6-Oxoestradiols from Estradiols: Exploiting Site Selective Metalation of Aralkyl Systems with Superbases

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Received 19 June 1995

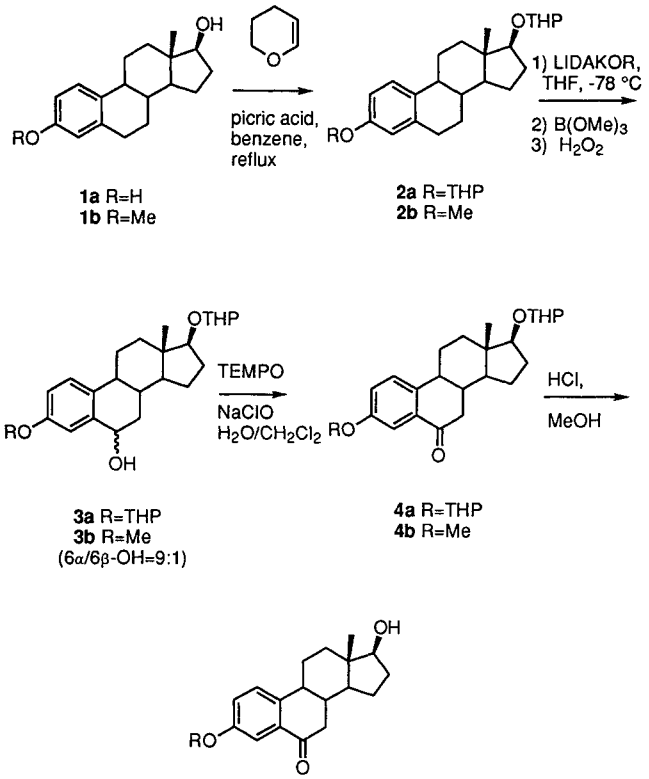
3-*O*-Protected estradiol derivatives undergo metalation at C-6 when exposed to a fourfold excess of the reagent consisting of an equimolar mixture of lithium diisopropylamide and potassium 1,1-dimethylpropoxide (3 h, THF, −78 °C). The metalated intermediates can be oxidized by quenching with trimethyl borate followed by treatment with hydrogen peroxide, thus allowing the introduction of a 6-hydroxy group into the estradiol framework. Further oxidation of the 6-hydroxy group gives *O*-protected 6-oxoestradiols.

6-Oxoestradiols **5** are key intermediates in the synthesis of a variety of valuable and interesting estradiol derivatives. They are precursors to estradiol 6-(*O*-carboxymethyl)oxime and other similar compounds employed as haptens to induce high specificity antibodies for radioimmunoassay of steroidal estrogens.[1,2] More importantly, 6-oxoestradiol **5b** is the starting material in a recent method for the stereospecific synthesis of 7α-alkylestradiols,[3] which are a class of compounds endowed with a variety of important properties: they encompass drugs with pure antiestrogen activity,[4] reagents for the derivatization of adsorbents used in affinity chromatography,[5] and ligands of the estrogen receptor whose affinity is higher or more selective than that of the corresponding 7-unsubstituted analogues.[6]

The most frequently employed approach to 6-oxoestradiols is oxidation of the 6-benzyl position of *O*-protected estradiol or estrone derivatives using chromium(VI) compounds.[1,7,8] Despite the many reagents and reaction conditions devised in the attempt to improve the process, yields are generally low, owing to the unwanted parallel oxidation of ring C.[8] Only by use of a large molar ratio of chromium reagents to estradiol diacetate has the introduction of a 6-oxo group been made efficient;[9] however, these conditions are impractical in large scale preparations, owing to the problem of disposal of chromium-containing wastes. Microsomal oxidation has been also adopted for the introduction of a hydroxy group in the 6-position,[10] but this method in not suitable for occasional laboratory syntheses. The oxidation of organometallic derivatives obtained by hydrogen–metal exchange is a general method for hydrocarbon functionalization, whose main limitation is the problem of achieving metalation at a specific position within a complex molecule. A variation of this approach was followed some time ago in a synthesis of the 6-oxoestradiols in which the C-6 was converted to an active methylene by temporary reduction of the aromatic ring A to a 4-en-3-one system; after deprotonation at C-6 and reaction with oxygen (resulting in the introduction of a carbonyl group) ring A was re-aromatized. The overall process, however, was not very efficient.[11] Activation of the C-6 of estradiols toward hydrogen–metal exchange has also been accomplished by complexation of the aromatic ring A with chromium tricarbonyl;[12] however, metalated chromium complexes of estradiols have not been exploited to oxidize the 6-position, although benzylchromium anions have been condensed with nitriles to convert a benzyl methylene into the corresponding oxime in fair yields.[13] We report herein that the position 6 of estradiol derivatives can be deprotonated and functionalized more conveniently and directly (without requiring any alteration of the aromatic ring A) by using the appropriate superbase.[14]

When exposed to fourfold excess of the LIDAKOR reagent prepared by mixing equimolar amounts of lithium diisopropylamide and potassium 1,1-dimethylpropoxide in a THF–cyclohexane mixture (−78 °C, 3 h), protected estradiols **2a, b** underwent selective deprotonation at C-6, as demonstrated by the isolation of the corresponding oxidation products **3a, b** in more than 85% yield after quenching the dark-brown solution from the metalation step with trimethyl borate, followed by reaction with excess hydrogen peroxide. Small amounts of ketones **4a, b**, evidently resulting from over-oxidation of the intermediate organometallics, were detected besides traces of unreacted starting materials in crude **3a, b**, which were generally of suitable purity to be processed in the subsequent oxidation to **4a, b**. The hydroxylation turned out to be remarkably stereoselective; in fact an approximate 90:10 ratio of 6α-OH to 6β-OH epimer was generally obtained. The two epimeric alcohols were not separated, but the composition of the mixture **3a** was established by comparison of its NMR spectrum with that of authentic samples of each isomer obtained as reported in the literature.[2] The identity of **3b** is based on the similarity of NMR spectra of **3a** and **3b**. The protection of 17-OH is not necessary to bring about the oxidation; in fact, estradiol 3-*O*-methyl ether **1b** was converted into the corresponding 6-hydroxy derivative by direct reaction with a 6-fold excess of the above LIDAKOR reagent, followed by the same treatment with trimethyl borate and hydrogen peroxide. The partially protected estriols **3a, b** were oxidized to the corresponding ketones **4a, b** by reaction with sodium hydrochlorite in the presence of tetramethylpyrrolidin-1-oxyl (TEMPO) as catalyst.[15] A cleaner conversion of **3a, b** into **4a, b** was obtained by pyridinium chlorochromate oxidation. Short exposure to methanolic HCl cleanly removed the tetrahydropyranyl group to convert **4a, b** into **5a, b**.

Estradiol derivatives can in principle undergo hydrogen–metal exchange with strong bases at the aromatic position 2 and at the benzyl position 6 (or at the sterically more hindered positions 4 and 9). Although benzyl protons are intrinsically more acidic than ring protons in benzene derivatives, aromatic rings containing an electronegative atom such as a methoxy group can seriously



1a R=H
1b R=Me

2a R=THP
2b R=Me

3a R=THP
3b R=Me
(6α/6β-OH=9:1)

4a R=THP
4b R=Me

5a R=H
5b R=Me

compete with the benzyl position for deprotonation. In fact, lithiation at C-2 is favored not only when protected **1** (R = methoxymethyl) is exposed to *sec*-butyllithium,[16] but also, as we observed, when estradiol 3-*O*-methyl ether was allowed to react either with a LICKOR reagent (prepared from potassium butoxide and butyllithium) or with trimethylsilylmethylpotassium.[17] The strong preference of LIDAKOR reagents for benzyl deprotonation has already been demonstrated by Schlosser in the metalation of some toluene derivatives bearing electronegative substituents on the ring, and an explanation for their selective behaviour has been given.[14] However, the reactions of **1b** and **2a, b** here reported constitute an extreme case of selectivity in benzyl versus ring deprotonation, since, in each case, position 6 is relatively hindered and far from heteroatoms, while the aromatic ring bears a substituent (particularly **2a**) which is generally a good promoter of *ortho*-lithiation.[18]

In conclusion, the superbase LIDAKOR proved to be effective in the selective deprotonation of position 6 of 3-*O*-protected estradiol derivatives. This reaction was exploited to prepare 6-hydroxy- and 6-oxoestradiols. The sequence, starting from commercially available materials, is short, efficient and avoids the use of environmentally unsafe reagents, thus providing a satisfactory solution to a relevant synthetic problem and opening an easier access to many interesting estradiol derivatives.

Mps were taken with a Kofler hot-plate apparatus and are uncorrected. For analytical TLC Merck silica gel F-254 on aluminum plates were used; for the visualization of the spots, the plates were soaked with an ethanol solution containing phosphomolybdic acid (5%) and sulfuric acid (5%), and heated with a heat gun. For column chromatography, the technique described by Still was adopted,[19] using mixtures of hexane and EtOAc as eluants. ^{1}H NMR (200 MHz) spectra were obtained using a Bruker AC200 for samples dissolved, unless otherwise indicated, in $CDCl_3$; chemical shifts (δ) are relative to tetramethylsilane as internal standard; the term "m" (multiplet) in the description of the spectra of some tetrahydropyranyl ethers refers sometimes to the complex signal arising from the presence of different epimers. Elemental analyses were performed by the microanalytical laboratory of the Faculty of Pharmacy of the University of Pisa. All the reactions involving organometallic reagents were performed under nitrogen in solvents distilled from sodium benzophenone ketyl. Unless otherwise stated, solutions were dried with $MgSO_4$ and evaporated in a rotary evaporator under diminished pressure. Starting materials **1a, b** were purchased from Sigma.

Compounds **2a, b**, **3a, b** and **4a, b** gave C, H analysis $\pm$ 0.21%.

The solution of potassium 1,1-dimethylpropoxide was prepared as follows: A mixture of potassium (10 g, 256 mmol), 1,1-dimethylpropan-1-ol (17.71 g, 22 mL, 201 mmol), cyclohexane (35 mL), and THF (35 mL) was heated to reflux under nitrogen for 8 h and left at r.t. for 18 h to allow any precipitate to settle. The solution was decanted and stored in a bottle under nitrogen; the alcoholate content was determined by hydrolysis of a portion and titration with standard 2 N aq H_2SO_4.

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5-triene (2a):
A mixture of benzene (20 mL), **1a** (1.2 g, 4.4 mmol), picric acid (20 mg), dihydropyran (3.68 g, 4 mL, 43.7 mmol; added last) was heated to reflux until **1a** was totally converted into a less polar compound (TLC; 3:1, hexane–EtOAc as eluant). Triethylamine (1 mL) was added and the mixture filtered through a short pad of silica; the silica was washed with Et_2O (50 mL) and the combined filtrates were evaporated to yield crude **2a** (98% yield) of suitable purity to be used in the next step. A sample of **2a** was purified by chromatography (4:1, hexane–EtOAc). Oil.

^{1}H NMR: δ = 0.79, 0.81 (2 s, 3 H), 2.72–2.92 (m, 2 H), 3.41–3.62 (m, 2 H), 3.71 (t, J = 7.5 Hz, 1 H), 3.81–4.01 (m, 2 H), 4.60–4.70 (m, 1 H), 5.38 (s, 1 H), 6.73–6.89 (m, 2 H), 7.10–7.24 (m, 1 H).

3-Methoxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5-triene (2b):
The compound was obtained in 100% yield from **1b** as detailed for the obtainment of **2a** from **1a**. The analytical sample was chromatographed (3:1, hexane–EtOAc). Oil which solidified on standing, mp 70–75 °C.

^{1}H NMR: δ = 0.80, 0.82 (2 s, 3 H), 2.78–2.92 (m, 2 H), 3.40–3.53 (m, 1 H), 3.72 (t, J = 7.5 Hz, 1 H), 3.77 (s, 3 H), 3.82–3.98 (m, 1 H), 4.61–4.70 (m, 1 H), 6.62 (d, J = 2.5 Hz, 1 H), 6.71 (dd, J = 2.5, 8.5 Hz, 1 H), 7.21, 7.20 (2 d, J = 8.5 Hz, 1 H).

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-ol (3a):
To a cooled (– 78 °C) solution of BuLi (2.72 mL, 2 M in cyclohexane) in THF (7 mL) were added potassium 1,1-dimethylpropoxide (2.72 mL, 2 M in cyclohexane/THF, see above), diisopropylamine (0.71 mL, 0.55 mg, 5.44 mmol), and, after 5 minutes, **2a** (600 mg, 1.36 mmol) dissolved in the minimum amount of hexane. A dark-red solution resulted, which was stirred for 3 h at – 78 °C; trimethyl borate (2 mL, 18 mmol) was then added and the dry-ice bath was replaced by an ice/water bath. The mixture was stirred for 1–2 h (during which time the dark-red solution turned milky) and then 30% aq H_2O_2 (5 mL) was added. After 1 h stirring at r.t., the reaction mixture was partitioned between EtOAc and 10% aq sodium thiosulfate. The organic phase was washed with water, dried (Na_2CO_3), and evaporated to afford a residue from which **3a** (0.55 g, 88% yield) was obtained by chromatography (3:2, hexane–EtOAc). Glass.

^{1}H NMR: δ = 0.81, 0.83 (2 s, 0.9 × 3 H, 18-H_3 of 6α-hydroxy epimer), 0.84, 0.86 (2 s, 0.1 × 3 H, 18-H_3 of 6β-hydroxy epimer), 3.40–3.78 (m, 3 H), 3.81–3.98 (m, 2 H), 4.60–4.70 (m, 1 H), 4.70–4.89 (m, 1 H), 5.37–5.45 (m, 1 H), 6.92 (dd, J = 2.5, 8.5 Hz, 0.9 × 1 H, 2-H of 6α-hydroxy epimer), 7.05 (d, J = 2.5 Hz, 0.1 × 1 H, 4-H of 6β-hydroxy epimer), 7.15–7.24 (m, 1 H), 7.24–7.29 (m, 1 H).

3-Methoxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-ol (3b):
The compound was obtained in 90% yield from **2b** as detailed for the conversion of **2a** into **3a**. Final chromatography (2:1, hexane–EtOAc). Oil which solidified on standing; mp 105–110°C.

^{1}H NMR: δ = 0.79, 0.81 (2 s, 0.9 × 3 H, 18-H_3 of 6α-hydroxy epimer), 0.82, 0.84 (2 s, 0.1 × 3 H, 18-H_3 of 6β-hydroxy epimer), 3.42–3.55 (m, 1 H), 3.64–3.76 (m, 1 H), 3.78 (s, 3 H), 3.83–3.97 (m, 1 H), 4.63–4.69 (m, 1 H), 4.70–4.87 (m, 1 H), 6.77 (dd, J = 2.5, 8.5 Hz, 0.9 × 1 H, 2-H of 6α-hydroxy epimer), 6.90 (d, J = 2.5 Hz, 0.1 × 1 H, 4-H of 6β-hydroxy epimer), 7.10–7.21 (m, 2 H).

3,17β-Bis(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-one (4a):
To a mixture of **3a** (1.10 g, 2.41 mmol), TEMPO (20 mg), aq KBr (30 mg in 1 mL of H_2O), and CH_2Cl_2 (10 mL), vigorously stirred at 0°C, 15% aq sodium hypochlorite (whose pH was adjusted to 8.5 by addition of $NaHCO_3$) was added in portions until **3a** was converted into a less polar compound (TLC hexane–EtOAc, 3:1). The organic phase was washed with 10% aq sodium thiosulfate and then with water, dried (Na_2CO_3), and evaporated to leave a residue from which **4a** (800 mg, 73% yield) was obtained by chromatography (3:1, hexane–EtOAc). Oil.

^{1}H NMR: δ = 0.80, 0.82 (m, 3 H), 2.73 (dd, J = 3.1, 16.6 Hz, 1 H), 3.42–3.77 (m, 3 H), 3.78–3.98 (m, 2 H), 4.61–4.79 (m, 1 H), 5.47 (m, 1 H), 7.22 (dd, J = 2.9, 8.5 Hz, 1 H), 7.30–7.39 (m, 1 H), 7.71 (m, 1 H).

3-Methoxy-17β-(2-tetrahydropyranyloxy)estra-1,3,5-trien-6-one (4b):
The compound was obtained with 76% yield from **3b** as detailed for the conversion of **3a** into **4a**. Final chromatography (3:1, hexane–EtOAc). Oil which solidified on standing, mp 87–92°C.

^{1}H NMR: δ = 0.81, 0.83 (2 s, 3 H), 2.74 (dd, J = 3.3, 16.7 Hz, 1 H), 3.43–3.55 (m, 1 H), 3.69–3.81 (m, 1 H), 3.84 (s, 3 H), 3.84–4.03 (m, 1 H), 4.62–4.72 (m, 1 H), 7.10 (dd, J = 2.9, 8.5 Hz, 1 H), 7.30–7.41 (m, 1 H), 7.55 (d, J = 2.9 Hz, 1 H).

6-Oxoestra-1,3,5-triene-3,17β-diol (5a):
A mixture of **4a** (110 mg, 0.24 mmol) and a solution obtained by adding acetyl chloride (0.3 mL) in methanol (10 mL) was gently heated until **4a** was converted into a more polar compound. Water (20 mL) was added and **5a** was collected by filtration (56 mg, 80% yield). Mp 277–280°C (Lit.[2] mp 282–283°C).

^{1}H NMR (DMSO-d_6): δ = 0.63 (s, 3 H), 3.50 (m, 1 H), 4.54 (d, J = 4.8 Hz, 1 H), 6.98 (dd, J = 2.9, 8.5 Hz, 1 H), 7.25 (d, J = 2.9 Hz, 1 H), 7.29 (d, J = 8.5 Hz, 1 H), 9.6 (s, 1 H).

3-Methoxy-6-oxoestra-1,3,5-trien-17β-ol (5b):
The compound was obtained in 85% yield from **4b** following the same procedure described to convert **4a** into **5a**. Mp 60–65°C (after trituration with Et_2O).

^{1}H NMR: δ = 0.79 (s, 3 H), 2.75 (dd, J = 3.3, 16.7 Hz, 1 H), 3.76 (t, J = 8 Hz, 1 H), 3.84 (s, 3 H), 7.11 (dd, J = 2.9, 8.5 Hz, 1 H), 7.35 (d, J = 8.5 Hz, 1 H), 7.56 (d, J = 2.9 Hz, 1 H).

Helpful discussion with Prof. J. A. Katzenellenbogen is gratefully acknowledged. The work was supported by the Ministero della Ricerca Scientifica e Tecnologica (MURST-Roma) and by the Consiglio Nazionale delle Ricerche (CNR-Roma).

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