ISOLATION OF PREGN-4-ENE-17α,20β-DIOL-3-ONE FROM THE PLASMA OF PACIFIC SALMON (ONCORHYNCHUS NERKA)

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Introduction

The isolation of cortisol, cortisone and 17-hydroxyprogesterone from salmon plasma and the high blood levels of the first two, particularly in post-spawned fish, was recently described (Idler et al., 1959). Three ultraviolet absorbing substances less polar than corticosterone were found. Fraction 6B, the substance of intermediate polarity when chromatographed in heptane:benzene 1:1-formamide (HBF), was 17-hydroxyprogesterone. The most polar of the three steroids, fraction 6A, has now been identified as pregn-4-ene-17α,20β-diol-3-one. To our knowledge this represents the first reported isolation of this steroid from a natural source.

Materials and Methods

Approximately 80 post-spawned female sockeye salmon, taken at Chilko Lake, B.C. in September, 1959, were bled by severing the caudal artery. Precautions were taken to exclude slime and excreta from the blood. Plasma (1640 ml) was prepared from the heparinized blood and frozen on dry ice. The thawed plasma was extracted 3 times with 2.5 volumes of CH_2Cl_2. The chilled extract was washed with 0.05N NaOH, 0.1N acetic acid and H_2O and the CH_2Cl_2 removed at 30° in vacuo. The residue was taken up in 70% methanol and non-steroid fatty material removed by partition with hexane.

Chromatography paper was pre-washed (Idler et al., 1959). Solvent

systems used were hexane–propylene glycol (HPG) (Savard, 1953), hexane–ethylene glycol (HEG), benzene:hexane:methanol:water 33:66:80:20 (BHMW) (Bush and Sandberg, 1953) and HBF.

The C-20 epimers of pregn-4-ene-17α,20-diol-3-one were located on paper chromatograms with the aid of their absorption of ultraviolet light and their fluorescence under ultraviolet light after the paper was sprayed with 15% aqueous H_3PO_4 and heated at 90°C for 10 minutes. They were prepared by selective reduction with $NaBH_4$ of the C-20 ketone of chromatographically pure 17α-hydroxyprogesterone under conditions similar to those described for other corticosteroids (Norymberski and Woods, 1955). The 20α-epimer was also prepared by microbiological reduction of 17α-hydroxyprogesterone with Rhodotorula longissima. The conditions were as those described by Carvajal et al., (1959) except that 2-day old cells were used and 50 mg of steroid were added per 100 ml of Edamin-dextrose-cornsteep liquor medium and incubated at 25-26° on a rotary shaker.

Infrared spectra were recorded of micro KBr pellets, containing 20-50 μg of steroid, using a Beckman IR-4 equipped with a beam-condensing system.

Results

The new steroid had its max. at 240 mμ in methanol. It gave a negative test for the dihydroxyacetone side-chain (Silber and Porter, 1954) and could be detected on paper chromatograms with a modified Zimmerman reagent (Bongiovanni et al., 1957). The unknown and synthetic pregn-4-ene-17α,20β-diol-3-one both had an R_f relative to 17α-hydroxyprogesterone (R_{HP}) of 0.21 in HPG and 0.40 in HEG. The R_f of both substances was 0.18 in the HBF system.

The bismuthate oxidation products (Bush and Sandberg, 1953) of the unknown and the synthetic 20β-epimer both had an R_f of 0.58 in BHMW identical to that of androst-4-ene-3,17-dione and all three compounds gave positive Zimmerman reactions.

The unknown and synthetic pregn-4-ene-17α,20β-diol-3-one gave identical sulfuric acid chromogens with maxima and minima at 298 mμ and 238 mμ after

4 hours and 305 mμ and 242 mμ after 24 hours at room temperature.

The infrared spectrum of the unknown exhibited absorption maxima at 1612, 1660, 1333, 1263, 1231, 1183, 958, 943, 869 and 776 cm^{-1} characteristic of a Δ^4-3 ketone (Jones and Herling, 1954). The absorption intensity at 3460 cm^{-1} indicated more than one OH group in the molecule. The spectrum corresponded in all respects with the published spectrum of pregn-4-ene-17α,20β-diol-3-one (Dobriner et al., 1953) which was probably synthesized by the reduction of 17α-hydroxyprogesterone 3-benzylthioenol ether with $LiAlH_4$ (Romo et al., 1951). The infrared spectra of both products were identical with that of the synthetic 20β-epimer prepared by borohydride reduction of 17α-hydroxyprogesterone. The 20α-epimer preparations made by reduction of 17α-hydroxyprogesterone with $NaBH_4$ and with R. longissima gave identical infrared spectra which were quite distinct from the spectrum of the 20β-epimer.

From the free steroid extract of 1640 ml of post-spawned female plasma 14.4 μg/100 ml of 17-hydroxyprogesterone and 5.5 μg/100 ml of the 20β-dihydro-epimer were recovered following chromatography in the HPG system. The concentration of 17-hydroxyprogesterone was comparable to that found previously (Idler et al., 1959) in plasma of pre-spawned females. Plasma levels of these steroids in the two sexes at various stages of the spawning migration will be the subject of a future report.

Discussion

The reduction of 17α-hydroxyprogesterone with $NaBH_4$ gave two ultraviolet absorbing compounds which were readily separable in the HEG solvent system. The 20β-dihydro-epimer predominated (90-95%) as expected from previous findings with other corticosteroids, (Norymberski and Woods, 1955; Southcott et al., 1956). There was a possibility that the minor component arose from a contaminant in the 17α-hydroxyprogesterone and that the 20α and 20β-dihydro-epimers of 17α-hydroxyprogesterone were not separable in the HEG solvent system. This possibility was eliminated by the synthesis of the 20α-epimer using a stereospecific microbiological reduction of 17α-hydroxyprogesterone. After a

one-day incubation 80% of the added Δ^4-3-ketone was recovered by CH_2Cl_2 extraction of the media and cells without prior grinding. The ultraviolet absorbing products were pregn-4-ene-17α,20α-diol-3-one (95%) and unchanged 17α-hydroxyprogesterone (5%). The yield was appreciably greater than that reported for longer time reduction of steroids with a dihydroxyacetone sidechain under similar conditions.

The reduction of the C-20 keto group, without the reduction of the Δ^4-3-ketone, is well established in a variety of tissues and species but not in fish (Vermeulen and Caspi, 1959; Berliner et al., 1958; Wiest, 1959). Progesterone has been converted to pregn-4-ene-17α,20β-diol-3-one by testicular tissue (Lynn et al., 1958).

Since 17-hydroxyprogesterone occurs at a relatively high level in the plasma of salmon, and has not yet been found in the plasma of other species, it is the most logical precursor of the 20β-dihydro-epimer. The site of the transformation is not necessarily the liver or other tissues referred to above. Recently human adrenal incubates have been shown to produce 20α and 20β-dihydrocortisol (Touchstone et al., 1959). Adrenal vein blood of young calves contains pregn-4-ene-20α-ol-3-one and the concentration is much higher than in peripheral blood, indicating that it is an adrenal secretion (Balfour et al., 1959). The biological activity of pregn-4-ene-17α,20β-diol-3-one is not known to the authors. However, 20β-dihydrocortisone has the same activity as cortisone in the eosinophil test (Carvajal et al., 1959), 20β-dihydrocortisol is active in the glycogen assay (Abelson et al., 1955), and 20β-dihydroprogesterone has progestational activity (Zander et al., 1958).

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