Optically Active trans-Diethylstilbestrol Oxide Monomethyl Ether

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Diethylstilbestrol oxide is a metabolic intermediate of diethylstilbestrol. In order to elucidate the effects of optically active diethylstilbestrol oxides on microtubule assembly and cell culture, we synthesized (+)-diethylstilbestrol oxide (2a). Since 2a was not stable under moderately acidic and basic conditions, the monomethyl ether (2c) of diethylstilbestrol oxide, which was more stable than 2a, was separated by high-pressure liquid chromatography using a chiral column. The mono (4-bromobenzoate) of (−)-2c was analyzed by X-ray crystallography and its absolute structure was determined as C (1R,1′R).

Keywords: optical resolution; diethylstilbestrol oxide monomethyl ether; absolute structure; X-ray crystallography; pig liver esterase; diethylstilbestrol oxide

Diethylstilbestrol (1a) is one of the few substances for which a clear association with carcinogenicity has been established in man.1 However, 1a, in contrast to most other carcinogens, fails to induce mutations in the Salmonella/microsome test2 or malignant transformation of eukaryotic cells in culture.3 Recently, we reported the inhibition of microtubule polymerization and the effects on cell culture of trans-diethylstilbestrol (1a) and its metabolic analogues.4 On the other hand, it has been clearly demonstrated that the estrogenticity of diethylstilbestrol oxide (2a), a metabolic intermediate of 1a, is lower than that of 1a, although it shows much more active sister chromatid exchange induction.5 In this study, in order to elucidate the effects of 2a on microtubule assembly and cell culture,6 we synthesized the oxide (2a). However, 2a was transformed into diethylstilbestrol pinacolone (3a) under moderately acidic and basic conditions. Therefore, the enantiomers were separated to > 99% purity as the oxide of diethylstilbestrol monomethyl ether, by high-pressure liquid chromatography (HPLC) using a chiral column, and the absolute structure of (−)-2c was determined by X-ray crystallography of its mono (4-bromobenzoate).

Results

Synthesis of (+)-Diethylstilbestrol Oxide Although (+)-trans-diethylstilbestrol oxide (2a) was synthesized from trans-diethylstilbestrol (1a) by the method of Jellinek and Bowen,7 we attempted an alternate preparation of 2a from trans-diethylstilbestrol diacetate (1b), because 1a exists in a cis–trans equilibrium in some solvents.8

Epoxidation of 1b was performed very easily, but its hydrolysis product was not identical with the authentic sample (2a); it was determined to be diethylstilbestrol pinacolone (3a) (Chart 2) from its 1H- and 13C-NMR

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(proton and carbon-13 nuclear magnetic resonance) data. Moreover, all of the $^1$H- and $^{13}$C-NMR signals of 3a, were assigned on the basis of two-dimensional Fourier-transform data and a long-range selective proton decoupling (LSPD) experiment.

Accordingly, we attempted the enzymatic hydrolysis of the acetate oxide (2b) using a cell-free system of Streptomyces cinereocrocatus$^{23}$ or porcine liver esterase (PLE)$^{10}$ resulting in the formation of (±)-2a and its monoacetate.

**Attempted Optical Resolution of trans-Diethylstilbestrol Oxide** trans-Diethylstilbestrol oxide (2a) possesses chiral centers at the C-1, 1’ positions. Therefore, 2a was derivatized to the dicamphanates (4a and 4b), in order to separate its enantiomers (Chart 2). Hence, repeated recrystallization of the dicamphanates from acetone afforded 4a as crystals (mp 259.5—261.5°C). Further, the product of the mother liquid from the first recrystallization was recrystallized from benzene to give 4b as crystals (245—248°C). Moreover, the $^1$H- and $^{13}$C-NMR spectra of 4a and 4b were similar, and a mixture of 4a and 4b could not be separated by HPLC (μBondapak NH$_2$ column).

Next, we attempted the hydrolysis of a mixture of 4a and 4b using the cell-free system of S. cinereocrocatus, or PLE, but no hydrolysis product was formed. Hydrolysis of the dicamphanates was performed, under various conditions (0.003—0.004% aqueous NH$_3$ in MeOH). However, the hydrolysis of 4a was slow, and the yield of the pinacolone (3a) increased with increasing reaction time.

**Properties of trans-Diethylstilbestrol Oxide (2a) and Its Analogs (2c and 2d) under Acidic and Basic Conditions** In order to elucidate the stability of trans-diethylstilbestrol oxide (2a), its monomethyl ether (2c) and dimethyl ether (2d) were prepared by methylation of 1a with dimethyl sulfate using the method of Jellinck and Bowen,$^{7}$ except that the reaction temperature was elevated to reflux temperature; the cis-isomer (6b) was also formed in 10% yield (Chart 3). Under acidic and basic conditions, 2a was transformed to diethylstilbestrol pinacolone (3a).$^{7,11}$ We examined the properties of 2a, 2c, and 2d (Table 1), determining the products to be 3a and its methyl analogs (3b and 3c). The results showed that 2a was more easily converted to 3a than were 2c and 2d to 3b and 3c, respectively.

**Optical Resolution of Monomethyl Ether of trans-Diethylstilbestrol Oxide (2e) by HPLC** Since the above results indicated that 2a is not stable under moderately acidic and basic conditions, we planned optical separation by HPLC of diethylstilbestrol oxide monomethyl ether (2e). Chromatographic separation of the individual enantiomers of 2c was achieved by HPLC using a Chiralcel OJ column ($^{12}$Daicel Chemical Co.). The chromatographic profile and conditions are shown in Fig. 1. It was possible to separate the 2e enantiomers on a preparative scale to >99% purity by HPLC. The $^1$H-NMR spectra and mass spectra (MS)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Products</th>
<th>2a (Rec.%)</th>
<th>2b (Proc.%)</th>
<th>2c (Rec.%)</th>
<th>2d (Proc.%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01% H$_2$SO$_4$</td>
<td>0 100</td>
<td>0 100</td>
<td>48 52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.0% AcOH$^a$</td>
<td>34 66</td>
<td>42 58</td>
<td>100 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.4% AcOH$^b$</td>
<td>47 53</td>
<td>53 47</td>
<td>100 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.4% AcOH$^c$</td>
<td>85 15</td>
<td>100 0</td>
<td>100 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.0% KOH$^d$</td>
<td>0 100</td>
<td>87 13</td>
<td>100 0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2% NaHCO$_3$$^e$</td>
<td>0 100</td>
<td>100 0</td>
<td>100 0</td>
<td></td>
<td></td>
</tr>
</tbody>
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| a) Rec.: recovered starting material. | b) Proc.: produced compound. | c) In acetone/24 h/room temperature. | d) In MeOH/1 h/reflux. | e) In MeOH/2 h/room temperature. |

Fig. 1. HPLC Chromatogram of the Monomethyl Ether of (±)-trans-Diethylstilbestrol Oxide (2e)

A 100-μl sample (5 mg) of 2c was injected onto a Chiralcel OJ column. The sample was eluted with a 20% 2-propanol/n-hexane solution at a flow rate of 1 ml/min. Sample detection was monitored by UV absorption at 254 nm.
of the individual samples were identical with those of (+)-2e. Moreover, samples collected from the two 2e peaks in HPLC gave opposite optical rotations; the 1st peak showed $[\alpha]_D$ +12.6° (EtOH) and the 2nd peak, $[\alpha]_D$ +13.7° (EtOH). In the circular dichroism spectra, (+)-2e showed a positive Cotton effect and the (−)-isomer a negative one (Fig. 2).

Crystal Structure of the Mono(4-bromobenzoate) of (−)-2e

The absolute structures of the 2e enantiomers were determined unequivocally by X-ray analysis. The highly purified 1st peak obtained from HPLC was derivatized to form the mono(4-bromobenzoate) of (−)-2e for X-ray analysis. A colorless needle crystal was obtained from EtOH and mounted on an automated Rigaku AFC-5 X-ray diffractometer using Cu Kα radiation. The unit cell parameters were $a=25.276$ (3), $b=8.083$ (1), $c=5.768$ (1) Å, $\alpha=90.77$ (1)°, $\beta=96.58$ (1)°, and $\gamma=87.58$ (1)° in space group $P_1$ ($Z=2$). Of the 3999 reflections measured with $2\theta \leq 130$° employing a $2\theta/\omega$ scan, 2838 were observed independently at the level of $F > 3\sigma (F)$. The size of the crystal used measured $0.4 \times 0.1 \times 0.1$ mm, but no correction was carried out for absorption. The structure was solved by MULTAN 78(13) and successive Fourier syntheses, and refined using the block-diagonal least-squares technique with anisotropic temperature factors for non-hydrogen atoms. All hydrogen atoms were included at the calculated positions with the equivalent isotropic temperature factors of the bound atoms, but not refined.

The refinement was terminated at $R=0.073$. Calculations were performed with the Direct-search program system.14 Twenty Bijvoet pairs which exhibited large anomalous scattering effects from the bromine atoms were selected and used to determine the absolute configuration. All observed Bijvoet ratios were in agreement with those calculated for the chosen enantiomer shown in Fig. 3, and the observed and calculated Bijvoet ratios are shown in Table II. Some bond lengths and angles differed from typical values because of the disorder and/or absorption. Four tables of atomic fractional coordinates, temperature factors, bond lengths, and bond angles have been deposited as supplementary material.

In the present study, we synthesized (+)-2a and its mono- and dimethyl ethers (2e and 2d). Moreover, (+)-2e was separated into (+)- and (−)-2e, and their absolute structures were determined as C(1S,1′S) and C(1R,1′R), respectively. Although the final purpose of this work is to determine the absolute configuration of a putative dialkylstilbestrol metabolite, (+)- or (−)- or (±)-diethylstilbestrol oxide, we have not yet been successful as the oxide is not stable under the conditions studied.7,15

Experimental

Apparatus for Structural Determination

All melting points were obtained on a Shimadzu MM2 micro-melting point apparatus. All 1H-NMR data were recorded in deuterioacetonite and are reported as parts per million downfield from Me$_4$Si ($\delta=0$). 13C-NMR spectra were determined at 67.8 MHz using a JEOL JNM-GX 270FT NMR spectrometer with 32 kilo data points for acquisition of free induction decays. For measurement of carbon-proton coupling constants, the coupling information was acquired using a gated decoupling facility, which permitted retention of the nuclear Overhauser effect (NOE). Abbreviations used: $s$-singlet, $d$-doublet, $t$-triplet, $br$-broad, $m$-multiplet, $dd$-doublet of doublets, $q$-quartet. MS and high-resolution MS (HRMS) were performed on a JEOL JMS-DX303 mass spectrometer at an ionizing potential of 70eV. The optical rotations were measured on a JASCO DIP-140 digital polarimeter using a cell with a 10-cm light path, and circular dichroism (CD) spectra were taken in ethanol using a 0.5-mm cell at room temperature (25°C) on a JASCO J-20 recording spectropolarimeter. Column chromatography was performed with Kanto Kagaku silica gel (100 mesh). The plates [precoated thin-layer chromatography (TLC) plates, Silica gel 60F-254, Merck] were developed in benzene–acetone (8:2,
Hydrolysis of 2b Using a Cell-Free System of Streptomyces cinereoroseus

The incubation and separation were carried out essentially as described in the previous paper except that 10 mg of 2b was used as a substrate in the cell-free system (20 ml). Column chromatography of the residue from the incubation mixture on neutral alumina (activity III) afforded 2a (7 mg), and either (±)-2a [α]D 25° = 0 (c = 0.08, CHCl3).

Hydrolysis of 2b Using Porcine Liver Esterase

A solution of 2b (53 mg) in acetone (2 ml) and PLE (2 mg) in 0.03 M phosphate buffer (pH 7.0, 20 ml) was incubated at 28°C for 2 h. After extraction of the reaction mixture with chloroform, the organic solution was evaporated to dryness and the residue was used for the PLE incubation described above. The crude product from benzene was chromatographed on neutral alumina (activity III, 10 g). From the methylethyl chloride eluate, the starting material (2b), the monocarboxylic acid (2a), and (±)-2a were obtained in the amounts of 20, 2, and 25 mg, respectively. (±)-2a [α]D 25° = 0 (c = 0.12, CHCl3).

Hydrolysis of trans-Diethylstilbestrol oxide (4a) and 4b

A solution of trans-diethylstilbestrol oxide (4.5 g, 16 mmol) and (−)-camphoracetic acid chloride (12 g, 75 mmol) in anhydrous pyridine was stirred for 3.5 h at room temperature. The reaction mixture was filtered with no precipitate collected. The crystals were extracted with ethyl acetate and the solution was washed with water, dried (Na2SO4), and concentrated in vacuo (49 mg). The crude product in benzene was chromatographed on neutral alumina (activity III, 10 g). From the ethylacetate eluate, the starting material (2b), the monocarboxylic acid (2a), and (±)-2a were obtained in the amounts of 20, 2, and 25 mg, respectively. (±)-2a [α]D 25° = 0 (c = 0.12, CHCl3).

Separation of 4a and 4b

1) Recrystallization from acetone of a mixture of 4a and 4b gave 4a colorless needles, mp 259.5–261.5°C [α]D 22° = 0.10 (CHCl3). Anal. Calcd for C23H30O8: C, 69.99; H, 6.70. CH3NO5: 884 (724, t, 7.3 Hz, 9.9′-CH3). 1.13 (6H, s, 16′-CH3). 1.16 (2H, m, 8′- or 8′-CH2). 1.71 (2H, m, 8′- or 8′-CH2). 1.82 (2H, m, 14a′- or 14b′-H). 2.01 (2H, dd, J = 13.6, 10.9). 2.49 (2H, d, J = 14.6, 14-b′-H). 2.21 (2H, d, J = 13.8, 9.3, 4.6 Hz, 13a′- or 13s-H). 2.68 (2H, dd, J = 13.5, 10.6, 4.3 Hz, 13a′- or 13s-H). 7.29 (4H, brd, J = 8.6 Hz, 4, 4′-b′-H). 7.56 (4H, brd, J = 8.6 Hz, 3, 3′-b′-H).

2) The mother liquor from the second recrystallization of a mixture of 4a and 4b was recrystallized from benzene to give 4b as colorless needles, mp 245–248°C [α]D 22° = 14.6° (c = 0.10, CHCl3). Anal. Calcd for C23H30O8, C70.79; H, 6.88. Found: C 70.72; H, 6.80. CH3NO5: m.p. 645 (M′), 514, 463, 125, 97, 43 (base peak). 1H-NMR δ ppm: 0.71 (6H, t, J = 7.3 Hz, 9,9′-CH3). 1.09 (6H, s, 16,16′-CH3). 1.13 (6H, s, 17,17′-CH3). 1.15 (2H, m, 8, 8′-CH2). 1.22 (6H, s, 18′-or 18′-CH3). 1.70 (2H, m, 8′- or 8′-CH2). 1.82 (2H, m, 14a′- or 14b′-H). 2.01 (2H, dd, J = 13.6, 10.9). 2.49 (2H, d, J = 14.6, 14-b′-H). 2.21 (2H, d, J = 13.8, 9.3, 4.6 Hz, 13a′- or 13s-H). 2.68 (2H, dd, J = 13.5, 10.6, 4.3 Hz, 13a′- or 13s-H). 7.29 (4H, brd, J = 8.6 Hz, 4, 4′-b′-H). 7.56 (4H, brd, J = 8.6 Hz, 3, 3′-b′-H).
and 20 ml of MeOH. The mixture was stirred for a few hours, then poured into ice-water, and extracted with methylene chloride (30 ml x 3). The methylene chloride extract was washed with water, dried (Na₂SO₄) and concentrated under reduced pressure. The aqueous phase for mass was made for the material, the reaction products (2a and 3a) were determined by H-NMR analysis. The reaction products in benzene were chromatographed on neutral alumina (activity III). Elution with benzene afforded a product, which was recrystallized from benzene-n-hexane to afford amyl carbamate (5) as colorless needles, mp 109.5°C. Anal. Calc. for C₁₂H₂₃O₃: C 72.25; H 7.66. Found: C 72.21; H 7.64. 1H-NMR δ (ppm) 0.96 (3H, s, 3-C₃), 1.06 (3H, s, 8-C₃), 1.12 (3H, s, 9-C₃), 1.70 (1H, Jdd = 13.12, 9.1, 4.3 Hz, A, 1.92) (1H, Jdd = 13.14, 10.8, 4.6 Hz, 4-F, 2.03) (1H, Jdd = 13.4, 10.6, 4.3 Hz, 5-H), 2.43 (1H, Jdd = 13.4, 10.6, 4.3 Hz, 5-H), 3.84 (3H, s, O-CH₃). 13C-NMR δ (ppm) 79.0 (Jq = 127.4 Hz, C-9), 167.7 (s, C=O). The ratio of starting material: 2a : 3a = 40 : 16.35 with 0.04% aqueous NH₄OH in MeOH/20 min. 2) The ratio of starting material: 2a : 3a = 56 : 15.7 under 0.01% NH₄OH in MeOH/1 h. 3) The ratio of starting material: 2a : 3a = 43 : 43.3 with 0.01% aqueous NH₄OH in MeOH/5 h. 4) The ratio of starting material: 2a : 3a = 77 : 13.2 with 0.003% aqueous NH₄OH in MeOH/4 h. 5) The ratio of starting material: 2a : 3a = 80 : 7.0 with 0.003% aqueous NH₄OH in MeOH/2h.

**Treatment of 4a with Ammonium Hydroxide in MeOH**

The reaction products (2a and 3a) from 4a with 0.003% aqueous NH₄OH in MeOH for 4–8 h were determined by 1H-NMR analysis. 1) The ratio of 4a : 2a : 3a = 100 : 60 : 15 (40,000 mg) in benzene-n-hexane (4:1) 2) The ratio of 4a : 2a : 3a = 70 : 7 : 34 with 0.003% aqueous NH₄OH in MeOH/4 h. 3) The ratio of 4a : 2a : 3a = 54 : 27 : 9 with 0.003% aqueous NH₄OH in MeOH/8 h. 4) The reaction mixture was concentrated and the residue was chromatographed on silica gel (50 g). The eluate from benzene gave the cis-dimethyl Ether (1d) and 6b of 1a were prepared from 1a (2g) by methylation with dimethyl sulfate in MeOH except that the reaction temperature was 85°C. The reaction mixture was concentrated and the residue was concentrated in vacuo. The residue (2.2 g) was chromatographed on silica gel (150 g). 1) Elution with benzene and recrystallization of the product from MeOH gave the trans-dimethyl ether (1e) of 1a as colorless needles, mp 150°C (1H-NMR δ (ppm) 4.08 (3H, s, 3-C₃), 1.05 (3H, s, 3-C₃), 1.12 (3H, s, 9-C₃), 1.70 (1H, Jdd = 13.12, 9.1, 4.3 Hz, 9-C₃), 2.54 (4H, brd, J = 7.3 Hz, 8.8-C₃), 3.68 (6H, s, 5-OCH₃), 6.44 (4H, brd, J = 8.9 Hz, 4.4', 6.6'), 6.89 (4H, brd, J = 8.9 Hz, 3.7', 7.7'), 13C-NMR δ (ppm) 126.5, 5.0 Hz, C-9, 16.5). 1H-NMR δ (ppm) 3.08 (3H, s, 5-OCH₃), 6.83 (2H, brd, J = 9.2 Hz, 4.6', 6.6'). 2) Elution with benzene and recrystallization of the product from methanol gave the trans-dimethyl ether (1e) (1c) of 1a. The MS and 1H-NMR data were identical with those of the authentic sample (1c).

The mother liquid of the above recrystallization from methanol was concentrated in vacuo and the residue was chromatographed on silica gel (15 g). The eluate from benzene gave the cis-dimethyl ether (6b) of 1a as an oil. MS m/z 296 (M⁺) (base peak), 267, 159, 121. 1H-NMR δ (ppm) 0.93 (6H, t, J = 7.3 Hz, 9.9-C₃), 2.54 (4H, brd, J = 7.3 Hz, 8.8-C₃). 3,68 (6H, s, 5-OCH₃), 6.44 (4H, brd, J = 8.9 Hz, 4.4', 6.6'), 6.89 (4H, brd, J = 8.9 Hz, 3.7', 7.7'), 13C-NMR δ (ppm) 126.5, 5.0 Hz, C-9, 16.5. 1H-NMR δ (ppm) 3.08 (3H, s, 5-OCH₃), 6.83 (2H, brd, J = 9.2 Hz, 4.6', 6.6'). 2) Elution with benzene and recrystallization of the product from methanol gave the trans-dimethyl ether (1e) (1c) of 1a. The MS and 1H-NMR data were identical with those of the authentic sample (1c). The mother liquid of the above recrystallization from methanol was concentrated in vacuo and the residue was chromatographed on silica gel (15 g). The eluate from benzene gave the cis-dimethyl ether (6b) of 1a as an oil. MS m/z 296 (M⁺) (base peak), 267, 159, 121. 1H-NMR δ (ppm) 0.93 (6H, t, J = 7.3 Hz, 9.9-C₃), 2.54 (4H, brd, J = 7.3 Hz, 8.8-C₃). 3,68 (6H, s, 5-OCH₃), 6.44 (4H, brd, J = 8.9 Hz, 4.4', 6.6'), 6.89 (4H, brd, J = 8.9 Hz, 3.7', 7.7'), 13C-NMR δ (ppm) 126.5, 5.0 Hz, C-9, 16.5. 1H-NMR δ (ppm) 3.08 (3H, s, 5-OCH₃), 6.83 (2H, brd, J = 9.2 Hz, 4.6', 6.6'). 2) Elution with benzene and recrystallization of the product from methanol gave the trans-dimethyl ether (1e) (1c) of 1a. The MS and 1H-NMR data were identical with those of the authentic sample (1c).

1. The Dimethyl Ether (7) of cis-Diethylstilbestrol Oxide

The reaction product from 3a was obtained as an oil. MS m/z 256 (M⁺) (base peak), 240, 147, 121, 91. 1H-NMR δ (ppm) 0.64 (3H, t, J = 7.3 Hz, 9.9-C₃), 0.82 (3H, t, J = 7.3 Hz, 9.9-C₃), 2.28 (2H, q, J = 7.3 Hz, 8.8-C₃), 2.34 (2H, q, J = 7.3 Hz, 8.8-C₃), 3.80 (6H, s, 5-OCH₃), 6.91 (4H, brd, J = 8.9 Hz, 4.4', 6.6', 7.7'). 13C-NMR δ (ppm) 79.0 (Jq = 127.4 Hz, C-9), 167.7 (s, C=O). The ratio of starting material: 7a : 7b = 1 : 20. 2) Elution with water and recrystallization of the product from methanol gave the trans-dimethyl ether (7e) (7c) of 7a. The MS and 1H-NMR data were identical with those of the authentic sample (7c).

**Treatments of 2a, 2b, and 2d under Acidic and Basic Conditions**

The reaction products from 2a, 2c, and 2d under acidic and basic conditions were determined by H-NMR analysis. The results and conditions are described in Table 1.
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References


