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Practical one-pot conversion of 17β-estradiol to 10β-hydroxy- (*p*-quinol) and 10β-chloro-17β-hydroxyestra-1,4-dien-3-one

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Sodium chlorite

Abbreviations:
NMR, nuclear magnetic resonance; COSY, $^{1}H,^{1}H$ Correlation spectroscopy; DQF-COSY, double quantum filtered correlation spectroscopy; HMQC, $^{1}H,^{13}C$ heteronuclear multiple quantum coherence; HMBC, $^{1}H,^{13}C$ heteronuclear multiple bond correlation; ROESY, rotating Overhauser effect spectroscopy; ESIMS, electrospray ionization mass spectrometry; HRESI, high resolution electrospray ionization; TLC, thin layer chromatography; CD, circular dichroism

ABSTRACT

An efficient one-pot procedure for the preparation of 10β,17β-dihydroxyestra-1,4-dien-3-one (*p*-quinol, **1**, 75%) is reported, involving oxidation of 17β-estradiol with potassium permanganate. Similar treatment of 17β-estradiol with sodium chlorite led to 10β-chloro-17β-hydroxyestra-1,4-dien-3-one (**2**) in 44% yield along with smaller amounts 4-chloro-10β,17β-dihydroxyestra-1,4-dien-3-one (**3**), 2,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one (**4**), and 4,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one (**5**).

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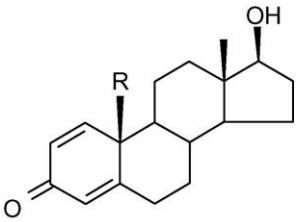
1. Introduction

10β-Substituted-17β-hydroxyestra-1,4-dien-3-ones are an interesting class of estradiol-related compounds that have found application in estrogen replacement therapy, prevention, and treatment of osteoporosis [1], in the detection and treatment of hormone dependent tumors [2], or for the prevention and therapy of ophthalmic diseases [3]. In addition, the identification of the 10-hydroxy derivative (*p*-quinol, **1**) in the redox cycling mechanisms underlying the putative antioxidant and cytoprotective properties of 17β-estradiol [4], suggested its employment as prodrug of antioxidants [5], while the halogenated derivatives have been shown to be valuable tools for probing interactions at estrogen receptors [6].

A number of synthetic approaches to 10β-substituted-17β-hydroxyestra-1,4-dien-3-ones have been reported [7–10], but they often require manipulation of estrone and estradiol derivatives, lengthy protection/deprotection steps or functional group modifications, resulting in complex mixtures of products. The best procedure for preparation of *p*-quinol derivatives involves oxidation of 17β-estradiol monoacetate with *m*-chloroperbenzoic acid and a catalytic amount of $(BzO)_2$ for 1.5 h under irradiation with a 60 W tungsten lamp leading to the product in 52% yield, along with an epoxy-derivative [11]. Other procedures are also available that afford the desired product, but in lower yields.

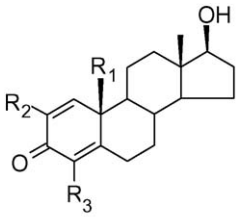
Access routes to 10β-halo derivatives of 17β-estradiol have also been reported [12–14], though expensive organic chlorinating agents are employed and complex mixtures of products are usually obtained requiring separation and purification steps. For example, access to the 10β-chloro derivative **2** is based on the reaction of 17β-estradiol with 2,3,4,5,6,6-hexachloro-2,4-cyclohexadienone in DMF which affords the desired compound in ca 75% yield together with 25% of other chlorinated derivatives [13] (Schemes 1 and 2).

As part of our studies [15–17] toward the preparation of functionalized steroidal scaffolds of potential investigative value and/or to be evaluated as pharmaceutical agents, we report herein operationally simple and convenient procedures for the one-pot conversion of 17β-estradiol to 10β,17β-dihydroxyestra-1,4-dien-3-one (**1**) and 10β-chloro-17β-hydroxyestra-1,4-dien-3-one (**2**).



1: R = OH
2: R = Cl

Scheme 1



3: R_1 = OH, R_2 = H, R_3 = Cl
4: R_1 = R_2 = Cl, R_3 = H
5: R_1 = R_3 = Cl, R_2 = H

Scheme 2

2. Experimental

2.1. Materials and methods

17β-Estradiol, potassium permanganate, sodium chlorite, were from Aldrich Chemie. Melting points were obtained with a Gallenkamp apparatus. Elemental analyses were performed with a Perkin-Elmer CHN analyzer mod. 2400. Ultraviolet spectra were performed using a diode array Hewlett Packard spectrophotometer model 8453E. CD spectra were taken on Spectropolarimeter Jasco J-715 at 25 °C using solutions of the products in ethanol exhibiting absorbance values in the range 0.1–0.2 at 220 nm. ^1H (^{13}C) NMR spectra were recorded at 400.1 (100.6) MHz using a Bruker DRX–400 MHz instrument fitted with a 5 mm ^1H broadband gradient probe with inverse geometry. ^1H,^1H Correlation spectroscopy (COSY), ^1H,^{13}C heteronuclear multiple quantum coherence (HMQC), ^1H,^{13}C heteronuclear multiple bond correlation (HMBC), rotating Overhauser effect spectroscopy (ROESY) and double-quantum-filtered correlation spectroscopy (DQF-COSY) experiments were run at 400.1 MHz using standard pulse programs from the Bruker library. Electrospray ionization mass spectrometry (ESIMS) spectra were recorded in negative or positive ion mode with a Waters ZQ quadrupole mass spectrometer on samples dissolved in methanol. High resolution electrospray ionization (HRESI) mass spectra were obtained with a Finnegan MAT 90 instrument. Analytical and preparative thin layer chromatography (TLC) analyses were performed on F_{254} 0.25 and 0.5 mm silica gel plates using cyclohexane/ethyl acetate 40:60 (v/v) as the eluant. HPLC was carried out on an Agilent mod. 1100 apparatus equipped with a UV detector set at 280 nm using a Sphereclone ODS(2) (5 μm, 4.6 mm × 250 mm) using 1% acetic acid/acetonitrile 50:50 (v/v) as the eluant, at a flow rate of 1 mL/min.

2.2. 10β,17β-dihydroxyestra-1,4-dien-3-one (1)

A solution of 17β-estradiol (100 mg, 0.37 mmol) in ethyl acetate (16 mL) was added under vigorous stirring to a solution of potassium permanganate (116 mg, 0.74 mmol) in 0.05 M aqueous HCl (16 mL). After 30 s, when substrate consumption was complete (TLC evidence), the mixture was extracted with ethyl acetate (3 × 75 mL), the organic layers were collected, and taken to dryness to afford pure **1** (80 mg, 75% yield, R_f = 0.30) as crystals from ethyl acetate, m.p. 217–219 °C; UV (MeOH): λ_{max} 243 nm; ^1H and ^{13}C NMR (see Table 1); ESI(−)MS *m*/*z*: 287

([M − H]$^-$). Anal. calcd. for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 75.00; H, 8.42.

2.3. 10β-Chloro-17β-hydroxyestra-1,4-dien-3-one (2), 4-chloro-10β, 17β-dihydroxyestra-1,4-dien-3-one (3), 2,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one (4), 4,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one (5)

17β-Estradiol (100 mg, 0.37 mmol) in methanol (7 mL) was added under vigorous stirring to 0.01 M aqueous HCl (20 mL) containing $NaClO_2$ (66 mg, 0.74 mmol). After 30 min reaction time, when substrate consumption was complete (HPLC evidence), the mixture was extracted with ethyl acetate (3 × 75 mL), the organic layers were collected, taken to dryness, and the residue fractionated on silica plates to afford pure **2** (50 mg, 44% yield, R_f = 0.49, R_T = 12.7 min) as colourless crystals from ethyl acetate, **3** (2 mg, 2% yield, R_f = 0.17, R_T = 5.5 min), **4** (7 mg, 6% yield, R_f = 0.55, R_T = 19.3 min), **5** (12 mg, 9% yield, R_f = 0.52, R_T = 21.2 min).

2: m.p. 158–160 °C [14]; UV (CH_3OH): λ_{max} 241, 280 (sh) nm; ^{1}H and ^{13}C NMR [14] (see Table 1); ESI(+)MS *m/z*: 307 ([M + H]$^+$, 100), 309 ([M + 2 + H]$^+$, 35). Anal. calcd. for $C_{18}H_{23}O_2Cl$: C, 70.46; H, 7.56. Found C, 70.89; H, 7.45.

3: UV (CH_3OH): λ_{max} 249, 289 nm; ^{1}H NMR ($CDCl_3$), δ (ppm) 0.85 (3H, s), 1.03 (1H, m), 1.07 (2H, m), 1.09 (1H, m), 1.34 (1H, m), 1.45 (1H, m), 1.50 (1H, m), 1.64 (1H, m), 1.65 (1H, m), 1.86 (1H, m), 2.00 (2H, m), 2.09 (1H, m), 2.60 (1H, m), 3.17 (1H, m), 3.64 (1H, m), 6.31 (1H, d, *J* = 10.4 Hz), 7.12 (1H, d, *J* = 10.4 Hz); ^{13}C NMR ($CDCl_3$), δ (ppm) 11.0 (CH_3), 22.8 (CH_2), 23.6 (CH_2), 28.7 (CH_2), 30.4 (CH_2), 31.9 (CH_2), 35.0 (CH), 36.1 (CH_2), 43.0 (C), 49.7 (CH), 55.3 (CH), 72.8 (C), 81.5 (CH), 126.9 (CH), 127.0 (C), 150.6 (CH), 160.6 (C), 178.7 (C); ESI(−)MS *m/z*: 321 ([M − H]$^-$, 100), 323 ([M + 2 − H]$^-$, 35).

4: UV (CH_3OH): λ_{max} 253, 288 (sh) nm; ^{1}H NMR [14]; ESI(+)MS *m/z*: 341 ([M + H]$^+$, 100), 343 ([M + 2 + H]$^+$, 63), 345 ([M + 4 + H]$^+$, 12).

5: UV (CH_3OH): λ_{max} 247, 289 nm; ^{1}H NMR [14]; ESI(+)MS *m/z*: 341 ([M + H]$^+$, 100), 343 ([M + 2 + H]$^+$, 63), 345 ([M + 4 + H]$^+$, 15).

Table 1 – Selected NMR data for compounds 1–2[a]

	1		2	
	δ_C	δ_H	δ_C	δ_H
C-1	151.1	6.98	147.9	7.13
C-2	131.3	6.34	126.6	6.18
C-3	186.0	–	185.0	–
C-4	126.1	6.17	123.8	6.06
C-5	165.2	–	161.1	–
C-6	32.3	2.45 (β), 2.33	32.3	2.85 (β), 2.41
C-7	33.4	1.95 (β), 1.06	32.3	1.96 (β), 1.03
C-8	35.1	1.97	35.8	1.96
C-9	55.6	1.20	53.4	1.34
C-10	76.3	–	67.7	–
C-11	22.7	1.83 (β), 1.68	22.9	1.89 (β), 1.80
C-12	36.3	1.99 (β), 1.05	35.9	1.91 (β), 1.12
C-13	43.2	–	43.0	–
C-14	49.9	0.92	49.4	1.02
C-15	23.6	1.33 (β),1.62	23.5	1.38 (β), 1.61
C-16	30.4	1.45 (β), 2.04	30.4	1.50 (β), 2.09
C-17	81.6	3.65	81.4	3.65
C-18	11.0	0.83	11.0	0.85

[a] Spectra were taken in $CDCl_3$.

3. Results and discussion

Several oxidants, such as potassium permanganate, lead tetraacetate, diacetoxyiodobenzene, sodium periodate, potassium persulfate were tested for their ability to bring about direct conversion of 17β-estradiol to the quinol **1**. Of these, potassium permanganate proved to be the most efficient in producing the desired quinol in good yield. The reaction was investigated under different experimental conditions, and an optimized procedure was eventually developed, using an acidic water/ethyl acetate 1:1 as solvent and 2 molar equivalents of the oxidant. By this method, complete substrate consumption was observed in less than 1 min, and the desired quinol **1** was obtained in 75% isolated yield in pure form in the organic phase. Product identity was determined by spectral analysis and comparison with literature data; the stereochemistry of the C-10 centre was confirmed from the CD spectrum [18] showing a negative Cotton effect similar to that reported for 10β-substituted compounds [14,18–20]. To the best of our knowledge, this is the first method for preparing **1** by direct oxidation of 17β-estradiol without functional group protection and chromatographic separation.

10β-Chloro-17β-hydroxyestra-1,4-dien-3-one (**2**) was prepared in 44% isolated yield (48% formation yield as determined by HPLC) by treating 17β-estradiol with 2 molar equivalents of $NaClO_2$. After 30 min, substrate consumption was complete, and compound **2** was obtained by extraction of the mixture with ethyl acetate, followed by chromatographic purification. Configuration at C-10 was determined by CD analysis [14]. Under these reaction conditions no formation of **1** was observed.

For both products **1** and **2** complete resonances assignment (Table 1) was obtained by 2D (COSY, HMQC, HMBC, and ROESY) NMR analysis. Coupling constants (Table 2) were obtained by DQF-COSY experiments.

Careful analysis of the reaction mixture of 17β-estradiol with $NaClO_2$ revealed the presence of minor components which were purified by preparative TLC and subjected to complete spectral analysis. The most polar compound was characterized as the novel 4-chloro-10β, 17β-dihydroxyestra-1,4-dien-3-one (**3**). The CD spectrum supporting the assignment of configuration at C-10 is shown in Fig. 1. The other two products were identified as 2,10β-dichloro-17β-hydroxyestra-1,4-

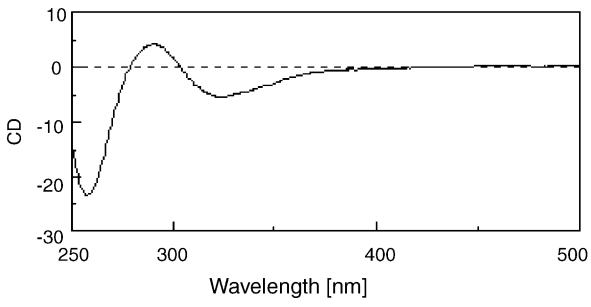


Fig. 1 – CD spectrum of compound 3 taken in methanol.

Table 2 – J(H,H) absolute values (Hz)

Atoms	1	2
1, 2	10.4	10.4
2, 4	2.0	2.0
4, 6β	1.7	1.6
6α, 6β	13.2	13.2
6α, 7α	4.4	4.4
6α, 7β	2.6	2.4
6β, 7α	13.4	13.6
6β, 7β	4.4	4.8
7α, 7β	13.0	12.0
7α, 8β	11.2	11.2
7β, 8β	n.d.	n.d.
8β, 9α	12.9	12.0
8β, 14α	10.8	11.0
9, 11α	4.0	4.8
9, 11β	11.6	n.d.
11α, 11β	13.2	13.2
11α, 12α	4.5	4.5
11α, 12β	2.2	2.0
11β, 12α	12.0	12.0
11β, 12β	n.d.	4.4
12α, 12β	13.4	11.2
14α, 15α	7.4	6.8
14α, 15β	12.5	11.6
15α, 15β	11.8	11.6
15α, 16α	10.2	8.8
15α, 16β	3.4	3.6
15β, 16α	5.4	5.6
15β, 16β	11.4	12.0
16α, 16β	12.9	9.2
17α, 16α	8.6	7.6
17α, 16β	8.3	8.0

n.d.: not determined.

dien-3-one (**4**), and 4,10β-dichloro-17β-hydroxyestra-1,4-dien-3-one (**5**) previously obtained by chlorination of 17β-estradiol with *N*-chloro imide reagents [14].

The procedures here disclosed for the preparation of the 10β-substituted 17β-hydroxyestra-1,4-dien-3-ones **1** and **2** are amenable to gram scale preparations and represent valuable alternatives to previous methodologies because of the lack of protection/deprotection steps, simple work-up, and use of cheap, non-toxic reagents.

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