The Mass Spectra of Diethylstilbestrol and Related Compounds†‡

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The low resolution mass spectra of *E*-3,4-bis-(*p*-hydroxyphenyl)-hex-3-ene (diethylstilbestrol), *E*-[1,1,1-$^3H_3$]3,4-bis-(*p*-hydroxyphenyl)-hex-3-ene, *E*-2,3-bis-(*p*-hydroxyphenyl)-but-2-ene (dimethylstilbestrol), *E,E*-3,4-bis-(*p*-hydroxyphenyl)hexa-2,4-diene (dienestrol) and 3,4-bis-(*p*-hydroxyphenyl)-hexane (hexestrol) were examined as the parent compounds, their diacetates, dimethyl ethers, and bis-trimethylsilyl ethers. In addition, the mass spectra of the diethyl ether and the hexadeuteriodimethyl ether of *E*-3,4-bis-(*p*-hydroxyphenyl)-hex-3-ene were studied. Each compound gives rise to several sets of characteristic fragment ions associated with loss of alkyl groups, loss of aryl groups and rearrangements. An ion of *m/e* 165 ($C_{13}H_9$) was found in the spectra of all the compounds studied. With the aid of high resolution mass spectrometry empirical formulae were assigned to major ions of the free diphenols.

INTRODUCTION

The occurrence of vaginal adenocarcinoma in adolescent daughters of mothers who had received diethylstilbestrol (**1a**) during pregnancy[1,2] stimulated our interest in the study of the metabolism of diethylstilbestrol by mammalian tissues. A suitable method for studying its metabolism was thought to be the twin ion technique[3] which was developed for the detection of steroid hormone metabolites by gas chromatography-mass spectrometry. In order to provide data for the identification of possible metabolites, as well as for the selection of characteristic ions suitable for quantitative estimation of diethylstilbestrol and related compounds by selected ion monitoring, a study was made of the mass spectra of diethylstilbestrol, related compounds and some derivatives, using both low and high resolution techniques.

EXPERIMENTAL

Diethylstilbestrol, dienestrol, hexestrol and diethylstilbestrol dimethyl ether were obtained from commercial sources. Dimethylstilbestrol was the gift of the Cancer Chemotherapy National Service Center, National Cancer Institute. The dimethyl ethers of dimethylstilbestrol, dienestrol and hexestrol were prepared by treatment of the phenols with potassium hydroxide and dimethyl sulfate in refluxing ethanol. All compounds were homogeneous as judged by thin-layer chromatography and all melting points were in agreement with values reported in the literature. Nuclear magnetic resonance spectra were in agreement with the assigned structures.

Trideuterated diethylstilbestrol ([1,1,1-$^2H_3$]*E*-3,4-bis-(*p*-hydroxyphenyl)-hex-3-ene) was prepared from α-ethyldesoxyanisoin and [2,2,2-$^2H_3$] ethyl iodide as described elsewhere.[4]

Diethylstilbestrol diethyl ether[5] was prepared from the diphenol and ethyl iodide by the Hakomori method.[6] The bis-(trideuteromethyl) ether of diethylstilbestrol was prepared in a similar manner from [$^2H_3$]methyl iodide and diethylstilbestrol.

Samples were prepared for introduction into the GCMS by deposition on stainless steel gauzes as described by Engel and Orr.[7] Gauzes were removed from the flash heater chamber daily as accumulated gauzes tend to catalyze pyrolysis of sensitive compounds.

The GCMS analyses were performed on an LKB 9000 gas chromatograph mass spectrometer. The column (2.5 m × 4 mm i.d.) was packed with 1% OV-1 on Gas Chrom Q. All samples were introduced into the instrument at a column temperature of 160 °C, and after 20 min the temperature was raised at 3 °C min^{-1} to 230 °C. The injection temperature was 260 °C and the separator temperature was 250 °C. Helium flow through the column was maintained at 30 ml min^{-1}, and spectra were taken at 70 eV; ion source temperature 275 °C.

High resolution mass spectra were determined with a CEC-110 dual focusing photoplate instrument (Dupont Instruments, Monrovia, California, 91016). Samples were introduced by the solid probe inlet with the ion source maintained at 210 °C.

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‡ Abbreviations: Diethylstilbestrol (DES) = *E*-3,4-bis (*p*-hydroxyphenyl)-hex-3-ene; dimethylstilbestrol (DMS) = *E*, 2,2-bis-(*p*-hydroxyphenyl)-but-2-ene; dienestrol = *E*, *E*-3,4-bis-(*p*-hydroxyphenyl)-hexa-2,4-diene and hexestrol for 3,4-bis-(*p*-hydroxyphenyl--hexane.

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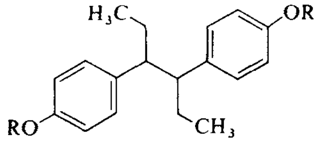
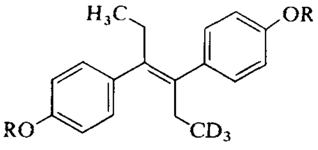
RESULTS AND DISCUSSION

The compounds studied were diethylstilbestrol (**1a**) (Fig. 1), [1,1,1-$^2H_3$] diethylstilbestrol (**2a**) (Fig. 2), dimethylstilbestrol (**3a**), dienestrol (**4a**) (Fig. 3) and hexestrol (**5a**) (Fig. 4) and their diacetates, dimethyl and bis-trimethylsilyl ethers. In addition, diethylstilbestrol diethyl ether (**1e**) and hexadeuterodimethyl ether (**1f**) were examined. Mass spectra of diethylstilbestrol dimethyl ether,[8] trimethylsilyl ether[9,10] and the trimethylsilyl ethers of dienestrol[10] and hexestrol[10] have been reported.



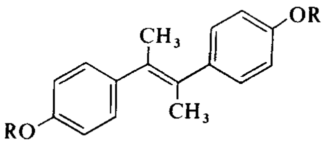
1

	R
1a	-H
1b	$-CH_3$
1c	$-Si(CH_3)_3$
1d	$-COCH_3$
1e	$-CH_2CH_3$
1f	$-CD_3$



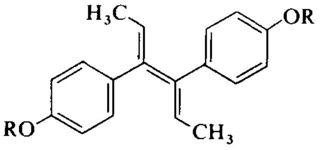
2

	R
2a	-H
2b	$-CH_3$
2c	$-Si(CH_3)_3$
2d	$-COCH_3$



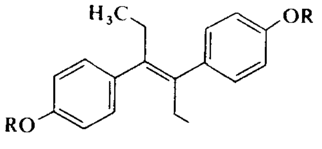
3

	R
3a	-H
3b	$-CH_3$
3c	$-Si(CH_3)_3$
3d	$-COCH_3$



4

	R
4a	-H
4b	$-CH_3$
4c	$-Si(CH_3)_3$
4d	$-COCH_3$



5

	R
5a	-H
5b	$-CH_3$
5c	$-Si(CH_3)_3$
5d	$-COCH_3$

The mass spectra of all the compounds studied here exhibit characteristic fragment ions that represent losses of alkyl (Table 1) and a combined loss of alkyl and aryl (Table 2) fragments from the molecular ion. Except in the bases of the diacetates (Table 1, Figs 5 and 8), in both the diethylstilbestrol (**1**) and hexestrol series (**5**), ions were seen at M − 15 ($M-CH_3$), M − 29 ($M-C_2H_5$), and M − (15 + 29); loss of two methyl groups is only observed in the dimethylstilbestrol series.

The facile elimination of ketene upon electron impact dominates the mass spectrum of all the diacetates except that of hexestrol (Fig. 8, e.g. diethylstilbestrol diacetate (**1d**) (Fig. 5).

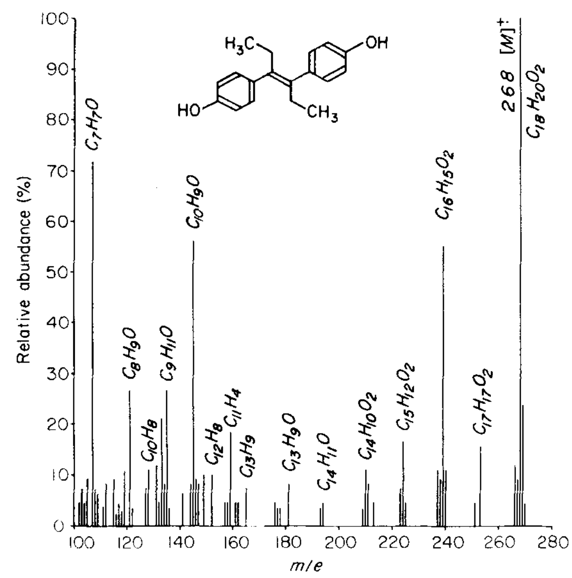


Figure 1. Mass spectrum of diethylstilbestrol (**1a**). The empirical formulae are those of ions whose exact masses were determined by high resolution mass spectrometry.

Dienestrol (**4a**) (Fig. 3) and its dimethyl (**4b**) (Fig. 6) and bis-trimethylsilyl ethers (**4c**) (Fig. 7) yield fragments resulting from the loss of a methyl (M − 15) and an ethyl group (M − 29), as well as loss of two C_2H_4 fragments (M − 56). In the spectrum of dienestrol there is also an ion *m/e* 238 with intensity approximately 5% that of the parent ion that results from the loss of the elements CO. This loss was not observed in the other diphenols examined in this study, but has been reported in the fragmentation pattern of quinones.[11] This point is discussed further in connection with the structure of a phenazine derived from a monohydroxydiethylstilbestrol.[12]

When fragmentation of the trideuterated diethylstilbestrol (**2a**) (Fig. 2) occurs, a group containing one of the

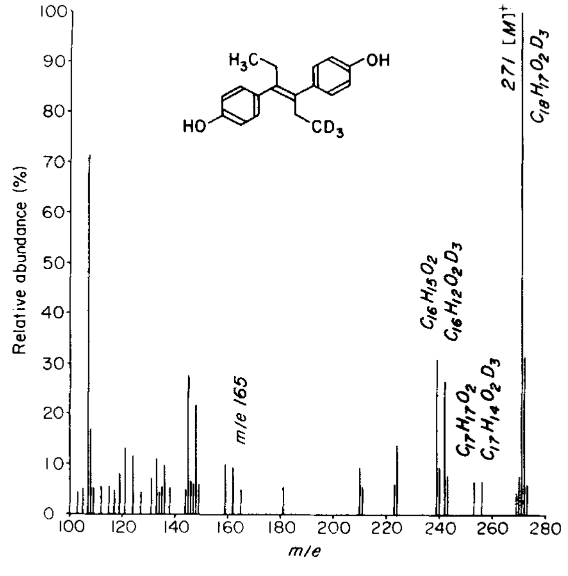


Figure 2. Mass spectrum of [$^2H_3$]diethylstilbestrol (**2a**). The empirical formulae are those of ions whose exact masses were determined by high resolution mass spectrometry.

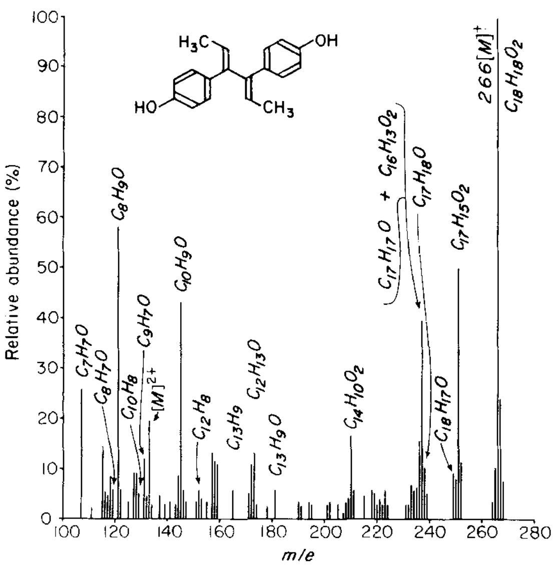


Figure 3. Mass spectrum of dienestrol (**4a**). The empirical formulae are those of ions whose exact masses were determined by high resolution mass spectrometry.

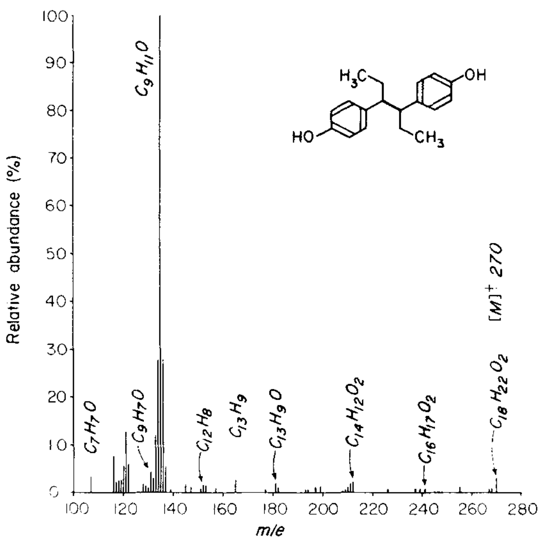


Figure 4. Mass spectrum of hexestrol (**5a**). The empirical formulae are those of ions whose exact masses were determined by high resolution mass spectrometry.

two methyl groups is lost and the distinctive configuration of twin ions 3 amu apart is seen (Table 1). Thus, the molecular ion is single (*m/e* 271) while those ions resulting from loss of a methyl group are twins (*m/e* 253/256), as are those resulting from loss of an ethyl group *m/e* 239/242).

Dimethyl and bis-trimethylsilyl ethers (e.g. Figs. 6 and 7) fragment like the parent compounds; little cleavage of the ether groups was observed.

Fragment ions due to loss of an aryl group in addition to one or more carbon atoms of the carbon chain are presented in Table 2. The ion corresponding to *m/e* 159 ($C_{11}H_{11}O$) in the spectrum of diethylstilbestrol (**1a**) (Fig. 1) appears as twins (*m/e* 159/162) in the spectrum of [$^2H_3$]diethylstilbestrol (**2a**) (Fig. 2), at *m/e* 173 (159+14 amu) in the dimethyl ether (**1b**), and at *m/e* 176 in the bis-(trideuteromethyl) ether (**1f**). These ions we believe to result from loss of an aryl group and C-1 (or C-6). A similar argument can be advanced for the ion *m/e* 145 ($C_{10}H_9O$) in diethylstilbestrol, which could arise by loss of an aryl group and two carbons. The ions *m/e* 135 ($C_9H_{11}O$) and 133 (C_9H_9O) could arise from cleavage of the ethylenic bond and a hydrogen migration or, alternatively, from loss of an aryl plus three carbon units and a rearrangement. Examination of the high resolution mass spectrum of diethylstilbestrol and [$^2H_3$]diethyl-

Table 1. Mass spectrometric fragmentation of diethylstilbestrol and related compounds; ions resulting from loss of alkyl groups

Compound		$[M]^{+\cdot}$	$[M-15-CH_3]^+$	$[M-29-C_2H_5]^+$	$[M-30-2CH_3]^+$	$[M-44-(CH_3+C_2H_5]^+$	$[M-56-2C_2H_4]^+$	$[M-58-2C_2H_5]^+$
Diethylstilbestrol	**(1a)**	268^a	253^a	239*		224^a		210^a
Dimethyl ether	**(1b)**	296	281	267		252		238
Bis-TMS ether	**(1c)**	412	397	383		368		354
Diacetate	**(1d)**	352						
[$^2H_3$]Diethylstilbestrol	**(2a)**	271^a	253^a/256^a	239^a/242^a		225^a		210^a
Dimethyl ether	**(2b)**	299	281/284	267/270		252		238
Bis-TMS ether	**(2c)**	415	397/400	383/386		368		354
Diacetate	**(2d)**	355						
Dimethylstilbestrol	**(3a)**	240	224		210			
Dimethyl ether	**(3b)**	268	253		238			
Bis-TMS ether	**(3c)**	384	369		354			
Diacetate	**(3d)**	324						
Dienestrol	**(4a)**	266^a	251^a	237^b			210^a	
Dimethyl ether	**(4b)**	294	279	265			238	
Bis-TMS ether	**(4c)**	410	395	381			354	
Diacetate	**(4d)**	350						
Hexestrol	**(5a)**	270^a	255^a	241^a		226		212^a
Dimethyl ether	**(5b)**	298	283	269		254		240
Bis-TMS ether	**(5c)**	414	399	385		370		356
Diacetate	**(5d)**	354						

[a] Confirmed by high resolution mass spectrometry.

[b] $C_{17}H_{17}O$ and $C_{16}H_{13}O_2$ in the ratio 14:86.

Table 2. Mass spectral fragmentation of diethylstilbestrol and related compounds: Aryl cleavages and rearrangements

	DES	[1,1,1-$^2H_3$]DES	DES $(OCH_3)_2$ m/e	DES $(OCD_3)_2$	DMS	DMS $(OCH_3)_2$
$[M]^{+\cdot}$	268 ($C_{18}H_{20}O_2$)[a]	271	296	302	240	268
$[M-Aryl]^+$					145	159
$[M-(Aryl+C\text{-}Unit)]^+$	159 ($C_{11}H_{11}O$)	159/162	173	176	135	
$[M-(Aryl+C_2\text{-}Unit)]^+$	145 ($C_{10}H_9O$)	145/148	159	162		
Symmetrical cleavage of ethylenic bond and H transfer[b]	135 ($C_9H_{11}O$)	134/137	149	152	121	135
$[M-(Aryl+C_3\text{-}Unit)]^+$	133 (C_9H_9O)	133/136	147	150	119	133
$[M-(Aryl+C_4-Unit)]^+$	121 (C_8H_9O)	121/124	135	138		
$[M-(Aryl+C_5-Unit)]^+$	107 (C_7H_7O)	107	121	124		

The abbreviations used: Aryl = p-hydroxyphenyl, p-methoxyphenyl or p-[$^2H_3$]methoxyphenyl.
[a] Empirical formulae established by high resolution mass spectrometry.
[b] = $C_9H_{10}O$.

stilbestrol reveals cleavage processes in which fragments containing one of the two methyl groups are lost with approximately equal probability. Thus, one finds ions of m/e 134 ($C_9H_{10}O$) and 137 (C_9H_7O $^2H_3$), m/e 133 (C_9H_9O) and 136 ($C_9H_6O_6{}^2H_3$) in addition to the doubly charged molecular ion, (m/e 135.58278). The ions at m/e 121 (C_8H_9O) and 107 (C_7H_7O) (121 − 14 amu) are thought to be hydroxytropylium ions, the former with one extra carbon atom. Since the ions corresponding to m/e 121 in diethylstilbestrol are twinned in [$^2H_3$]-diethylstilbestrol (Fig. 2) and 3 amu apart, one of the remaining carbon atoms must be C-1 or C-6 with hydrogen or deuterium intact.

Hexestrol diacetate (**5d**) (Fig. 8) is the only diacetate studied that shows only minor ions derived from loss of ketene (m/e 312 and 270). One of its most prominent ions is at m/e 177 and could either result from symmetrical cleavage of the molecule or be a doubly charged molecular ion.

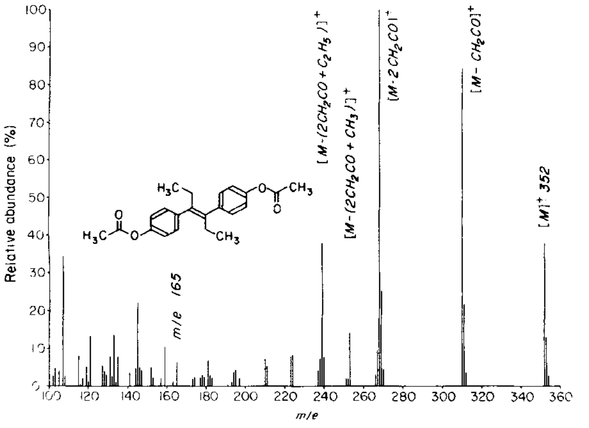


Figure 5. Mass spectrum of diethylstilbestrol diacetate (1d).

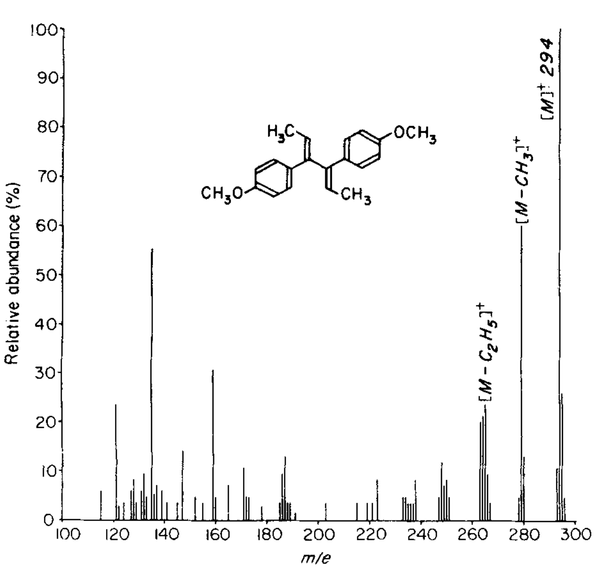


Figure 6. Mass spectrum of dienestrol dimethyl ether (4b).

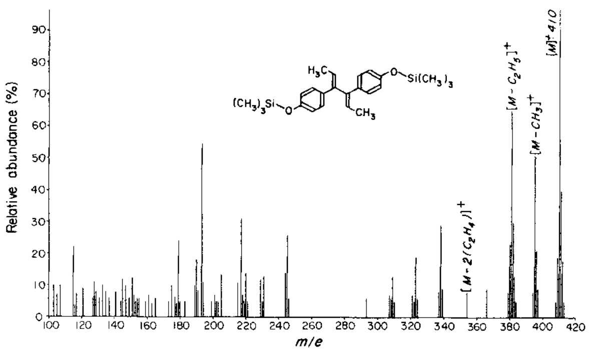


Figure 7. Mass spectrum of dienestrol bis-trimethylsilyl ether (4c).

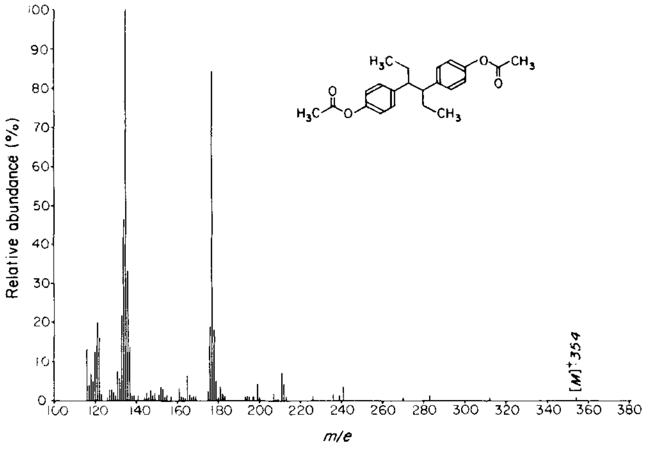
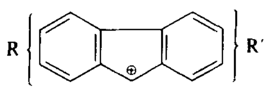


Figure 8. Mass spectrum of hexestrol diacetate (5d).

Table 3 lists the compositions of ions of m/e 181 and less, that are common to at least two of the group: diethylstilbestrol, hexestrol and dienestrol. These fragments, in conjunction with characteristic alkyl cleavages could serve to distinguish among the three compounds.

An ion of m/e 165 ($C_{13}H_9$) is seen in the spectra of nearly all the compounds studied (e.g. Fig. 1). All the free diphenols show an ion at m/e 181 ($C_{13}H_9O$), and dienestrol (**4a**) (Fig. 3) and hexestrol (**5a**) (Fig. 4) both show an ion at m/e 197 ($C_{13}H_9O_2$). The spectra of the dimethyl ethers (**4b** and **5b**) have the ion, m/e 165, and additional ions at m/e 195 (+OCH_2) and 225 (−2(OCH_2)). The ion of nominal mass 165 in the mass spectrum of stilbene is believed by Johnston and Millard[13] to be the fluorene carbonium ion (*a*). It is likely that the ion, m/e 165, observed in the compounds presently under study is the same and that $[C_{13}H_9]^+$ may be characteristic not only of stilbenes but also of 1,2-dialkyl-1,2-diarylethanes and 3,4-diarylhexa-2,4-dienes and perhaps other compounds as well. Support for this formulation is given by the mass spectra of diethylstilbestrol diethyl ether (**1e**) and bis-trideuteromethyl) ether (**1f**). The former has an ion at m/e 209 (165+44) and the latter gave an ion at m/e 198 (165+33), indicating the presence of ethoxy and trideuteromethoxy groups. Formation of the postulated fluorene carbonium ion must occur by the loss of at least one phenolic oxygen atom.



a (R=R′=H)

Table 3. Mass spectral fragmentation of diethylstilbestrol, dienestrol and hexestrol; Ions resulting from rearrangements and loss of aryl groups

Ions	m/e	Dienestrol	DES	Hexestrol
$C_{13}H_9O$	181	+	+	+
$C_{12}H_8O$	168	+	−	+
$C_{13}H_9$	165	+	+	+
$C_{11}H_{11}O$	159	+	+	−
$C_{12}H_8$	152	+	+	+
$C_{10}H_9O$	145	+	+	−
$C_9H_{11}O$	135	−	+	+
C_9H_9O	133	+[a]	+	+
C_9H_7O	131	+	+	+
$C_{10}H_8$	128	+	+	−
C_8H_9O	121	+	+	−
C_8H_7O	119	+	−	+
C_7H_7O	107	+	+	+
C_7H_7	91	+	+	+

[a] Same mass as $[M]^+$.

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