Novel 10β-Thiiranyl Steroids as Aromatase Inhibitors

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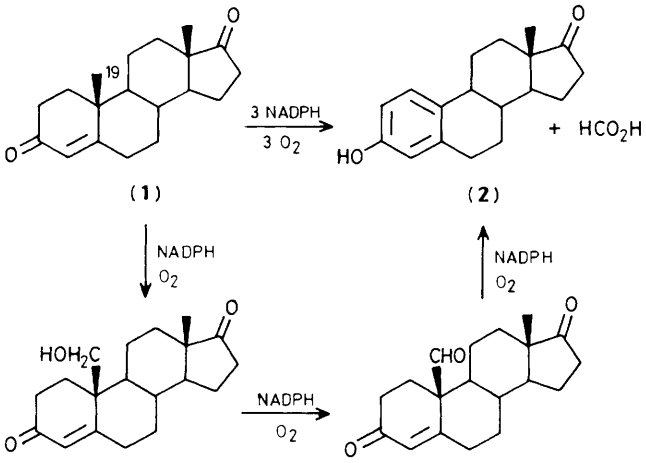
The novel 10β-thiiranyl-4-estrene-3,17-diones (**5**) and (**6**) have been synthesized by the action of triphenylphosphine sulphide–picric acid on oxirane precursors and show potent inhibition of placental aromatase; the (19*R*)-isomer (**5**) is 75 times more effective than the (19*S*)-isomer (**6**).

The conversion of androgens (**1**) into estrogens (**2**) is catalysed by aromatase, a cytochrome P-450 enzyme system. The transformation (Scheme 1) involves three successive oxidations, each requiring 1 mol of NADPH and 1 mol of O_2 with the sequential formation of 19-hydroxy and 19-oxo intermediates and the eventual loss of C-19 as formic acid.[1] There has been considerable recent interest in the development of inhibitors of aromatase, in part because of their potential value in treatment of estrogen-sensitive breast tumours.[2]

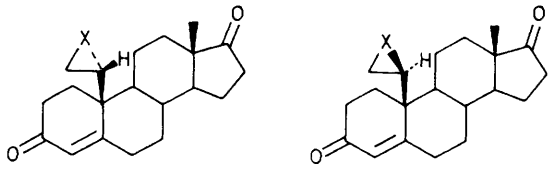
This laboratory recently described[3] the synthesis of the C-19 diastereoisomers of 10β-oxiranyl-4-estrene-3,17-dione (**3**, **4**). Each of these compounds is a powerful inhibitor of human placental aromatase, and the stereochemistry at C-19 plays an important role, as the (19*R*)- and (19*S*)-isomers (**3**) and (**4**) showed K_i values of 7 and 75 nM, respectively. Consequently, we embarked on the synthesis of the novel 10β-thiiranyl steroids (**5**) and (**6**). It was thought that the thiirane grouping would be susceptible to nucleophilic attack or that, alternatively, it might be oxidized by the enzyme to give reactive species.[4] In either event, covalent attachment to the enzyme might occur.[5] Furthermore, the different bond lengths and bond angles in thiiranes *vs.* oxiranes could result in differences in binding at the enzyme active site.

We synthesized the desired thiiranes by a modification of the triphenylphosphine sulphide–trifluoroacetic acid procedure which has been reported to generate thiiranes from oxiranes.[6] Surprisingly, treatment of the 10β-oxiranyldione[3] (**3**) with these reagents failed to give thiirane, but gave instead a new non-u.v. absorbing product tentatively identified as compound (**7**).† The sulphur heterocycle (**7**) presumably arises *via* intramolecular Michael addition of the expected[6] intermediate thiol to the 4-ene-3-one system, as an alternative path to the normal closure to a thiirane. Analogy for the formation of (**7**) is found in the intramolecular Michael addition of a 19-hydroperoxy group[7] or a 10β-(2-hydroxyethyl)[8,9] group to the steroidal 4-ene-3-one system. We were finally able to generate the desired 10β-thiiranyl steroid (**6**)‡ stereospecifically in good yield (75%) by treatment of (**3**) with triphenylphosphine sulphide (10 equiv.) and picric acid (10 equiv.) in benzene at 80 °C under nitrogen for 16 h. The stereochemical assignment at C-19 was made on the basis of the proposed[6] mechanism of the reaction which would require inversion at C-19, and by comparison with the spectroscopic data and chromatographic behaviour of (**3**) and (**4**), whose structures are securely established by *X*-ray crystal structure determinations.[3]

Likewise, exposure of (*S*)-epoxydione (**4**) to picric acid–triphenylphosphine sulphide under the conditions described above gave (19*R*)-10β-thiiranyl-4-estrene-3,17-dione (**5**)‡ in 40% yield. Approximately 30% starting material was recovered in this case, and these results suggest greater chemical reactivity for the (*R*)-epoxydione (**3**) *vs.* the (*S*)-epoxydione (**4**). Treatment of (*S*)-epoxydione (**4**) with trifluoroacetic acid–triphenylphosphine sulphide, on the other hand,

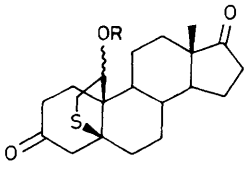


Scheme 1



(19*R*)-isomer (**3**); X = O (**5**); X = S

(19*S*)-isomer (**4**); X = O (**6**); X = S



(**7**); R = $COCF_3$

† Analytical and spectroscopic data for compound (**7**): m.p. 126—127 °C; $\nu_{max.}$ ($CHCl_3$) 1780, 1735, 1715, 1220 cm^{-1}; ^1H n.m.r. (400 MHz, $CDCl_3$) δ 0.86 (s, 3H, 18-Me), 2.25 (s, 2H), 2.79 (dd, 1H, *J* 12.4, 6.8 Hz), 2.90 (d, 1H, *J* 16 Hz), 3.62 (dd, 1H, *J* 12.4, 9.2 Hz), 6.05 (dd, 1H, *J* 9.2, 6.8 Hz); ^{19}F n.m.r. (75.4 MHz, $CDCl_3$, Freon 11 standard) −75.8 p.p.m. (s, CF_3CO_2); M^+ *m/z* 444.1614 (calc. 444.1582).

‡ Analytical and spectroscopic data for compounds (**5**) and (**6**): compound (**5**), m.p. 162—163 °C; $\nu_{max.}$ (KBr) 1740, 1670, 1620 cm^{-1}; ^1H n.m.r. (400 MHz, $CDCl_3$) δ 0.96 (s, 3H, 18-Me), 2.44 (dd, 1H, *J* 6.4, 2 Hz), 2.52 (m, 1H), 3.27 (t, 1H, *J* 6.4 Hz), 5.81 (s, 1H, 4-CH vinylic), $\lambda_{max.}$ (MeOH) 234 nm (11 500); M^+ *m/z* 330. Compound (**6**), m.p. 155—156 °C; $\nu_{max.}$ (KBr) 1740, 1665, 1615 cm^{-1}; ^1H n.m.r. (400 MHz, $CDCl_3$) δ 0.98 (s, 3H, 18-Me), 2.56 (dd, 1H, *J* 6.8, 2 Hz), 2.77 (dt, 1H, *J* 14, 2 Hz), 3.22 (t, 1H, *J* 6.8 Hz), 5.89 (s, 1H, 4-CH vinylic); $\lambda_{max.}$ (MeOH) 232 nm (12 500); M^+ *m/z* 330.

gave a non-u.v. absorbing adduct analogous to that obtained from (*R*)-epoxydione (**3**) under the same conditions. As a final proof of the structure, the thiiranes (**5**) and (**6**) were each smoothly desulphurized to the known[10] 10β-vinyl-4-estrene-3,17-dione by treatment with triphenylphosphine in refluxing toluene.

Both the (*R*)- and (*S*)-thiiranes (**5**) and (**6**) are excellent competitive inhibitors of placental microsomal aromatase, with K_i values of 1 and 75 nM, respectively (K_m for androstenedione = 39 nM). The difference in inhibitory potency due to C-19 stereochemistry which was seen for the epoxides is observed in the thiiranes to an even greater degree. Two recent reports describe effective inhibition of aromatase by 19-thiosteroids.[5,11] In one case,[11] spectroscopic studies implicated co-ordination of sulphur to heme iron. The exact nature of the inhibition by our thiiranes is presently under study.

Very few reports of thiiranes as enzyme inhibitors can be found in the literature, and those examples proved to be weak inhibitors of enzymes such as 2,3-oxidosqualene cyclase[12] and a mammalian 5-lipoxygenase.[13] We believe that compounds (**5**) and (**6**) represent the first examples of effective inhibition of a cytochrome P-450 system by thiiranes.

In conclusion, we have synthesized (19*R*)- and (19*S*)-10β-thiiranyl-4-estrene-3,17-diones and have shown them to be extremely powerful inhibitors of aromatase. As in the case of the analogous epoxides, these thiiranes show a significant difference in inhibitory potency due to the configuration at C-19.

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