Biosynthesis of Estrogens by Microsomal Placental Aromatase; Isolation and Metabolism of 10β-Hydroxyestr-4-ene-3,17-dione

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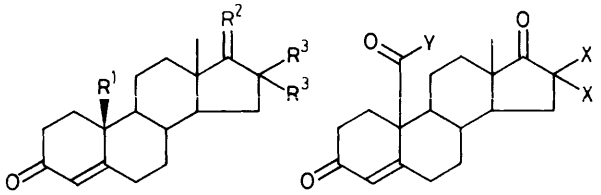
Incubation of [16,16,19-$^2H_3$][19-^{3}H]-19-oxoandrost-4-ene-3,17-dione (**2a**) with microsomal aromatase gave *inter alia* [16,16-$^2H_2$]-10β-hydroxyestr-4-ene-3,17-dione (**1**), whose analogue (**1b**) on incubation with the aromatase gave 10β,17β-dihydroxyestr-4-en-3-one (**1d**) (*ca.* 10%) and no estrogens which would indicate that (**1b**) is not an obligatory estrogen precursor.

For the past several years we have systematically explored the scope of transformations of [16,16,19-$^2H_3$][19-^{3}H]-19-oxoandrost-4-ene-3,17-dione (**2a**) by human microsomal placental aromatase.[1—4] In the course of our recent studies,[1] we isolated several 'new' products. One of these products showed an HPLC† retention time (R_t) of 7.8 min, and the GC-MS of its bis-*O*-methyloxime trimethylsilyl (MO–TMS) derivative showed *m/z* 420 (M^+), 389, 299, 226, *etc.* The mass spectrum was consistent with a $C_{18}H_{22}{}^2H_2O_3$ compound, *e.g.*, [$^2H_2$]-(ξ-hydroxy)-estr-4-ene-3,17,dione. These results were most interesting in view of the hypothesis that the 'third' mole of oxygen is utilized for the oxidative transformation of (**2b**) to 10β-hydroxyestr-4-ene-3,17-dione (**1b**) which, following dehydration, yields estrone.[5] This hypothesis seemed rather unusual since Covey *et al.*[6] demonstrated that the 10β-hydroxy compound (**1b**), and Akhtar *et al.*[8] showed that the 10β-hydroxy formate (**1e**), are not metabolized to estrogens by microsomal aromatase.

Considering the gross composition of the unknown, the mechanistic implications of the proposed scheme[5] and the contradicting evidence,[6,7] prompted us to determine the structure of the metabolite. As a working hypothesis, we assumed that the metabolite is [16,16-$^2H_2$]-10β-hydroxyestr-4-ene-3,17-dione (**1a**) and we undertook the synthesis of an authentic reference sample. To this end, a moderate stream of



(**1a**) R^1=OH; R^2=O; R^3=D
(**1b**) R^1=OH; R^2=O; R^3=H
(**1c**) R^1=OOH; R^2=O; R^3=H
(**1d**) R^1=OH; R^2=β-OH; R^3=H
(**1e**) R^1=HCOO; R^2=O; R^3=H

(**2a**) X=D; Y=D(T)
(**2b**) X=H; Y=H

dry air was bubbled (72 h) through a toluene solution (5 ml) of a mixture of (**2b**) (150 mg) and azoisobutyronitrile (AIBN); (7.5 mg) maintained at 50 °C (reflux condenser).[8] The toluene was removed in a stream of N_2, and the residue was purified by PLC (silica; EtOAc–cyclohexane, 3:1) to yield (**1b**) (30 mg) and the 10β-hydroperoxide (**1c**) (75 mg). The hydroperoxide (**1c**) showed m.p. 176—178 °C (from MeOH); NMR (δ) 9.23 (br., OOH,), 6.0 (4-H), 0.9 (13-Me); mass spectrum of MO–TMS *m/z* 434, 417, 403, 386, 334, 320, 313, *etc.* Treatment of a solution[9] of (**1c**) (40 mg in 2.0 ml of EtOH) with PPh_3 (70 mg) for 3 h at room temperature gave, following PLC (as above), (**1b**) (25 mg), m.p. 198—200 °C (from acetone); NMR (δ) 5.76 (4-H), 0.94 (13-Me); mass spectrum of MO–TMS gave *m/z* 418, 387, 328, 297, *etc.* The HPLC R_t of

† Altech Co. column; Nucleosil 50, 5μ; i.d. 4.6 mm × 25 cm; 20% propan-2-ol in iso-octane; flow rate 1 ml/min; the eluates were monitored at 240 and 280 nm.

authentic (**1b**) (7.8 min) was identical to that of the metabolite. Except for the shift by two mass units, the mass spectrum of the metabolite and of the synthetic (**1b**) were identical. This established the structure of the metabolite as the 10β-hydroxy compound (**1a**).

We then undertook the evaluation of intermediacy of (**1b**) in the biosynthesis of estrone. The experiments were carried out at pH 6 and 7.2 exactly as previously described for the incubations of (**2a**) with placental aromatase from which estrone and several other metabolites including (**1a**) were isolated.[1] The 10β-hydroxy compound (**1b**) (1 mg) was incubated (pH 7.2) with placental microsomal aromatase, from the same batch employed above, in the usual manner and the products were recovered with chloroform.[1] The residue obtained was analysed by GC-MS of the derived MO-TMS. In addition to the starting material, (**1b**) MO–TMS, only one product (*ca.* 10%) which showed *m/z* 463 (M^+), 431, 416, 342, 252, *etc.* was detected. The metabolite was identified as 10β,17β-dihydroxyestr-4-en-3-one (**1d**).

The absence of detectable amounts of estrogenes indicates that (**1b**) is not an obligatory intermediate in the biosynthesis of estrogens.

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