hydroxy[12α-^3H]pregnanedione and [11α,12α-$^3H_2$]progesterone to [11α,12α-$^3H_2$]corticosterone and [11α,12α-$^3H_2$]hydrocortisone, establishing this point*.

A second set of data has now been compiled in an experiment involving enzymic hydroxylation at the 12β position of steroids. [11α,12α-$^3H_2$]progesterone was incubated with *Calonectria decora*[2] for 24 h, the medium thereafter extracted with CH_2Cl_2. Purification of the residue from this extract was effected by silica-gel chromatography using elution mixtures of benzene and ethyl acetate. 12β,15α-Dihydroxyprogesterone was eluted with ethyl acetate. Mild chromic acid oxidation of this product to the 11,15-diketo analogue was carried out after the method of Poos[3]. Silica-gel chromatography yielded this product in the benzene–ethyl acetate (2:1) eluate. All substances were rechromatographed when necessary and crystallized from acetone to a constant count. Identifications were established by comparisons of melting points and/or infrared spectra with those of known standards.

In Table I is a presentation of the data obtained. The results show essentially no loss (2 %) of the original count of the progesterone on 12β-hydroxylation. Oxidation of 12β,15α-dihydroxyprogesterone to the 11,15-diketo analogue gave a loss of 30 % of the counts (Table II). These results are precisely as expected from the previous observations of enzymic hydroxylations at C-11α and C-11β of steroids from the same parent source. A schematic representation summarizing all findings from these laboratories is presented in Fig. 1.

TABLE I

HYDROXYLATION OF [11α,12α-$^3H_2$]PROGESTERONE BY *Calonectria decora*

All counts were determined in a Packard Tri-Carb liquid scintillation counter Model 314. Accuracy ± 3 %. All melting points were observed on a Fisher-Johns hot stage and are corrected.

	Counts/min/μmole
Starting material:	
Progesterone, m.p. 124–125°	$7.53 \cdot 10^6$
Isolated product:	
12β,15α-Dihydroxyprogesterone, m.p. 220–223°	$7.38 \cdot 10^6$

TABLE II

CHROMIC ACID OXIDATION OF 12β,15α-DIHYDROXY[11α,12α-$^3H_2$]PROGESTERONE

	Counts/min/μmole
12β,15α-Dihydroxyprogesterone, m.p. 220–223°	$3.58 \cdot 10^6$
12,15-Diketoprogesterone, m.p. 217–220°	$2.50 \cdot 10^6$

* [11α,12α-$^3H_2$]pregnane-3,20-dione → [11α,12α-$^3H_2$]progesterone. Synthesis and discussion of isotope ratio at C-11 and C-12 to be published.

Thus another instance confirming the fact that enzymic steroid hydroxylations occurs by way of a simple displacement of the hydrogen of the position oxygenated has now been documented. Previous instances are those at carbons 11α and 11β from these laboratories[1], and C-11α[4] and C-7α[5] from other laboratories.

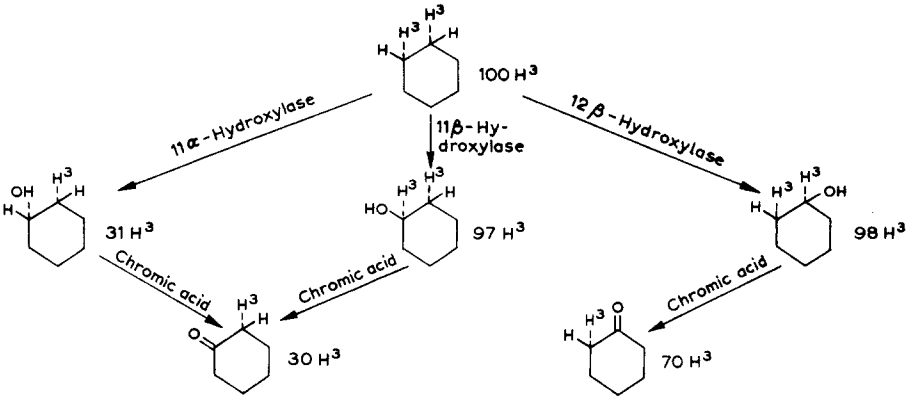

Fig. 1.

Supported in part by Research Grant C-2207 from the Research Grants and Fellowships, National Institutes of Health, U.S. Public Health Service.

Worcester Foundation for Experimental Biology, Shrewsbury, Mass. (U.S.A.)
MIKA HAYANO
MARCEL GUT
R. I. DORFMAN

Wissenschaftliche Laboratorien VEB Jenapharm, Jena (Germany)
A. SCHUBERT
R. SIEBERT

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Received August 12th, 1958

The role of cell sap in the incorporation of ^{14}C-labelled leucine into proteins of isolated rat-liver mitochondria

The rate of incorporation of intravenously injected amino acid into the microsomal nucleoproteins of rat liver greatly exceeds that into other subcellular fractions and it has been suggested that most or all of the proteins of the living cell are synthesised in this particulate fraction[1]. This hypothesis has received support from the fact that