Stereoselective Inhibition of Aromatase by Novel Epoxysteroids

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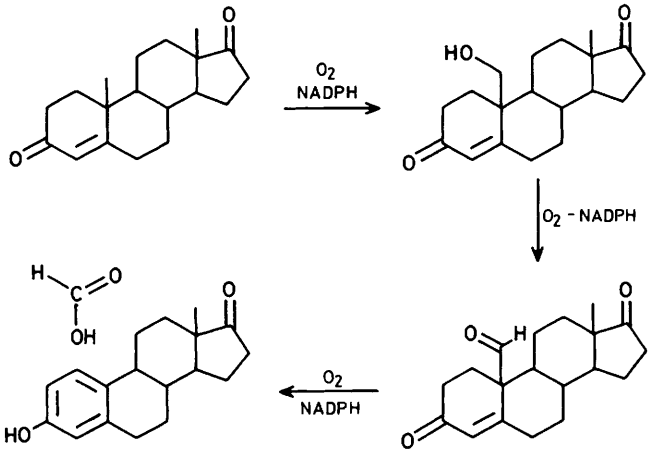
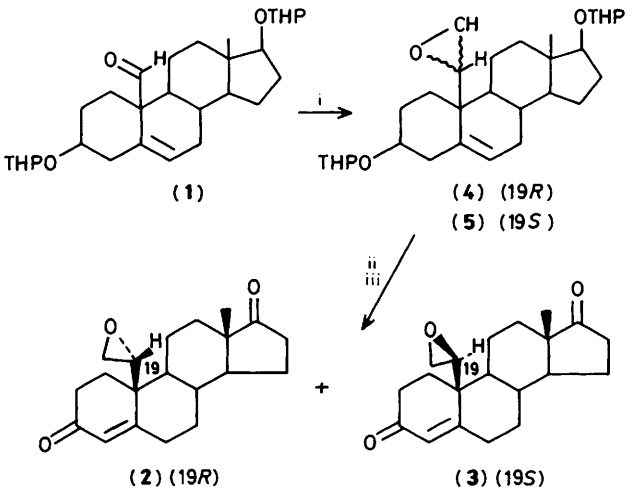
The diastereoisomeric 10-(epoxyethyl)estr-4-ene-3,17-diones (**2**) and (**3**) have been synthesized, their conformations and configurations have been established by *X*-ray crystallographic analysis, and they have been shown to be powerful inhibitors of human placental aromatase.

Human placental aromatase is a cytochrome P-450 enzyme complex which catalyses the important biosynthetic transformation of androgens to estrogens. The process is exemplified by the conversion of androst-4-ene-3,17-dione to estrone *via* three steps, each of which requires 1 mol of O_2 and 1 mol of NADPH (Scheme 1).[1] The possible therapeutic usefulness of selective and powerful aromatase inhibitors in processes such as estrogen-dependent breast cancer has led to much recent work in this area.[2] We report here the synthesis of the diastereoisomeric 10-(epoxyethyl)estr-4-ene-3,17-diones (**2**) and (**3**), the establishment of their conformations and configurations by *X*-ray crystallographic analysis, and the demonstration that they are powerful inhibitors of aromatase. The synthesis of (**2**) and (**3**) (Scheme 2) involved reaction of 19-oxo-androst-5-ene-3β,17β-diol 3,17-bistetrahydropyranyl (THP) ether (**1**) with either dimethylsulphonium methylide or dimethyloxosulphonium methylide.[3] Thus a solution of (**1**) (5 mmol) in dry tetrahydrofuran (THF) was added to dimethyloxosulphonium methylide (50 mmol) in THF under nitrogen at 60 °C and the mixture kept for 24 h at 60 °C, followed by 48 h at 25 °C. The THP ether groups in the crude product were cleaved (pyridinium toluene-*p*-sulphonate–methanol, 25 °C, 4 h) to give the (19*R*)- and (19*S*)-isomers, (**4**) and (**5**) respectively, of 10-(epoxyethyl)estr-5-ene-3β,17β-diol in the ratio 26:74 (determined by h.p.l.c.). Alternatively, reaction of (**1**) (4.47 mmol) with dimethylsulphonium methylide (20 mmol) in dimethyl sulphoxide (DMSO)–THF under N_2 at ice-bath temperature for 1 h, followed by 19 h at 25 °C, gave the isomers (**4**) and (**5**) in the ratio 89:11 respectively after cleavage of the THP groupings. The epoxides (**4**) and (**5**) were separated (silica gel, h.p.l.c.) and were then each oxidized (Oppenauer procedure) to give the desired 10-(epoxyethyl)-estr-4-ene-3,17-diones (**2**) and (**3**).†



Scheme 1

† New compounds gave satisfactory analytical and spectroscopic data. (**2**): m.p. 211—214 °C; i.r. ($CHCl_3$) 1735, 1665, and 1620 cm^{-1}; n.m.r. (400 MHz, $CDCl_3$) δ 0.93 (s, 3H, 18-Me), 2.57 (dd, 1H, *J* 3.1, 4.6 Hz, epoxide CH), 2.74 (dd, 1H, *J* 4.6, 4.1 Hz, epoxide CH), 3.28 (dd, 1H, *J* 3.1, 4.1 Hz, epoxide CH), 5.85 (s, 1H, 4-H); $\lambda_{max.}$ (MeOH) 239 nm (ε 12 400); *m/z* 314 (M^+). (**3**): m.p. 202—203 °C; i.r. ($CHCl_3$) 1732, 1665, and 1620 cm^{-1}; n.m.r. (400 MHz, $CDCl_3$) δ 0.96 (s, 3H, 18-Me), 2.53 (dd, 1H, *J* 2.8, 5 Hz, epoxide CH), 2.74 (dd, 1H, *J* 4, 5 Hz, epoxide CH), 3.26 (dd, 1H, *J* 2.8, 4 Hz, epoxide CH), 5.90 (s, 1H, 4-H); $\lambda_{max.}$ (MeOH) 242 nm (ε 12 600), *m/z* 314 (M^+).



Scheme 2. THP = tetrahydropyran-2-yl. *Reagents:* i, $Me_2S^+CH_2^-$ or $Me_2S^+OCH_2^-$; ii, $C_5H_6N^+p\text{-}MeC_6H_4SO_3^-$, MeOH; iii, Oppenauer oxidation.

X-Ray crystallographic analysis of (**2**) and (**3**) has established the configuration and conformation of the epoxide groupings.‡ The structures are shown in Figure 1, together with the known structure for the aromatase substrate and reaction intermediate 19-hydroxyandrost-4-ene-3,17-dione.[4] The oxirane system in the (19*R*)-isomer (**2**) is positioned over the steroid A-ring in a manner very similar to the arrangement of the 19-hydroxymethyl group in 19-hydroxyandrost-4-ene-3,17-dione,[4] the first intermediate in the aromatization sequence. In the (19*S*)-isomer (**3**) on the other hand, the oxygen of the oxirane group is positioned over the B-ring which is an arrangement unlike that of 19-hydroxyandrost-4-ene-3,17-dione. Consequently, if these solid state conformations can be extrapolated to the arrangements at the enzyme active site, the (19*R*)-epoxide (**2**) might be expected to bind more effectively than the (19*S*)-isomer (**3**). The (19*R*)-compound (**2**) proved to be a very powerful competitive inhibitor at 37 °C of human placental microsomal aromatase (K_i = 7 nM) whereas the (19*S*)-isomer (**3**) was less effective (K_i = 75 nM). Time-dependent assays at 37 and 25 °C revealed a 25 °C time-dependent loss of enzyme activity in the absence of NADPH during the initial 8 min. The limiting $t_{1/2}$ values for the (*R*)- and (*S*)-isomers (**2**) and (**3**) were 1.6 and 20 min, respectively. The exact nature of these brief temperature-dependent processes is not known but they may represent conformation changes and new interactions at the active site.

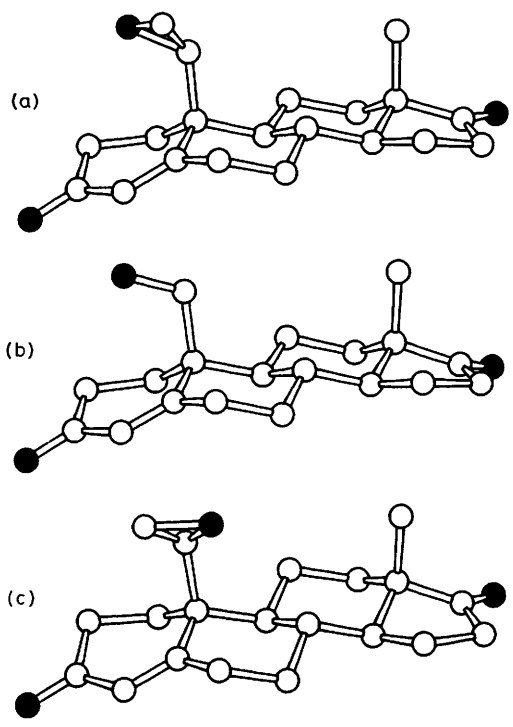


Figure 1. Molecular structures of (a) (19*R*)-oxirane (**2**); (b) 19-hydroxyandrost-4-ene-3,17-dione,[4] and (c) (19*S*)-oxirane (**3**). Diagrams produced using VIEW (H. L. Carrell, Computer Program from producing Molecular Diagrams, Philadelphia, Institute for Cancer Research, The Fox Chase Cancer Center, 1976).

The epoxide compounds reported above are of considerable interest both as active site probes of aromatase, and as inhibitors of estrogen synthesis. They are also very unusual in that they inhibit a cytochrome P-450 system. Epoxides are often biosynthesized by such systems, but are not commonly viewed as potential cytochrome P-450 enzyme inhibitors.

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‡ *Crystal data.* (**2**): $C_{20}H_{26}O_3$, M = 314.4, space group $P2_1$, a = 11.413(3), b = 10.487(3), c = 7.246(2) Å, β = 103.03(2)°, Z = 2, D_c = 1.234 g cm^{-3}, μ(Mo-K_α) = 0.453 cm^{-1}, λ = 0.7107 Å, graphite monochromated, $2\theta_{max.}$ = 60°, 2601 unique reflections, $R(R_w)$ = 0.047 (0.051) for 2230 reflections with $I > 2\sigma(I)$.

(**3**): $C_{20}H_{26}O_3$, M = 314.4, space group $P2_12_12_1$, a = 11.148(4), b = 16.345(5), c = 9.284(4) Å, Z = 4, D_m = 1.236 g cm^{-3}, μ(Cu-K_α) = 5.657 cm^{-1}, λ = 1.5418 Å, graphite monochromated, $2\theta_{max.}$ = 139°, 1833 unique reflections, $R(R_w)$ = 0.041 (0.044) for 1581 reflections with $I > 2\sigma(I)$.

Crystal structures for both (**2**) and (**3**) were solved using MULTAN80 (P. Main, S. J. Fiske, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. N. Woolfson, 1980, MULTAN80. A System of Computer Programs for the Automatic Solution of Crystal Structures from *X*-Ray Diffraction Data, Univs. of York, England and Louvain, Belgium). In both structures, full-matrix least squares refinement was carried out first with isotropic thermal parameters factors then anisotropic thermal parameters for the non-hydrogen atoms. The hydrogen atoms were located in the difference electron density maps and further full-matrix least-squares refinement carried out with isotropic thermal parameters for the hydrogen atoms. Refinement was carried out using the program ICRFMLS (H. L. Carrell, Computer Program ICRFMLS, Modification of UCLALS4, Program from The Institute for Cancer Research, The Fox Chase Cancer Center, Philadelphia, PA 19111; P. K. Gantzel, R. A. Sparks, R. E. Long, and K. N. Trueblood, 1969, UCLALS4 program in FORTRAN IV). Atomic scattering factors used are those in 'International Tables for *X*-Ray Crystallography,' Vol. 4, 1974, Kynoch Press, Birmingham, pp. 72—102. Atomic co-ordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre. See Notice to Authors, Issue No. 1.

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