THE SYNTHESIS OF TRIDEUTERATED DIETHYLSTILBESTROL ([1,1,1-D_3] E-3,4-DI-(4-HYDROXYPHENYL)-HEX-3-ENE)*

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SUMMARY

[1,1,1-D_3] E-3,4-Di-(4-hydroxyphenyl)-hex-3-ene has been synthesized from 1,2-di-(4-methoxyphenyl)-butan-1-one. Reaction of this ketone with [2,2,2-D_3] ethyl magnesium iodide yields [1,1,1-D_3] 3,4-di-(4-methoxyphenyl)-hexan-3-ol, which on dehydration with potassium hydrogen sulfate and demethylation with methyl magnesium iodide yields isotopically pure trideuterated diethylstilbestrol. Isotopically pure [2,2-D_2] 3,4-di-(4-dydroxyphenyl)-hex-3-ene cannot be prepared by this route from [1,1-D_2] ethyl magnesium iodide.

Introduction

The occurrence of vaginal adenocarcinoma in adolescent daughters of mothers who received diethylstilbestrol during pregnancy (1,2) stimulated our interest in the metabolism of diethylstilbestrol by mammalian tissues. In order to study its metabolism by means of the twin ion technique (3), it was necessary to synthesize deuterated diethylstilbestrol. In addition, we propose to use deuterated diethylstilbestrol as a carrier in the estimation of diethylstilbestrol by mass fragmentography.

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We now report the synthesis of a trideuterated species, [1,1,1-D_3] E-3,4-di-(4-hydroxyphenyl)-hex-3-ene.

Results and Discussion

The synthetic route to diethylstilbestrol is based on that of Twombly and Schoenewaldt (4), which is itself an adaptation of the original method of Dodds *et al.* (5). In this procedure the Grignard addition of ethyl iodide to α-ethyldesoxyanisoin (1)*** yields 3,4-dianisylhexan-3-ol (2). Dehydration is accomplished by heating the carbinol with potassium hydrogen sulfate at 200° for one hour; 3,4-dianisylhex-3-ene (5) is obtained in good yield. The Grignard addition and acid catalyzed dehydration were found to proceed in yields of 90% and 80% respectively.

Although Twombly and Schoenewaldt (4) reported that demethylation of the dimethyl ether (5) to give diethylstilbestrol (9) may be achieved by refluxing it with excess potassium hydroxide in ethylene glycol for four hours, considerable difficulty was encountered in this step. Refluxing the dimethyl ether for up to 12 hours with excess potassium hydroxide in dry, freshly distilled ethylene glycol (4) produced a mixture of products from which diethylstilbestrol was obtained in only 20% yield after extensive purification. Other methods for the cleavage of methyl ethers included treatment with hydrobromic acid in refluxing acetic acid (6), aluminum chloride in refluxing benzene (7), and pyridine hydrochloride (8); none gave satisfactory results. Finally, methyl magnesium iodide was found to be an effective demethylating agent (9). The dimethyl ether (5) was treated with an ethereal solution of the Grignard reagent, the solvent evaporated, and the residual material heated to 180° for

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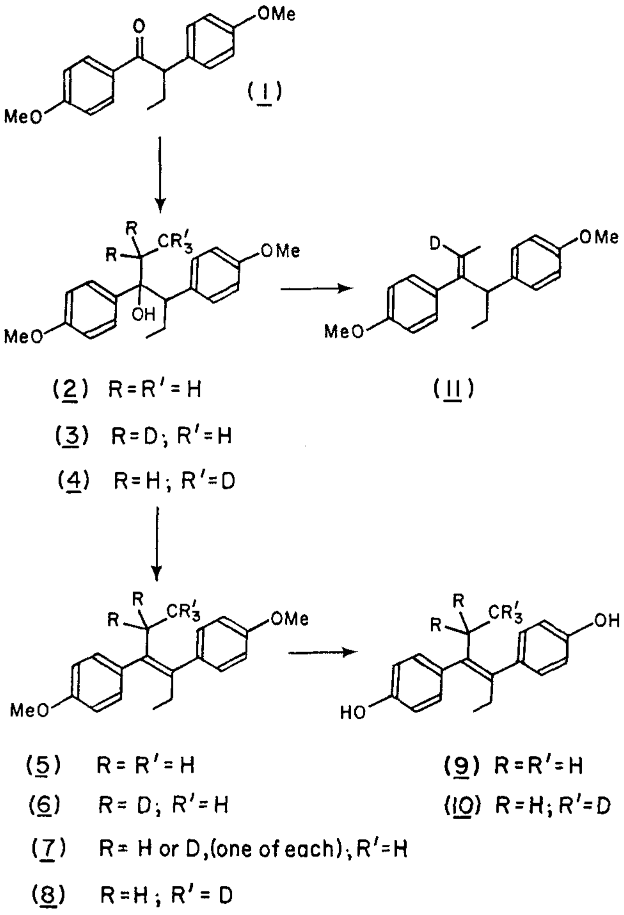
***The following trivial names and non-standard abbreviations are employed: α-ethyldesoxyanisoin for 1,2-di-(4-methoxyphenyl)-butan-1-one; 3,4-dianisylhexan-3-ol for 3,4-di-(4-methoxyphenyl)-hexan-3-ol; 3,4-dianisylhex-3-ene for 3,4-di-(4-methoxyphenyl)-hex-3-ene; diethylstilbestrol for E-3,4-di-(4-hydroxphenyl)-hex-3-ene; gc-ms for gas chromatography-mass spectrometry; uv for ultraviolet; tlc for thin layer chromatography; EtOH for ethanol; nmr for nuclear magnetic resonance spectroscopy.



(1)

(2) R=R'=H
(3) R=D; R'=H
(4) R=H; R'=D

(11)

(5) R=R'=H
(6) R=D; R'=H
(7) R=H or D,(one of each); R'=H
(8) R=H; R'=D

(9) R=R'=H
(10) R=H; R'=D

one hour. Pure diethylstilbestrol (9) was obtained in 50% yield after minimal purification.

[2,2-D_2] 3,4-Dianisylhexan-3-ol (3) was prepared by the Grignard addition of [1,1-D_2] ethyl iodide to α-ethyldesoxyanisoin (1). Although the [D_2] carbinol (3) was isotopically pure, dehydration with potassium hydrogen sulfate caused loss of deuterium. When the resulting diethylstilbestrol dimethyl ether was examined by mass spectrometry, it was found to consist of a mixture of 23% of the D_2 species (6), 41% of a monodeuterated species (7), and 36% of the undeuterated species (5). The loss of deuterium may be explained by the intermediary formation of [2D] 3,4-dianisylhex-2-ene (11). This compound, designated "ψ-diethylstilbestrol dimethyl ether", was first noted as a by-product in the acidic dehydration of 3,4-dianisylhexan-3-ol (2)(5) and thought to be the cis dimethyl ether (5), as it was converted to the trans isomer on irradiation (5) or treatment with iodine (10) or p-toluene sulfonic acid (10). Since ozonolysis yields acetaldehyde and α-ethyldesoxyanisoin (11) the "ψ" compound is now thought to be a mixture of the two geometrical isomers of 3,4-dianisylhex-2-ene. The reversible, acid-catalyzed migration of the double bonds via (7) explains the loss of deuterium. Examination by uv of the resulting mixture of deuterated and nondeuterated dimethyl ethers showed the absence of "ψ" isomers.

Dehydration of [1,1,1-D_3] 3,4-dianisylhexan-3-ol (4) gave [1,1,1-D_3] 3,4-dianisylhex-3-ene (8) of 98% isotopic purity. Isotopically pure trideuterated diethylstilbestrol (10) was formed by demethylation with methyl magnesium iodide in 36% overall yield.

Experimental

The gc-ms analyses were performed on an LKB 9000 Gas Chromatograph-Mass Spectrometer. The column (2.5m x 4 mm id) was packed with 1% OV-1 on Gas-Chrom Q and operated at a temperature of 200^o with a helium flow of 27ml/min. The samples were deposited on stainless steel gauzes (3). Spectra were taken at 70eV.

Nmr spectra were taken using a Varian A-60 Nmr Spectrometer. Solvents were as noted and 2% tetramethylsilane was used as internal standard; absorptions are recorded in δ values. Ultraviolet spectra were recorded on a Cary 11 spectrophotometer. Melting points were taken on a Kofler hot-block apparatus, and are uncorrected. Prepared silica gel plates for preparative thin layer chromatography were obtained from Quantum Industries (Quanta Gram PQ1F, 1mm thickness).

Deuterated ethyl iodide

Ethyl iodide deuterated at the 1 or 2 position (i.e. [1,1-D_2] or [2,2,2-D_3]) was obtained from Stohler Isotope Chemicals. Isotopic purity was checked in each case by Grignard addition to α-ethyldesoxyanisoin (1) and mass spectrometric examination of the resultant carbinol, [2,2-D_2] or [1,1,1-D_3] dianisylhexan-3-ol ((3) or (4) respectively). 99% Purity was found in each case.

α-Ethyldesoxyanisoin (1)

α-Ethyldesoxyanisoin was the generous gift of Dr. Victor Drill of the Searle Company. It was purified by column chromatography on alumina, eluting with benzene-petroleum ether:1-4 (10). The resultant colorless viscous oil was crystallized from petroleum ether-benzene at -70° to yield white cyrstals, melting at 46-49° to a cloudy melt, clearing at 54-56° (lit. (12) m.p. 46-48°, clearing at 50-53°); M^+ 284; nmr ($CDCl_3$) δ 0.76-1.02 (3H, t, J=7Hz, methyl), 1.80-2.24 (2H, m, methylene), 3.73-3.79 (6H, d, J=4Hz, methoxy) 4.16-4.55 (1H, m, methine), 6.70-6.98 (4H, m, aromatic), 7.11-7.33 (2H, m, aromatic), 7.84-8.10 (2H, m, aromatic).

[2,2-D_2] 3,4-Dianisylhexan-3-ol (3)

Onto a stirred mixture of magnesium turnings (510 mg, 21 mg atoms, 2.0 equiv.) and dry ether (10 ml) was dropped [1,1-D_2] ethyl iodide (2.16 ml, 26 mmole, 2.5 equiv.) in dry ether (10 ml) at such a rate as to maintain refluxing without external heating. After the reaction was completed, α-ethyldesoxyanisoin (3.0 g, 11 mmole, 1.0 equiv.) was added in dry ether (20 ml) and the mixture stirred at room temperature overnight. After addition of aqueous ammonium chloride, the ether solution was washed, dried (Na_2SO_4) and the ether evaporated under reduced pressure to yield (3) as a colorless oil (3.3 g, 99% yield) that was pure by tlc and gc. Trituration with ethanol yielded white crystals, m.p. 65-95° (lit. (10) 70-100°). Apparent M^+ 298 (i.e. M^+-H_2O) due to ready dehydration in the flash heater; 99% isotopic purity; nmr ($CDCl_3$) δ0.59-1.13 (6H, m, methyl), 1.36-2.26 (3H, m, methylene + OH), 3.17-3.36 (1H, m, methine) 3.84 (6H, s, methoxy), 6.59-7.20 (8H, m, aromatic).

[1,1,1-D_3] 3,4-Dianisylhexan-3-ol (4)

The method of preparation of [1,1,1-D_3] 3,4-dianisylhexan-3-ol was the same as that described for (3) except that [2,2,2-D_3] ethyl iodide was used. For the product: m.p. (EtOH) 75-90° (lit. (10) 70-100°); M^+299 (dehydration in mass spectrometer), isotopic purity 99%; nmr ($CDCl_3$) δ 0.32-0.80 (3H, m, methyl), 1.59-1.96 (5H, m, methylene + OH), 2.60-2.96 (1H, m, methine), 3.78 (6H, s, methoxy), 6.70-7.28 (8H, m, aromatic).

[2,2-D_2] 3,4-Dianisylhex-3-ene (6)

[2,2-D_2] 3,4-Dianisylhexan-3-ol (457 mg, 1.5 mmole, 1.0 equiv.) and potassium hydrogen sulfate (240 mg, 1.8 mmole, 1.2 equiv.) were stirred together at 200° for one hour. After cooling, the reaction mixture was extracted with ether, and the ether extract washed with aqueous sodium bicarbonate and water, and dried (Na_2SO_4). Removal of the solvent yielded a dark brown oil that was purified by column chromatography on Florisil (20g, 1.5 x 12.5cm column). The pale yellow oil that was eluted with benzene was crystallized from ethanol to give clumps of cream colored needles (350 mg, 82% yield) m.p. 122-3° (lit. (5) 123-4°); λ_{max} 242 (ε 19,500)) and 280 nm (ε 5,500),[(lit. (13) λ_{max} 240 (ε 20,000) and 280 nm (ε 5,500)]; nmr ($CDCl_3$) δ 0.59-0.95 (6H, triplet on doublet, methyl), 1.92-2.39 (2H, m, methylene), 3.85 (6H, s, methoxy), 6.84-7.33 (8H, m, aromatic). Mass spectrometry showed a mixture of m/e 298 (23%), 297 (41%) and 296 (36%).

[1,1,1-D_3] 3,4-Dianisylhex-3-ene (8)

[1,1,1-D_3] 3,4-Dianisylhexan-3-ol (640 mg, 2.0 mmole, 1.0 equiv.) and potassium hydrogen sulfate (400 mg, 2.9 mmole, 1.5 equiv.) were stirred together at 200° for one hour. The reaction mixture was worked up as for (6) to yield [1,1,1-D_3] 3,4-dianisylhex-3-ene as pale yellow crystals (520 mg, 86% yield), m.p. (EtOH) 123° (lit. (5), 123-4°), λ_{max} 240 (ε 20,500) and 280 nm (ε 5,500), [lit. (13) λ_{max} 240 (ε 20,000) and 280 nm

(ε 5,500)];m/e 299 with 98% isotopic purity; nmr ($CDCl_3$) δ 0.60-0.94 (3H, m, methyl), 2.04-2.25 (4H, m, methylene), 3.83 (6H, s, methoxy), 6.77-7.30 (8H, m, aromatic).

[1,1,1-D_3] 3,4-Di-(4-hydroxyphenyl)-hex-3-ene (10)

Methyl magnesium iodide was prepared by adding methyl iodide (0.5 ml, 8.0 mmole, 6.0 equiv.) to magnesium turnings (100 mg, 4.2 mg atoms, 3.1 equiv.) in dry ether (10 ml). After the reaction was completed, [1,1,1-D_3] 3,4-dianisylhex-3-ene (400 mg, 1.3 mmole, 1.0 equiv.) was added and, after evaporation of the ether, the mixture was heated at 170° for one hour.

The cooled reaction mixture was treated with 1N HCl and ether, and the ether layer extracted twice with 5% KOH. The basic solution was then acidified (1N HCl) and extracted twice with ether. After drying (Na_2SO_4), removal of the solvent gave a pale yellow oil. Purification by preparative tlc, (benzene-ether 1-2) on silica plates yielded a pale yellow solid. Recrystallization from benzene gave colorless needles of [1,1,1-D_3] 3,4-di-(4-hydroxyphenyl)-hex-3-ene (183 mg, 50% yield), m.p. 167-8° (lit (5), m.p. 167-8°);m/e 271 with 98% isotopic purity; nmr($(CD_3)_2CO$) δ 0.54-0.94 (3H, m, methyl), 1.93-2.30 (4H, m, methylene), 2.88-3.02 (2H, broad, OH), 6.76-7.46 (8H, m, aromatic).

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