

SYNTHESIS OF HEXADEUTERATED DIETHYLSTILBESTROL

(E - 3,4-DI-(4-HYDROXYPHENYL)HEX-3-ENE-1,1,1,6,6,6-²H₆)

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SUMMARY

We describe the synthesis of deuterated diethylstilbestrol isomers, starting from commercially available ketones. Attempts to synthesize E-3,4-di-(4-hydroxyphenyl)hex-3-ene-2,2,5,5-²H₄ from 4-methoxypropiophenone were not successful. Starting from 4-methoxyacetophenone, isotopically pure E-3,4-di-(4-hydroxyphenyl)hex-3-ene-1,1,1,6,6,6-²H₆ was obtained. The 3-steps synthesis involved alkylation of the ketone with iodomethane-1,1,1-²H₃, reductive coupling with titanium trichloride-lithium reagent, and demethylation with trimethylsilyliodide. The possible use of the hexadeuterated diethylstilbestrol in isotope dilution mass spectrometry is demonstrated.

KEY WORDS : ²H₆- diethylstilbestrol, deuterium labelling, isotope dilution gas chromatography/mass spectrometry.

INTRODUCTION

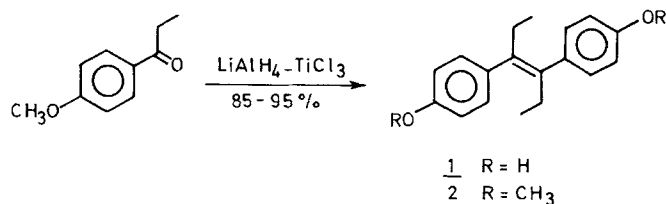
The medical use of the synthetic estrogen diethylstilbestrol (DES) has been restricted strongly because of its possible carcinogenic effects (1,2). In view of these health risks, also the veterinary use of DES in the raising and fattening of livestock and poultry is prohibited in most countries. Techniques to trace illegal DES use via analysis of cattle meat, urine or animal feeds have been reviewed (3). Unambiguous quantitation, however, requires the use of isotope dilution mass spectrometry, which necessitates the availability of isotopically labelled DES.

This study describes our attempts at synthesizing deuterium labelled DES-isotopomers. In view of their possible use in twin-ion techniques (4) and bioavailability studies (5) E (trans) isotopomers had to be obtained as the Z (syn) configuration is not used as an estrogen since it has much less biological activity (6).

RESULTS AND DISCUSSION

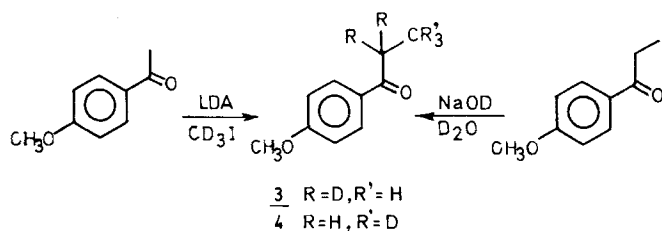
A synthesis of a trideuterated DES derivative, i.e., E-3,4-di-(4-hydroxyphenyl)hex-3-ene-1,1,1-²H₃, has been reported previously (7). The route was based on the method of Dodds and coworkers (8), starting from commercial desoxyanisoin, and involved the use of 1-iodoethane-2,2,2-²H₃.

For the purpose of developing a direct synthesis of DES (1) and in view of its symmetry, we studied the reductive dimerization of ketones to coupled olefins (9). In fact, the one-step synthesis of dimestrol (2), the corresponding dimethyl ether of DES, has been reported by McMurry, using the low valent tita-



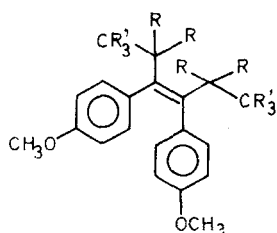
mium induced coupling of 4-methoxypropiophenone ($\text{LiAlH}_4\text{-TiCl}_3$ reagent, 85-95 % yield) (10-11). Other procedures for the reductive coupling of aromatic ketones to olefins with titanium-based reagents are known (12-14).

As a rule, mixtures of \underline{E} - and \underline{Z} -isomers are obtained ; e.g., from acetophenone \underline{E} - and \underline{Z} -2,3-diphenyl-2-butenes are obtained in ratios of 18:82 ($\text{TiCl}_4\text{-Zn}$; 92 % yield) (12), 59:41 ($\text{TiCl}_3\text{-Mg}$; 71% yield) (13), and 90:10 ($\text{TiCl}_3\text{-Li}$; 94% yield) (14), respectively. The latter method was chosen in the present study for effecting the coupling of 1-(4-methoxyphenyl)propan-1-one-2,2- $^2\text{H}_2$ (3) and of 1-(4-methoxyphenyl)propan-1-one-3,3- $^2\text{H}_3$ (4).



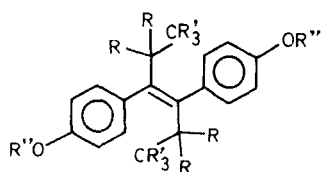
The isotopically pure deuterated ketones 3 and 4 were readily obtained from 4-methoxypropiophenone and 4-methoxyacetophenone, respectively. Treatment of the former ketone with NaOD in D_2O -THF (reflux, 24h) gave the corresponding dideuterated 3 in 87% yield (isotopically pure by ^1H NMR). In contrast, treatment with sodium carbonate in D_2O -THF (reflux, 24h) led to no incorporation of deuterium. Alkylation of the enolate, derived from 4-methoxyacetophenone with lithium-diisopropylamide (LDA), with iodomethane-1,1,1- $^2\text{H}_3$ gave after HPLC purification the desired trideuterated derivative 4 in 73% yield (isotopically pure by ^1H NMR).

The titanium-induced coupling of 3 using McMurry's $\text{TiCl}_3\text{-Li}$ reagent gave, after purification by column chromatography on silica gel, a mixture of \underline{Z} - and \underline{E} -3,4-di-(4-methoxyphenyl)hex-3-ene-2,2,5,5- $^2\text{H}_4$ largely in favor of the \underline{Z} -isomer (58% yield; \sim 95:5 ratio of 5 and 6, respectively). In a similar way a mixture of \underline{Z} - and \underline{E} -3,4-di-(4-methoxyphenyl)hex-3-ene-1,1,1,6,6,6- $^2\text{H}_6$ (7 and 8 ; ratio \sim 95:5, respectively) was obtained from ketone 4. The assignment of the \underline{Z} -geometry to the major isomers 5 and 7 follows from the observed chemical shifts in the ^1H NMR spectrum of 5 (δ 0.93 ppm for H at C-1 and at C-6) and of 7 (δ 2.50 ppm for H at C-2 and at C-5) (15-18). Both 5 and 7 were found isotopically pure by ^1H NMR (360 MHz).



5 R = D, R' = H

7 R = H, R' = D

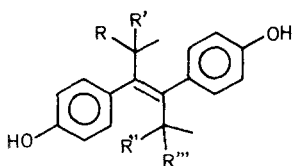


6 R = D, R' = H, R'' = CH₃

8 R = H, R' = D, R'' = CH₃

15 R = H, R' = D, R'' = H

The demethylation of dimestrol 2 usually requires harsh conditions and/or proceeds in low or moderate yields (7,19,20). The use of trimethylsilyl iodide (21), however, allows for smooth dealkylation of 5 (CHCl₃, room temperature, 24h). After work-up (CH₃OH) and purification by column chromatography on silica gel a mixture was obtained (89% yield; 1:1 ratio of E- and Z-DES derivatives according to TLC), from which the E-isomer (higher R_f value (22) in isooctane-ethyl acetate, 7:3) was selectively obtained by crystallization from benzene (31% isolated yield). Mass spectrometric analysis, however, showed the product to consist mainly of undeuterated 1 (32.6%), monodeuterated 9 (39.4%), dideuterated 10 and/or 11 (20.1%), trideuterated 12 (6.2%), and 1.1% tetradeuterated diethylstilbestrol (13).



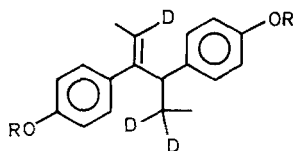
9 R = D, R' = R'' = R''' = H

10 R = R' = D, R'' = R''' = H

11 R = R'' = D, R' = R''' = H

12 R = R' = R'' = D, R''' = H

13 R = R' = R'' = R''' = D



14 R = H or CH₃

The isomerization of the double bond upon demethylation of 5 is not surprising in view of the reaction conditions used (18,22,23). The concomitant loss of deuterium indicates the intermediary formation of Ψ -diethylstilbestrol derivatives, e.g., 3,4-di-(alkoxyphenyl)hex-2-ene-2,5,5-²H₃ (14), during the isomerization process (24).

Under the same reaction conditions hexadeuterated dimestrol 7 gave, after purification on silica gel and crystallization from benzene, the corresponding labelled DES derivative 15 (m.p. 169°C) isotopically pure by ^1H NMR (360 MHz). Analysis by mass spectrometry (selected ion monitoring) indicated a 100% incorporation yield.

The use of 15 as labelled internal standard for isotope dilution mass spectrometry was investigated. No mutual interferences were observed during selected ion monitoring because of the excellent isotopic purity and high mass increment of the synthesized molecule. Hence the molecule is extremely suitable as internal standard to detect and quantitate trace amounts of diethylstilbestrol.

EXPERIMENTAL

The melting points are uncorrected. The ^1H NMR spectra were recorded at 90 MHz (Varian EM-390) or at 360 MHz (WH-Bruker) with TMS as internal standard. Chemical shifts (δ) are given in ppm.

Mass spectral data were obtained on a HP5992B or HP5985B instrument. Spectra of the underivatized compounds were obtained via direct inlet (250°C ballistically). For high resolution gas chromatography-mass spectrometry, an OV1 fused silica column (FSOT 12m x 0.33mm ID) was installed. Temperature settings were: injector (moving needle) 290°C, oven 200°C and transfer line 220°C. Helium (2ml min^{-1}) was used as carrier gas. All spectra were obtained at 70 eV electron impact.

Rf values are quoted for Merck silica gel 60 GF₂₅₄ TLC plates of 0.25 mm thickness.

4-Methoxypropiophenone, 4-methoxyacetophenone, deuterium oxide (100.0 atom % D), iodomethane-D₃ (99 + atom % D), n-butyllithium (1.6 M in hexane) and trimethylsilyliodide were purchased from Janssen Chimica and used without further purification. Tetrahydrofuran and 1,2-dimethoxyethane were freshly distilled from sodium-benzophenone before use. Diisopropylamine (Janssen Chimica) was distilled from lithium aluminium hydride before use. Lithium wire was obtained from Merck and titanium trichloride from Ventron.

For chemical derivatizations prior to gas chromatography, heptafluorobutyric anhydride (HFBA) was used (Macherey and Nagel).

Authentic diethylstilbestrol was obtained from Serva.

1-(4-Methoxyphenyl)propan-1-one-2,2-²H₂ (3)

To cooled deuterium oxide (35 ml) was added, under argon, sodium cut into small pieces (3,5 g; 0.14 mol). After stirring for 30 min at room temperature a solution of 4-methoxypropiophenone (6.99 g; 42 mmol) in tetrahydrofuran (12 ml) was added and the resulting solution was stirred under reflux for 24 hrs. The reaction mixture was extracted with ether, the combined organic phases dried over magnesium sulfate and concentrated in vacuo after filtration to yield ketone 3 (6 g; 87% yield) as an oil which solidified upon standing. The product was found isotopically pure by ¹H NMR. Rf (isooctane-ethyl acetate, 95:5) 0.08. ¹H NMR (90 MHz, CDCl₃) : 1.20 (3H,s), 3.87 (3H,s), 6.95 (2H, A₂B₂), 7.99 (2H, A₂B₂).

1-(4-Methoxyphenyl)propan-1-one-3,3-²H₃ (4)

To a cooled (0°C) solution of lithium diisopropylamide (from 7.57 ml of diisopropylamine, 33.7 ml of a 1.6 molar n-butyllithium solution in hexane and 100 ml of tetrahydrofuran) was added, under argon, a solution of 4-methoxyacetophenone (8.1 g; 54 mmol) in tetrahydrofuran. After stirring for 30 min at 0°C iodomethane-1,1,1-²H₃ (10 ml, 0.162 mol) was added. After stirring for 3 hrs the solution was poured into a saturated ammonium chloride solution and extracted with ether. After drying over magnesium sulfate, the filtrate was concentrated in vacuo and the residue purified on HPLC (Waters Ass., Prep LC/System 500) with isooctane-ethyl acetate (85:15) as eluent to yield ketone 4 (6.54 g, 73% yield) as an oil which solidified upon standing. The product was found isotopically pure by ¹H NMR. Rf (isooctane-ethyl acetate, 95:5) 0.08. ¹H NMR (90 MHz, CDCl₃) : 2.90 (2H, s), 3.80 (3H, s), 6.90 (2H, A₂B₂), 7.95 (2H, A₂B₂).

Z-3,4-di-(4-methoxyphenyl)hex-3-ene-2,2,5,5-²H₄ (5) and Z-3,4-di(4-methoxyphenyl)hex-3-ene-1,1,1,6,6,6-²H₆ (7)

A suspension of lithium (1.74 g; 0.252 mol) and titanium trichloride (12.8 g; 83 mmol) in 1,2-dimethoxyethane (300 ml) was stirred under argon at reflux for 3 hrs. To the cooled (20°C) suspension a solution of ketone 3 (3 g; 18 mmol) in 1,2-dimethoxyethane (100 ml) was added and the reaction mixture refluxed for 16 hrs. The cooled suspension was poured onto a florisil bed and washed with hexane. After concentration in vacuo the residue was purified by column chromatography on silica gel (isooctane-ethyl acetate, 95 : 5) to yield the Z-isomer 5 as an oil (1.59 g; 58% yield), somewhat contaminated (<5% by TLC and ¹H NMR) by the E-isomer 6. R_f (isooctane-ethyl acetate): 0.21 for 5; 0.29 for 6. ¹H NMR (360 MHz, CDCl₃) : 0.93 (6H, s), 3.71 (6H, s), 6.62 (4H, A₂B₂), 6.86 (4H, A₂B₂) ppm.

Under the same conditions ketone 4 gave the Z-isomer 7 (contaminated by 5% of 8) as an oil. R_f (isooctane-ethyl acetate) : 0.21 for 7; 0.29 for 8. ¹H NMR (360 MHz, CDCl₃) : 2.50 (4H, s), 3.71 (6H, s), 6.62 (4H, A₂B₂), 6.86 (4H, A₂B₂) ppm.

E-3,4-di-(4-hydroxyphenyl)hex-ene-1,1,1,6,6,6-²H₆ (15)

A solution of Z-olefin (1.59 g; 5.3 mmol) and trimethylsilyl iodide (3.6 ml; 25 mmol) in chloroform (18 ml) was stirred at room temperature for 24 hrs. After filtration of the black suspension, methanol (9 ml) was added to the filtrate and the solution stirred for 24 hrs. The solid residue, obtained upon concentration in vacuo, was purified by column chromatography on silica gel (isooctane-ethyl acetate, 70:30) to yield a mixture of two products (1.29 g, 89% yield; ratio 1:1 by TLC, R_f values 0.11 and 0.17). Recrystallization from benzene (2 times) gave colorless needles (m.p. 169-172°C) in 31% isolated yield (0.45 g). The product showed the same TLC behavior as authentic diethylstilbestrol. R_f (ethyl acetate-isooctane) : 0.17

Mass spectrometric analysis showed the following distribution of relative intensities around the molecular ion region : 268, 71.1; 269, 100; 270, 62.4; 271, 24.3; 272, 6.3; 273, 1.4; 274, 0.5; 275, 0.3 (m/z value, rel. int.).

Under identical conditions isotopically pure E -3,4-di(4-hydroxyphenyl)hex-3-ene-1,1,1,6,6,6- 2H_6 (15) was obtained from Z -olefin 7. M.p. (benzene): 169°C. Rf (ethyl acetate-isooctane): 0.17. 1H NMR (360 MHz, CD_3OD) : 2.10 (4H, s), 4.90 (s), 6.77 (4H, A_2B_2), 7.00 (4H, A_2B_2). The mass spectrum of 15 (M.W. 274) via direct inlet is given in comparison with authentic DES (M.W. 268) in Figure 1.

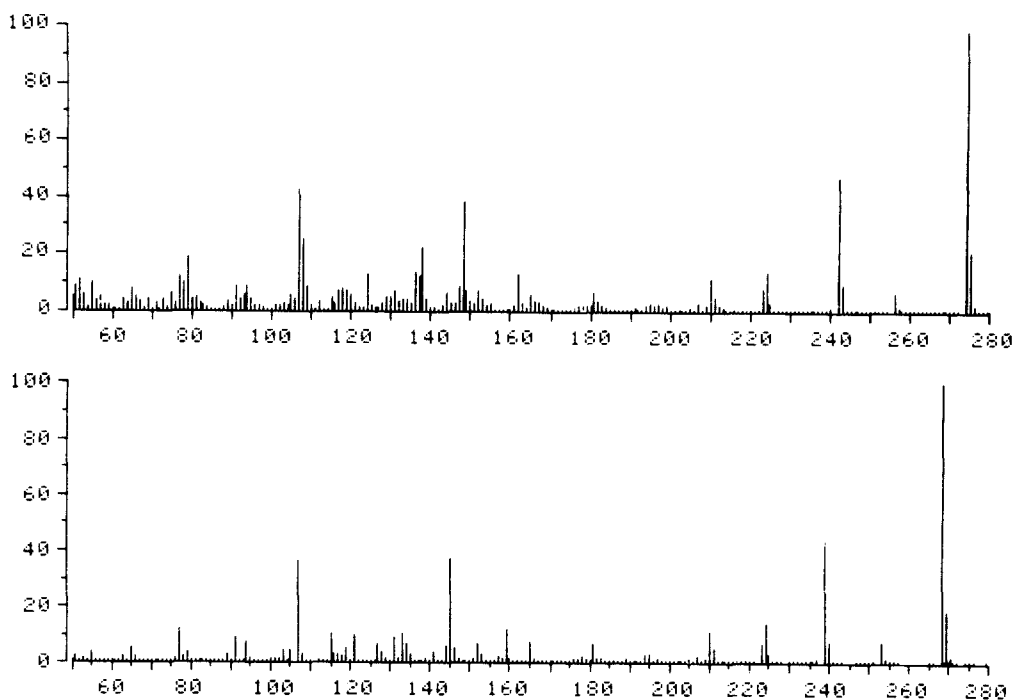


Fig.1. Mass spectrum of the synthesized DES- d_6 (15) (top) in comparison with unlabelled DES (1) (bottom).

Isotope Dilution Gas Chromatography Mass Spectrometry (ID-GCMS)

Prior to gas chromatography, derivatisation took place by adding 20 μL HFBA in 100 μL acetonitrile and heating for 60 min at 60 °C. The mass spectrometer was focussed for detection of the

molecular ions of di-HFB-DES (m/z 660.1) and di-HFB-DES- 2H_6 (m/z 666.1) using 100 msec dwell times. Figure 2 shows the selected ion chromatograms of an extract of 1 g meat, to which 1 ng DES and 2 ng DES- 2H_6 were added.

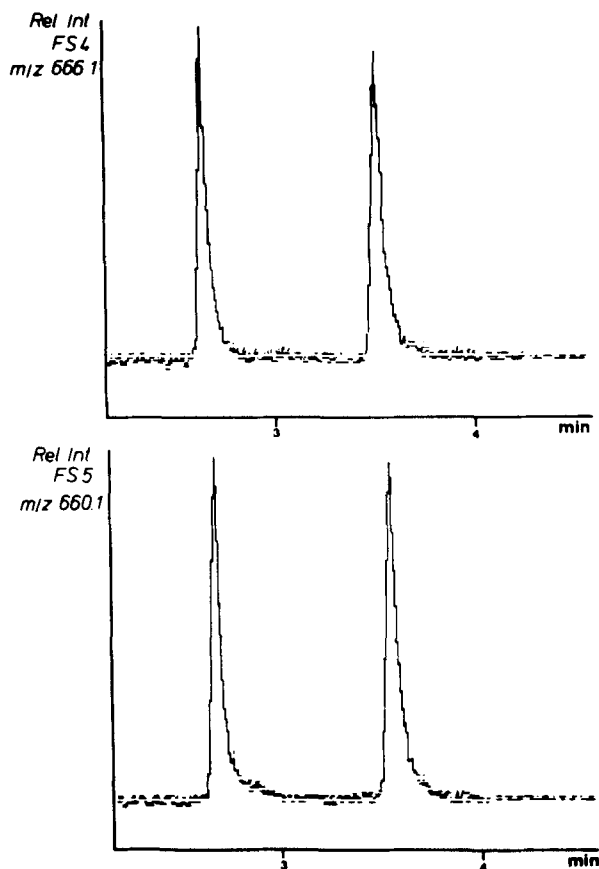


Fig. 2. Selected ion chromatograms after processing 1 g meat spiked which 1 ng DES (m/z 660.1) and 2 ng DES-d6 (m/z 666.1) .

Acknowledgements

This work was supported trough the NFSR (grant 3.0011.81), and the IWONL. Author P.J.D.C. is a research associate of the NFSR.

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