Synthesis of hexadeuterated diethylstilbestrol ([1,1,1,6,6,6-d6]-E-3,4-di-(4-hydroxyphenyl)-hex-3-ene)*

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Summary

[1,1,1,6,6,6-d6]-E-3,4-di-(4-hydroxyphenyl)-hex-3-ene was synthesized from [1,1,1-d3]-4-methoxypropiophenone by reductive coupling . The preparation of [1,1,1-d3]-4-methoxypropiophenone involved reaction of p-anisoylchloride with perdeuterated diethylcadmium followed by back-exchange of the alpha protons.

Introduction

Diethylstilbestrol (DES) is an anabolic drug which can be used to improve the growth rate and the feed conversion efficiency of animals in livestock breeding.

Because of the carcinogenicity of DES its use is prohibited by law in The Netherlands. Therefore, sensitive and reliable methods of analysis are necessary in order to control the illegal use of DES. Isotope dilution-gas chromatography-mass speetros-

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copy (ID-GC-MS) offers a sensitive and reliable tool excellently suited for this purpose. In this connection hexadeuterated DES was required as an internal standard. Its preparation is reported in this paper.

Results and discussion

The synthetic route leading to DES-d6 reported in this paper differs from the ones used to prepare DES-d3 (1) and DES-d5 (2). The present route is delineated in figure 1.

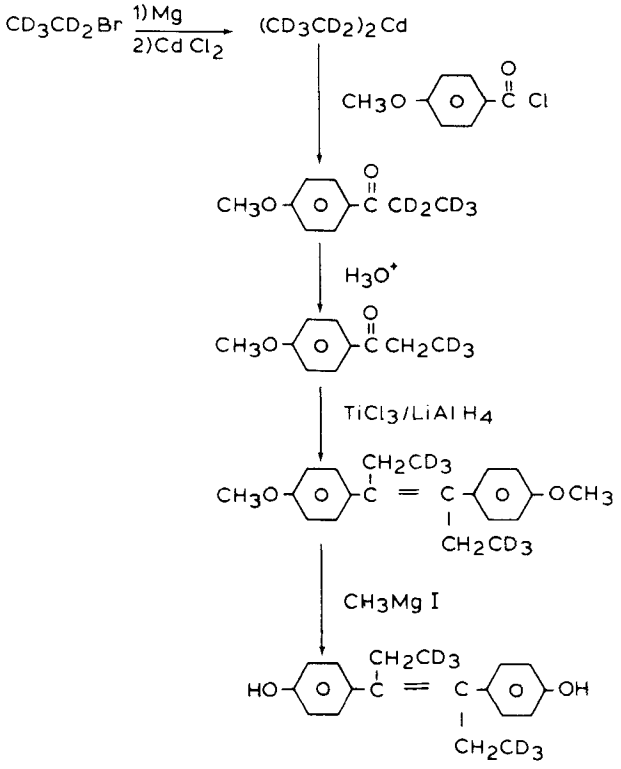

Fig 1

Perdeuterated diethylcadmium was prepared from pentadeuterated ethylbromide via the Grignard reagent. Reaction of the organocadmium compound with p-anisoylchloride yielded [1,1,1,2,2-d5]-4-methoxypropiophenone in 50% yield. Back-exchange with diluted hydrochloric acid afforded [1,1,1-d3]-4-methoxypropiophenone.

Coupling of [1,1,1-d3]-4-methoxypropiophenone with titaniumtrichloride- lithiumaluminiumhydride (3) gave a mixture of cis- and trans DES-d6-dimethylether (cis : trans ~ 85 : 15) in 50% yield. Demethylation was accomplished in 90% yield using methylmagnesiumiodide. The product, a mixture of cis- and trans DES-d6 (cis : trans ~ 4 : 1) was isomerized in boiling chloroform to afford the equilibrium mixture of cis- and trans DES-d6 (cis : trans ~ 1 : 3), from which trans DES-d6 was obtained by recrystallization from benzene.

Experimental

NMR spectra were taken using a Varian EM 360 NMR spectrometer. Absorptions are recorded in δ-values. GC analyses were performed on a Varian 3700 gas chromatograph, equiped with a 3 % OV-1 on Supelcoport (100-120 mesh) column (2 m x 2 mm) operating at a temperature of 200 °C and a flow-rate of 25 ml/min. HPLC analysis were performed with the aid of a Kipp Analytica 9208 HPLC pump on an 150 x 4.6 mm hypersil ODS column. (Eluent 65 % (v/v) methanol in water, UV detection at 254 nm).

Pentadeuterated ethyl bromide

[1,1,2,2,2-d5]-ethyl bromide was obtained from E. Merck AG (Darmstadt, West Germany). According to the manufacturer the isotopic purity was better than 98%.

[1,1,1-d3]-4-Methoxypropiophenone

Perdeuterated diethylcadmium was prepared from 8 g of ethyl bromide-d5, 1.8 g of magnesium and 6.8 g of cadmium(II)chloride in 50 ml of benzene. To this solution was added with stirring 11.9 g of p-anisoylchloride dissolved in 40 ml of benzene. The addition caused the temperature to rise to about 45 °C. After stirring the reaction mixture for two hours at 45 °C it was poured on ice and acidified with concentrated sulfuric acid. The resulting mixture was left overnight. Extraction with diethyl ether, washing of the organic layers with water, drying and evaporation of the solvents gave deuterated 4-methoxypropiophenone. According to NMR the alpha deuterium atoms had not completely exchanged. The crude product was refluxed in dioxane containing 20 ml of 1 N HCl for one hour. Work-up as described above yielded crude [1,1,1-d3]-4-methoxypropiophenone. It was purified by distillation to afford 5.7 g (50%) of pure [1,1,1-d3]-4-methoxypropiophenone (b.p. 138-140 °C / 14 mm) as a colourless oil that solidified completely after one night at 6 °C. NMR ($CDCl_3$) : δ2.6 (broad s., 2H), 3.5 (s., 3H), 6.7-7.8 ppm (AB-system, 4H)

[1,1,1,6,6,6-d6]-E-3,4-di-(4-methoxyphenyl)-hex-3-ene

Titaniumtrichloride (10 g) in 60 ml of dry tetrahydrofuran (THF) was stirred during 15 minutes. Lithiumaluminiumhydride (1.25 g) was added carefully in small portions under a nitrogen atmosphere. A vigorous reaction occurred (gas evolution, generation of heat) depositing a black precipitate. To complete the reaction the mixture was refluxed for 30 minutes. After cooling the reaction mixture to room temperature a solution of 5.7 g of [1,1,1-d3]-4-methoxypropiophenone in 20 ml of dry THF was added in 10 minutes. The resulting mixture was refluxed for four hours

under nitrogen. The cooled reaction mixture was diluted with 100 ml of water and extracted with dichloromethane. The organic layers were washed with water, dried and evaporated to give an oil. This oil was adsorbed on silica and eluted with petroleum ether bp 40-60 °C / ethylacetate, 20 / 1 to yield 2.2 g (50%) of dimethyl-DES-d6 as a mixture of cis- and trans DES-d6 as judged by NMR. NMR : δ2.4 (broad s., 2H, trans DES) 2.7 (broad s., 2H, cis DES) 3.8 (s.,3H), 6.7-7.2 ppm (AB-system, 4H).

[1,1,1,6,6,6-d6]-E-3,4-di-(4-hydroxyphenyl)-hex-3-ene

Methylmagnesiumiodide (150 mmol) was prepared in diethyl ether. [1,1,1,6,6,6-d6]-3,4-di-(4-methoxyphenyl)-hex-3-ene (2.2 g) was dissolved in this solution. The diethyl ether was removed by distillation. The resulting mixture was heated under nitrogen at 160-180 °C for one hour. After cooling, diethyl ether (100 ml) was added followed by the careful addition of water (10 ml). The mixture was acidified with 10% sulfuric acid and stirred until both layers were clear. The aqueous layer was extracted with diethyl ether, the combined ether layers were dried and evaporated. There was obtained 1.8 g (90%) of a mixture of cis- and trans DES-d6 (cis : trans ~ 4 : 1). Isomerization was accomplished by refluxing the crude product in 100 ml of chloroform until NMR indicated the presence of a cis /trans ratio of 1/3. Pure [1,1,1,6,6,6-d6]-E-3,4-di-(4-hydroxyphenyl)-hex-3-ene was obtained by crystallization of the crude product from benzene. The purity of the product was judged to be > 95% by NMR, HPLC and GC. NMR (deuteroacetone) : δ2.1 (bs,4H), 6.7-7.1 (AB-system,8H), 8.3 ppm (s,2H).

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