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

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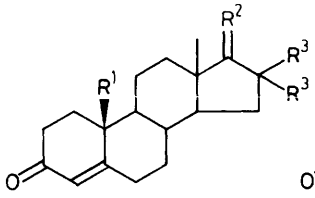
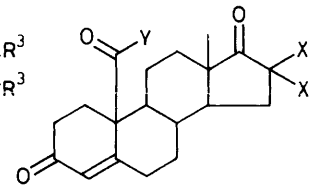
Incubation of [16,16,19-$^2H_3$][19-^{3}H]-19-oxoandrost-4-ene-3,17-dione (**2a**) with microsomal aromatase gave *inter alia* [16,16-$^2H_2$]-10β-hydroxyestr-4-ene-3,17-dione (**1**), whose analogue (**1b**) on incubation with the aromatase gave 10β,17β-dihydroxyestr-4-en-3-one (**1d**) (*ca.* 10%) 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.

The work at the Worcester Foundation is supported by NIH Grant DK 39300. The work at the Children's Hospital is supported by Grant DK 34400.

Received, 11th June 1989; Com. 9/02467K

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