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Oxidative Coupling of 17β-Estradiol: Inventory of Oligomer Products and Configuration Assignment of Atropoisomeric C4-Linked Biphenyl-Type Dimers and Trimers

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The oxidation chemistry of 17β-estradiol (**1**) is of central relevance to the nongenomic effects of estrogens and offers valuable prospects in the search for novel steroidal scaffolds of academic and industrial interest. Herein, we report the results of a detailed investigation into the nature of the oligomer products formed by phenolic oxidation of **1**. Of the oxidants tested, the peroxidase/H_2O_2 system proved to be the most effective in inducing conversion of **1** to a complex mixture of oligomer species. Repeated chromatographic fractionation followed by extensive 2D NMR and mass spectrometric analysis allowed identification of a series of phenolic coupling products comprising, besides the C_2-symmetric dimers **2** and **3,** a 2,4′ dimer (**4**), two O-linked dimers (**5**, **6**), and the novel trimers **7**−**9**. All 4-linked biphenyl-type oligomers, i.e., **3** and **7**−**9**, occurred as couples of atropoisomers, reflecting steric hindrance at biphenyl linkages. For all atropoisomers, absolute configuration was established by the exciton chirality method and the interconversion energy was determined by dynamic NMR. These results provide the first systematic inventory of oxidative coupling products of **1** and lay the foundation for future studies aimed to develop novel estrogen derivatives based on oligomeric scaffolds.

Introduction

17β-Estradiol (**1**) and structurally related estrogens possess both carcinogenic[1] and neuroprotective[2] properties that have been attributed to the inherent susceptibility of the phenolic A-ring to enzymatic or chemical oxidation.[3] In particular, the involvement of **1** in breast and other human cancers would be the result of metabolic conversion to the 2- and 4-hydroxy derivatives, termed the catechol estrogens, and the corresponding *o*-quinones, which can induce the critical initiation step of tumorigenesis via adduct formation with DNA and depurination processes.[4] On the other hand, the neuroprotective action of estrogens would be related to, or at least complemented by, their potent antioxidant[5] and free radical scavenging capacity.[6] In view of that, a detailed elucidation of the structural modifications suffered by **1** in oxidative settings is central for the understanding of the nongenomic effects of estrogens. In addition, beyond the specific relevance to the steroid sector, the oxidation of estrogen compounds represents an attractive research issue because of its potential as convenient entry to complex functionalized scaffolds of academic and industrial interest, e.g. in asymmetric synthesis[7] and supramolecular chemistry,[8] in the quest for innovative lead compounds in anticancer therapy,[9] or for liquid crystal preparations,[10] where **1** and related compounds are commonly

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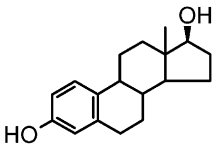
employed. The close bearing on the field of phenolic coupling, which is central to several areas of organic chemistry, further warrants exploration of the oxidation of **1**.

Yet, despite the many prospects offered by oxidative manipulation of estrogens, current knowledge in the field is surprisingly limited. The only known oxidation products include, besides the catechol estrogens, a 10β-hydroxyestra-1,4-dien-3-one derivative arising by peracid-induced photooxygenation or oxidation by Fenton reagent,[6b] a series of benzylic oxidation species of estrone methyl ether,[11] and two dimers obtained by chemical and enzymatic oxidation of **1**, namely, the symmetric 2,2′ and 4,4′ dimers.[12] Other studies have appeared reporting formation of oligomer species by oxidation of **1**, but their characterization relied only on evaluation of chemical physical properties.[13] More recently, a convenient synthetic access to O-linked dimers of **1** was reported[14] in the frame of a study of the NADPH-dependent metabolism of **1** by human liver microsomes and cytochrome P450 enzymes. These latter studies and the vast body of literature on the oxidative coupling of phenols[15] suggest that oxidative conversion of **1** and related estrogens in vivo can lead to an array of oligomeric products, yet their nature and biological properties have remained so far poorly elucidated. This study was therefore concerned with an investigation of the reaction behavior of **1** with various oxidizing systems. Specific goals were to gain a systematic insight into the modes of oxidative coupling of **1** and to provide a detailed structural characterization of the oligomer products for future in vitro and in vivo studies.



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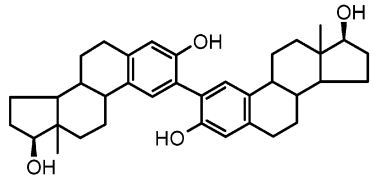
Results and Discussion

In preliminary experiments, the ability of various chemical and enzymatic oxidants to induce conversion of **1** to oligomer products was briefly investigated under different reaction conditions. With the chemical oxidants tested, i.e., persulfate, ferricyanide, and ceric ammonium nitrate, little or no substrate conversion was observed (HPLC and TLC evidence) in aqueous buffers or biphasic media in a broad range of pH values. With ferricyanide, slow substrate consumption was obtained only in 0.1 M NaOH, as previously reported.[12]

By contrast, a substantial substrate consumption was observed with the peroxidase/H_2O_2 system, with formation of a number of products whose chromatographic and spectral properties were suggestive of oligomer species. The mechanistic background provided by the extensive literature on peroxidase-catalyzed oxidation of phenols[16] and the occurrence of peroxidase in mammalian tissues responsive to estrogen activity, such as uterus,[17] warranted investigation of such reaction as a paradigm to detail the behavior of this estrogen on oxidation. Accordingly, we decided to embark on the isolation and detailed characterization of the products formed by peroxidase/H_2O_2 oxidation of **1**. As the medium, phosphate buffer at pH 7.4 with little methanol to favor estrogen solubilization was preferably used. Two-phase systems, e.g. water−ethyl acetate,[12] proved of limited utility and were not pursued further.

In a typical preparative scale reaction, **1** at 0.3 mM concentration was allowed to react with peroxidase (1 U/mL) and hydrogen peroxide (2 mol equiv). After 60 min, with >98% substrate consumption, the mixture was extracted with ethyl acetate following careful acidification to pH 5.0. PLC fractionation afforded seven main chromatographic bands at R_f = 0.68, 0.55, 0.45, 0.43, 0.33, 0.22, and 0.10 (eluant A) designated A−G, in that order. Of these, only fraction D consisted of a single species pure enough for spectroscopic analysis, whereas fractions A, B, and D−F required further fractionation. The most polar band G was made of chromatographically ill-defined products, and their identity was not investigated.

Spectral data (^{1}H and ^{13}C NMR) of the product from band D were in agreement with a C_2-symmetric dimer (molecular ion peak at *m*/*z* 542). Homo- and heteronuclear correlation experiments allowed straightforward formulation of the product as **2**.



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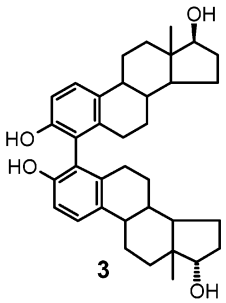

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Chromatographic band A consisted of an intimate mixture of two closely related species which could be

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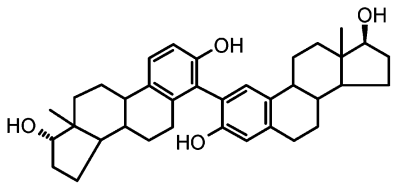
separated by preparative HPLC. The products showed nearly identical ^{1}H NMR spectra featuring in the aromatic region two doublets (J = 8.8 Hz) at around δ 7.3 and 6.9, suggesting the C_2-symmetric 4,4′ dimer **3**. On this basis, the two products were regarded as atropoisomers arising by a restricted rotation around the sterically crowded biphenyl 4,4′ linkage. No appreciable interconversion of the rotational isomers was observed by heating to 110 °C at which temperature the products began to decompose significantly. This implies that the activation energy barrier is greater than 22.5 kcal mol^{-1}.

The constituents of chromatographic band C as purified by HPTLC displayed very similar ^{1}H NMR spectra showing in the aromatic region two singlets at about δ 7.0 and 6.8 and two doublets (J = 8.4 Hz) at about δ 7.3 and 6.9, suggesting two atropoisomers of a 2,4′-linked dimer (**4**). This view was confirmed by dynamic ^{1}H NMR experiments. Line shape analysis at a temperature around coalescence allowed calculation of the mean lifetime of the atropoisomers, and a free energy of activation of 21.5 ± 0.5 kcal mol^{-1} was determined by application of the Eyring equation.



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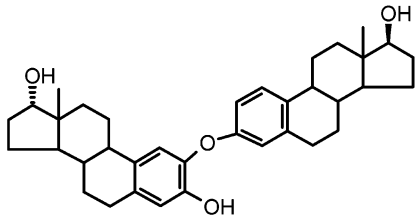
The two components of chromatographic band B were separated by preparative HPLC. The mass and NMR data led to straightforward formulation of the compounds as the O-linked dimers **5** and **6**.[14] Extensive 2D proton−proton and proton−carbon correlation experiments allowed complete assignment of the aromatic resonances (see Table 1).



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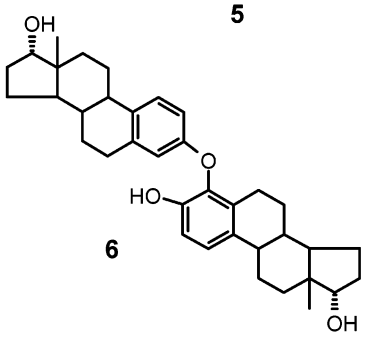
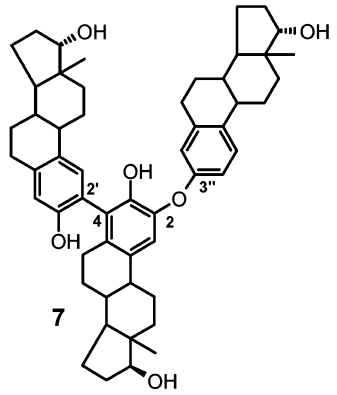


6

All products from chromatographic bands E and F exhibited molecular ion peaks at *m*/*z* 812 in the EI-MS spectrum indicating trimeric structures. Products from band E as obtained in pure form by HPLC separation showed very close proton spectra and were regarded as atropoisomers. Both displayed in the aromatic region an ABX spin system and three singlets around δ 6.7, 6.9, and 7.0, consistent with a trimer in which a central estradiol unit is linked to the 2-position of an outer moiety and to the oxygen of the other unit. On the basis of the HMBC correlation data, it was possible to assign the signals at δ 7.04 and 6.75, for the faster HPLC eluted compound, to the H-1 and H-4 protons of the same estradiol unit. The shielding effect caused by the oxygen bridge, observed also in dimers **5** and **6**, and the presence of a weak but well discernible cross-peak between the proton resonance at δ 7.04 and a substituted C-4 carbon resonance at δ 124.3 (see Table 2) allowed straightforward assignment of the former signal to the H-1 proton of the central unit leading eventually to assign the trimers structure **7**. In these, the atropoisomerism is apparently the result of the restricted rotation around the 2,4′ linkage. NMR line shape analysis around the coalescence temperature allowed calculation of a free energy of activation of 20.9 ± 0.4 kcal mol^{-1}.

TABLE 1. Selected NMR Data for Compounds 5 and 6

	5		6	
	δ_C	δ_H	δ_C	δ_H
C-1	117.6	6.87	123.6	7.10
C-2	141.5		113.8	6.86
C-3	146.2		147.4	
C-4	116.7	6.74	139.7	
C-5	134.2		131.5	
C-10	133.7		134.4	
C-1′	127.4	7.22	127.5	7.17
C-2′	114.8	6.76	112.8	6.62
C-3′	156.0		155.7	
C-4′	117.7	6.70	115.6	6.60
C-5′	139.3		139.4	
C-10′	135.8		135.5	



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Of the four main HPLC-separable products in band F, those eluting at 24 and 80 min (eluant II) interchanged on heating, suggesting again an atropoisomer relationship, whereas those eluting at 34 and 37 min (eluant II) were not affected by heating to 100 °C.

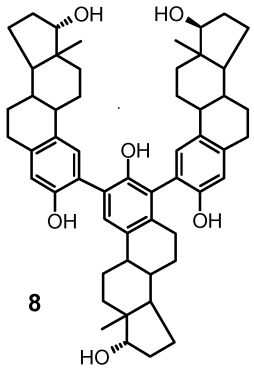
The aromatic region of the ^{1}H NMR spectrum of the products eluted at 24 and 80 min displayed five singlets, a feature which was compatible with the trimeric structure **8**. Complete assignment of the proton and carbon signals in the aromatic region was achieved on the basis of the data of the correlation experiments. In particular, in the case of the slower eluting isomer (**8b**), the singlets

TABLE 2. Selected ^{1}H−^{13}C NMR Correlation Data for Compound 7a[18]

	δ_C	one-bond correlatn δ_H	multiple-bond correlatn δ_H
C-1[a]	117.8	6.98	
C-2	142.2		6.98
C-3	144.6		6.98
C-4	124.3		7.04
C-5	133.9		6.98
C-10	133.6		6.98
C-1′	128.3	7.04	
C-2′	119.8		6.75
C-3′	151.6		6.75, 7.04
C-4′	115.4	6.75	
C-5′	132.3		7.04
C-10′	134.3		6.75
C-1″	127.4	7.23	
C-2″	116.4	6.80	
C-3″	155.9		7.23
C-4″	118.4	6.76	6.80
C-5″	139.3		7.23
C-10″	136.0		6.76, 6.80

[a] Numbering as shown in formula **7**.

at δ 7.21 and δ 6.76 were assigned to H-1 and H-4 protons of the same unit, respectively, on the basis of the ^3J and ^2J long-range contacts exhibited with C-3 and C-2 at δ 151.1 and 123.0, respectively. A ^3J contact between the latter carbon and the H-1′ proton at δ 7.32 of another unit provided support to the 2,2′ linkage between two of the trimer units (see Table 3). The free energy activation for the interconversion was calculated as 21.3 ± 0.5 kcal mol^{-1}.



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The ^{1}H and ^{13}C spectra of the other two constituents of band F were likewise very similar. The aromatic regions of the proton spectra diplayed three singlets and two doublets (J = 8.4 Hz), a pattern of resonance that

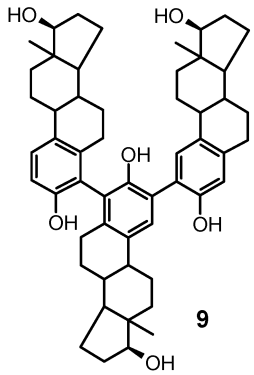

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TABLE 3. Selected ^{1}H−^{13}C NMR Correlation Data for Compound 8b[18]

	δ_C	one-bond correlatn δ_H	multiple-bond correlatn δ_H
C-1	128.9	7.21	
C-2	123.0		6.76, 7.32
C-3	151.1		7.21, 6.76
C-4	118.2	6.76	
C-5	138.3		7.21
C-10	133.3		6.76
C-1′	130.1	7.32	
C-2′	122.5		7.32
C-3′	150.9		7.32
C-4′	121.9		7.04
C-5′	137.4		7.32
C-10′	133.9		
C-1″	128.6	7.04	
C-2″	121.9		7.04
C-3″	150.9		7.04
C-4″	116.9	6.78	
C-5″	140.0		7.04
C-10″	134.5		6.78

TABLE 4. Selected ^{1}H−^{13}C NMR Correlation Data for Compound 9b[18]

	δ_C	one-bond correlatn δ_H	multiple-bond correlatn δ_H
C-1	128.2	7.22	
C-2	122.6		7.22, 6.76
C-3	151.5		7.22, 6.76
C-4	118.3	6.76	
C-5	138.2		7.22
C-10	132.6		6.76
C-1′	130.2	7.36	
C-2′	122.7		7.36, 7.22
C-3′	148.0		7.36
C-4′			
C-5′	136.5		7.36
C-10′			
C-1″	130.1	7.33	
C-2″	118.6	6.89	
C-3″	152.1		7.33, 6.89
C-4″	118.9		6.89
C-5″	136.3		7.33
C-10″	134.3		6.89

was compatible with either of the two trimeric structures in which the estradiol units were linked through the 2,4′:2′,4″ or the 2,2′:4′,4″ positions. The lack of appreciable interconversion on heating, observed also for the atropoisomers of the 4,4′ dimer **3**, strongly argued in favor of structure **9**. This assignment was confirmed by analysis of the proton−carbon correlation spectra showing contacts matching those of the 2,2′ subunit of trimer **8** (see Table 4).

Interestingly, when the oxidation of **1** was carried out with the substrate at 0.3 μM concentration, that is, at a concentration close to physiological values, substrate consumption was >95% at 1 h and the main reaction products were the dimers **2** and **4**, whereas the O-linked dimers **5** and **6** were formed only in very small amounts and dimer **3** and trimers **7**−**9** were below detection limits.

For comparative purposes, the oxidation of **1** with manganese dioxide in chloroform was briefly investigated. Under these conditions, a smooth oxidation of **1** (>99% consumption after 20 h) was observed with formation of dimers **3** and **4** as main species (about 30% overall formation yields) but with no detectable **2**, **5**, and **6**. The different product patterns obtained at lower

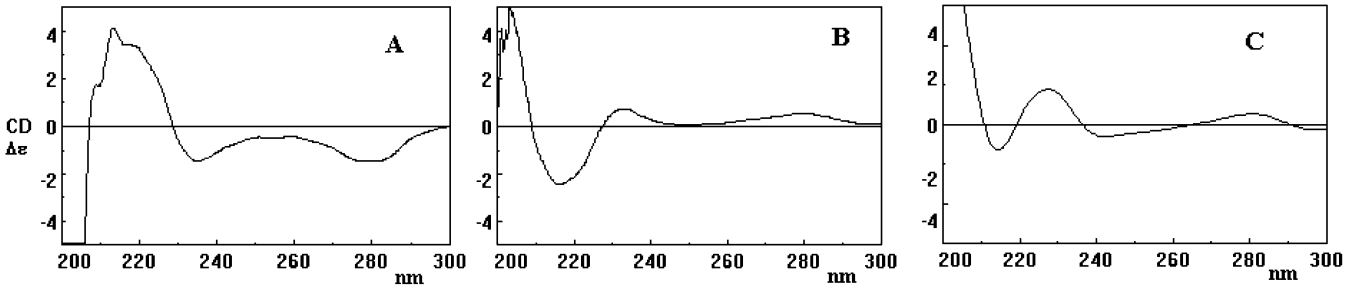

FIGURE 1. CD spectra of compounds **3a** (A), **4a** (B), and **7a** (C).

substrate concentration, or using manganese dioxide in chloroform, suggest that the generation and mode of coupling of phenoxyl radicals is under the influence of several factors. For example, tenuous steric factors may become significant under high dilution conditions, thus accounting for the lack of formation of the relatively hindered dimer **3**, whereas solvent effects may explain the prevalence of C-coupling products, i.e. dimers **3** and **4** in chloroform, also furnishing a suggestion for preparative purposes requiring regiochemically more restricted products patterns.

The sterically hindered biphenyl linkage in **3, 4,** and **7**−**9** represents a stereogenic element which adds to those already present in **1**. For all isolated products featuring such structural system, configuration at the biphenyl linkage (and thus absolute stereochemistry) was established by the exciton chirality method on the basis of the Cotton effect associated with the phenolic transition $1L_a$, whose vector nearly overlaps that joining the C10−C3−O centers.[19] This transition is observed at around 220 nm in **1** in EtOH, but the formation of biphenyl linkages and the presence of other substituents, such as the O-linked unit in **7a**,**b**, cause shift to longer wavelengths. The relative directions of the $1L_a$ transition dipoles and of the biphenyl bonds allowed assignment of positive screwness configuration (*P*) to those isomers exhibiting positive Cotton effect independently from the regiochemistry of the biphenyl linkage. Indeed, geometry optimization of the oligomer structures (MM+) showed that the dihedral angle between the planes of the aromatic rings of the biphenyl system has an absolute value ranging from 43° 22′ to 45° 15′ for 2,4′ linkages and from 90° 02′ to 94° 92′ for 4,4′ linkages. On the basis of the angles between $1L_a$ transition vectors and the dihedral intersection line, trigonometric calculations gave the range +37 to +77° for the angle between the dipole transition moments in the case of a positive dihedral angle. These values are significantly smaller than 110°, which is the theoretical zero point at which for polyphenyl systems featuring right-handed screwness[19c] the sign of the exciton split of CD Cotton effect changes from positive to negative, allowing straightforward molecular configuration assignment.

The choice of $1L_a$ transition arises also from the clearly defined monosignated Cotton effect at around 230 nm in all isolated products, whereas the Cotton effect at ca. 280 nm (transition $1L_b$) was less defined for nearly all products, with the exception of those featuring 4,4′-biphenyl linkages.

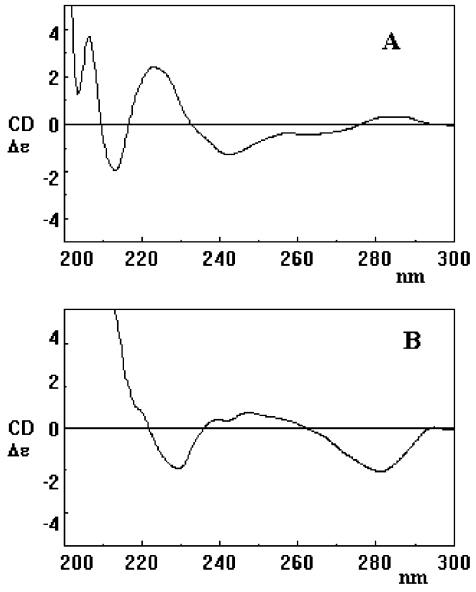

FIGURE 2. CD spectra of compounds **8a** (A) and **9a** (B).

On this basis, the negative Cotton effect of the first HPLC eluted atropoisomer of **4** and **7** (i.e. **4a** and **7a**) indicates a negative helical orientation of phenol transition moments that means an *M* molecular chirality, while the first eluted isomer of **3** has the *P* configuration (Figure 1 A−C).

In the case of **8** (i.e. 2,2′:4′,2″-triestradiol) and **9** (i.e. 2,2′:4′,4″-triestradiol) the first eluted isomers share the *M* configuration at the 4′,2″ biphenyl linkage and at the 4′,4″ linkage, respectively (Figure 2 A,B).

Mechanistically, formation of oligomer products **3**−**9** by peroxidase/H_2O_2 promoted oxidation of **1** can be interpreted as involving generation and coupling of phenoxyl radicals from **1**. In the presence of H_2O_2, ferric peroxidase (ground state) generates the ferryl π cation (compound I) via two electron oxidation. Compound I can then be reduced to compound II, the ferryl form of the enzyme, which has higher oxidative equivalents than the resting ferric form.[17b] Both compounds I and II can oxidize the phenolic moiety of **1** to give the phenoxyl radical.

From inspection of the SOMO and Mulliken spin densities of the phenoxyl radical of **1** reported in a previous study,[20] no appreciable difference was antici-

(18) "a" and "b" lettering denotes the faster and the slower HPLC eluted isomer, respectively

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pated in the reactivity of **1** through the 2 and 4 positions, in accord with experimental evidence. Coupling through the oxygen center is clearly a reflection of the high spin density at this site, in conformity with the known patterns of oxidative coupling of phenols.

Conclusions

The analytical and structural undertaking described herein fills an important gap in the current knowledge of the oxidation chemistry of estrogens and, more in general, of natural phenolic compounds. Highlights of this study include (a) the first isolation and complete characterization of trimeric steroids linked through C−C and C−O−C bonds, (b) the first example, to the best of our knowledge, of atropoisomerism in steroidal systems, generated by steric hindrance to free rotation at 2,4′- and 4,4′-biphenyl linkages, and (c) the exploitation of peroxidase/H_2O_2 as an efficient and clean oxidizing system in estrogen chemistry.

From the biomedical point of view, the present results offer an improved background to elucidate the chemical nature and fate of the products derived from the antioxidant and radical scavenging reactions or from oxidative changes of the estrogens at sites of inflammation and active metabolic transformation. In the light of the suggested role of **1** as OH radical scavenger, generation of these oligomers may represent an alternative outcome of the radical scavenging action in addition to quinol formation.[6b] Oligomers **5** and **6** resemble the photodegradation products of ethinyl estradiol,[21] and their formation by autoxidation and photodegradation of estradiol-containing drugs can be predicted. C_2-symmetric dimers bear considerable similarity to stereochemically related products[22] currently under scrutiny because of their antiestrogenic activity and may represent attractive prototypes/leads for the rational design of new bioactive steroids.

Finally, atropoisomeric estradiol oligomers are analogous to *para*-polyaryls, which exhibit attractive structural features, such as helicity, and other connected unusual chemical−physical properties underlying a number of applications in material science.[23]

The extension of the scope and utility of the oxidation chemistry of estrogens is currently a matter of concern to our laboratory.

Experimental Section

General Methods. 17β-Estradiol (**1**), manganese(IV) dioxide activated 5 μm (85%), and hydrogen peroxide (30% w/w solution in water) were used as obtained. Horseradish peroxidase (donor:H_2O_2 oxidoreductase; EC 1.11.1.7) type II and mushroom tyrosinase (EC 1.14.18.1) were used.

UV spectra were performed using a diode array spectrophotometer. CD spectra were taken on spectropolarimeter at 25 °C using solutions of the products in ethanol exhibiting absorbance values in the range 0.1−0.2 at 220 nm. ^{1}H (^{13}C) NMR spectra were recorded at 400.1 (100.6) MHz. ^{1}H−^{1}H COSY, ^{1}H−^{13}C HMQC, and ^{1}H−^{13}C HMBC experiments were run at 400.1 MHz using standard pulse programs from the Bruker library. For electron impact (EI-MS) and high resolution (HR-MS) mass spectra samples were ionized with a 70 eV beam and the source was taken at 180−280 °C.

Analytical and preparative TLC analyses were performed on F254 0.25 and 0.5 mm silica gel plates or high performance TLC (HPTLC) using 40:60 cyclohexanes−ethyl acetate (eluant A) or 98:2 chloroform−methyl alcohol (eluant B).

Analytical and preparative HPLC was performed with an instrument equipped with a UV detector set at 280 nm. Octadecylsilane-coated columns, 4.6 × 250 mm or 22 × 250 mm, 5 μm particle size, were used for analytical or preparative runs, respectively. Flow rates of 1 or 15 mL/min were used. Different isocratic and gradient elution conditions were used as follows: 70:30 H_2O−acetonitrile (eluant I); 60:40 H_2O−acetonitrile (eluant II); 90:10 H_2O−acetonitrile (solvent A), acetonitrile (solvent B), 0−5 min 30% B, 5−30 min 30−55% B, 30−40 min 55% B (eluant III).

Oxidation of 1 by the Peroxidase/H_2O_2 System: General Procedure. To a solution of **1** (5 mg, 1.9×10^{-5} mol) in methanol (5 mL) were added 0.1 M phosphate buffer, pH 7.4 (60 mL), and peroxidase (1 U/mL) sequentially. The mixture was then treated with hydrogen peroxide in aliquots ($8 \times 2.5 \times 10^{-6}$ mol) every 10 min while being kept under stirring at room temperature. At different time intervals the reaction was carefully acidified at pH 5.0 and extracted three times with ethyl acetate (3 × 60 mL). The combined organic layers were dried over sodium sulfate and analyzed by HPLC (eluant III) and TLC (eluant A). In other experiments the reaction was carried out as above with the substrate at 3×10^{-7} M concentration using peroxidase (0.02 U/mg) and hydrogen peroxide (1 mol equiv)

Oxidation of 1 by MnO_2. A solution of **1** (10 mg, 3.7×10^{-5} mol) in chloroform (10 mL) was treated with MnO_2 (64 mg, 8×10^{-4} mol) and kept overnight at room temperature. The solid was removed by centrifugation, and the mixture was taken to dryness, taken up in methanol, and analyzed by HPLC (eluant III) and TLC (eluant A).

Isolation of Compounds 2−9. For preparative purposes, reaction of **1** with peroxidase/H_2O_2 was run as described above using 500 mg (1.84×10^{-3} mol) of the starting material at 3.0 $\times 10^{-4}$ M concentration. After workup of the reaction mixture, the residue obtained (480 mg) was fractionated by PLC (eluant A) to give seven fractions. Fraction A (15 mg, $R_f = 0.68$ eluant A) was further purified by preparative HPLC (eluant II) to give pure **3a** (5 mg, $t_r = 8$ min, eluant II, 1% yield) and **3b** (5 mg, $t_r = 17$ min, eluant II, 1% yield). Fraction B (25 mg, $R_f = 0.55$ eluant A) was fractionated by PLC (eluant I) to give pure **5** (8 mg, $t_r = 27$ min, eluant II, 1.6% yield) and **6** (8 mg, $t_r = 29$ min, eluant II, 1.6% yield). Fraction C (15 mg, $R_f = 0.45$ eluant A) was purified by HPTLC (eluant B) to afford **4a** (5 mg, $t_r = 9$ min, eluant II, 1% yield) and **4b** (5 mg, $t_r = 10$ min, eluant II, 1% yield). Fraction D (20 mg, $R_f = 0.43$, eluant A) consisted of pure **2** ($t_r = 14$ min, eluant II, 4% yield). Fraction E (22 mg, $R_f = 0.33$, eluant A) was purified by preparative HPLC (eluant II) to afford **7a** (3 mg, $t_r = 31$ min, eluant II, 0.6% yield) and **7b** (3 mg, $t_r = 32$ min, eluant II, 0.6% yield). Fraction F (14 mg, $R_f = 0.22$, eluant A) was fractionated by preparative HPLC (eluant II) to give four bands corresponding to pure **8a** (3 mg, $t_r = 24$ min, eluant II,, 0.6% yield), **8b** (3 mg, $t_r = 80$ min, eluant II, 0.6% yield), **9a** (3 mg, $t_r = 34$ min, eluant II, 0.6% yield), and **9b** (3 mg, $t_r = 37$ min, eluant II, 0.6% yield). Fraction G (35 mg, $R_f = 0.10$ eluant A) was found to consist of a complex pattern of species and was not further purified.

2,2′-Bis[estra-1,3,5(10)-trien-3,17β-diol] (2). UV [λ_{max} (CH_3-OH)]: 288 nm. ^{1}H NMR (CD_3OD), δ (ppm): 0.75 (s, 3H × 2), 1.0−1.8 (m, 8H × 2), 1.9−2.1 (m, 4H × 2), 2.15 (m, 1H × 2), 2.25 (m, 1H × 2), 2.85 (m, 1H × 2), 3.64 (m, 1H × 2), 6.31 (s,

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(22) (a) Rabouin, D.; Perron, V.; N'Zemba, B.; C−Gaudreault, R.; Berube, G. *Bioorg. Med. Chem. Lett.* **2003**, 13, 557−560 (b) Portoghese, P. S. *J. Med. Chem.* **1992**, *35*, 1927−1937.
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1H × 2), 7.14 (s, 1H × 2). ^{13}C NMR (CD_3OD), δ (ppm): 12.6 (2 × CH_3), 24.1 (2 × CH_2), 29.3 (2 × CH_2), 31.5 (2 × CH_2), 31.8 (4 × CH_2), 38.8 (2 × CH_2), 41.1 (2 × CH), 45.1 (2 × C), 46.1 (2 × CH), 52.1 (2 × CH), 83.3 (2 × CH), 118.1 (2 × CH), 127.0 (2 × C), 130.3 (2 × CH), 134.7 (2 × C), 139.1 (2 × C), 153.0 (2 × C). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3401.

4,4′-Bis[estra-1,3,5(10)-trien-3,17β-diol] (3a). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.80 (s, 3H × 2), 1.1−1.7 (m, 8H × 2), 1.78 (m, 1H × 2), 1.95 (m, 1H × 2), 2.11 (m, 1H × 2), 2.15−2.30 (m, 2H × 2), 2.30−2.40 (m, 2H × 2), 3.73 (t, J = 8.2 Hz, 1H × 2), 6. 87 (d, J = 8.8 Hz, 1H × 2), 7.30 (d, J = 8.8 Hz, 1H × 2). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.9 (2 × CH_3), 23.9 (2 × CH_2), 27.1 (2 × CH_2), 27.8 (2 × CH_2), 28.1 (2 × CH_2), 31.4 (2 × CH_2), 37.5 (2 × CH_2), 39.1 (2 × CH), 44.0 (2 × C), 45.0 (2 × CH), 50.9 (2 × CH), 82.6 (2 × CH), 113.6 (2 × CH), 119.9 (2 × C), 128.0 (2 × CH), 134.4 (2 × C), 137.8 (2 × C), 152.0 (2 × C). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3393.

3b. UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.81 (s, 3H × 2), 1.1−1.7 (m, 8H × 2), 1.79 (m, 1H × 2), 1.99 (s, 1H × 2), 2.12 (m, 1H × 2), 2.15−2.30 (m, 2H × 2), 2.30−2.40 (m, 2H × 2), 3.74 (t, J = 8.2 Hz, 1H × 2), 6. 86 (d, J = 8.8 Hz, 1H × 2), 7.32 (d, J = 8.8 Hz, 1H × 2). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3397.

2,4′-Bis[estra-1,3,5(10)-trien-3,17β-diol] (4a). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.79 (s, 6H), 1.1−1.8 (m, 16H), 1.9−2.0 (m, 4H), 2.10 (m, 2H), 2.25 (m, 2H), 2.32 (m, 2H), 2.50 (m, 2H), 2.85 (m, 2H), 3.73 (m, 2H), 6.77 (s, 1H), 6.85 (d, J = 8.4 Hz, 1H), 7.00 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.9 (CH_3), 23.9 (CH_2), 27.3 (CH_2), 27.8 (CH_2), 28.0 (CH_2), 28.3 (CH_2), 30.4 (CH_2), 31.4 (CH_2), 31.7 (CH_2), 37.50 (CH_2), 37.52 (CH_2), 39.0 (CH), 39.5 (CH), 44.0 (C), 44.8 (CH), 45.0 (CH), 50.8 (CH), 83.3 (CH), 113.6 (CH), 116.7 (CH), 117.4 (C), 120.5 (C), 127.9 (CH), 128.3 (CH), 133.3 (C), 134.5 (C), 136.9 (C), 140.1 (C), 152.3 (C). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3403.

4b. UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.78 (s, 6H), 1.1−1.8 (m, 16H), 1.9−2.1 (m, 4H), 2.1−2.3 (m, 4H), 2.3−2.5 (m, 4H), 2.92 (m, 2H), 3.72 (m, 2H), 6.78 (s, 1H), 6.87 (d, J = 8.4 Hz, 1H), 6.99 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS: calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3401.

2-[[(17β)-17-Hydroxy-19-norpregna-1,3,5(10)-trien-3-yl]-oxy]estra-1,3,5(10)-trien-3,17β-diol (5). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.77 (s, 6H), 1.1−1.6 (m, 16H), 1.6−1.8 (m, 2H), 1.90 (m, 2H), 1.95 (m, 1H), 2.0−2.2 (m, 5H), 2.32 (m, 1H), 2.83 (m, 3H), 3.71 (m, 2H), 6.70 (d, J = 2.4 Hz, 1H), 6.74 (s, 1H), 6.76 (dd, J = 8.4, 2.4 Hz, 1H), 6.87 (s,1H), 7.22 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.8 (CH_3), 23.9 (CH_2), 27.0 (CH_2), 27.1 (CH_2), 27.9 (CH_2), 28.0 (CH_2), 29.9 (CH_2), 30.4 (CH_2), 31.4 (CH_2), 37.4 (CH_2), 37.5 (CH_2), 39.40 (CH), 39.45 (CH), 44.0 (C), 44.8 (CH), 50.8 (CH), 82.6 (CH), 114.8 (CH), 116.7 (CH), 117.6 (CH), 117.7 (CH), 127.4 (CH), 133.7 (C), 134.2 (C) 135.8 (C), 139.3 (C), 141.5 (C), 146.2 (C), 156.0 (C). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3399.

4-[[(17β)-17-Hydroxy-19-norpregna-1,3,5(10)-trien-3-yl]-oxy]estra-1,3,5(10)-trien-3,17β-diol (6). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.78 (s, 6H), 1.1−1.8 (m, 16H), 1.8−1.9 (m, 2H), 1.9−2.0 (m, 2H), 2.0−2.2 (m, 4H), 2.33 (m, 2H), 2.40 (m, 1H), 2.73 (m, 1H), 2.81 (m, 2H), 3.73 (m, 2H), 6.60 (d, J = 2.4 Hz, 1H), 6.62 (dd, J = 8.4, 2.4 Hz, 1H), 6.86 (d, J = 8.4, 1H), 7.10 (d, J = 8.4 Hz, 1H), 7.17 (d, J = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.8 (CH_3), 23.9 (CH_2), 24.6 (CH_2), 27.0 (CH_2), 28.0 (CH_2), 28.2 (CH_2), 30.5 (CH_2), 31.4 (CH_2), 37.4 (CH_2), 39.0 (CH), 39.5 (CH), 44.0 (C), 44.8 (CH), 50.8 (CH), 82.7 (CH), 112.8 (CH), 113.8 (CH), 115.6 (CH), 123.6 (CH), 127.5 (CH), 131.5 (C), 134.4 (C), 135.5 (C), 139.4 (C), 139.7 (C), 147.4 (C), 155.7 (C). EI/MS (*m*/*z*): 542, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{36}H_{46}O_4$, 542.3396; found, 542.3402.

2-[[(17β)-17-Hydroxy-19-norpregna-1,3,5(10)-trien-3-yl]-oxy]-4,2′-bis[estra-1,3,5(10)-trien-3,17β-diol] (7a). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.78 (s, 3H), 0.79 (s, 3H) 0.80 (s, 3H), 1.1−1.7 (m, 21H), 1.70−1.85 (m, 3H), 1.85−2.00 (m, 5H), 2.1−2.2 (m, 5H), 2.2−2.3 (m, 3H), 2.35 (m, 2H), 2.45 (m, 1H), 2.55 (m, 1H), 2.85 (m, 4H), 3.74 (m, 3H), 6.75 (s, 1H), 6.76 (d, J = 2.4 Hz, 1H), 6.80 (dd, J = 8.4, 2.4 Hz, 1H), 6.98 (s, 1H), 7.04 (s, 1H), 7.23 (d, J = 8.4, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.9 (CH_3), 23.9 (CH_2), 27.1 (CH_2), 27.3 (CH_2), 27.9 (CH_2), 30.5 (CH_2), 31.4 (CH_2), 31.7 (CH_2), 37.5 (CH), 38.9 (C), 39.5 (C), 44.0 (CH), 44.8 (CH), 45.1 (CH), 50.9 (CH), 82.7 (CH), 115.4 (CH), 116.4 (CH), 117.8 (CH), 118.4 (CH), 119.8 (C), 124.3 (C), 127.4 (CH), 128.3 (CH), 132.3 (C), 133.6 (C), 133.9 (C), 134.3 (C), 136.0 (C), 139.3 (C), 142.2 (C), 144.6 (C), 151.6 (C), 155.9 (C). EI/MS (*m*/*z*): 812, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5035.

7b. UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.77 (s, 3H), 0.78 (s, 3H) 0.79 (s, 3H), 1.1−1.7 (m, 24 H), 1.7−2.0 (m, 5H), 2.0−2.3 (m, 8H), 2.40 (m, 2H), 2.50 (m, 2H), 2.87 (m, 4H), 3.72 (m, 3H), 6.75 (d, J = 2.4 Hz, 1H), 6.77 (s, 1H), 6.81 (dd, J = 8.4, 2.4 Hz, 1H), 6.98 (s, 1H), 7.03 (s, 1H), 7.24 (d, J = 8.4, 1H). EI/MS (*m*/*z*): 812, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5026.

2,2′:4′,2″-Tris[estra-1,3,5(10)-trien-3,17β-diol] (8a). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.78 (s, 3H), 0.80 (s, 6H), 1.1−1.9 (m, 24H), 1.9−2.0 (m, 6H), 2.1−2.4 (m, 10H), 2.42 (m, 2H), 2.90 (m, 3H), 3.73 (m, 3H), 6.76 (s, 1H), 6.79 (s, 1H), 7.04 (s, 1H), 7.20 (s, 1H), 7.32 (s, 1H). EI/MS (*m*/*z*): 812, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5005.

8b. UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.77 (s, 3H), 0.78 (s, 6H), 1.1−1.9 (m, 24H), 1.9−2.0 (m, 5H), 2.0−2.2 (m, 5H), 2.2−2.5 (m, 8H), 2.90 (m, 3H), 3.73 (m, 3H), 6.76 (s, 1H), 6.78 (s, 1H), 7.04 (s, 1H), 7.21 (s, 1H), 7.32 (s, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.8 (CH_3), 23.9 (CH_2), 24.0 (CH_2), 24.5 (CH_2), 27.2 (CH_2), 27.9 (CH_2), 29.3 (CH_2), 30.1 (CH_2), 30.4 (CH_2), 30.5 (CH_2), 31.4 (CH_2), 37.5 (CH_2), 39.1 (CH), 39.4 (CH), 39.6 (CH), 44.0 (C), 44.8 (CH), 45.1 (CH), 50.9 (CH), 82.7 (CH), 116.9 (CH), 118.2 (CH), 121.9 (C), 122.5 (C), 123.0 (C), 128.6 (CH), 128.9 (CH), 130.0 (CH), 133.3 (C), 133.9 (C), 137.4 (C), 138.3 (C), 140.0 (C), 150.9 (C), 151.1 (C). EI/MS (*m*/*z*): $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5027.

2,2′:4′,4″-Tris[estra-1,3,5(10)-trien-3,17β-diol] (9a). UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.79 (s, 3H), 0.80 (s, 3H), 0.81 (s, 3H), 1.2−1.8 (m, 24H), 1.8−2.0 (m, 6H), 2.0−2.2 (m, 4H), 2.2−2.4 (m, 8H), 2.89 (m, 3H), 3.74 (m, 3H), 6.76 (s, 1H), 6.88 (d, J = 8.4 Hz, 1H), 7.22 (s, 1H), 7.32 (d, J = 8.4, 1H), 7.34 (s, 1H). EI/MS (*m*/*z*): $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5003.

9b. UV [λ_{max} (CH_3OH)]: 288 nm. ^{1}H NMR ($CDCl_3$), δ (ppm): 0.79 (s, 3H), 0.80 (s, 6H), 1.1−1.8 (m, 24H), 1.8−2.0 (m, 5H), 2.1−2.2 (m, 5H), 2.2−2.5 (m, 8H), 2.8−2.9 (m, 3H), 3.73 (m, 3H), 6.76 (s, 1H), 6.89 (d, J = 8.4 Hz, 1H), 7.22 (s, 1H), 7.33 (d, J = 8.4, 1H), 7.36 (s, 1H). ^{13}C NMR ($CDCl_3$), δ (ppm): 11.8 (CH_3), 11.9 (CH_3), 23.8 (CH_2), 24.0 (CH_2), 24.5 (CH_2), 26.5 (CH_2), 28.1 (CH_2), 29.7 (CH_2), 30.5 (CH_2), 31.1 (CH_2), 31.4 (CH_2), 37.5 (CH_2), 38.6 (CH), 39.5 (CH), 42.1 (C), 44.0 (CH), 44.9 (CH), 48.8 (CH), 50.8 (CH), 82.7 (CH), 118.3 (CH), 118.6 (CH), 118.9 (C), 122.6 (C), 122.7 (C), 128.2 (CH), 130.1 (CH), 130.2 (CH), 132.6 (C), 134.3 (C), 136.3 (C), 136.5 (C), 138.2 (C), 148.0 (C), 151.5 (C), 152.1 (C). EI/MS (*m*/*z*): 812, $[M]^+$. HREIMS (*m*/*z*): calcd mass for $C_{54}H_{68}O_6$, 812.5016; found, 812.5024.

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Supporting Information Available: ^{1}H NMR spectra or selected regions of compounds **2**−**9** and $^{1}H-^{13}C$ HMBC spectra of compounds **3a, 4a, 5, 7a, 8a,** and **9a.** This material is available free of charge via the Internet at http://pubs.acs.org.

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