

## ISOMERIZATION OF TRANS-DIETHYLSTILBESTROL TO PSEUDO-DIETHYLSTILBESTROL

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### ABSTRACT

This study reports the formation and isolation of a diethylstilbestrol-dimethylsulfoxide (DES-DMSO) adjunct and Z-3, 4-di(p-hydroxyphenyl)-2-hexene ( $\psi$ -DES) from trans-DES. The presence of  $\psi$ -DES was indicated by NMR and mass spectrometry and confirmed by direct comparison to a reference sample. High resolution NMR(360 MHz) along with the comparison of the chemical shift values of methine and methyl protons attached to carbon-carbon double bonds in Z and E isomers of 3-substituted-2-pentenes and dienestrol derivatives were used in postulating the Z-stereochemistry for  $\psi$ -DES. A NMR additive increment method was useful for the comparison of the chemical shift values of methine protons in  $\psi$ -DES and other literature compounds. Nuclear Overhauser Enhancement (NOE) confirmed the Z-stereochemistry of  $\psi$ -DES.

### INTRODUCTION

Metabolic activation is an important consideration in chemical toxicity. Many ethylenic compounds result in the formation of reactive epoxides which have been assumed to be possible active metabolites. Since DES is a human carcinogen, it was of interest to include its trans and cis epoxide derivatives in our mutagenicity study of aliphatic epoxides reported earlier (1). Therefore, the trans to cis isomerization of DES (2) was reexplored under various reaction conditions. As part of this

investigation, a DES-DMSO adjunct and  $\psi$ -DES were isolated from trans-DES under mild conditions.

## EXPERIMENTAL

### Apparatus

Melting points were measured on a Mel-Temp melting point apparatus and were uncorrected. IR spectra were recorded on Perkin Elmer 281 spectrophotometer in KBr pellets. NMR spectra were obtained with a Varian EM-360 at 60 MHz using tetramethylsilane as an internal standard. High resolution proton NMR spectra were recorded with Bruker WM-360 at 360 MHz. For NOE experiments, the dissolved oxygen from air was thoroughly removed from the specimen in the NMR tube. This was accomplished by first freezing the specimen in liquid nitrogen followed with careful thawing under vacuum. The above step was repeated eight times until air bubbles ceased coming out of the frozen specimen. The NMR tube was then carefully sealed. The solid state carbon-13 NMR spectra were obtained by Dr. Warner R. Woolfenden (4). Differential scanning calorimetry (DSC) were recorded from a Perkin Elmer DSC-2C instrument with the following settings: heating rate 20<sup>o</sup> K/min.; range setting 10mcal/sec.; Indium standard was used to calibrate the calorimeter. Thermogravimetric analysis (TGA) data were recorded with Perkin Elmer TGS-2 instrument with the following settings: heating rate 5<sup>o</sup>C/min.; range setting 0.1. Mass spectra (70eV) were obtained with Finigan 4023 GC-MS with INCOS data acquisition system. UV spectrum was measured in methanol with Hewlett Packard 8450 A spectrophotometer.

### Z-3,4-di(p-hydroxyphenyl)-2-hexene ( $\psi$ -DES)

Trans-DES (ICN Pharmaceutical, Inc.) (3.0g) was added to dimethylsulfoxide (25ml) and the mixture was heated on a steam bath for three minutes. The contents were diluted with distilled water (50ml) and filtered immediately. The filtrate was then left for over three weeks where fine crystals of  $\psi$ -DES was isolated and dried under vacuum. Yield 178mg (5.9%); m. p. 151-152<sup>o</sup>C (lit., 153<sup>o</sup>C and 143.5<sup>o</sup>C (5) reported for unspecified stereo-isomers of  $\psi$ -DES); IR (KBr) 3280 (broad), 2960, 2920, 2860, 1870 (weak, shoulder), 1610, 1595, 1500, 1440, 1365 (with shoulder), 1230 (broad), 1170, 1120, 1010, 940, 925, 815 cm<sup>-1</sup>.

360 MHz NMR ( $d_6$ -acetone);  $\delta$ 0.85(t,  $J=7.3$ Hz, 6- $CH_3$ ), 1.48 (d, d,  $J=6.7$ Hz,  $J=0.9$ Hz, 1- $CH_3$ ), 1.65 (m, 5-H), 1.77 (m, 5-H), 3.31 (t,  $J=7.3$ Hz, 4-H), 5.57 (q, q,  $J=6.7$ Hz,  $J=0.9$  Hz, 2-H), 6.70 (m, aromatic, 6H), 6.93 (m, aromatic, 2H), 8.07 (s, phenolic OH), 8.18 (s, phenolic OH). Double irradiation at  $\delta$ 3.31 ppm; observed at  $\delta$ 1.48 (d, 1- $CH_3$ ) and  $\delta$ 5.57 (q, 2-H). Mass spectrum m/e (relative intensity) 268 ( $M^+$ , 32), 239 (80), 145 (57), 135 (35), 134 (12), 133 (79), 121 (13), 107 (100), 105 (18), 91 (18), 77 (30), 55 (15).

Diethylstilbestrol-Dimethylsulfoxide Adjunct (DES-DMSO)

The initial residue from the above experiment was thoroughly washed with carbon tetrachloride until the washings did not show the presence of DMSO as checked by proton NMR. The residue was dried under vacuum (0.5mm of Hg) at 110°C for 18 hours. Yield 3.6g(93%); m.p. 144-145°C; 60MHz NMR ( $d_6$ -acetone);  $\delta$ 0.75 (t,  $J=7.0$ Hz,  $CH_3$ ), 2.17 (q,  $J=7.0$ Hz,  $CH_2$ ), 2.60 (s,  $CH_3$  of DMSO), 6.90 (m, aromatic, 8H), 8.43 (s, phenolic OH); carbon-13 NMR (acetone); 13.7, 29.1, 40.8 (DMSO), 115.6, 130.4, 134.3, 139.4, 156.6; solid state carbon-13 MAS/CP NMR (4); 14.5, 27.3, 37.6, 115.3, 116.6, 129.8, 133.2, 135.1, 138.2, 155.2 ppm. IR (KBr); 3410, 3270, 2980, 2940, 2875, 1992, 1610, 1591, 1515, 1455, 1440, 1430, 1370, 1355, 1338, 1282, 1248, 1235, 1205, 1175, 1115, 1010, 956, 850, 830, 800, 745, 732, 718, 640  $cm^{-1}$ . Mass spectrum m/e(relative intensity); 268( $M^+$ , 52), 239 (36), 159 (22), 145 (76), 133 (21), 121 (29), 115 (19), 107 (100), 91 (20), 77 (26).

Anal. (Midwest Microlab, Indianapolis, IN) Calcd for  $C_{20}H_{26}O_3S$ : C, 69.36; H, 7.51; S, 9.24%. Found: C, 69.77, H, 6.88, S, 9.47%.

RESULTS AND DISCUSSION

In our modification of the literature procedure of the isomerization in DMSO of trans-DES to cis-DES (2), a DES-DMSO adjunct can be isolated immediately upon addition of water to the reaction mixture. This is followed after several weeks by precipitation of  $\psi$ -DES from the filtrate. Even under conditions

of washing and drying to remove any free DMSO, the initial precipitate showed the presence of sulfur by elemental analysis. Mass spectra of the precipitate and trans-DES were the same. Solution spectra were consistent with either a mixture of DMSO and trans-DES or an adjunct of the two which was unstable in solution. That is, UV analysis in methanol gave a spectrum consistent with that of trans-DES and NMR in deuterated methanol or acetone gave spectra indicating a 1:1 ratio of DMSO to trans-DES. An IR spectrum obtained from a solid state sample in KBr confirmed the presence of DMSO with several bands not present in a trans-DES spectrum. A second OH stretching band at  $3270\text{ cm}^{-1}$  and a new band at  $1235\text{ cm}^{-1}$  (hydrogen bonded OH bending (3)) as well as a strong increase in absorbance at  $1010\text{ cm}^{-1}$  (bonded S=O stretching (3)) all suggest a DES-DMSO adjunct. Also the precipitate in question behaved like a single compound exhibiting a sigmoidal curve in TGA without any weight loss until  $116.5^{\circ}\text{C}$  while DMSO under the same conditions exhibited 99.5% loss of its total weight at  $116^{\circ}\text{C}$ . DSC analysis for the precipitate showed a sharp endotherm characteristic for a pure compound at  $147^{\circ}\text{C}$  as compared to  $173^{\circ}\text{C}$  for the trans-DES.

Solid state carbon-13 magic angle spinning/cross polarization (MAS/CP) nuclear magnetic resonance spectra for the

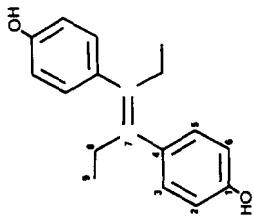
DES-DMSO adjunct as well as for trans-DES were obtained (4) and are summarized in Table 1. The DES-DMSO adjunct exhibited the greatest difference in chemical shift value ( $\Delta\delta=2.70\text{ppm}$ ) for the carbon attached to a phenolic group. This is consistent with hydrogen bonding of the phenolic protons to the oxygen of DMSO. The spectrum of the DES-DMSO adjunct also confirmed a 1:1 ratio of DMSO to DES.

Initial IR, NMR and mass spectra of the second precipitate led us to the conclusion that we were dealing with  $\psi$ -DES. This was confirmed by comparison of our 60MHz NMR spectrum to the one furnished by K.S. Korach for the  $\psi$ -DES used in his study (6) as well as by direct comparison of 360MHz NMR and mass spectra of our compound to that of a reference sample (7) of  $\psi$ -DES.

The mass spectrum of our isolated  $\psi$ -DES indicated important peaks not included in an abridged spectrum previously reported (8). We rationalize the fragmentation pattern as outlined in Scheme 1. Various investigators (5, 8-12) have reported the preparation of  $\psi$ -DES but without evidence for its E or Z stereochemistry. In the present study the stereochemistry of the isolated and reference  $\psi$ -DES was predicted on the basis of comparison to literature NMR data for mono-methyl-trisubstituted alkenes

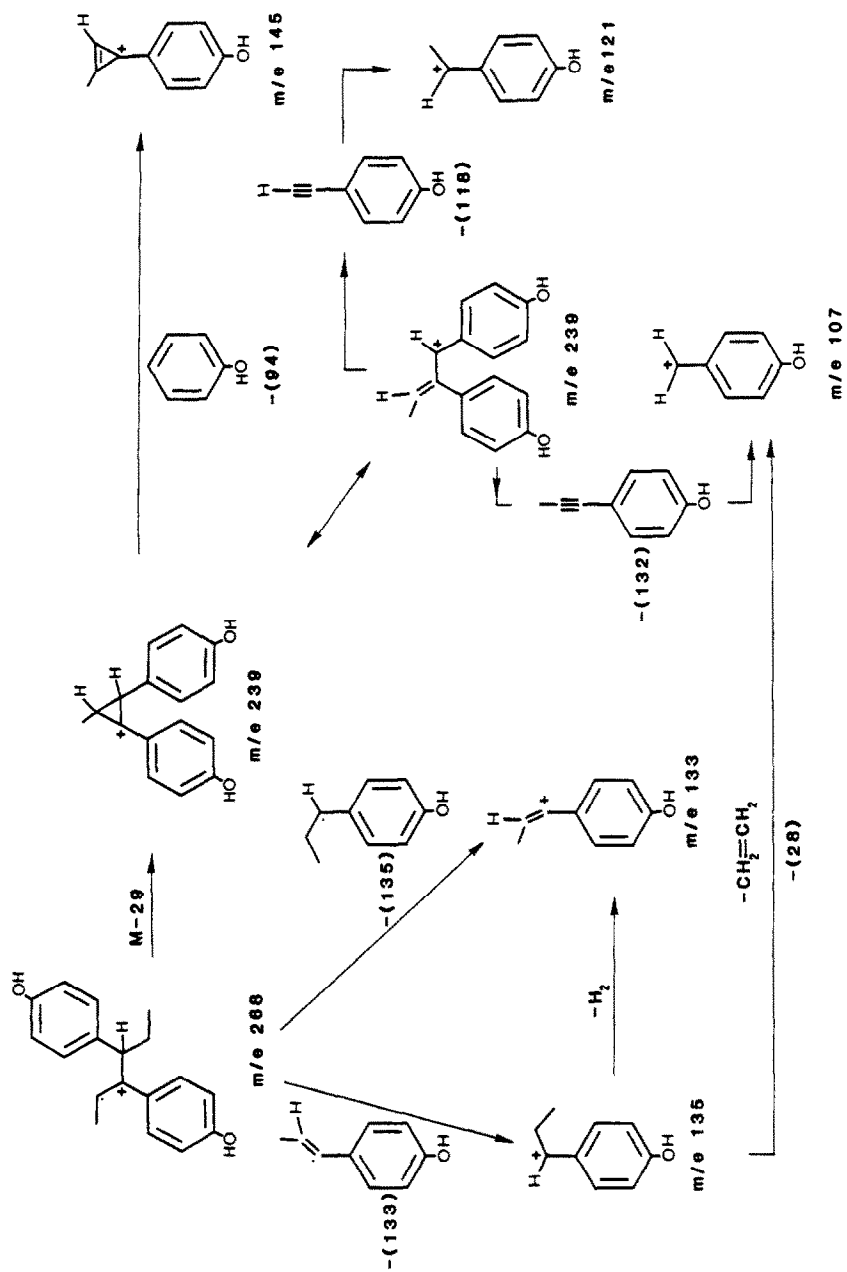
TABLE I  
 CARBON-13 SHIFTS<sup>a</sup> (ppm)

Carbon NO.	<u>trans-DES</u>	DES-DMSO Adjunct	$\Delta\delta^b$
1	152.5	155.2	2.7
2 or 6	115.0	115.3	
3 or 5	115.0	116.6	
	132.4	133.2	
	131.2	129.8	
4	134.5	135.1	0.6
7	137.3	138.2	0.9
8	27.6	27.3	0.3
9	13.8	14.5	0.7
		37.6 (DMSO)	



(a) see ref. 4

SCHEME 1



and confirmed by NOE measurements to be the Z-isomer.

We applied the additive increment method of Pascual et al. (13) and Matter et al. (14, 15) to  $\psi$ -DES and predicted the chemical shift for the methine proton to be  $\delta 5.77$ ppm for the E-isomer and  $\delta 5.41$ ppm for the Z-isomer. As is generally the case in the observed (16, 17) and our calculations for the methine protons in the configuration about the double bonds of this series of trisubstituted alkenes, the predicted value for the Z-isomer was less than that of the E-isomer. Thus, our observed value of  $\delta 5.57$ ppm best fits the calculated value of  $\delta 5.41$ ppm for the Z-isomer of  $\psi$ -DES.

Also our observed value of  $\delta 1.48$ ppm for the methyl group attached to the double bond of  $\psi$ -DES corresponds more closely to the observed value of  $\delta 1.45$  to  $\delta 1.56$  for these protons of the Z-isomers in the same series of trisubstituted alkenes (16) than values of  $\delta 1.78$  to  $\delta 1.88$  for E-isomers. This Z assignment is further supported by methyl proton values of  $\delta 1.45$  vs  $\delta 1.70$  for conformation about each double bond in dienestrol diacetate in a study in which Metzler (17) noted the differences in chemical shifts of both the methyl and methine protons in a series of dienestrol derivatives.

The presence of a single asymmetric center at the C-4 position (Fig. 1) is clearly shown by the non-equivalence display



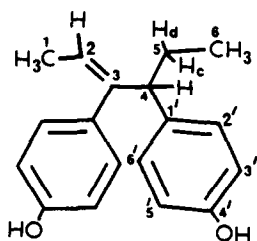


Figure 1

of the protons of the adjacent methylene group. Thus a noteworthy feature of the NMR spectrum of  $\psi$ -DES is that each methylene protons exhibited different chemical shift ( $\delta$ 1.77 and  $\delta$ 1.65) with multiple splitting.

In  $\psi$ -DES long range proton-proton coupling constants were also detected. Our double irradiation experiments show that the olefinic hydrogen atom and methyl protons attached to the carbon-carbon double bond are coupled with the methine proton at C-4 (Fig. 1). The above protons are split by the methine proton with an equal coupling constant ( $J=0.9\text{Hz}$ ). However, after decoupling at  $\delta$ 3.3ppm (methine proton), the methyl as well as olefinic protons exhibited a sharp doublet and a quartet respectively.

NOE measurements (Table 2) were extremely useful in the confirmation of the stereochemistry of the olefinic side chain in  $\psi$ -DES. The Z-stereochemistry is supported by comparing the

TABLE 2  
NUCLEAR OVERHAUSER ENHANCEMENTS IN  $\psi$ -DES

Irradiate	Observe	NOE, %
H-2	H-5d	40.9
H-2	H-5c	21.7
CH <sub>3</sub> -1	H-5d	27.3
CH <sub>3</sub> -1	H-5c	17.4
H-4	(H-2'+H-6')	5.0

magnitude of the NOEs for (CH<sub>3</sub>-1), H-5d=27.3% (read as saturation of CH<sub>3</sub>-1 protons caused a 27.3% increase of the signal from proton H-5d), (CH<sub>3</sub>-1), H-5c=17.4% and (H-2), H-5d=40.9%, (H-2), H-5c=21.7%. These NOE data clearly imply that CH<sub>3</sub>-1 group is cis oriented in relation to the phenyl group and the olefinic proton (H-2) is more proximate to the methylene protons (Fig. 1). Additional virtues of the NOE data are shown in the spectral assignments that could be made for the aromatic protons at  $\delta$ 6.93 (2H) which is shown by the observance of the NOE (H-4), H-2'+H-6'=5%.

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