PN 1194

The conversion of 17α-hydroxyprogesterone into pregn-4-en-3α,17α-diol-20-one and other substances by perfusions through bovine adrenals and ovaries

We have reported the conversion of 17α-hydroxyprogesterone (I) by a single-cycle perfusion through a bovine adrenal gland into cortisol and 2 unidentified substances, unknown 1, m.p. 250–257°, and unknown 2, m.p. 268–270°; and, by a 2-cycle perfusion, into cortisol and an unknown 3 (II), m.p. 192–195° (ref. 1). The unknown substance 1 has been identified as allopregnan-17α-ol-3,20-dione and the unknown 2 as allopregnan-3β,17α-diol-20-one by comparison of the infrared spectrum of unknown 1 (taken in $CHCl_3$), and of the acetate of unknown 2 (from acetic anhydride and pyridine), taken in CS_2, with those of the respective known substances*. An additional quantity of II has since been obtained, for a total of 11.9 mg, and the m.p. raised to 199–200°. Further, another substance has since been isolated from the second perfusion. This was obtained as prisms, 10.6 mg, m.p. 202–204°; λ_{max} (in methanol) 242.5 mμ; ν_{max} (film) 3448, 1653 and 1613 cm^{-1}, from certain benzene – ethyl acetate (2:1 and 1:1) eluates of the silica gel adsorption column (somewhat less mobile than II) and has been identified as pregn-4-en-17α,20β-diol-3-one by comparison of its infrared spectrum with that of the known compound**.

In one ovarian perfusion, 17α-hydroxy[4-^{14}C]progesterone, 37 mg, in 3.5 ml of propylene glycol and 750 ml of citrated whole cow blood was circulated through 3 ovaries (from non-pregnant cows) for 50 cycles in 2 h 26 min***. In a second experiment, 32 mg in 3 ml of propylene glycol and 650 ml of blood were perfused through 4 ovaries for 25 cycles in 3.8 h. Each perfusate was extracted with 8–9 vol. of isopropyl acetate and the extract residues chromatographed on adsorption columns of silica gel (Grade 62 or 70, Davison Chem. Corp.). The residues of the benzene – ethyl acetate (95:5) through acetone eluates from both perfusates were combined and rechromatographed on a partition column of diatomaceous earth (Celite 545) using methanol – water (4:1) as the stationary phase (0.67 ml/g). From certain of the hexane – benzene (3:1) eluate residues, 31.7 mg of crystalline 17α-hydroxy[4-^{14}C]-progesterone were recovered. Later hexane – benzene (3:1 and 1:1) eluates led to a residue of 2.2 mg from which 0.8 mg of a radioactive substance, needles, m.p. 198–201°, could be crystallized in ethyl acetate. Its infrared spectrum was identical with that of the unknown substance, II, from the adrenal perfusates.

Later hexane – benzene (1:1 and 1:3) eluates led to the crystallization, from ethyl acetate, of a third radioactive substance, 6.6 mg, m.p. 198–205°. Recrystallization achieved the purified substance, m.p. 204–210°; ν_{max} (KBr) 3538, 3400, 1655, 1607 cm^{-1}, whose infrared spectrum was identical with that of authentic pregn-4-en-17α, 20α-diol-3-one§.

Substance II had ν_{max} (KBr) 3680 (hydroxyl), 3375 (hydroxyl), 1692 (unconjugated ketonic carbonyl), 1660 (isolated double bond), 1013 and 993 cm^{-1}; λ_{max} (conc. H_2SO_4, 1 h at room temp.) 308.5, 415 and 490 mμ plus very weak peaks at 282 and 446 mμ. Reaction with acetic anhydride and pyridine at room temperature

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* We are grateful to Dr. T. F. GALLAGHER of Sloan-Kettering Institute for these comparisons.
** The known compound was kindly supplied by Ciba Pharmaceutical Co.
*** Method of E. B. ROMANOFF AND G. PINCUS, *Endocrinology*, in the press.
§ We are indebted to Dr. E. P. OLIVETO of the Schering Corp. for this reference sample.

yielded a substance whose infrared spectrum, ν_{max} (film) 3375 and 1265 cm^{-1}, indicated the presence of hydroxyl and acetate groups. The free compound, II, was rapidly converted at room temperature by acetic acid containing about 6% of conc. HCl into a substance with λ_{max} (methanol) 227, 234.5 and 243 $m\mu$. This spectrum is characteristic of a $\Delta^{3,5}$-diene group and its ready formation indicated that one hydroxyl group in II is allylic to the double bond. Pregn-4-en-3β-ol-20-one was also dehydrated under these conditions but not androst-5-en-3α-ol-17-one*. These data, as well as its mobility in the partition column, suggested that II is pregn-4-en-3α,17α-diol-20-one or the 3β-ol epimer. Since it did not form an insoluble digitonide, while pregn-4-en-3β-ol-20-one did, the conclusion was that it is the former. This conclusion was supported by the nuclear-magnetic-resonance spectrum of II, taken in $CDCl_3$, which had peaks at τ (relative to $(CH_3)_4Si$) 4.54 (J = 5 cycles/sec; hydrogen on a double-bonded carbon), 5.97 (hydrogen on the carbon of a secondary alcohol), 9.00 (hydrogens on C-19) and 9.25 (hydrogens on C-18). A detailed analysis of the spectrum, which is too extended for this communication, led to the conclusion that the secondary alcohol is allylic to the double bond and that, if the latter is between C-4 and C-5, then this hydroxyl is at 3α**.

The conclusion that II is a Δ^4-3-hydroxysteroid was corroborated by mild oxidation with 1.5 equiv of 2,3-dichloro-5,6-dicyanobenzoquinone in *tert.*-butanol at room temperature for 17 h. These conditions are sufficient for oxidizing an allylic alcohol but not the alcohol function of a Δ^5-3-hydroxylated steroid[2]. The reaction product was a crystalline substance, m.p. 205–216°, whose infrared spectrum was identical with that of 17α-hydroxyprogesterone.

Finally, pregn-4-en-3β,17α-diol-20-one-17-acetate*** was hydrolyzed in 0.06 M aq. methanolic KOH at room temperature for 16 h to obtain the free alcohol, m.p. 219–222°; ν_{max} (KBr) 3390, 1690, 1665, 1062 and 1034 cm^{-1}, whose infrared spectrum was different from that of II§. Therefore, II is the isomer, pregn-4-en-3α,17α-diol-20-one. It is interesting that neither pregn-4-en-3β,17α-diol-20-one nor its 17-acetate formed an insoluble digitonide.

Until recently, the biological conversion of a Δ^4-3-ketosteroid into a Δ^4-3-hydroxysteroid had not been observed. The first indication of such a reaction was recorded by NEEMAN *et al.*[3] who isolated crystalline androst-3,5-dien-11β-ol-17-one from the urine of a normal male to whom androst-4-en-11β-ol-3,17-dione had been administered and obtained suggestive evidence for the presence of a Δ^4-3-hydroxysteroid. RINGOLD *et al.*[4] gave evidence for the reduction of 6β-fluorotestosterone, by incubation with supernatant fractions of male rat liver, into the Δ^4-3α- and the Δ^4-3β-hydroxysteroids. FARNSWORTH *et al.*[5] incubated testosterone with prostate minces and stated that chromatographic data indicated the presence of a 4-androsten-3,17-diol among the products.

The isolation of II, a new substance, is the first example of the reduction of a Δ^4-3-ketosteroid to a Δ^4-3-hydroxysteroid by adrenals or ovaries and is the first instance in which such a substance (α or β) has been isolated in crystalline form

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from any biochemical system. It is likely that this type of reduction is more common than the present number of examples indicates and that additional instances will appear when the extreme lability of a Δ^4-3-hydroxysteroid to acid is appreciated. Rate studies of the dehydrations of II and pregn-4-3β-ol-20-one, under the mild conditions above, showed that the reactions were almost 75% complete within 5 min.

The reduction of the carbonyl group of a Δ^4-3-ketosteroid without prior reduction of the double bond reflects an additional degree of complexity in the metabolism of steroids. It will be worth while to test a Δ^4-3-hydroxysteroid as a substrate in various biochemical systems or *in vivo* to learn whether the double bond rearranges to form a Δ^5-3-hydroxysteroid. If this occurs, then the two steps—reduction of the 3-carbonyl group and rearrangement of the double bond—would constitute a reversal of the oxidation of a Δ^5-3-hydroxysteroid to a Δ^4-3-ketosteroid. This reversal has not been hitherto observed.

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Heme dissociation and autoxidation of myoglobin*

The autoxidation of HbO_2 and MbO_2 has been studied by BROOKS[1,2], GEORGE AND STRATMAN[3–5] and TSUSHIMA[6] among others. The evidence accumulated supports the concept that reduced, deoxygenated heme pigment reacts with oxygen to yield oxidized heme pigment. BROOKS[2] and GEORGE AND STRATMAN[4] showed that the rate constants for autoxidation of HbO_2 and MbO_2, respectively, increase with decreasing partial pressure of oxygen and go through a maximum at a partial pressure of oxygen corresponding to half saturation of the particular heme pigment. As WATTS[7] has pointed out, the differences in reduced heme pigment molecules which allow them

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