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Carbon-13 Nuclear Magnetic Resonance Study of *meso*-Hexestrol and Its Derivatives

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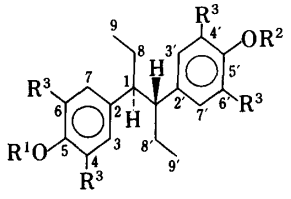
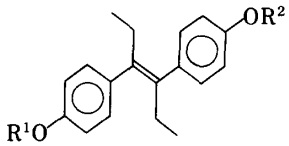
The carbon-13 nuclear magnetic resonance chemical shift assignments for *meso*-hexestrol (**1a**), made on the basis of two-dimensional ^{13}C–^{1}H chemical shift correlation, long-range selective ^{1}H decoupling experiment, and a reported two-dimensional Fourier-transform experiment for long-range proton–carbon-13 spin coupling constants, are reported. For measurement of carbon–proton coupling constants of *meso*-hexestrol derivatives (**1b—e**, **2a—d**, **3a—c**, and **4**), the coupling information was detected by using a gated decoupling facility which permitted retention of the nuclear Overhauser enhancement and a long-range selective ^{1}H decoupling experiment. The results showed that the aromatic carbon resonances are influenced by the structure (no double bond, or one or two double bond(s)) of the hexane framework in the central portion.

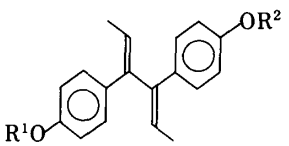
Keywords——hexestrol derivative; ^{13}C-NMR; ^{1}H-NMR *meso*-hexestrol; diethylstilbestrol; dienestrol; isodienestrol

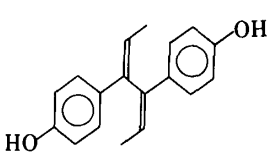
meso-Hexestrol (**1a**) is one of the first active synthetic estrogens, and diethylstilbestrol (DES) (**2a**) and dienestrol (**3a**) are frequently used as synthetic estrogens in the clinic. In the previous paper,[1] we presented direct evidence that DES is active in inhibiting microtubule assembly *in vitro*. Sharp and Parry[2] and Hartley-Asp *et al.*[3] also reported the effects of DES on microtubules. Recently we described the structure–activity relationship of *meso*-hexestrol (**1a**), dienestrol (**3a**), isodienestrol (**4**), *dl*-hexestrol, and their methyl ether derivatives, for inhibition of microtubule assembly *in vitro*, and electron microscopic observation revealed that twisted ribbon structures are formed from microtubule proteins in the presence of some synthetic estrogens (**1a**, **3a**, and *dl*-hexestrol).[4]

Since no carbon-13 nuclear magnetic resonance (^{13}C-NMR) study of *meso*-hexestrol and its analogues has yet been done, we report here the assignment of the ^{13}C-NMR spectra of these compounds as a basis for investigations of the interaction with target molecules such as microtubules. The ^{13}C-NMR assignments for *meso*-hexestrol (**1a**), made on the basis of two-dimensional ^{13}C–^{1}H chemical shift correlation and a reported two-dimensional Fourier-









1a: $R^1=R^2=R^3=H$
1b: $R^1=R^2=H$, $R^3=^2H$
1c: $R^1=Me$, $R^2=R^3=H$
1d: $R^1=R^2=Me$, $R^3=H$
1e: $R^1=R^2=Ac$, $R^3=H$

2a: $R^1=R^2=H$
2b: $R^1=Me$, $R^2=H$
2c: $R^1=R^2=Me$
2d: $R^1=R^2=Ac$

3a: $R^1=R^2=H$
3b: $R^1=Me$, $R^2=H$
3c: $R^1=R^2=Me$

4

Chart 1

transform experiment for long-range proton–carbon-13-spin coupling constants, are reported. Moreover, ^{13}C-NMR chemical shift assignments for *meso*-hexestrol derivatives (**1b**—**e**, **2a**—**d**, **3a**—**c**, and **4**) are determined. For measurement of carbon–proton coupling constants, the coupling information was detected by using a gated decoupling facility which permitted retention of the nuclear Overhauser enhancement (NOE) and a long-range selective ^{1}H decoupling experiment.

Results and Discussion

Determination of Chemical Shifts of *meso*-Hexestrol (1a)

The conformational analysis[5] of *meso*-hexestrol has been studied on the basis of proton nuclear magnetic resonance spectroscopy (^{1}H-NMR) and force field calculations. Since individual carbon resonances can be correlated with the corresponding proton resonances, it is desirable to study exactly the ^{1}H-NMR spectrum of *meso*-hexestrol.

In this study, we have achieved the complete signal assignment for *meso*-hexestrol based on the chemical shift, multiplicity, coupling constant, and selective proton decoupling data. The carbon numbering of all of the synthetic estrogens was according to Smiley and Rossmann.[6] Thus, the ^{1}H-NMR spectrum of **1a** showed signals due to the 9,9′-methyl groups (0.51 ppm), methylene protons (1.25 and 1.40 ppm), 1,1′-methine protons (2.49 ppm), and the eight aromatic protons (6.80 and 7.05 ppm). The signals at 1.25 and 1.40 ppm were tentatively assigned to 8,8′-*pro*-*S*- and 8,8′-*pro*-*R*-protons, respectively, by selective proton decoupling, on the basis of the conformational analysis[5] and examination of the Büchi model, with reference to the results of X-ray analysis[6,7] of DES. Aromatic protons were assigned by comparison with those of [4,4′,6,6′-$^2H_4$]*meso*-hexestrol (**1b**).[8] The ^{1}H-NMR spectrum of **1b** was identical with that of **1a**, except that the signal at 6.80 ppm disappeared and the signal at 7.05 ppm turned into a broad singlet. Thus, the signals at 6.80 and 7.05 ppm of **1b** were confirmed to be due to the 4,4′,6,6′- and 3,3′,7,7′-protons, respectively.

On the other hand, the ^{13}C–^{1}H shift correlation of *meso*-hexestrol was determined (data not shown). The ^{13}C chemical shifts (Table I) and one-bond ^{13}C–^{1}H coupling constants were determined. Moreover, in order to confirm the ^{13}C assignments, we attempted a ^{13}C-NMR long-range selective proton decoupling (LSPD) experiment[9] (Table II). We also utilized reported two-dimensional Fourier-transform data[10] (not shown).

The ^{1}H-NMR Spectra of *meso*-Hexestrol Derivatives

The structures of DES (**2a**)[6,7] and dienestrol (**3a**)[11] have been reported by Smiley and Rossmann,[6] Hospital *et al.*,[7] and Doyle *et al.*[11] The proton chemical shifts of *meso*-hexestrol derivatives are described in the experimental section. The signals of the methylene protons at the 8,8′-positions of *meso*-hexestrol (**1a**) and its derivatives (**1b**—**e**) appeared at different regions as described above, but the corresponding 8,8′-proton signals of DES (**2a**) and its derivatives (**2b**—**d**) appeared without any separation in their ^{1}H-NMR spectra. The chemical shifts of aromatic protons of **1a** and **2a** were observed at similar values, but those of the acetylated and methyl ether derivatives appeared at lower field (*ca.* 0.1 ppm) than those of the corresponding mother compounds (**1a** and **2a**). On the other hand, comparison of the ^{1}H-NMR spectra of dienestrol (**3a**) and isodienestrol (**4**) revealed that the signal patterns were identical but their chemical shifts were considerably different, and the 8,8′-proton signals of **4** appeared at lower field (0.91 ppm) than those of **3a**. The ^{1}H chemical shifts of the monomethyl derivatives (**1c**, **2b**, and **3b**) were assigned by comparison with those of hydroxy and dimethyl derivatives (**1a**, **2a**, **3a**, and **1d**, **2c**, **3c**, respectively).

The ^{13}C-NMR Spectra of *meso*-Hexestrol Derivatives

The ^{13}C chemical shifts of *meso*-hexestrol derivatives are summarized in Table I. A

comparison of the chemical shifts of aromatic carbons of **1a**, **2a**, **3a**, and **4** revealed that the chemical shifts of C-3,3′,7,7′ differed from one another but those of C-4,4′,6,6′ were almost the same. On the other hand, the results of LSPD experiments proved that the related compounds have almost the same coupling constants. Acetylation (**1e** and **2d**) of phenolic groups caused downfield shifts (*ca.* 6.6 ppm) at C-4,4′,6,6′ which are alpha from the phenolic carbon, but no shift was observed at C-3,3′,7,7′ which are in the beta positions with respect to the phenolic carbon. Methylation (**1c**, **1d**, **2b**, **2c**, **3b**, and **3c**) of phenolic groups caused *ca.* 1.5 ppm upfield shift at the alpha carbons but no shift was observed at the beta carbons. It is of interest that the chemical shifts at C-1,1′ of DES (**2a**), dienestrol (**3a**) and isodienestrol (**4**) are 134.5, 146.5 and 139.6 ppm, respectively. This result demonstrates that the difference of molecular geometry (*trans–cis*) produces a difference (6.7 ppm) in chemical shifts as observed in the latter two compounds (**3a** and **4**), brought about by the steric interactions. Further, comparison of the chemical shifts at C-2,2′ provided the interesting information that the signal of **2a** appears at the lowest field (139.5 ppm) followed by **1a** (136.2 ppm) and the dienes **4** and **3a** (132.6 and 131.6 ppm, respectively), indicating that the presence of a double bond at the 1,1′-position in **2a** is associated with an increase in the deshielding, whereas that of the diene in **4** and **3a** is associated with an increase in the shielding. The assignment of ^{13}C chemical shifts of the monomethyl derivatives (**1c**, **2b**, and **3b**) was determined by comparison with those of the hydroxy and dimethyl derivatives (**1a**, **2a**, **3a**, and **1d**, **2c**, **3c**, respectively).

The synthetic estrogen analogues described here are classified into (A) *meso*-hexestrols which have no double bond at the 1,1′-position, (B) DESs which have one double bond at the 1,1′-position, and (C) dienestrols and isodienestrol which have two double bonds at the 1,8- and 1′,8′-positions. The one-bond ^{13}C–^{1}H coupling constant values ($^{1}J_{CH}$) at the C-9,9′ positions were almost the same (124.6–126.5 Hz) in the all compounds examined. The $^{1}J_{CH}$ at C-8,8′ were within the ranges of 124.6—126.7 Hz in the A and B groups and of 152.1—154.0 Hz in the C group. The $^{1}J_{CH}$ at the 3,3′, 7,7′-positions were within the range of 155.8—160.8 Hz in all the groups of compounds, and the $^{1}J_{CH}$ at the 4,4′,6,6′-positions were within the ranges of 156.7—158.5, 159.5—159.9, and 162.7—163.1 Hz in the phenols (**1a**, **2a**, **3a**, and

TABLE I. ^{13}C-NMR Spectral Data for *meso*-Hexestrol (**1a**) and Its Derivatives (**1c—e**, **2a—d**, **3a—c**, and **4**)

Carbon	Chemical shift (ppm)											
	1a	**1c**	**1d**	**1e**	**2a**	**2b**	**2c**	**2d**	**3a**	**3b**	**3c**	**4**
C-1	54.2	54.2	54.2	54.2	134.5	135.6	135.6	140.4	146.4	146.2	146.2	139.6
C-1′	54.2	54.2	54.2	54.2	134.5	134.3	135.6	140.4	146.4	146.4	146.2	139.6
C-2	136.2	137.4	137.3	142.5	139.5	139.3	139.6	139.7	131.6	132.7	132.7	132.6
C-2′	136.2	136.0	137.3	142.5	139.5	139.7	139.6	139.7	131.6	132.7	132.7	132.6
C-3,3′	129.9	129.9	129.9	129.9	130.4	130.4	130.5	130.3	131.6	131.6	131.6	127.8
C-4	115.8	114.4	114.4	122.3	115.7	114.2	114.3	122.3	115.7	114.2	114.3	115.9
C-4′	115.8	116.4	114.4	122.3	115.7	115.7	114.3	122.3	115.7	115.7	114.3	115.9
C-5	156.3	158.9	159.0	150.2	156.7	159.1	159.2	150.5	156.8	159.3	159.4	157.3
C-5′	156.3	155.9	159.0	150.2	156.7	156.7	159.2	150.5	156.8	156.9	159.4	157.3
C-6	115.8	114.4	114.4	122.3	115.7	114.2	114.3	122.3	115.7	114.2	114.3	115.9
C-6′	115.8	115.9	114.4	122.3	115.7	115.7	114.3	122.3	115.7	115.7	114.3	115.9
C-7,7′	129.9	129.9	129.9	129.9	130.4	130.4	130.5	130.3	131.6	131.6	131.6	127.8
C-8,8′	28.1	28.1	28.1	28.0	29.1	29.1	29.2	29.1	124.9	125.1	125.3	122.0
5-OCH_3	—	55.3	54.9	—	—	54.9	54.9	—	—	54.9	54.9	—
5′-OCH_3	—	—	55.4	—	—	—	55.4	—	—	—	55.4	—
5,5′-$OCOCH_3$	—	—	—	169.6	—	—	—	169.6	—	—	—	—
5,5′-$OCOCH_3$	—	—	—	21.0	—	—	—	21.0	—	—	—	—
9,9′-CH_3	12.5	12.5	12.5	12.4	13.6	13.6	13.6	13.5	15.3	15.3	15.3	15.3

TABLE II. Fine Splitting Patterns and Long-Range ^{13}C–^{1}H Coupling Constants (Hz) of *meso*-Hexestrol (**1a**) and Its Derivatives (**1d**, **1e**, **2a**—**d**, **3a**, **3c**, and **4**)

Carbon		**1a**	**1d**	**1e**	**2a**	**2c**	**2d**	**3a**	**3c**	**4**
		br d	br d	br d	m	m	m	br s	m	br s
C-1,1′	^{2}J (C-1,1′, H-8,8′)	—	—	—	—	—	4.1	—	—	—
	^{3}J (C-1,1′, H-3,3′,7,7′)	—	—	—	6.9	7.5	10.5	2.8	—	6.9
		m	m	m	br s	br s	br s	dd	dd	dd
C-2,2′	^{3}J (C-2,2′, H-8,8′)	7.3	7.3	7.3	10.1	9.4	10.1	10.0	10.1	13.7
	^{3}J (C-2,2′, H-4,4′,6,6′)	7.3	7.3	7.3	4.6	4.7	4.6	7.3	7.3	7.3
		ddd	br dd	ddd	dd	dd	dd	dd	dd	dd
C-3,3′,7,7′	^{2}J (C-3,3′,7,7′, H-4,4′,6,6′)	7.3	7.3	7.3	7.3	7.3	7.8	7.3	7.3	7.3
	^{3}J (C-3,3′,7,7′, H-1,1′)	3.7	2.3	4.6	—	—	—	—	—	—
		dd	dd	dd	dd	dd	dd	dd	dd	dd
C-4,4′,6,6′	^{2}J (C-4,4′,6,6′, H-3,3′,7,7′)	3.4	5.0	5.0	5.0	4.6	4.6	4.6	4.6	4.6
		br dd	m	dddd	dddd	m	dddd	dddd	m	dddd
C-5,5′	^{2}J (C-5,5′, H-4,4′,6,6′)	2.8	2.8	3.7	2.8	4.4	3.7	2.8	2.8	2.8
	^{2}J (C-5,5′, H-5,5′-OCH_3)	—	—	—	—	4.6	—	—	4.6	—
	^{3}J (C-5,5′, H-3,3′,7,7′)	9.2	9.2	10.1	9.6	9.7	10.1	9.2	9.2	9.6
		br dt	br dt	br dt	tq	tq	tq	dq	dq	dq
C-8,8′	^{2}J (C-8,8′, H-9,9′-CH_3)	2.8	2.8	3.7	5.0	4.1	4.6	7.3	7.3	7.3
		dtq	dtq	dtq	tq	tq	tq	dq	dq	dq
C-9,9′	^{2}J (C-9,9′, H-8,8′)	2.8	3.7	3.7	4.1	5.0	4.1	4.6	4.6	4.1
		—	—	q	—	—	q	—	—	—
5,5′-$OCOCH_3$	^{2}J (C-5,5′-$OCOCH_3$, H-5,5′-$OCOCH_3$)	—	—	7.3	—	—	7.3	—	—	—

4), the dimethyl ethers (**1d**, **2c**, and **3c**), and the acetates (**1e** and **2d**), respectively.

The present results should be useful for further studies of the interaction of synthetic estrogens with tubulin and/or microtubules and also for the structural elucidation of metabolites of synthetic estrogens.

Experimental

All melting points were obtained on a Shimadzu MM2 micro-melting point apparatus and are uncorrected. ^{1}H-NMR spectra were obtained at 270 MHz on a JEOL JNM-GX 270 FT NMR spectrometer. All ^{1}H-NMR data were recorded in deuterioacetone and are reported as parts per million downfield from Me_4Si ($\delta=0$). ^{13}C-NMR spectra were determined at 67.8 MHz using a JEOL JNM-GX 270 FT NMR spectrometer with 32 k data points for acquisition of free induction decays. For measurement of carbon–proton coupling constants, the coupling information was retained by using a gated decoupling facility which permitted retention of the NOE. The ^{13}C-NMR spectra for *meso*-hexestrol derivatives were obtained in deuterioacetone. Spectra were referenced to the solvent signal, known separations from Me_4Si being employed in order to present chemical shift data in the conventional manner. Abbreviations used: s=singlet, d=doublet, t=triplet, br=broad, m=multiplet, dd=doublet of doublets, q=quartet. Mass spectra (MS) were recorded on a JEOL D-100 spectrometer at 75 eV ionizing potential.

***meso*-Hexestrol, DES and Dienestrol**——*meso*-Hexestrol (**1a**) was obtained from Wako Pure Chemical Industries Ltd. (Osaka). DES (**2a**) and dienestrol (**3a**) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo) and were checked for the purity by ^{1}H-NMR spectroscopy. **1a**: ^{1}H-NMR δ (ppm): 0.51 (6H, t, $J=7.3$ Hz, 9,9′-CH_3), 1.25 (2H, dqd, $J=14.2$, 7.3, and 4.0 Hz, 8.8′ *pro-S*-H), 1.40 (2H, dqd, $J=14.2$, 7.3, and 3.3 Hz, 8.8′ *pro-R*-H), 2.49 (2H, m, 1,1′-H), 6.80 (4H, br d, $J=6.9$ Hz, 4,4′,6,6′-H), 7.05 (4H, br d, $J=6.7$ Hz, 3,3′,7,7′-H), 8.06 (2H, br s, 5,5′-OH). **2a**: ^{1}H-NMR δ (ppm): 0.76 (6H, t, $J=7.3$ Hz, 9,9′-CH_3), 2.14 (4H, q, $J=7.3$ Hz, 8,8′-H), 6.86 (4H, br d, $J=8.7$ Hz, 4,4′,6,6′-H), 7.05 (4H, br d, $J=8.7$ Hz, 3,3′,7,7′-H), 8.25 (2H, br s, 5,5′-OH). **3a**: ^{1}H-NMR δ(ppm): 1.46 (6H, d, $J=6.6$ Hz, 9,9′-CH_3), 5.29 (2H, q, $J=6.6$ Hz, 8,8′-H), 6.84 (4H, br d, $J=8.8$ Hz, 4,4′,6,6′-H), 6.98 (4H, br d, $J=8.8$ Hz, 3,3′,7,7′-H), 8.29 (2H, br s, 5,5′-OH).

[4,4′,6,6′-$^{2}H_4$]*meso*-Hexestrol (1b)[8]——*meso*-Hexestrol (600 mg) was dissolved in a mixture of 1.5 ml of deuterium chloride/deuterium oxide (20 wt.% solution in D_2O, 99 atom%) and 5.1 ml of methanol-*d* (CH_3OD, 99.5% atom% D). The mixture was heated to 110 °C for 2 d. After the mixture had cooled to room temperature, the solvents

were evaporated off in a stream of nitrogen, the residue was dried *in vacuo*, and the deuteration procedure was repeated. After evaporation of the solvents and drying, the deuterated hexestrol was recrystallized from methanol as colorless needles, mp 187—188 °C. MS: $^1H_0$ 1%, $^2H_4$ 99%. ^{1}H-NMR δ (ppm): 0.51 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.25 (2H, dqd, J=14.2, 7.3, 4.0 Hz, 8,8′ *pro-S*-H), 1.40 (2H, dqd, J=14.2, 7.3, 3.3 Hz, 8,8′ *pro-R*-H), 2.49 (2H, m, 1,1′-H), 7.05 (4H, br s, 3,3′,7,7′-H), 8.06 (2H, br s, 5,5′-H).

Mono- and Dimethyl Ether Derivatives of *meso*-Hexestrol, DES, and Dienestrol——*meso*-Hexestrol monomethyl ether (**1c**) was prepared by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as small white needles, mp 119.5—120.5°C (lit.,[12] mp 118.5—120 °C). MS *m/z*: 284 (M^+), 149 (base peak), 135, 131, 107. *Anal.* Calcd for $C_{19}H_{24}O_2$: C, 80.24; H, 8.51. Found: C, 79.89; H, 8.84. ^{1}H-NMR δ (ppm): 0.51 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.25—1.45 (4H, m, 8,8′-H), 2.55 (2H, m, 1,1′-H), 3.79 (3H, s, 5-OCH_3), 6.81 (2H, br d, J=7.6 Hz, 4′,6′-H), 6.89 (2H, br d, J=7.6 Hz, 4, 6-H), 7.06 (2H, br d, J=7.6 Hz, 3′,7′-H), 7.15 (2H, br d, J=7.6 Hz, 3,7-H), 8.12 (1H, br s, 5′-OH).

meso-Hexestrol dimethyl ether (**1d**) was prepared, with some modification, by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as colorless needles, mp 144.5—145.0 °C. MS *m/z*: 298 (M^+), 149 (base peak), 121. *Anal.* Calcd for $C_{20}H_{26}O_2$: C, 80.49; H, 8.78. Found: C, 80.69; H, 8.68. ^{1}H-NMR δ (ppm): 0.51 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.20—1.46 (4H, m, 8,8′-H), 2.55 (2H, m, 1,1′-H), 3.79 (6H, s, 5,5′-OCH_3), 6.89 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.16 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H).

DES monomethyl ether (**2b**) was prepared by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as small white needles, mp 114–116.5 °C (Lit.,[13] 112—114 °C and 116—117.5 °C). MS *m/z*: 282 (M^+, base peak), 267, 253, 238, 159, 121, 107. *Anal.* Calcd for $C_{19}H_{22}O_2$: C, 80.81; H, 7.55. Found: C, 81.13; H, 7.98. ^{1}H-NMR δ (ppm): 0.76 (6H, t, J=7.3 Hz, 9,9′-CH_3), 2.13 (2H, q, J=7.3 Hz, 8′-H), 2.16 (2H, q, J=7.3 Hz, 8-H), 3.82 (3H, s, 5-OCH_3), 6.86 (2H, br d, J=8.6 Hz, 4′, 6′-H), 6.94 (2H, br d, J=8.9 Hz, 4,6-H), 7.06 (2H, br d, J=8.6 Hz, 3′,7′-H), 7.14 (2H, br d, J=8.9 Hz, 3,7-H), 8.25 (1H, br s, 5′-OH).

DES dimethyl ether (**2c**) was prepared, with some modification, by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as small colorless needles, mp 124—124.5 °C (Lit., [13] 124 °C). MS *m/z*: 296 (M^+), 281, 267, 252, 173, 159, 121. *Anal.* Calcd. for $C_{20}H_{24}O_2$: C, 81.04; H, 8.16. Found: C, 81.06; H, 8.12. ^{1}H-NMR δ (ppm) 0.76 (6H, t, J=7.3 Hz, 9,9′-CH_3), 2.15 (4H, q, J=7.3Hz, 8,8′-H), 3.82 (6H, s, 5,5′-OCH_3), 6.94 (4H, br d, J=8.9 Hz, 4,4′, 6,6′-H), 7.14 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H).

Dienestrol monomethyl ether (**3b**) was prepared by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as small colorless needles, mp 145–148°C. MS *m/z*: 280 (M^+, base peak), 265, 251, 159, 135, 121. *Anal.* Calcd for $C_{19}H_{20}O_2$: C, 81.39; H, 7.19. Found: C, 81.89; H, 7.44. ^{1}H-NMR δ (ppm): 1.46 (6H, d, J=6.3 Hz, 9,9′-CH_3), 3.81 (3H, s, 5-OCH_3), 5.27 and 5.32 (2H, q, J=6.3 Hz, 8,8′-H), 6.86 (2H, br d, J=8.9 Hz, 4′,6′-H), 6.94 (2H, br d, J=8.9 Hz, 4,6-H), 6.99 (2H, br d, J=8.9 Hz, 3′,7′-H), 7.07 (2H, br d, J=8.9 Hz, 3,7-H), 8.25 (1H, br s, 5′-OH).

Dienestrol dimethyl ether (**3c**) was prepared by the method of Wilds and McCormack.[12] The product was recrystallized from benzene as small colorless needles, mp 128—130 °C. MS *m/z*: 294 (M^+, base peak), 279, 265, 159, 135, 121. *Anal.* Calcd for $C_{20}H_{22}O_2$: C, 81.60; H, 7.53. Found: C, 81.33; H, 7.38. ^{1}H-NMR δ (ppm): 1.46 (6H, d, J=6.3 Hz, 9,9′-CH_3), 3.81 (6H, s, 5,5′-OCH_3), 5.29 (2H, q, J=6.3 Hz, 8,8′-H), 7.01 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 7.08 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H).

Isodienestrol——Isodienestrol (**4**) was prepared by the method of Liao and Williams-Ashman.[14] MnO_2 6 g, (prepared according to Mancera *et al.*[15]) was added to 1 g of diethylstilbestrol dissolved in 20 ml of acetone. The mixture was stirred at room temperature for 5 h. It was then filtered and the filtrate was dried *in vacuo*. The residue (989 mg) was dissolved in a small amount of diethyl ether and chromatographed on Fluorisil (15 g), by eluting with benzene and then with benzene–diethyl ether (90 : 10). The residue from the eluate, after recrystallization from benzene, gave isodienestol (**4**) as pale brawn needles, mp 192—194°C. *Anal.* Calcd for $C_{18}H_{18}O_2$: C, 81.17; H, 6.81. Found: C, 81.15; H 7.02. MS *m/z*: 266 (M^+), 251, 236, 145, 121, 107 (base peak). The above melting point and the ultraviolet-absorption spectra of **4** agree well with those described in the literature.[14] ^{1}H-NMR δ (ppm): 1.68 (6H, d, J=6.6 Hz, 9,9′-H), 6.20 (2H, q, J=6.6 Hz, 8,8′-H), 6.70 (4H, br d, J=8.8 Hz, 4,4′,6,6′-H), 7.22 (4H, br d, J=8.8 Hz, 3,3′,7,7′-H), 8.24 (2H, br s, 5.5′-H).

Diacetyl Derivatives of *meso*-Hexestrol and DES——**1a** was acetylated with acetic anhydride–pyridine at room temperature by standing overnight. The product was recrystallized from methanol to give *meso*-hexestrol diacetate (**1e**) as colorless needles, mp 135—136 °C. *Anal.* Calcd for $C_{22}H_{26}O_4$: C, 74.55; H, 7.39. Found: C, 74.50; H, 7.37. MS *m/z*: 354 (M^+), 177, 135, 107. ^{1}H-NMR δ (ppm): 0.53 (6H, t, J=7.3 Hz, 9.9′-CH_3, 1.33—1.48 (4H, m, 8,8′-H), 2.25 (6H, s, 5,5′-$OCOCH_3$), 2.70 (2H, m, 1,1′-H), 7.09 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.30 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H).

DES was acetylated with acetic anhydride–pyridine at room temperature by standing overnight. The product was recrystallized from methanol to give DES diacetate (**2d**) as colorless needles, mp 121—124 °C. *Anal.* Calcd for $C_{22}H_{24}O_4$: C, 74.97; H, 6.86. Found: C, 75.01; H, 6.95. MS *m/z*: 352 (M^+), 310, 268, 239. ^{1}H-NMR δ (ppm): 0.78 (6H, t, J=7.3 Hz, 9,9′-CH_3), 2.16 (4H, t, J=7.3 Hz, 8,8′-H), 2.28 (6H, s, 5,5′-$OCOCH_3$), 7.16 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.28 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H).

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References

1) Y. Sato, T. Murai, M. Tsumuraya, H. Saitô, and M. Kodama, *Gann*, **75**, 1046 (1984).
2) D. C. Sharp and J. M. Parry, *Carcinogenesis*, **6**, 865 (1985).
3) B. Hartley-Asp, J. Deinum, and M. Wallin, *Mutat. Res.*, **143**, 231 (1985).
4) Y. Sato, T. Murai, T. Oda, H, Saitô, M. Kodama, and A. Hirata, *J. Biochem.* (Tokyo), **101**, 1247 (1987).
5) M. R. Kilbourn, A. J. Arduengo, J. T. Park, and J. A. Katzenellenbogen, *Mol. Pharmacol.*, **19**, 388 (1981).
6) I. E. Smiley and M. G. Rossmann, *J. Chem. Soc., Chem. Commun.*, **1969**, 198.
7) M. Hospital, B. Busetta, C. Courseille, and G. Precigoux, *J. Steroid Chem.*, **6**, 221 (1975).
8) J. G. Liehr and A. M. Ballatore, *Steroids*, **40**, 713 (1982).
9) P. W. Westermann, S. P. Gunasekera, M. Uvia, S. Sultanbawa, and R. Kazlauskas, *Org. Magn. Reson.*, **9**, 631 (1977); A. K. Sen, K. K. Sarkar, P. C. Mazumder, N. Banerji, R. Uusvuori, and T. A. Hase, *Phytochemistry*, **19**, 2223 (1980).
10) A. Bax and R. Freeman, *J. Am. Chem. Soc.*, **104**, 1099 (1982).
11) T. D. Doyle, J. M. Stewart, N. Filipescu, and W. R. Benson, *J. Pharm. Sci.*, **64**, 1525 (1975).
12) A. L. Wilds and W. B. McCormack, *J. Am. Chem. Soc.*, **70**, 4127 (1948).
13) E. E. Reid and E. Wilson, *J. Am. Chem. Soc.*, **64**, 1625 (1942).
14) S. Liao and H. G. Williams-Ashman, *Biochim. Biophys. Acta*, **59**, 705 (1962).
15) O. Mancera, C. Rosencranz and F. Sondheimer, *J. Chem. Soc.*, **1953**, 2189.