

Synthesis of 6-Hydroximino-3-oxo Steroids, a New Class of Aromatase Inhibitor

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(*E*)-3 β ,17 β -Dihydroxyandrost-4-en-6-one oxime has been converted into (*E*)-6-hydroximinotestosterone and (*E*)-6-hydroximinoandrost-4-ene-3,17-dione by chemical methods, and into (*E*)-6-hydroximinoandrosta-1,4-diene-3,17-dione by biotransformation using *Arthrobacter simplex* ATCC 6946.

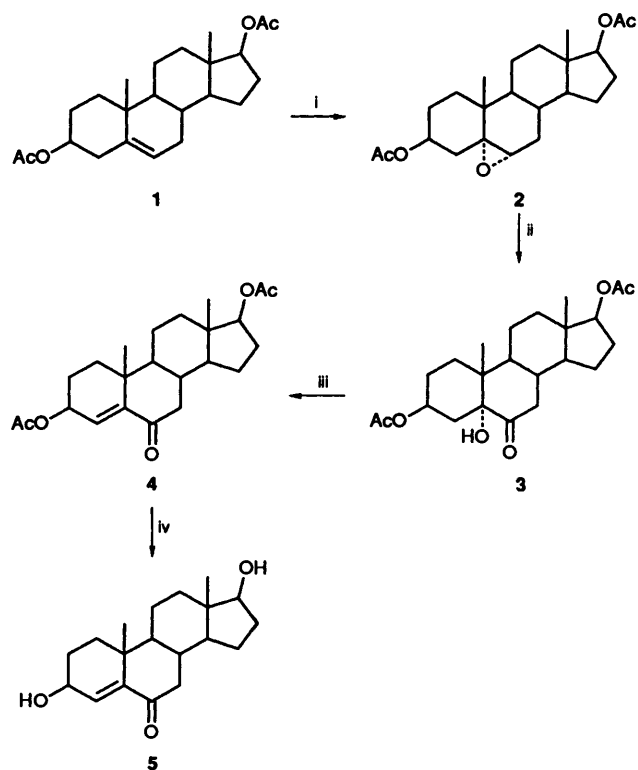
The enzyme aromatase (estrogen synthetase), responsible for the production of the estrogen hormones in human females, is currently a priority target for the development of active-site directed inhibitors which may have potential for the control of breast cancer. Among the steroids which have been studied in this context are 'suicide substrates' whose effect is dependent upon activation by the enzyme, such as 10 β -prop-2-ynyl,¹ 10 β -mercapto,² 19-oxirane³ and 19-thiirane³ steroids, and steroids substituted at C-4,⁴ -6,⁵ -7,⁶ -14⁷ and -19.⁸

We report here the synthesis of a group of steroids, the 6-hydroximinoandrost-4-en-3-ones, which show a high affinity for human placental aromatase, and function as competitive inhibitors of this enzyme.⁹ Although 6-hydroximino steroids are known,¹⁰ and 3-hydroximino-6-oxo-4-enes functionally isomeric with the compounds described herein have been prepared,¹¹⁻¹³ the 6-hydroximino-3-oxo-4-enes appear to constitute a class of steroid not hitherto reported.

The key intermediate in our synthesis, 3 β ,17 β -dihydroxyandrost-4-en-6-one **5**, was prepared by routine procedures as outlined in Scheme 1. Hitherto unreported spectral data are presented in the Experimental section: ¹³C NMR chemical shifts were assigned by comparison with reported values for closely related compounds.¹⁴⁻¹⁶ Treatment of **5** with hydroxylamine hydrochloride gave the (*E*)-oxime **6** in good yield, together with traces of an isomeric compound tentatively proposed to be the *Z* oxime, but not obtained in sufficient quantity for satisfactory characterization.

The stereochemistry of oxime **6** about the C=N bond was determined by selective ¹H/¹³C decoupling experiments in which irradiation of the ¹H signal at δ 3.32 caused, in the ¹³C spectrum, collapse of one of the triplet signals at δ 30.5 to a doublet. Since the latter signal is attributable to C-7, this experiment confirms that the signal shifted downfield to δ 3.32 in the ¹H spectrum is one of the C-7 hydrogens, assigned to C-7 β , and thus demonstrates that the oxime possesses the *E* geometry in which the C-7 β hydrogen is proximate to the oxime OH. That the downfield shift of this hydrogen signal is not simply attributable to the anisotropy of the exocyclic π system at C-6 is apparent from analysis of the ¹H NMR spectrum of the 6-oxo derivative **5**, in which such a downfield resonance is absent.

Oxidation of the oxime **6** to the testosterone derivative **7** (Scheme 2) was achieved in moderate yield by the use of activated manganese dioxide, which also gave as a minor product the enamide **10**, presumably formed as a result of a Beckmann rearrangement of **6** or **7**. The structure of **10** follows from ¹H NMR spectral analysis, in particular the absence of olefinic proton absorptions and the presence of four downfield



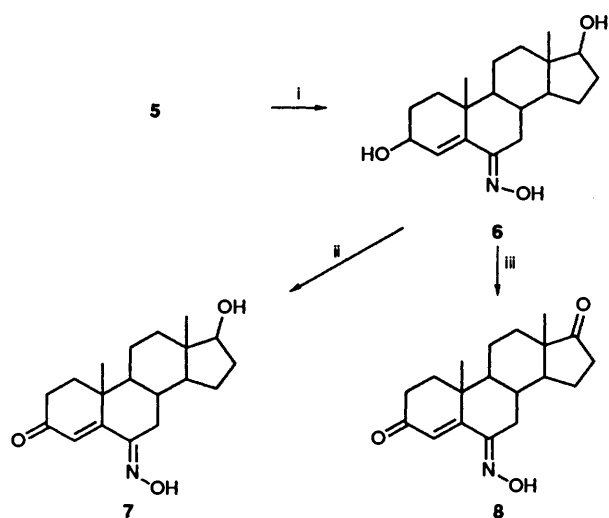
Scheme 1 i, MCPBA/CH₂Cl₂; ii, CrO₃/H₂O; iii, SOCl₂/pyridine; iv, KOH/MeOH

hydrogens at δ 2.6–3.1 ppm, assignable to the C-4 and C-7 hydrogens; it also provides further evidence for the geometry of the oxime group in **6** and **7**. Oxidation of **6** to the 4-ene-3,17-dione **8** was carried out using chromium trioxide in pyridine, other oxidizing agents (pyridinium chlorochromate, Jones' reagent, and tetrapropylammonium perruthenate/*N*-methylmorpholine *N*-oxide) giving either none or very little of the desired product.

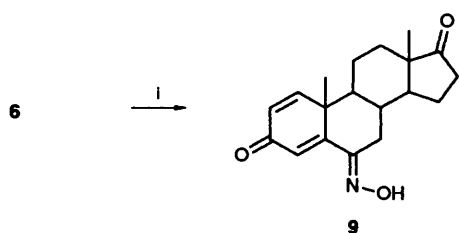
The 1,4-diene-3,17-dione **9** (Scheme 3) was obtained as the sole product of biotransformation of the oxime diol **6** by *Arthrobacter simplex* ATCC 6946, an organism known to be capable of 1(2) desaturation of a range of 3-oxygenated steroids.¹⁷ None of the standard methods of desaturation of compounds **7** or **8** using quinone reagents¹⁸ gave any detectable amount of 1,4-diene product.

Both compounds **7** and **8** have been found to bind to human placental aromatase with high efficiency, inducing Type-1 difference spectra: with for **7** $K_i = 4.71 \mu\text{mol dm}^{-3}$ and for **8** $K_i = 0.08 \mu\text{mol dm}^{-3}$.⁹ The 1,4-diene **9** remains to be examined for enzyme activity.

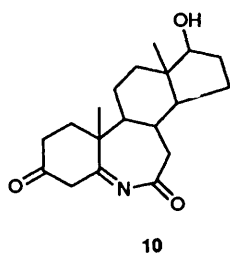
† Medical Research Council of Canada Invited Scientist, 1990.



Scheme 2 i, $\text{NH}_2\text{OH}\cdot\text{HCl}$; ii, $\text{MnO}_2/\text{CHCl}_3$; iii, $\text{CrO}_3/\text{pyridine}$



Scheme 3 (i) *Arthrobacter simplex* ATCC 6946



Experimental

General procedures and techniques were identical with those described.¹⁹ *Arthrobacter simplex* ATCC 6946 was maintained at 26 °C on slopes of nutrient agar.

5,6 α -Epoxy-5 α -androstane-3 β ,17 β -diyl Diacetate 2.—This compound, prepared by a literature method,²⁰ provided spectral and analytical data identical with those reported.^{15,20}

5-Hydroxy-6-oxo-5 α -androstane-3 β ,17 β -diyl Diacetate 3.—This compound, prepared as reported,²¹ provided spectral and analytical data as described.^{16,21}

6-Oxoandrost-4-ene-3 β ,17 β -diyl 3,17-Diacetate 4.—Thionyl chloride (1.5 cm³) was added at 0 °C to a solution of compound 3 (2.5 g) in dry pyridine (30 cm³) and the mixture was stirred for 20 min at 0 °C. It was then poured onto water (200 cm³) and the resulting mixture extracted with ethyl acetate (2 \times 75 cm³). The combined extracts were washed (10% HCl followed by aqueous NaCl), dried and evaporated, and the residue was crystallized from methanol to give the title compound 4 (2.32 g, 90%), m.p. 169–172 °C (lit.,²² 172–174 °C from MeOH); δ_{H} 0.83 (3 H, s, 18-H₃), 1.03 (3 H, s, 19-H₃), 2.03, 2.04 (each 3 H, s, OAc), 4.63 (1 H, t, 17 α -H), 5.32 (1 H, m, 3 α -H), 6.08 (1 H, br s, 4-H); δ_{C} 12.0 (C-18), 19.6 (C-19), 20.3 (C-11), 21.1 (2C, acetyl CH₃), 23.2, 24.1 (C-2, -15), 27.4 (C-16), 34.0 (C-8), 34.8 (C-1), 36.4 (C-12), 38.3

(C-10), 42.7 (C-13), 45.7 (C-7), 51.1 (2C, C-9, -14), 69.2 (C-3), 82.2 (C-17), 129.0 (C-4), 147.8 (C-5), 171.0, 171.6 (acetyl C=O) and 201.7 (C-6).

3 β ,17 β -Dihydroxyandrost-4-ene-6-one 5.—The diacetate 4 (2.5 g) was dissolved in methanol (25 cm³) under an inert atmosphere, and the resulting solution treated with 10% methanolic potassium hydroxide (85 cm³). The mixture was stirred at room temperature for 45 min, and then concentrated under reduced pressure without heating to a volume of 15 cm³. This solution was then poured into ice-water (200 cm³), and the product extracted with ethyl acetate (3 \times 75 cm³). The combined extracts were washed with saturated brine, dried and evaporated. The residue was crystallized from aqueous ethanol to give 5 (1.6 g, 82%), m.p. 114–117 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1679; $\delta_{\text{H}}[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$ 0.72 (3 H, s, 18-H₃), 0.96 (3 H, s, 19-H₃), 3.30 (1 H, t, 17 α -H), 4.06 (1 H, m, 3 α -H) and 6.13 (1 H, br s, 4-H); $\delta_{\text{C}}[\text{CDCl}_3 + (\text{CD}_3)_2\text{SO}]$ 10.7 (C-18), 19.4 (C-19), 20.0 (C-11), 22.6 (C-15), 27.6 (C-2), 29.6 (C-16), 33.7 (C-8), 34.4 (C-1), 35.8 (C-12), 37.7 (C-10), 42.5 (C-13), 45.4 (C-7), 50.9 (2C, C-9,14), 66.0 (C-3), 80.4 (C-17), 133.8 (C-4), 145.1 (C-5) and 202.0 (C-6); m/z (%) 304 (M^+ , 100) (Found: C, 73.7; H, 9.45. $\text{C}_{19}\text{H}_{28}\text{O}_3 \cdot 0.5\text{C}_2\text{H}_5\text{OH}$ requires C, 73.36; H, 9.54%).

(E)-6-Hydroxyiminoandrost-4-ene-3 β ,17 β -diol 6.—A solution of the ketone 5 (1.4 g) in ethanol (30 cm³) was treated with a solution of hydroxylamine hydrochloride (1.1 g) and anhydrous sodium acetate (1.1 g) in 50% aqueous ethanol (50 cm³), and the resulting mixture was stirred at room temperature for 24 h. The solvent was removed under reduced pressure at room temperature, and the residue diluted with water (50 cm³). The solution was extracted with ethyl acetate, and the extract dried and evaporated to give a white solid which, following crystallization from aqueous ethanol, afforded the oxime 6 (1.1 g, 75%), m.p. 227–229 °C; $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1560, 1660 and 3000–3600; $\delta_{\text{H}}(\text{CD}_3\text{OD})$ 0.76 (3 H, s, 18-H₃), 0.99 (3 H, s, 19-H₃), 3.32 (1 H, dd, 7 β -H), 3.60 (1 H, t, 17 α -H), 4.1 (1 H, t, 3 α -H) and 5.7 (1 H, br s, 4-H); $\delta_{\text{C}}(\text{CD}_3\text{OD})$ 11.6 (C-18), 19.2 (C-19), 21.6 (C-11), 24.0 (C-15), 29.0 (C-2), 30.5 (2C, C-7, -16), 35.6 (C-8), 35.7 (C-1), 37.4 (C-12), 39.0 (C-10), 44.0 (C-13), 52.2 (C-14), 54.2 (C-9), 67.8 (C-3), 82.0 (C-17), 129.1 (C-4), 143.1 (C-5) and 159.2 (C-6); m/z (%) 319 (M^+ , 6.6) relative to 302 (100) (Found: C, 71.25; H, 9.1; N, 4.12. $\text{C}_{19}\text{H}_{29}\text{NO}_3$ requires C, 71.44; H, 9.15; N, 4.38%). The mother liquors from the crystallization of compound 6 were concentrated and subjected to chromatography. Elution with chloroform–ethyl acetate (2:3) gave further 6, followed by a crude sample (9 mg) of a different material with [m/z 319 (M^+)] tentatively proposed to be (Z)-6-hydroxyiminoandrost-4-ene-3 β ,17 β -diol.

(E)-17 β -Hydroxy-6-hydroxyiminoandrost-4-en-3-one [(E)-6-Hydroxyiminotestosterone] 7.—A mixture of the oxime diol 6 (0.3 g) and activated MnO_2 (1.62 g) in dry chloroform (15 cm³) was shaken vigorously at room temperature for 17 h. The mixture was then filtered and the filtrate concentrated to give a pale yellow solid (0.26 g) which was subjected to chromatography over silica gel. Elution with chloroform–ethyl acetate (85:15) gave the enamide 10 (0.028 g, 9.5%) as an oil which resisted crystallization; $\nu_{\text{max}}(\text{film})/\text{cm}^{-1}$ 1625, 1702; δ_{H} 0.84 (3 H, s, 18-H₃), 1.31 (3 H, s, 19-H₃), 2.64 (1 H, dd), 2.88 (2 H, m), 3.07 (1 H, dd), (4- and 7-H's) and 3.67 (1 H, t, 17 α -H); m/z (%) 317 (M^+ , 9), 315 (21), 302 (54), relative to 260 (100). Further elution with chloroform–ethyl acetate (80:20) afforded the title compound 7 (0.133 g, 45%), m.p. 138–140 °C (from aqueous methanol); $\nu_{\text{max}}(\text{KBr})/\text{cm}^{-1}$ 1560, 1654 and 1682; δ_{H} 0.79 (3 H, s, 18-H₃), 1.15 (3 H, s, 19-H₃), 3.43 (1 H, dd, 7 β -H), 3.68 (1 H, t, 17 α -H), 6.42 (1 H, s, 4-H) and 9.9 (1 H, br s, OH); δ_{C} 11.0 (C-18), 16.6 (C-19), 20.5 (C-11), 23.2 (C-15), 29.1 (C-7), 30.5 (C-16), 32.9

(C-8), 33.6 (C-2), 34.9 (C-1), 36.2 (C-12), 38.8 (C-10), 43.0 (C-13), 51.3, 51.4 (C-9, -14), 81.5 (C-17), 122.9 (C-4), 155.7 (C-6), 161.8 (C-5) and 200.7 (C-3); m/z (%) 317 (21), relative to 301 (100) (Found: C, 68.25; H, 9.35; N, 4.0. $C_{19}H_{27}NO_3 \cdot CH_3OH$ requires C, 68.34; H, 9.46; N, 3.98%).

(E)-6-Hydroxyiminoandrosta-4-ene-3,17-dione **8**.—A solution of the oxime diol **6** (0.12 g) in pyridine (2 cm³) was added to the chromium trioxide–pyridine complex prepared by the addition of CrO₃ (0.33 g) to pyridine (3.5 cm³). The reaction mixture was stirred at room temperature for 1 h, and then diluted with ethyl acetate (30 cm³). The resulting precipitate was filtered off, and the filtrate was washed (10% HCl, 10% aq. NaHCO₃, saturated brine), dried and evaporated. The resulting solid was purified by preparative TLC (ethyl acetate–hexane, 1:1) to give the title compound (0.084 g, 70%), m.p. 260–263 °C; $\nu_{max}(KBr)/cm^{-1}$ 1669 and 1723; δ_H 0.92 (3 H, s, 18-H₃), 1.17 (3 H, s, 19-H₃), 3.58 (1 H, dd, 7 β -H), 6.53 (1 H, s, 4-H) and 10.9 (1 H, br s, OH); δ_C 13.7 (C-18), 16.6 (C-19), 20.1 (C-11), 21.7 (C-15), 28.4 (C-7), 31.0 (C-12), 32.2 (C-8), 33.5 (C-2), 34.8 (C-1), 35.7 (C-16), 38.7 (C-10), 47.6 (C-13), 51.1, 51.6 (C-9, -14), 122.8 (C-4), 154.6 (C-6), 161.8 (C-5), 201.2 (C-3) and 219.8 (C-17); m/z (%) 315 (15), relative to 272 (100) (Found: C, 72.5; H, 8.0; N, 4.84. $C_{19}H_{25}NO_3$ requires C, 72.35; H, 7.99; N, 4.44%).

(E)-6-Hydroxyiminoandrosta-1,4-diene-3,17-dione **9**.—*Arthrobacter simplex* ATCC 6946 was grown as a submerged culture on a rotary shaker at 26 °C in six 1000-cm³ Erlenmeyer flasks, each containing 250 cm³ of Difco nutrient broth. Following an initial growth period of 48 h, a solution of the oxime diol **6** (0.3 g) in 95% ethanol (12 cm³) was distributed equally among the flasks, and growth allowed to continue for a further 48 h. The culture was then extracted with dichloromethane (2 × 200 cm³), and the extract washed with water, dried and evaporated. The residue was subjected to chromatography over silica gel: elution with chloroform–ethyl acetate (4:1) gave **9** (0.14 g, 48%), m.p. 266 °C from chloroform–ethyl acetate; $\nu_{max}(KBr)/cm^{-1}$ 1603, 1624 and 1657; δ_H 0.95 (3 H, s, 18-H₃), 1.24 (3 H, s, 19-H₃), 3.62 (1 H, dd, 7 β -H), 6.38 (1 H, dd, 2-H), 6.78 (1 H, d, 4-H) and 7.17 (1 H, d, 1-H); δ_C 13.8 (C-18), 19.4 (C-19), 21.8 (C-15), 22.1 (C-11), 28.8 (C-7), 31.0 (C-12), 32.6 (C-8), 35.6 (C-16), 43.6 (C-10), 47.7 (C-13), 48.7 (C-14), 51.1 (C-9), 124.3 (C-4), 127.7 (C-2), 154.6 (C-6), 154.8 (C-1), 160.4 (C-5), 187.0 (C-3) and 219.3 (C-17); m/z (%) 313 (M⁺, 100) and 297 (53) (Found: C, 72.25; H, 7.2; N, 4.25. $C_{19}H_{23}NO_3$ requires C, 72.81; H, 7.40; N, 4.47%).

Acknowledgements

This work was supported by the Medical Research Council of Canada. We are grateful to Mr. T. Jones and Mrs. D. Vukmanic for their assistance in obtaining spectral data.

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Paper 1/05480E

Received 28th October 1991

Accepted 3rd December 1991