PREPARATION OF PENTADEUTERATED DIETHYLSTILBESTROL ([1,1,1,2,2-D_5] E-3,4-DI-(4-HYDROXYPHENYL)-HEX-3-ENE) *

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SUMMARY

[1,1,1,2,2-D_5] E-3,4-Di-(4-hydroxyphenyl)-hex-3-ene has been synthesized from 1,1-di-(4-benzyloxyphenyl)-butyraldehyde. The preparation of this aldehyde is described. Its reaction with pentadeuterated ethyl magnesium bromide yields [1,1,1,2,2-D_5] 4,4-di-(4-benzyloxyphenyl)-hexan-3-ol, which is debenzylated by catalytical hydrogenation. Upon heating of the resulting [1,1,1,2,2-D_5] 4,4-di-(4-hydroxyphenyl)-hexan-3-ol, a mixture of pentadeuterated diethylstilbestrol and pentadeuterated pseudo diethylstilbestrol is formed, from which the pentadeuterated diethylstilbestrol is obtained in pure form through fractional crystallization.

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Introduction

The synthetic estrogen diethylstilbestrol (DES)* has been associated with the occurrence of carcinogenic and teratogenic lesions of the genital tract in human offspring exposed to DES prenatally (1,2,3). In order to evaluate the role of metabolism for the mechanism underlying these fetotoxic effects, a study of the biotransformation of DES in different species was initiated (4,5). We prepared [monoethyl-D_5]-DES, to make use of the "twin ion technique"(6) in mass spectrometry, a most suitable analytical technique for metabolic studies, particularly if used in combination with radio gas chromatography. Several DES metabolites have been identified recently applying these methods (4,5). The synthesis of the pentadeuterated DES is described in this paper.

Results and Discussion

Methods so far employed for the synthesis of specifically labelled DES are adaptations of the original procedure of Dodds et al. (7). In this route, 1,2-di-(4-methoxyphenyl)-butan-1-one (α-ethyldeoxyanisoin) is reacted with ethylmagnesium iodide to yield 3,4-di(4-methoxyphenyl)-hexan-3-ol, which is dehydrated to 3,4-di-(4-methoxyphenyl)-hex-3-ene (DES dimethyl ether) by heating with potassium hydrogen sulfate. Finally, removal of the methyl groups yields DES.

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*The following abbreviations are used:
DES for diethylstilbestrol, 3,4-di-(4-hydroxyphenyl)-hex-3-ene; ψ-DES for pseudo diethylstilbestrol, 3,4-di-(4-hydroxyphenyl)-hex-2-ene; gc for gas chromatography; ms for mass spectrometry; tlc for thin layer chromatography; nmr for nuclear magnetic resonance spectroscopy; ir for infra red spectroscopy; tms for trimethylsilyl.

[2-^{14}C]3,4-Di-(4-hydroxyphenyl)-hex-3-ene (8) and [1,1,1-D_3] 3,4-di-(4-hydroxyphenyl)-hex-3-ene (9) were prepared by this synthesis. However, in an attempt to synthesize DES labelled with deuterium in the methylene group, it was found that more than 50% of the deuterium was lost during the dehydration step and the desired DES could not be obtained in an isotopically pure state (9). Furthermore, the removal of the protecting methyl groups appears to be difficult.

We wish to report a different synthesis (Fig.1), where the loss of labelled hydrogen from the methylene group is less extensive, and readily removable benzyl groups are used as protecting groups. As a first step, 4-benzyloxy-benzaldehyde (1) is condensed to 4,4'-di-(4-benzyloxy)-benzoin (2). Grignard addition of ethyl bromide yields 1,2-di-(4-benzyloxyphenyl)-butan-1,2-diol (3), which upon treatment with phosphorus pentoxide in benzene is dehydrated and rearranged to a mixture of 1,1-di-(4-benzyloxyphenyl)-butyraldehyde (4) and 1,1-di-(4-benzyloxyphenyl)-butan-2-one (5). The label is introduced at this stage by reacting 1,1-di-(4-benzyloxyphenyl)-butyraldehyde (4) with pentadeutero ethyl magnesium bromide to yield [1,1,1,2,2-D_5] 4,4-di-(4-benzyloxyphenyl)-hexan-3-ol (6), from which the benzyl groups are easily cleaved off by catalytical hydrogenation to give [1,1,1,2,2-D_5] 4,4-di-(4-hydroxyphenyl)-hexan-3-ol (7). Heating of (7) under nitrogen at 200°C for 90 minutes affords a mixture of the cis- and trans-isomers of [1,1,1,2,2-D_5] 3,4-di-(4-hydroxyphenyl)-hex-3-ene (D_5-DES, 8) and [1,1,1,2,2-D_5] 3,4-di-(4-hydroxyphenyl)-hex-4-ene (D_5-ψ-DES, 9). Trans-D_5-DES can be obtained in pure form from the mixture through crystallization from benzene. No attempt was made to

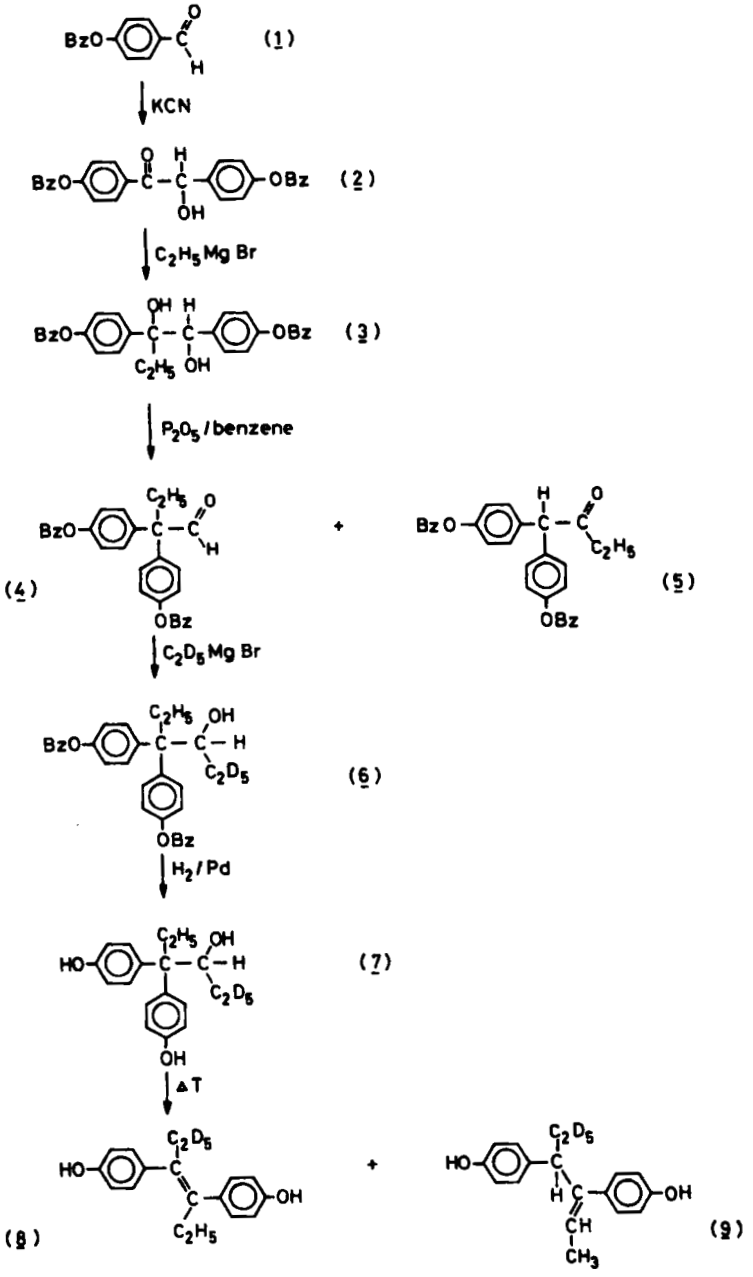

Fig. 1. Synthesis of pentadeuterated DES.

isolate the $D_5\psi$-DES, although this could be done by column chromatography or high pressure liquid chromatography (8). The pentadeuterated DES and most of the intermediates have not been reported in the literature so far. The identification of the new substances is mainly based on their mass spectra, nuclear magnetic resonance spectra and infra red spectra.

The mass spectrum of the pure trans-D_5-DES is shown in Fig.2. Although the pentadeuterated species (m/e 273) prevails, there are significant peaks at m/e 272 and 271. These are only partly accounted for by the facts that (M-1) and (M-2) ions are also found in the mass spectrum of unlabelled DES (Fig.2), and that the pentadeuterated ethyl bromide was not 100% isotopically pure. Thus, a certain loss of deuterium has to be assumed, and the trans-D_5-DES is estimated to consist of a mixture of 74% D_5 species, 16% D_4 species and 10% D_3 species. If it is assumed that the deuterium is exclusively released from the methylene groups, the loss can be calculated to be approximately 20%.

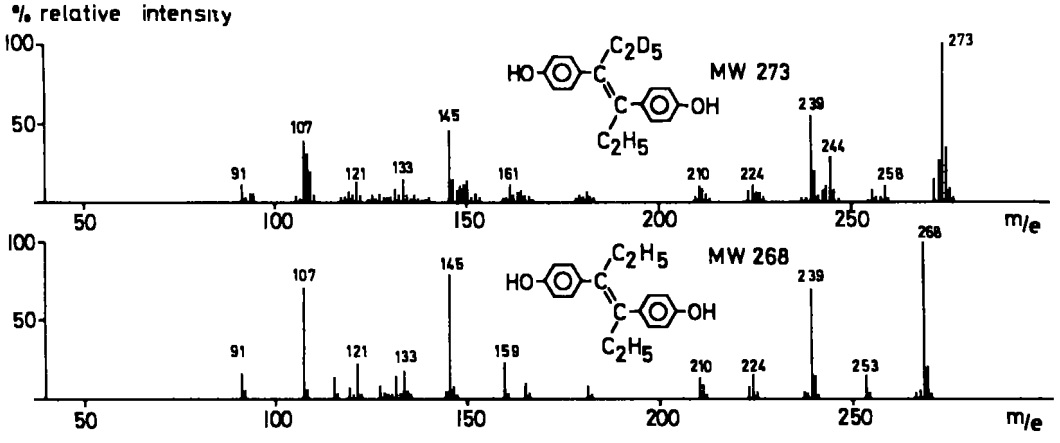

Fig. 2. Mass spectra of pentadeuterated and unlabelled DES.

It should be emphazised that during the final steps of the synthesis, where labelled material is involved, no purification of the intermediates is required, and labelled DES is obtained through crystallization from the reaction mixture. The simplicity of the reactions and the fair overall yield of 23% over the last three steps, together with the moderate exchange of hydrogen, advocate the synthesis for the introduction of radioactive and stable isotopes of hydrogen and carbon into the methyl and/or methylene groups of DES.

Experimental

Melting points (^{o}C) were taken on a Kofler hot-block apparatus and are not corrected. Silica gel 0.05-0.2 mm (70-325 mesh ASTM, E.Merck, Darmstadt, West Germany) was used for column chromatography. Tlc was carried out on precoated 250 µm silica gel HF_{254} glass plates (E.Merck, Darmstadt) in benzene-ethylacetate 9:1 v/v. For gc-ms analysis, a Varian 2700 gas chromatograph coupled to a Varian CH 7 mass spectrometer was used. The gc column (glass, 6 ft x 2 mm id) was packed with 3% OV-225 on GasChrom Q 100/120 mesh and operated at 200-260^{o}C with 4^{o}C/min and a helium flow of 30 ml/min. Temperatures were 260^{o}C for the injection port, separator and capillary connecting tubes (all-glass system). Samples analyzed by gc-ms were trimethylsilylated with N.O-bis-(trimethylsilyl)acetamide. All mass spectra were taken at an electron energy of 70 eV.

Nmr spectra were recorded on a Varian EM 360 Spectrometer. $CDCl_3$ containing 2% tetramethylsilane as internal standard was used as standard, and absorptions are given in δ values. Ir spectra were taken on a Beckmann IR 33 Spectrometer.

Perdeuterated ethyl bromide

[1,1,2,2,2-D_5] ethyl bromide was obtained from E.Merck AG (Darmstadt, West Germany). Isotopic purity according to mass spectrometric analysis was 96.2%.

4-Benzyloxy-benzaldehyde (1)

4-Hydroxy-benzaldehyde (91.5 g, 0.75 mole), benzylbromide (128 g, 0.75 mole) and K_2CO_3 (103.5 g, 0.75 mole) were stirred together in acetone (150 ml) for 20 hours. The reaction mixture was filtered and the acetone removed under reduced pressure. The residue was crystallized from ethanol to yield (1) (110 g, 0.52 mole, 69% yield) m.p. 73° (lit. (10), 72°).

4,4'-Di-(benzyloxy)-benzoin (2)

4-Benzyloxy-benzaldehyde (106 g, 0.5 mole) in ethanol (450 ml) and potassium cyanide (9 g, 0.14 mole) in water (25 ml) were combined and refluxed for 48 hours. The solvents were evaporated in vacuo and the residue distributed between chloroform (400 ml) and water (400 ml). The organic phase was washed with water and dried over Na_2SO_4 prior to evaporation of the chloroform. The remaining product according to tlc (benzene-ethylacetate 9:1 v/v) contained the starting material (R_F 0.46), the benzoin (2) (R_F 0.31) and two unknown compounds (R_F0.55 and 0.10). It was chromatographed in four portions on columns (55 x 4 cm) filled with silica gel and eluted subsequently with benzene (500 ml) and benzene-ethylacetate 98:2 v/v (4 l). Fractions (15 ml) were monitored by tlc. (2) was obtained in 19% yield and had m.p. 135° after crystallization from ethanol. nmr ($CDCl_3$) ∘ 4.50-4.60 (1H, d, hydroxy), 4.97 (2H, s, benzylic), 5.03 (2H, s, benzylic), 5.77-5.87 (1H, d, methine), 6.83-8.00 (8H, m, aromatic), 7.37 (10H, s, phenyl); ir (KBr) ν_{CO} 1660; M^+ 424 (Fig.3).

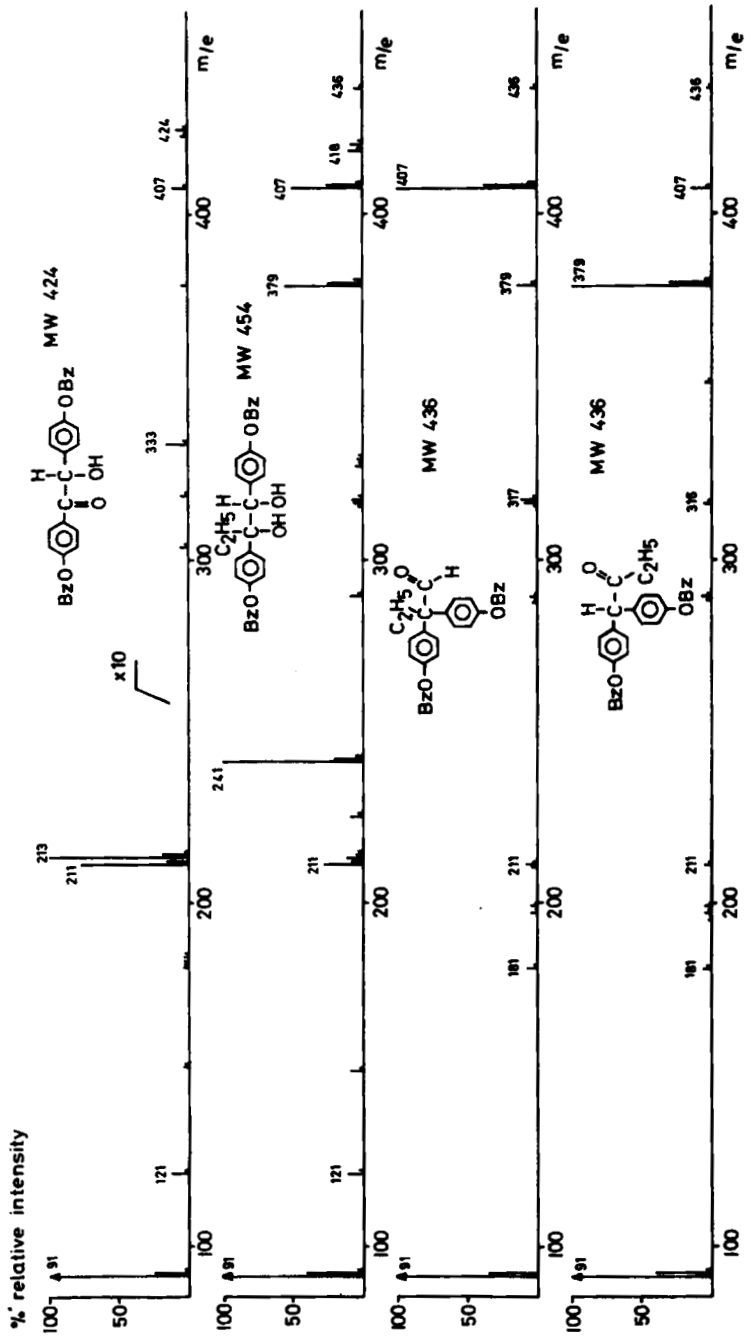

Fig. 3. Mass spectra of unlabelled compounds (obtained by direct inlet).

1,2-Di-(4-benzyloxyphenyl)-butan-1,2-diol (3)

4,4'-Di-(benzyloxy)-benzoin (4.24 g, 10 mmole) was dissolved in dry tetrahydrofurane (50 ml) and dropped into a stirred solution of ethyl magnesium bromide prepared from magnesium turnings (1.2 g, 50 mg atoms) and ethyl bromide (3.75 ml, 50 mmole) in dry ether (50 ml). The reaction mixture was stirred for 5 hours, then decomposed by adding water (30 ml) in small portions. Aqueous 25% ammonium chloride (30 ml) and ether (100 ml) were added and the ether phase washed with water, dried (Na_2SO_4) and evaporated in vacuo. Tlc revealed the main product (3) with R_F 0.16, starting material (2) R_F 0.31 and two other products (R_F 0.48 and 0.58). The mixture was chromatographed on a silica gel column (50 x 3 cm) and subsequently eluted with benzene-ethylacetate 95:5 v/v (100 ml), 9:1 (300 ml) and 2:1. Recrystallization from benzene gave pure (3) in 74% yield with m.p. 97-8°. nmr ($CDCl_3$) δ 0.63-0.90 (3H, t, J = 7 Hz, methyl), 1.80-2.20 (2H, q, J = 7 Hz, methylene), 2.47 (2H, s, broad, hydroxy), 5.03 (4H, s, benzylic), 6.77-7.17 (8H, m, aromatic), 7.40 (10 H, s, phenyl); mass spectrum Fig.3.

1,1-Di-(4-benzyloxyphenyl)-butyraldehyde (4) and

1,1-di-(4-benzyloxyphenyl)-butan-2-one (5)

1,2-Di-(4-benzyloxyphenyl)-butan-1,2-diol (4.54 g, 10 mmole) was dissolved in benzene (150 ml) and phosphorus pentoxide was added in small portions under magnetic stirring until no more diol could be detected by tlc. Ice cold water (150 ml) was added in portions and the benzene layer washed with water and dried (Na_2SO_4). The products (4) (R_F 0.65) and (5) (R_F 0.58) were separated on a silica gel column (55 x 4 cm) eluted with benzene. Both compounds can be crystallized from ethanol. (4) 47% yield, m.p. 110°; nmr ($CDCl_3$) δ 0.60-0.83 (3H, t, J = 7 Hz,

methyl), 2.07-2.43 (2H, q, J = 7 Hz, methylene), 5.05 (4H, s, benzylic), 6.83-7.23 (8H, m, aromatic), 7.40 (10 H, s, phenyl), 9.73 (1H, s, aldehyde); ir (KBr) ν_{CO} 1710 cm^{-1}; M^+ 436 (Fig.3). (5) 36% yield, m.p. 113°; nmr ($CDCl_3$) δ 0.90-1.17 (3H, t, J = 7 Hz, methyl), 2.37-2.90 (2H, q, J = 7 Hz, methylene), 5.03 (4H, s, benzylic), 6.83-7.23 (9H, m, aromatic and methine), 7.40 (10 H, s, phenyl); ir (KBr) ν_{CO} 1710 cm^{-1}; M^+ 436 (Fig.3).

[1,1,1,2,2-D_5] 4,4-Di-(4-benzyloxyphenyl)-hexan-3-ol (6)

1,1-Di-(4-benzyloxyphenyl)-butyraldehyde (1.09 g, 2.5 mmole) dissolved in dry tetrahydrofurane (10 ml) was added to the stirred solution of D_5-ethyl magnesium bromide prepared from magnesium turnings (0.24 g, 10 mmole) and pentadeutero ethyl bromide (0.75 ml, 10 mmole) in dry ether (20 ml). Stirring was continued for 2 hours, then water (10 ml) and 25% aqueous ammonium chloride (10 ml) were added and the ether phase washed with water and dried (Na_2SO_4). Evaporation of the ether in vacuo left a residue that was uniform in tlc (R_F 0.56), but was shown to consist of 2 compounds by gc-ms: (6), 91%, mass spectrum Fig.4, and another substance, 9%, which was tentatively identified by its mass spectrum as [1-D] 2,2-di-(4-benzyloxyphenyl)-butan-1-ol (spectrum not shown). Since this by-product did not affect the following reactions, no attempts to purify (6) were made.

[1,1,1,2,2-D_5] 4,4-Di-(4-hydroxyphenyl)-hexan-3-ol (7)

Crude [1,1,1,2,2-D_5] 4,4-di-(4-benzyloxyphenyl)-hexan-3-ol (ca. 2.5 mmole) was dissolved in ethylacetate (100 ml) and hydrogenated in the presence of palladium (10% on charcoal) for 2.5 hours. The catalyst was removed by filtration through celite and the solvent evaporated under reduced pressure. The product according to gc-ms contained 91% (7) and 9% of [1-D] 2,2-di-(4-hydroxyphenyl)-butan-1-ol (mass spectra Fig.4).

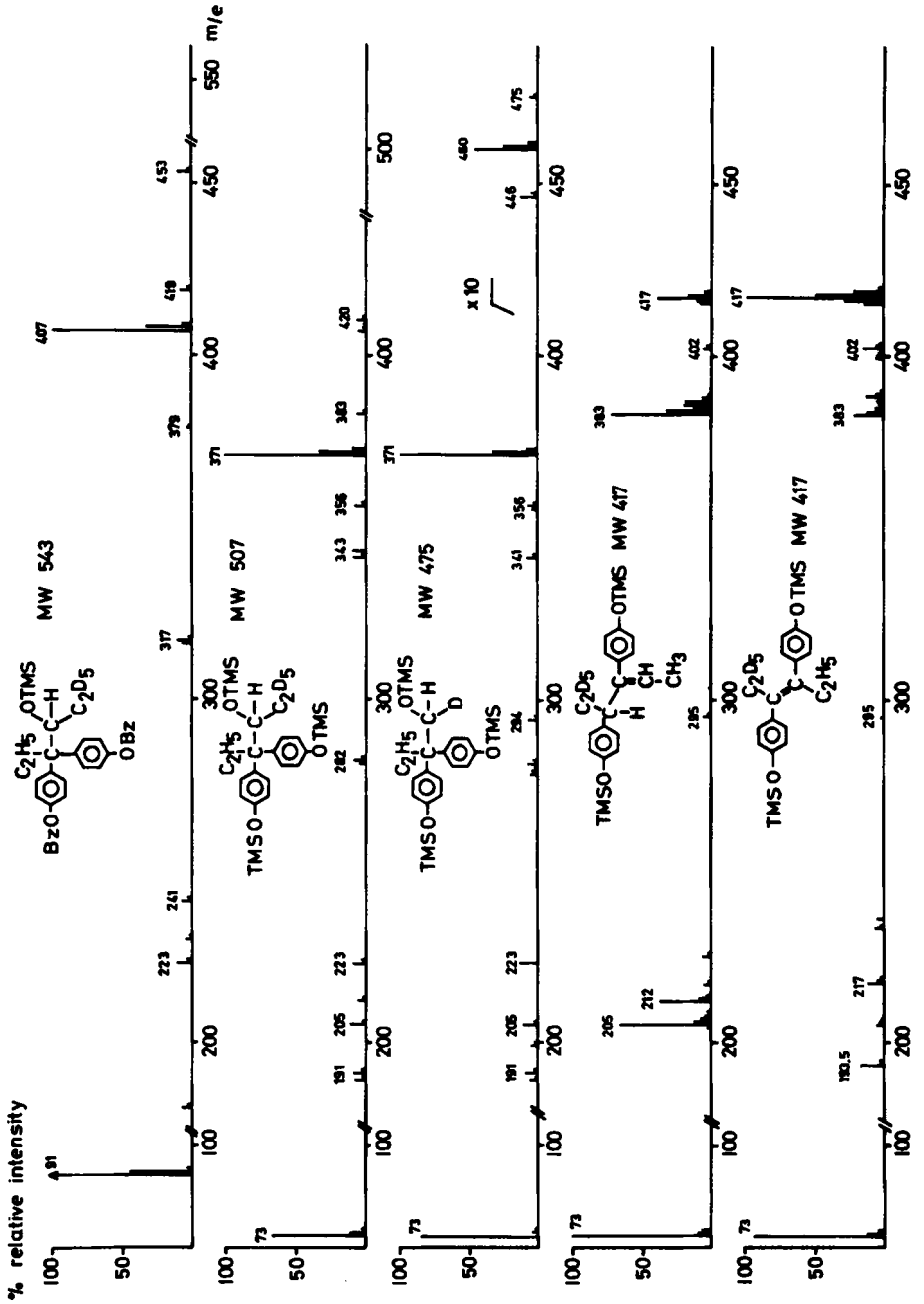

Fig. 4. Mass spectra of pentadeuterated compounds (obtained by gc-ms).

[1,1,1,2,2-D_5] 3,4-Di-(4-hydroxyphenyl)-hex-3-ene (8) and

[1,1,1,2,2-D_5] 3,4-di-(4-hydroxyphenyl)-hex-4-ene (9)

[1,1,1,2,2-D_5] 4,4-Di-(4-hydroxyphenyl)-hexan-3-ol (ca. 2.5 mmole) as obtained from the preceeding reaction was dissolved in a small volume of ether, filled into a thick walled glass tube and the solvent evaporated under a stream of nitrogen. After heating the sealed tube in an oil bath at 200° for 90 minutes, the reaction mixture consisted of 5 compounds, as was shown by gc-ms analysis (Fig.5). Pentadeuterated cis-DES (15% of the

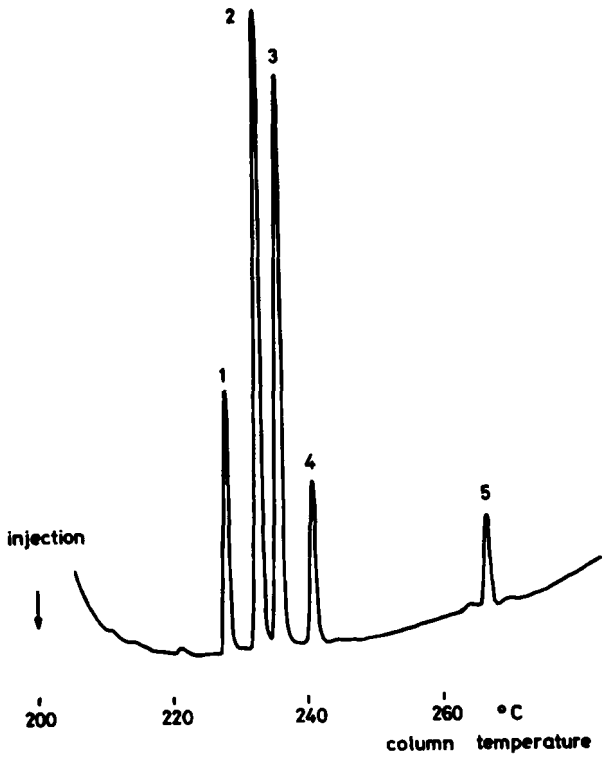

Fig. 5. Gas chromatogram of the crude D_5-DES: 1 cis-DES; 2 ψ-DES; 3 trans-DES; 4 2,2-di-(4-hydroxyphenyl)-butan-1-ol; 5 4,4-di-(4-hydroxyphenyl)-hexan-3-ol.

reaction mixture), trans-DES (8) (33%) and ψ-DES (37%) were extracted from the crude mixture by trituration with hot benzene (15 ml). The extract was subject to fractional crystallization from benzene to yield 155 mg (0.57 mmole, 23% yield) of (8). Purity according to gc was > 97%, the only impurity being (9) (< 3%); m.p. 169° (m.p. DES 171°); mass spectrum Fig.2. No attempt was made to isolate pure (9).

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