Synthesis and GC-MS of 6-Alkylestradiols, Possible Aromatase Reaction Products of 6-Alkylandrostenediones

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A series of 6α- and 6β-alkylestradiols (5 and 6) (alkyl: methyl, ethyl, *n*-propyl, *n*-pentyl, and *n*-heptyl) were synthesized as possible aromatase reaction products of 6-alkylandrost-4-ene-3,17-diones and their Δ^1-derivatives, potent competitive and mechanism-based inhibitors of aromatase. Treatment of 6-oxoestradiol with Grignard reagents followed by acid–catalyzed dehydration and subsequent catalytic hydrogenation over Pd–C gave the 6-alkylestradiols (5 and 6). GC-MS (electron impact mode) of trimethylsilyl derivatives of bis-trimethylsilyl derivatives of compounds 5 and 6 revealed that the 6α-alkyl compounds, 5, emerged with a longer retention time compared to the corresponding 6β-alkyl isomers, 6, where the retention time was in proportion to the length of the 6-alkyl chain in each series. In the MS, a molecular ion (M^+) peak was the base peak for all the 6-alkylestrogens, with strong and characteristic fragment ion peaks corresponding to $M^+ - 131$ and at *m/z* 325. A selected ion monitoring method using a molecular ion will be sensitive enough for analysis of the aromatization reaction of the 6-alkylandrogens.

Key words 6-alkylestradiol; synthesis; aromatase; GC-MS; analysis

Aromatase is a cytochrome P-450 (P-450 XIXA1) enzyme complex which catalyzes the conversion of androst-4-ene-3,17-dione (androstenedione) to estrone through three sequential oxygenations of the 19-methyl group.[1] Inhibitors of aromatase have recently become of interest not only in the treatment of advanced estrogen-dependent breast cancer[2] but also in the elucidation of the spatial aspects of the active-site of the enzymes as well as the still unresolved mechanism of the aromatase reaction.[3]

Several 6-substituted substrate analogs, 6-bromo-,[4] 6-bromoacetoxy-,[5] 6β-fluoro-,[6] and 6-hydroperoxy-[7] steroids, have been synthesized to act as probes of the aromatase active site. Recently, the structure–activity relationships of 6-alkylandrostenediones as aromatase inhibitors have revealed that aromatase has a hydrophobic binding pocket with limited accessible volume in the active site in the region corresponding to the C-6 position of the substrate.[8] In addition, Δ^1-analogs of the 6-alkylandrostenediones inactivate aromatase in a suicide manner, indicating that the Δ^1-steroids could be substrates for the enzyme.[9] On the basis of these previous findings, it seems to be important to determine whether or not these 6-alkylandrostenediones with or without a double bond at C-1 are aromatized by catalysis of aromatase. Thus, we needed authentic samples for the identification of possible aromatized products of the 6-alkylandrogens by GC-MS. A survey of the literature revealed one report of stereospecific synthesis of 6α- and 6β-alkylestradiols (alkyl: methyl, isopropyl, and *n*-$C_{12}H_{25}$) *via* $Cr(CO_3)$ complexes, but their physical data, except melting points, have not been described.[10] In this report, we report the synthesis and GC-MS of various 6α- and 6β-alkylestradiols (**5** and **6**).

Synthesis of 6-Alkylestradiols (5 and 6) The reaction of 6-oxoestradiol (**1**) with Grignard reagents (RMgBr; R = methyl, ethyl, *n*-propyl, *n*-pentyl, and *n*-heptyl) in tetrahydrofuran (THF) on heating under reflux gave the corresponding 6-alkyl-6-ols **2** in 32—45% yields (Chart 1). On the basis of the ^{1}H-NMR spectroscopy, the products were found to be *ca.* 1 : 1 mixtures of 6β-alkyl-6α-ols and their 6α-alkyl isomers in every case ($\Delta\delta$ of 18-methyl protons with resonances at 0.75—0.79 ppm between the two stereoisomers = 0.01 ppm). It has been reported that CH_3MgBr reacts with 5α-6-keto steroids which have a 19-methyl group, with a very pronounced preference for the formation of the tertiary alcohol with an equatorial methyl group, the 6α-methyl derivatives.[11] It seems likely that a phenolic A-ring of compound **1** might decrease the steric preference for the production of compound **2**. Since the mixtures could not be separated by silica gel column chromatography, the 6-ols **2** were, without further purification, subjected to dehydration with HCl in 95% EtOH (68—86% yield). ^{1}H-NMR spectroscopy of the dehydration products showed that the reaction produced *ca.* 3 : 1 to 6 : 1 mixtures of 6-ene steroids, **3**, and 6,6-methylene analogs, **4**, respectively, except the reaction with the 6-methyl compound **2a** in which the 6-ene compound **3a** was solely produced. [^{1}H-NMR δ: 5.75—5.76, 7-H (s) for **3**; 5.93—6.05, 6^1-H (m) for **4**]. The mixtures obtained from the ethyl and *n*-propyl steroids **2b** and **2c** were separated, respectively, by reverse-phase HPLC (C_{18} column, MeCN–H_2O); however, the other mixtures were used without separation for the next step. Catalytic hydrogenation of the dehydrated mixtures obtained from the *n*-pentyl and *n*-heptyl steroids **2d** and **2c**, as well as the 6-methyl-, 6-ethyl-, and 6-*n*-propyl-6-enes **3a**—**c** over Pd–C under H_2, gave *ca.* 1 : 5 to 1 : 7 mixtures of 6α-alkylestradiols, **5**, and their 6β-alkyl isomers, **6** (58—81%) (Tables 1 and 2). All of the 6α-alkyl- and 6β-alkyl steroids, **5** and **6**, were isolated in a pure form using reverse-phase HPLC (C_{18} column, MeCN–H_2O). Catalytic hydrogenation of the 6-alkyl-6-ene steroid, **3**, and its 6,6-methylene analog, **4**, under the condition used seems to occur preferentially from the α side,[12] suggesting that the major product would be the 6β-alkyl steroid **6**. Moreover, we have previously reported that the 18-methyl protons of 6β-alkylandrost-4-enes and their Δ^1 analogs exhibit resonance at a lower field compared to those of the corresponding 6α-alkyl analogs on their ^{1}H-NMR

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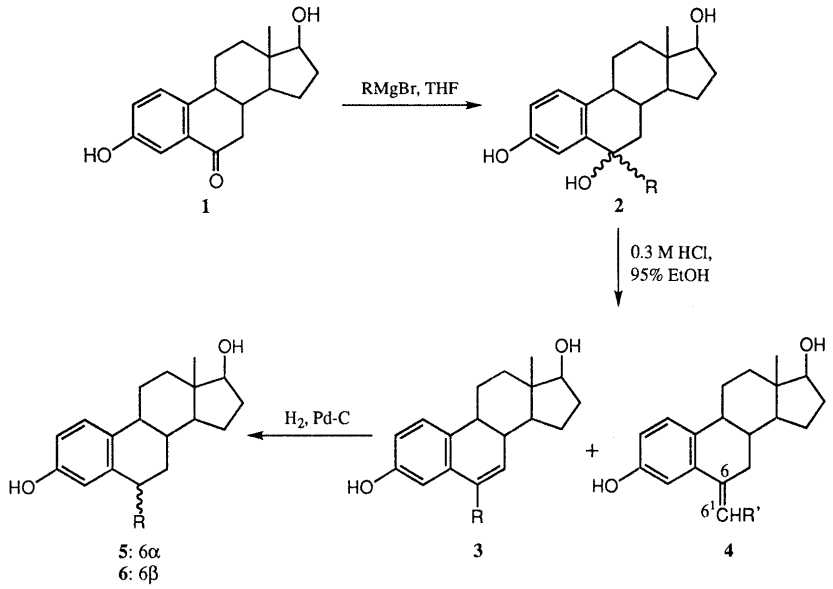

R : **a** = CH_3; **b** = C_2H_5; **c** = *n*-C_3H_7; **d** = *n*-C_5H_{11}; **e** = *n*-C_7H_{15}
R': **a** = CH_3; **b** = C_2H_5; **c** = *n*-C_4H_9; **d** = *n*-C_6H_{13}

Chart 1

Table 1. Physical Data for 6-Alkylestradiols **5** and **6**

Compound	R	Yield (%)	mp (°C)	Purification	Formula	Analysis (%) Calculated		Found	
						C	H	C	H
6α-Series									
5a	CH_3	6	Oil (130)[b]	HPLC	$C_{19}H_{26}O_2$	286.19330[a]		286.1979[a]	
5b	CH_2CH_3	10	Oil	HPLC	$C_{20}H_{28}O_2$	300.20900[a]		300.2083[a]	
5c	$(CH_2)_2CH_3$	10	Oil	HPLC	$C_{21}H_{30}O_2$	314.22460[a]		314.2251[a]	
5d	$(CH_2)_4CH_3$	11	Oil	HPLC	$C_{23}H_{34}O_2$	342.25590[a]		342.2554[a]	
5e	$(CH_2)_6CH_3$	8.6	Oil	HPLC	$C_{25}H_{38}O_2$	370.28720[a]		370.2850[a]	
6β-Series									
6a	CH_3	68	172—173 (oil)[b]	Recryst. (AcOEt)	$C_{19}H_{26}O_2$	79.68	9.15	79.46	9.21
6b	CH_2CH_3	48	Oil	HPLC	$C_{20}H_{28}O_2$	300.20900[a]		300.2126[a]	
6c	$(CH_2)_2CH_3$	71	91—93	Recryst. (MeOH–H_2O)	$C_{21}H_{30}O_2$	80.21	9.62	80.20	9.75
6d	$(CH_2)_4CH_3$	48	Oil	HPLC	$C_{23}H_{34}O_2$	342.25590[a]		342.2538[a]	
6e	$(CH_2)_6CH_3$	52	144—146	Recryst. (AcOEt–hexane)	$C_{25}H_{38}O_2$	81.03	10.34	80.99	9.87

a) Determined by High-resolution mass spectrometry. *b*) Reference 10.

spectroscopies.[8,9] Signals of 18-methyl protons of the major products, **6**, appeared at 0.79 or 0.80 ppm, while those of the minor ones, **5**, resonanced at 0.75 or 0.76 ppm. Taken together, it is reasonable to assume the major products, **6**, to have a 6β-configuration. Finally, the configuration of the 6-alkyl substituents were unambiguously determined on the basis of the results of the aromatization reaction with human placental microsomes; 6α-alkyl-substituted androstenediones were converted into the corresponding compounds, **5**. In contrast, the 6β-alkylandrogens were aromatized to the corresponding compounds **6** (to be reported elsewhere).

Gas Chromatography–Mass Spectrometry of 6-Alkylestradiols (5 and 6) Bis-trimethylsilyl (TMS) derivatives of compounds **5** and **6** were then analyzed by GC (column, fused silica DB 5)-MS (EI). Retention times (t_R) of the 6α-alkyl steroids, **5**, were longer than those of the corresponding 6β-isomers, **6**. In every case, the two stereoisomers were separated by more than 1.3 of resolution (R_s) under the given conditions (Table 3). The introduction of a methyl group at C-6β of estradiol decreased the t_R value (14.9 *vs.* 15.0 min); in contrast, the same chemical modification at C-6α increased it (15.1 *vs.* 15.0 min). The t_R value increased in proportion to the carbon number of the 6-alkylchain in both the 6α- and 6β-series. The results indicate that the bis-TMS derivatives of the 6β-alkyl compound having a quasi-axial conformation at C-6 are more hydrophobic and volatile than the corresponding quasi-equatorial 6α-isomer. Further study is required to understand the structure–retention time relationship, since there is currently no data concerning the conformational analysis of the alkylsteroids.

Table 2. Spectral Data for 6-Alkylestradiols **5** and **6**

Compd.	R	^{1}H-NMR ($CDCl_3$), δ					UV (EtOH) nm (ε)	IR (KBr or neat) cm^{-1}
		18-Me	6-$(CH_2)_n$Me	6-H	1-H	Other signals		
6α-Series								
5a	CH_3	0.76	1.29 (t, J=6.9 Hz)	2.92 (m)	7.15 (d, J=8.6 Hz)	6.63 (dd, J=8.6, 2.6 Hz, 2-H) 6.76 (d, J=2.6 Hz, 4-H)	280.8 (2160)	3415, 2923
5b	CH_2CH_3	0.76	0.87 (t, J=7.4 Hz)	2.83 (m)	7.14 (d, J=8.6 Hz)	6.63 (dd, J=8.6, 2.6 Hz, 2-H) 6.76 (d, J=2.6 Hz, 4-H)	278.8 (2160)	3423, 2928
5c	$(CH_2)_2CH_3$	0.75	0.92 (t, J=7.3 Hz)	2.85 (m)	7.14 (d, J=8.6 Hz)	6.61 (dd, J=8.6, 2.3 Hz, 2-H) 6.75 (d, J=2.3 Hz, 4-H)	281.2 (2250)	3414, 2955
5d	$(CH_2)_4CH_3$	0.75	0.88 (t, J=6.6 Hz)	2.85 (m)	7.14 (d, J=8.6 Hz)	6.62 (dd, J=8.6, 2.3 Hz, 2-H) 6.74 (d, J=2.3 Hz, 4-H)	281.2 (2380)	3412, 2926
5e	$(CH_2)_6CH_3$	0.75	0.88 (t, J=6.6 Hz)	2.84 (m)	7.14 (d, J=8.6 Hz)	6.62 (dd, J=8.4, 2.3 Hz, 2-H) 6.74 (d, J=2.3 Hz, 4-H)	280.9 (2210)	3418, 2927
6β-Series								
6a	CH_3	0.80	1.29 (d, J=7.3 Hz)	2.92 (m)	7.14 (d, J=9.6 Hz)	6.61—6.64 (2H, m, 2-H and 4-H)	280.8 (2100)	3309, 2958
6b	CH_2CH_3	0.79	1.00 (t, J=7.4 Hz)	2.61 (m)	7.14 (d, J=9.6 Hz)	6.61—6.64 (2H, m, 2-H and 4-H)	280.8 (2060)	3424, 2925
6c	$(CH_2)_2CH_3$	0.80	0.95 (t, J=7.3 Hz)	2.71 (m)	7.14 (d, J=9.6 Hz)	6.60—6.65 (2H, m, 2-H and 4-H)	281.2 (2060)	3402, 2927
6d	$(CH_2)_4CH_3$	0.80	0.91 (t, J=6.6 Hz)	2.69 (m)	7.14 (d, J=9.2 Hz)	6.60—6.65 (2H, m, 2-H and 4-H)	281.2 (2340)	3400, 2927
6e	$(CH_2)_6CH_3$	0.80	0.90 (t, J=6.3 Hz)	2.69 (m)	7.14 (d, J=9.2 Hz)	6.60—6.63 (2H, m, 2-H and 4-H)	281.2 (2200)	3400, 2926

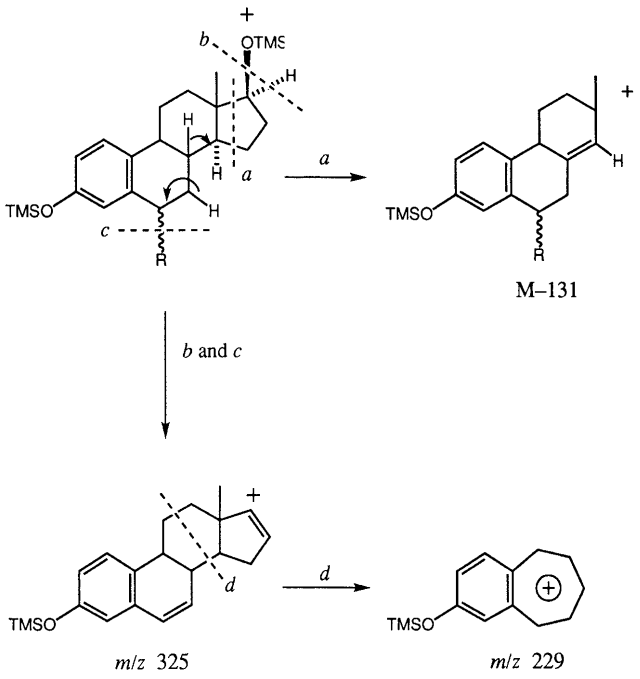

Chart 2

Table 3. Gas Chromatographic Retention Times for 6α- and 6β-Alkylestradiols **5** and **6**

R	Retention time, t_R, min	
	5 (6α)	**6** (6β)
Estradiol, H	15.0	—
a, CH_3	15.1	14.9
b, C_2H_5	15.9	15.7
c, n-C_3H_7	16.8	16.4
d, n-C_5H_{11}	20.1	19.5
e, n-C_7H_{15}	24.5	23.5

Table 4. Fragmentation of 6-Alkylestradiols (**5** and **6**) by Electron Impact Mass Spectrometry

Compounds	Fragment ion, m/z (relative abundance)[a]		
	M^+	M^+-131	Others
6α-Methyl **5a**	430 (100)	299 (100)	129 (28), 73 (60)
6β-Methyl **6a**	430 (100)	299 (43)	129 (10), 73 (10)
6α-Ethyl **5b**	444 (100)	313 (28)	325 (23), 207 (28)
6β-Ethyl **6b**	444 (100)	313 (19)	229 (11), 73 (10)
6α-n-Propyl **5c**	458 (100)	327 (66)	325 (68), 207 (31), 73 (70)
6β-n-Propyl **6c**	458 (100)	327 (66)	325 (68), 229 (28), 73 (68)
6α-n-Pentyl **5d**	486 (100)	355 (30)	207 (35), 147 (42), 73 (43)
6β-n-Pentyl **6d**	486 (100)	355 (45)	207 (26), 73 (32)
6α-n-Heptyl **5e**	514 8100)	383 (29)	325 (51), 73 (53)
6β-n-Heptyl **6e**	514 (100)	383 (29)	325 (41), 73 (59)

a) Fragment ions with more than 10% of relative intensity to the base peak were listed.

Electron impact mass spectra of the bis-TMS derivatives of all the 6-alkylestradiols (**5** and **6**) showed a molecular ion (M^+) as the base peak with two characteristic ions of M^+-131 and m/z 325 (Table 4). The fragment M^+-131 can be derived from the cleavage of the C(13)–C(17) and C(14)–C(15) bonds of the bis-TMS derivatives (*a*, Chart 2) followed by deprotonation at the C-8 position, as previously reported for the bis-TMS derivative of estradiol.[13] The fission of C–H and C–O bonds at the C-17 position (*b*), together with a C(6)–C(6^1) bond (*c*), as well as deprotonation at the C-7 position, may produce an other ion at m/z 325 which, when further fragmented, causes the rupture of the vinylogous benzylic C(8)–C(14) bond, followed by a second benzylic cleavage to furnish the siloxybenztropylium ion (m/z 229).[14] Using a selected ion monitoring (SIM) method with the M^+, we could quantitate the TMS

derivatives as low as 50 pg, respectively ($S/N > 10$). This sensitivity is sufficient for the detection of the aromatization reaction of the 6-alkylandrogens. Study of the aromatization reaction using this GC-MS method is now underway in our laboratory.

Experimental

Melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 1725X spectrophotometer and UV spectra in 95% EtOH solution on a Hitachi 150-20 spectrophotometer. ^{1}H-NMR spectra were obtained in $CDCl_3$ solution with JEOL EX 270 (270 MHz) spectrometers using tetramethylsilane as an internal standard, and MS (HR-MS) with a JEOL JMS-DX 303 spectrometer. GC-MS was carried out with a Finnigan MAT SSQ GC-MS instrument. TLC was performed on E. Merck pre-coated silica gel plates. Column chromatography was conducted with silica gel (E. Merck, 70—230 mesh). HPLC was carried out using a Waters Model 510 pump, YMC Pack-D-ODS-5 column (250 mm × 20 mm i.d.), and a UV detector (280 nm). Grignard reagents in THF solutions were purchased from Aldrich Chemical Co. Bis(trimethylsilyl)trifluoroacetamide (BSTFA) was obtained from Tokyo Kasei Kogyo Co. 6-Oxoestradiol was synthesized according to the method previously reported by Akanni and Marples.[15]

Grignard Reactions of 6-Oxoestradiol (1) 30 molar eq. of Grignard reagent (RMgBr: R = methyl, ethyl, *n*-propyl, *n*-pentyl, or *n*-heptyl) in THF (68 ml) was added to a solution of compound **1** (2.3 mmol) in THF (16 ml), and the mixture was heated under reflux for about 30 h in an N_2 stream. After the solution was cooled, saturated NH_4Cl solution (200 ml) was added and the product was extracted with AcOEt (250 ml × 2). The combined organic layer was washed with saturated $NaHCO_3$ solution and water to neutrality, dried with Na_2SO_4, and evaporated to dryness leaving a residue which was purified by column chromatography (hexane–AcOEt) to give 6-alkylestra-1,3,5(10)-triene-3,6,17β-triol (**2**) consisting of two 6α- and 6β-alkyl stereoisomers as a solid or oil (yield: methyl, 45%; ethyl, 43%; *n*-propyl, 38%; *n*-pentyl, 32%; *n*-heptyl, 35%). The isomers could not be separated by HPLC, therefore, compound **2** was used without further purification for the next step reaction.

Dehydration of the 6-Alkyl-6-ols 2 with HCl 0.3 M HCl (2.3 ml, 0.7 mmol) was added to a stirred solution of compound **2** (0.94 mmol) in 95% EtOH (20 ml) and the mixture was stirred at room temperature for 24 h. After this time, 0.1 M NaOH solution (6 ml) was added to the mixture and the solvent was concentrated under reduced pressure, diluted with AcOEt (100 ml), washed with water to neutrality, and dried with Na_2SO_4. Evaporation of the solvent gave an oily product which was purified by column chromatography to afford a mixture of 6-alkylestra-1,3,5(10),6-tetraene-3,17β-diol (**3**) and 6,6-alkylmethyleneestra-1,3,5(10)-triene-3,17β-diol (**4**), except the reaction of the 6-methyl compound **2a**, which gave only the 6-ene steroid **3a** (yield: methyl, 68%; ethyl, 71%; *n*-propyl, 66%; *n*-pentyl, 75%; *n*-heptyl, 86%). The mixtures obtained from the 6-ethyl and 6-*n*-propyl steroid **2b** and **2c** were respectively separated by reverse-phase HPLC [solvent, MeCN–H_2O (60 : 40, v/v), 5 ml/min for the separation of **2b** and MeCN–H_2O (65 : 35, v/v), and 5 ml/min for the separation of **2c**]. ^{1}H-NMR spectrum of the mixture produced from the 6-*n*-pentyl derivative **2d** revealed it to be a 6 : 1 mixture of the 6-ene **3d** and the 6,6-methylene analog **4c** [**3d**: δ 0.77 (s, 18-Me), 3.76 (t, J = 8.4 Hz, 17α-H), 5.76 (s, 7-H), 6.67 (dd, J = 2.6, 8.3 Hz, 2-H), 6.79 (d, J = 2.6 Hz, 4-H), 7.11 (d, J = 8.3 Hz, 1-H); **4c**: δ 0.76 (s, 18-Me), 3.74 (t, J = 8.4 Hz, 17α-H), 5.94 (m, C=C<u>H</u>CH$_2$–), 6.67 (dd, J = 2.6, 8.3 Hz, 2-H), 7.03 (d, J = 2.6 Hz, 4-H), 7.14 (d, J = 8.3 Hz, 1-H)], and the spectrum of the mixture from the 6-*n*-heptyl compound, **2e**, revealed it to be a 3 : 1 mixture of the 6-ene **3e** and the 6,6-methylene analog **4d** [**3e**: δ 0.77 (s, 18-Me), 3.76 (t, J = 8.4 Hz, 17α-H), 5.75 (s, 7-H), 6.67 (dd, J = 2.6, 8.3 Hz, 2-H), 6.78 (d, J = 2.6 Hz, 4-H), 7.12 (d, J = 8.3 Hz, 1-H); **4d**: δ 0.76 (s, 18-Me), 3.74 (t, J = 8.4 Hz, 17α-H), 5.95 (m, C=C<u>H</u>CH$_2$–), 6.67 (dd, J = 2.6, 8.1 Hz, 2-H), 7.03 (d, J = 2.4 Hz, 4-H), 7.15 (d, J = 8.1 Hz, 1-H)]. The other mixtures were used, without further purification, for the next step reaction.

6-Methylestra-1,3,5(10),6-tetraene-3,17β-diol (3a) Yield: 68% (oil). ^{1}H-NMR δ: 0.77 (3H, s, 18-Me), 3.75 (1H, t, J = 8.4 Hz, 17α-H), 5.78 (1H, s, 7-H), 6.68 (1H, dd, J = 2.6, 8.3 Hz, 2-H), 6.77 (1H, d, J = 2.6 Hz, 4-H), 7.12 (1H, d, J = 8.3 Hz, 1-H). FT-IR (neat): 3352, 2938 (OH) cm^{-1}. UV λ_{max}: 261 (ε = 8400), 300 (ε = 4000) nm. HR-MS m/z: Calcd for $C_{19}H_{24}O_2$ (M^+), 284.17760; found, 284.1777.

6-Ethylestra-1,3,5(10),6-tetraene-3,17β-diol (3b) Yield: 58% (oil). HPLC: t_R = 30.6 min. ^{1}H-NMR δ: 0.77 (3H, s, 18-Me), 1.13 (3H, t, J = 7.4 Hz, 6-CH$_2$<u>Me</u>), 5.76 (1H, s, 7-H), 6.67 (1H, dd, J = 2.6, 8.3 Hz, 2-H), 6.80 (1H, d, J = 2.6 Hz, 4-H), 7.13 (1H, d, J = 8.3 Hz, 1-H). FT-IR (neat): 3414, 2936 (OH) cm^{-1}. UV λ_{max}: 261 (ε = 7200), 303 (ε = 3000) nm. HR-MS m/z: Calcd for $C_{20}H_{26}O_2$ (M^+), 298.19330; found, 298.1920.

6-*n*-Propylestra-1,3,5(10),6-tetraene-3,17β-diol (3c) Yield: 53%. mp 160—162 °C. HPLC: t_R = 32.0 min. ^{1}H-NMR δ: 0.77 (3H, s, 18-Me), 0.93 (3H, t, J = 7.3 Hz, 6-CH$_2$CH$_2$<u>Me</u>), 3.76 (1H, t, J = 8.4 Hz, 17α-H), 5.76 (1H, s, 7-H), 6.67 (1H, dd, J = 2.6, 8.3 Hz, 2-H), 6.79 (1H, d, J = 2.6 Hz, 4-H), 7.13 (1H, d, J = 8.3 Hz, 1-H). FT-IR (KBr): 3405, 2955 (OH) cm^{-1}. UV λ_{max}: 262 (ε = 6900), 304 (ε = 2700) nm. *Anal.* Calcd for $C_{21}H_{28}O_2$: C, 80.73; H, 9.03. Found: C, 80.79; H, 8.86.

6,6-Ethyleneestra-1,3,5(10)-triene-3,17β-diol (4a) Yield: 13% (oil). HPLC: t_R = 28.9 min. ^{1}H-NMR δ: 0.76 (3H, s, 18-Me), 1.76 (3H, d, J = 6.9 Hz, C=CH<u>Me</u>), 3.74 (1H, t, J = 8.4 Hz, 17α-H), 6.05 (1H, m, C=C<u>H</u>Me), 6.67 (1H, dd, J = 2.6, 8.5 Hz, 2-H), 7.02 (1H, d, J = 2.6 Hz, 4-H), 7.15 (1H, d, J = 8.5 Hz, 1-H). FT-IR (neat): 3400, 2980 (OH) cm^{-1}. UV λ_{max}: 260 (ε = 10500), 302 (ε = 4300) nm. HR-MS m/z: Calcd for $C_{20}H_{26}O_2$ (M^+), 298.19330; found, 298.1922.

6,6-*n*-Propyleneestra-1,3,5(10)-triene-3,17β-diol (4b) Yield: 13%. mp 112—115 °C. HPLC: t_R = 30.5 min. ^{1}H-NMR δ: 0.76 (3H, s, 18-Me), 1.06 (3H, t, J = 7.6 Hz, 6-CHCH$_2$<u>Me</u>), 3.74 (1H, t, J = 8.2 Hz, 17α-H), 5.76 (1H, s, 7-H), 6.67 (1H, dd, J = 2.6, 8.3 Hz, 2-H), 5.94 (1H, dd, J = 5.4, 8.1 Hz, C=C<u>H</u>CH$_2$Me), 7.05 (1H, d, J = 2.6 Hz, 4-H), 7.14 (1H, d, J = 8.3 Hz, 1-H). FT-IR (KBr): 3403, 2960 (OH) cm^{-1}. UV λ_{max}: 259 (ε = 10800), 303 (ε = 4600) nm. *Anal.* Calcd for $C_{21}H_{28}O_2$: C, 80.77; H, 8.97. Found: C, 80.75; H, 9.11.

Catalytic Hydrogenation of the 6-Ene Steroids 3 5% Pd–C (80 mg) was added separately to a solution of the 6-ene steroids **3a**—**c** or the mixtures of the 6-ene steroids **3d** and **3e** and the 6,6-methylene derivatives **4c** and **4d** (*ca.* 0.54 mmol) in dry EtOH (15 ml). The mixture was vigorously stirred under H_2 for 2 to 3 h. The catalyst was removed by filtration and the filtrate was evaporated to give a mixture of 6α- and 6β-alkyl steroids, **5** and **6**, which was purified by column chromatography (hexane–AcOEt) followed by reverse-phase HPLC. Conditions: MeCN–H_2O (50 : 50, v/v), 5 ml/min for **5a** (t_R = 44.3 min) and **6a** (t_R = 41.4 min); MeCN–H_2O (70 : 30, v/v), 5 ml/min for **5b** (t_R = 23.0 min) and **6b** (t_R = 21.4 min) and for **5c** (t_R = 29.0 min) and **6c** (t_R = 26.5 min); MeCN–H_2O (80 : 20, v/v), 5 ml/min for **5d** (t_R = 32.0 min) and **6d** (t_R = 29.2 min) and 7 ml/min for **5e** (t_R = 42.2 min) and **6e** (t_R = 35.2 min).

GC-MS A Finnigan MAT SSQ GC-MS instrument was used. Gas chromatographic conditions: column, 30 m × 0.250 mm i.d. fused silica DB5 (J & W Scientific, CA, U.S.A.); column temperature, from 50 °C at 25 °C/min to 250 °C and then at 10 °C/min to 280 °C; carrier gas, He at a flow rate of 60 ml/min. Mass spectrometric conditions: ionization energy, 70 eV; ion source temperature, 150 °C.

Derivatization of the 6-Alkylestradiols 5 and 6 with BSTFA BSTFA (30 μl) was added separately to a solution of the estradiols **5** and **6** in dry pyridine (30 μl). The mixture was heated at 60 °C for 30 min and then the solvent was removed under a stream of N_2. The residue was dissolved in anhydrous hexane (25 μl), and 2 μl portions of the solution were subjected to analysis.

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