Metabolism in Vitro of 19-Nortestosterone in Female Rat Liver Homogenate*

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A study in vivo of 19-nortestosterone metabolism in the postmenopausal woman has revealed a metabolic pattern of products similar to that obtained after the administration of testosterone (1). In the present investigation the transformation of 19-nortestosterone by female rat liver homogenates was studied. The rate of reduction of ring A by this tissue system appeared to be extremely rapid. Four major products were isolated and identified. These were: 3α-hydroxy-19-nor-5α-androstane-17-one (19-norandrosterone) and the previously undescribed metabolites 3β-hydroxy-19-nor-5α-androstane-17-one (19-norepiandrosterone), 19-nor-5α-androstan-3α,17β-diol, and 19-nor-5α-androstan-3β,17β-diol.

METHODS

Thirteen female rats weighing approximately 200 g were killed and bled. The livers were removed, washed with distilled water, and placed immediately on ice until collection was complete. Ninety-eight grams (wet weight) of liver were homogenized in 100 ml of 0.154 M KCl solution for 30 seconds in a Virtis homogenizer at 5°C. Incubations were carried out in eighteen 125-ml Erlenmeyer flasks at 37°C in air for 1 hour. Each flask contained 10 mg (0.0364 mmole) of 19-nortestosterone dissolved in 0.2 ml of propylene glycol, 0.035 mmole of TPN, 0.375 mmole of phosphate buffer (pH 7.2), and 7.5 ml of liver homogenate in a final volume of 15 ml. After incubation the contents of the Erlenmeyer flasks were added to 300 ml of acetone. The resulting mixture was stirred for 1 hour and filtered through a Buchner funnel. The clear filtrate was concentrated under reduced pressure to a volume of 180 ml. Methanol, 420 ml, was added and the resulting solution extracted with 400 ml of petroleum ether. The petroleum ether phase was back extracted with 70% aqueous methanol and the aqueous methanolic phases were combined. The petroleum ether phase was discarded. The alcoholic extract was concentrated under reduced pressure to about 400 ml and then extracted with 800 ml of chloroform. The chloroform phase was washed once each with 200 ml of 5% sodium bicarbonate, 200 ml of 0.01 M NaOH, and water. The washed chloroform phase was dried over Na2SO4 and evaporated to dryness under vacuum.

Experimental Procedure

The crude residue was separated into ketonic and nonketonic fractions (2). The ketonic fraction (31.7 mg) was chromatographed on two 15-cm wide strips of paper and developed in the ligroin-propylene glycol system for 48 hours. The overflow was collected. The strips were dried in air at room temperature. Two-millimeter strips were cut along the length of the chromatograms and the steroid-containing zones were detected with the dinitrophenylhydrazine reagent, Zimmermann reagent, and ultraviolet absorption. Results are illustrated in Table I. Corresponding zones were cut off the paper strips and eluted with methanol-methylene chloride (1:1). Zone I contained a component in small quantity, identified by migration rate and color reactions as the unreacted 19-nortestosterone. The crude of zone II was evaporated to dryness (7 mg). Zone III was eluted, combined with the overflow and evaporated to dryness (substance A, 8 mg).

Substance A was chromatographed on paper for 24 hours yielding a single zone which gave a stable Zimmermann color, weak yellow dinitrophenylhydrazine reagent, and no ultraviolet absorption. The relative rate of migration of the zone with respect to 19-nor-5α-androstene-3,17-dione was 0.6. The rates of migration of zone II and substance A indicated that they were composed of substances which were less polar than 19-nortestosterone but more polar than 19-nor-5α-androstene-3,17-dione. The positive Zimmermann and negative ultraviolet tests indicated the absence of an α,β-unsaturated ketone and the presence of a 17-alkyl. Infra-red spectra of the crude material of zone II and substance A showed hydroxyl and cyclopentyl ketone absorptions, suggesting that both substances were monohydroxy-17-alkylandrosteroids.

Substance A was chromatographed on silica gel and eluted with benzene and benzene-ethyl acetate mixtures. Elution with benzene-ethyl acetate, 9:1, yielded 6 mg of a white amorphous substance, δmax 2.72 to 3.0 (free and bonded hydroxyl), 5.78 (cyclopentyl ketone), and 6.0 μ (isolated C=C). Crystallization from acetone-hexane yielded colorless needles with a double melt; 149°, 166-167°; [α]D 2° 110 (c, 0.763 in CHCl3); δmax 2.75 (hydroxyl), 5.75 (cyclopentyl ketone), 9.0, 9.35, 9.49, 9.65, 9.81, and 10 μ. The absorption band at 10 μ was good evidence for the presence of an axial hydroxyl (3). Positive identification of this compound was established after partial synthesis of 3α-hydroxy-19-nor-5α-androstane-17-one from 19-nor-5α-androstan-3β,17β-diol. The incubation product and the synthetic

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1 Incubations were carried out with a freshly prepared homogenate. On standing in the cold for 24 hours a total loss in activity resulted.

2 The reduction of ring A, measured by a decrease in absorbancy at 240 μ, was complete (98%).

3 This concentration gave maximal reduction in 20 minutes.

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The substance isolated from zone II was crystallized from methylene chloride-ether and yielded colorless plates, m.p. 149-151.5° and were identified as cholesterol.

The infrared spectrum of the compound was identical to that of cholesterol. 19-nor-5α-androstan-3α,17β-diol, which agreed well with the results of other investigators (1) in which 19-nortestosterone was administered to a female rat, and the synthetic material had identical infrared spectra, optical rotations and no depression in melting point was observed on admixture of the two substances.

Fraction 19 to 22 (6 mg) was chromatographed on 250 mg of silica gel and yielded a diol (m.p. 158-173°) which was identified by its infrared spectrum as being identical to an authentic sample of 19-nor-5α-androstan-3α,17β-diol. The infrared spectrum and the synthetic material had identical infrared spectra and optical rotations and no depression in melting point was observed.

Reduction of the C-3 carbonyl was predominantly axial (3α, 5α) which agreed well with the results of other investigators (6, 7). It should be noted that the ratio of 3β-hydroxyl to 3α-hydroxyl products was about 0.2 which was of the same order (0.12 and 0.16) reported by Rubin (8) for reduction of the C-3 carbonyl of androstan-3,17-dione by female rat liver homogenate. This is in contrast to the study in vivo of Engel et al. (1) in which 19-nortestosterone was administered to a female patient and both 5α and 5β products were isolated from urine.

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summary

three previously undescribed metabolites, 3β-hydroxy-19-nor-5α-androstane-17-one, 19-nor-5α-androstan-3α,17β-diol, 19-nor-5α-androstan-3β,17β-diol, and the previously reported 3α-hydroxy-19-nor-5α-androstane-17-one were isolated after the incubation of 9-nortestosterone with a female rat liver homogenate.

REFERENCES


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