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 (1*R*,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 test[2] 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 estrogenicity 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[4a] 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).

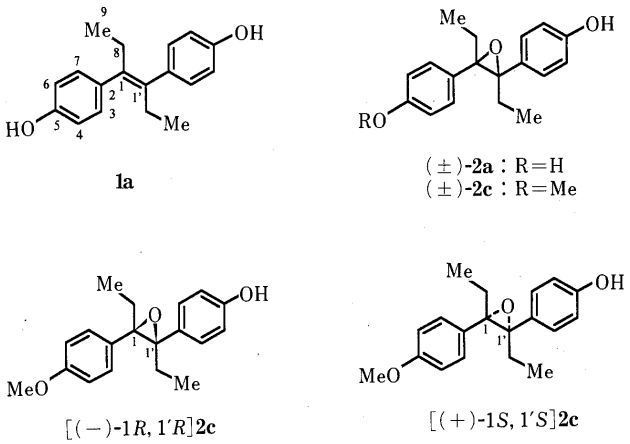

Chart 1

Results

Synthesis of (±)-Diethylstilbestrol Oxide Although (±)-*trans*-diethylstilbestrol oxide (**2a**) was synthesized from *trans*-diethylstilbestrol (**1a**) by the method of Jellinck 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 ^{1}H- and ^{13}C-NMR

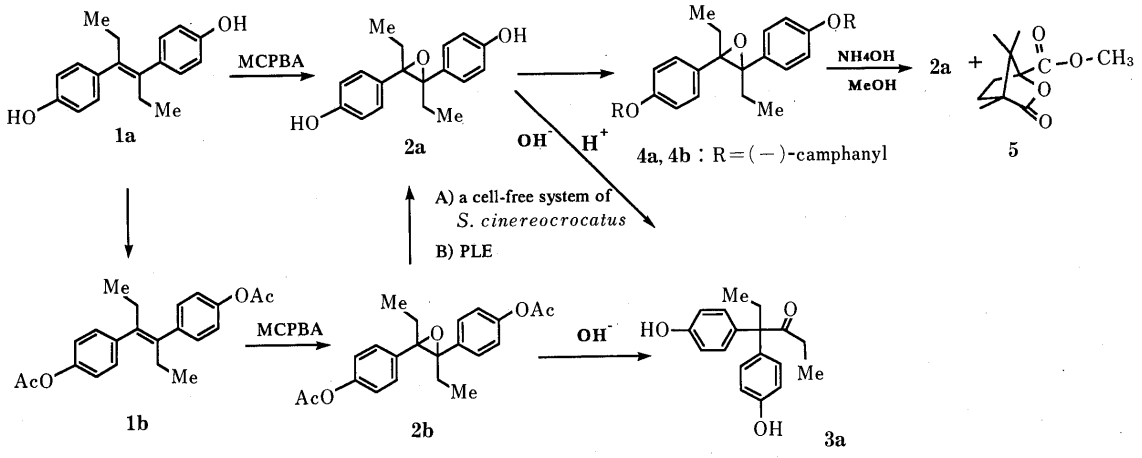

Chart 2

(proton and carbon-13 nuclear magnetic resonance) data.[7] 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*[9] 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 I), 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 (2c) 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 (**2c**). 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 **2c** enantiomers on a preparative scale to >99% purity by HPLC. The ^{1}H-NMR spectra and mass spectra (MS)

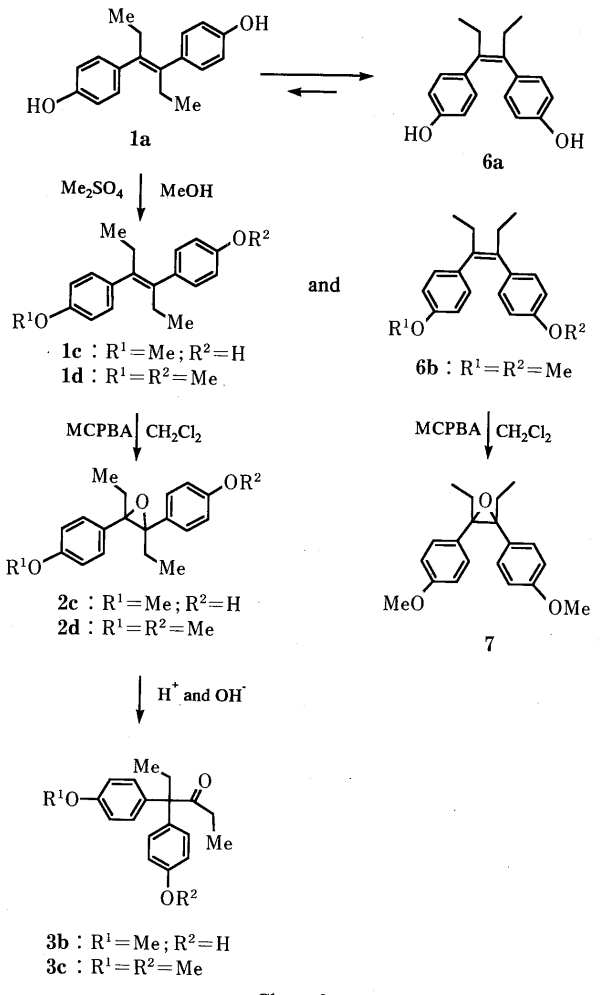

Chart 3

TABLE I. Reaction of (±)-Diethylstilbestrol Oxide (**2a**), the (±)-Monomethyl Ether (**2c**), and the (±)-Dimethyl Ether (**2d**) under Acidic and Basic Conditions

Condition	Products	Starting material: 2a Rec.[a] (%)	2a Pro.[b] (3a) (%)	2c Rec. (%)	2c Pro. (3b) (%)	2d Rec. (%)	2d Pro. (3c) (%)
0.01%	H_2SO_4[c]	0	100	0	100	48	52
2.0%	AcOH[c]	34	66	42	58	100	0
1.4%	AcOH[c]	47	53	53	47	100	0
0.4%	AcOH[c]	85	15	100	0	100	0
5.0%	KOH[d]	0	100	87	13	100	0
0.2%	$NaHCO_3$[e]	0	100	100	0	100	0

a) Rec.: recovered starting material. *b*) Pro.: produced compound. *c*) In acetone/24 h/room temperature. *d*) In MeOH/1 h/reflux. *e*) In MeOH/2 h/room temperature.

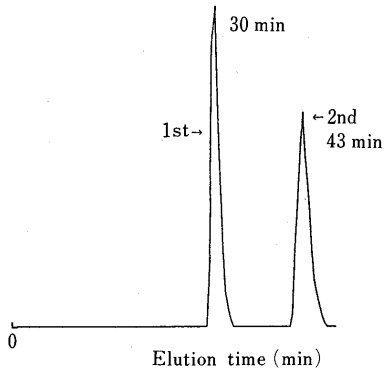

Fig. 1. HPLC Chromatogram of the Monomethyl Ether of (±)-*trans*-Diethylstilbestrol Oxide (**2c**)

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.

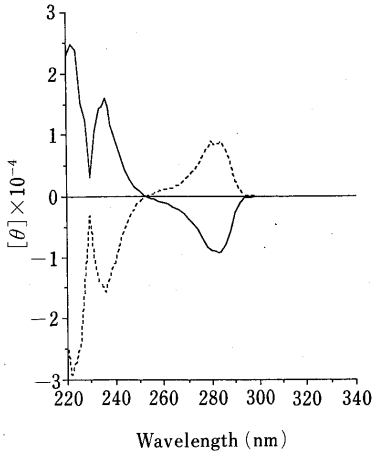

Fig. 2. CD Spectra of (+)-**2c** (——) and (−)-**2c** (---)

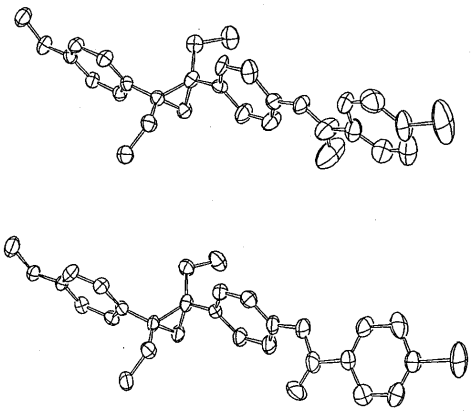

Fig. 3. Molecular Structures of the Two Independent Molecules in the Mono(4-bromobenzoate) of (−)-**2c**

of the individual samples were identical with those of (±)-**2c**. Moreover, samples collected from the two **2c** 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, (+)-**2c** showed a positive Cotton effect and the (−)-isomer a negative one (Fig. 2).

Crystal Structure of the Mono(4-bromobenzoate) of (−)-2c The absolute structures of the **2c** 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 (−)-**2c** 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 K_α 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 $2\theta\leq130°$ 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 $0.4\times0.1\times0.1$ 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.

Table II. Observed and Calculated Bijvoet Ratios

h	k	l	$F_o(+)/F_o(-)$	$F_o(+)/F_o(-)$
−1	0	1	1.077	1.062
2	−1	1	0.960	0.952
8	0	1	0.931	0.909
−3	−1	1	1.124	1.084
3	2	1	1.068	1.056
3	−1	1	1.106	1.142
5	1	1	0.959	0.936
−9	0	1	0.923	0.919
0	2	1	1.096	1.098
0	0	1	1.044	1.040
1	−2	1	0.936	0.940
−2	−3	1	1.106	1.091
1	2	1	0.928	0.925
11	−1	1	1.114	1.092
9	−1	1	0.903	0.907
−5	1	1	1.048	1.039
−1	−1	2	0.936	0.925
−10	1	1	0.921	0.941
−6	0	1	1.053	1.035
−11	2	1	1.110	1.078

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 (**2c** and **2d**). Moreover, (±)-**2c** was separated into (+)- and (−)-**2c**, and their absolute structures were determined as C(1*S*,1′*S*) and C(1*R*,1′*R*), respectively. Although the final purpose of this work is to determine the absolute configuration of a putative diethylstilbestrol 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 ^{1}H-NMR data were recorded in deuterioacetone and are reported as parts per million downfield from Me_4Si ($\delta=0$). ^{13}C-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 70 eV. 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,

v/v). The compounds were visualized under UV light and/or by spraying with concentrated H_2SO_4 and heating on an electric heater. HPLC was performed on an HPLC Chiralcel OJ column (10 mm × 25 cm; Daicel Chemical Co.), using a Waters pump (model 510) and a Waters detector (model 480 spectrophotometer, set at 254 nm). *n*-Hexane–2-propyl alcohol (80 : 20) was used as the eluent.

Materials *trans*-Diethylstilbestrol (**1a**) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo). (−)-Camphamic acid chloride was obtained from Fluka Chemie AG (Switzerland). All other reagents were purchased from commercial sources and were of analytical grade. PLE was obtained from Sigma Chemical Corporation (St. Louis, Mo., U.S.A)

(±)-*trans*-Diethylstilbestrol Oxide (2a) Compound **2a** was prepared from **1a** by epoxidation with *m*-chloroperbenzoic acid.[7] Compound **1a** (1.5 g, 5.6 mmol) in methylene chloride (120 ml) was mixed with *m*-chloroperbenzoic acid (1.0 g, 6 mmol) in methylene chloride (9 ml) for 1 h at room temperature. Next, 10% sodium sulfite (6 ml) was added to the reaction mixture, and the resulting residue was collected by filtration. The residue was extracted with ethyl acetate, and the ethyl acetate solution was washed with 5% $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated *in vacuo* (716 mg). Recrystallization of the product from ethyl acetate gave colorless needles of **2a**, mp 107—108 °C (lit., mp 102—104 °C),[7] $[\alpha]_D^{23}$ 0° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{18}H_{20}O_3 \cdot 1/2H_2O$: C, 71.50; H, 7.33. Found: C, 71.2; H, 7.19. MS *m/z*: 284 (M^+), 255, 227, 133 (base peak), 57. ^{1}H-NMR δ (ppm): 0.67 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.15 (2H, m, 8 or 8′-H), 1.71 (2H, m, 8 or 8′-H), 6.86 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.24 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H), 8.30 (2H, br s, 5,5′-OH). ^{13}C-NMR δ (ppm): 9.3 (tq, J=125.6, 4.1 Hz, C-9, 9′), 28.4 (tq, J=127.4, 4.6 Hz, C-8, 8′), 72.8 (br s, C-1, 1′), 115.6 (dd, J=157.6, 4.6 Hz, C-4, 4′, 6, 6′), 129.0 (dd, J=157.2, 7.8 Hz, C-3, 3′, 7, 7′), 131.3 (br dd, J=12.4, 6.0 Hz, C-2, 2′), 157.2 (br dd, J=10.1, 9.6 Hz, C-5, 5′).

(±)-*trans*-Diethylstilbestrol Oxide Diacetate (2b) Compound **1b** was prepared by acetylation of **1a** with acetic anhydride–pyridine at room temperature. The product was recrystallized from MeOH to give **1b** as colorless needles, mp 124—125 °C. MS *m/z*: 352 (M^+), 310, 268 (base peak), 239. ^{1}H-NMR δ (ppm): 0.78 (6H, t, J=7.3 Hz, 9.9′-CH_3), 2.17 (4H, dq, J=7.3 Hz, 8.8′-CH_2), 2.28 (6H, s, 5,5′-$OCOCH_3$), 7.16 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.28 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H). Then **2b** was prepared from **1b** by epoxidation as described above. The product was recrystallized from benzene to give **2b** as colorless needles, mp 94—95 °C. *Anal.* Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57. Found: C, 71.64; H, 6.53. MS *m/z*: 368 (M^+), 309, 134 (base peak), 57. ^{1}H-NMR δ (ppm): 0.69 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.14 (2H, m, 8, or 8′-CH_2), 1.81 (2H, m, 8, or 8′-CH_2), 2.27 (6H, s, 5,5′-$OCOCH_3$), 7.14 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 7.49 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 9.2 (tq, J=126.5, 4.6 Hz, C-9, 9′), 21.0 (q, J=130.1 Hz, 5,5′-$OCOCH_3$), 28.3 (tq, J=127.8, 4.6 Hz, C-8, 8′), 72.8 (br s, C-1, 1′), 122.2 (dd, J=126.7, 7.3 Hz, C-4, 4′, 6, 6′), 128.9 (dd, J=159.9, 6.9 H, C-3, 3′, 7, 7′), 137.5 (br dd), J=11.9, 6.4 Hz, C-2, 2′), 151.0 (m, J=10.1, 10.1, 3.7 Hz, C-5, 5′), 169.6 (q, J=7.3 Hz, 5,5′-$OCOCH_3$).

Diethylstilbestrol Pinacolone (3a) Compound **3a** was prepared by the method of Jellinck and Bowen.[7] The product was obtained as an oil. MS *m/z*: 227 (base peak), 133, 57. HRMS Calcd for $C_{15}H_{15}O_2$: 227.1072 ($M^+ - COCH_2CH_3$). Found: 227.1030. HRMS Calcd for C_3H_5O: 57.0340 ($-COCH_2CH_3$). Found: 57.0368. ^{1}H-NMR δ (ppm): 0.63 (3H, t, J=7.3 Hz, 9-CH_3), 0.81 (3H, t, J=7.3 Hz, 9′-CH_3), 2.25 (2H, q, J=7.3 Hz, 8-CH_2), 2.31 (2H, q, J=7.3 Hz, 8′-CH_2), 6.82 (4H, br dd, J=8.9 Hz, 4,4′,6,6′-H), 7.08 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H), 8.35 (2H, br s, 5,5′-OH). ^{13}C-NMR δ (ppm): 9.4 (tq, J=127.8, 4.1 Hz, C-9′), 9.9 (tq, J=125.6, 4.1 Hz, C-9), 30.9 (tq, J=124.2, 4.6 Hz, C-8), 32.5 (tq, J=126.5, 4.6 Hz, C-8′), 65.8 (br s, C-1), 115.7 (dd, J=159.5, 4.6 Hz, C-4, 4′, 6, 6′), 131.2 (dd, J=158.1, 7.8 Hz, C-3, 3′, 7, 7′), 133.4 (m, C-2, 2′), 156.9 (br ddd, J=9.2, 9.2, 2.8 Hz, C-5, 5′), 211.4 (m, C-1′).

Hydrolysis of 2b under Basic Conditions 1) A solution of **2b** (960 mg) in MeOH (20 ml) and 20% KOH (4.6 ml) was heated at 90 °C for 50 min. After dilution of the solution with water, the reaction mixture was extracted with ethyl acetate, and the extract was washed with water and concentrated *in vacuo* (690 mg). The crude product in benzene was chromatographed on silica gel (50 g). Elution with methylene chloride gave DES-pinacolone as an oil. The data MS, ^{1}H- and ^{13}C-NMR data were identical with those of **3a**. 2) A solution of **2b** (100 mg) in MeOH (6 ml) and 5% $NaHCO_3$ (0.3 ml) was stirred at room temperature for 2 h. After dilution with water, the reaction mixture was extracted with ethyl acetate, and the extract was washed with water and concentrated *in vacuo*. The crude product (97 mg) in benzene was chromatographed on neutral alumina (activity III, 10 g). From the methylene chloride eluate, the starting material (**2b**), and monoacetates of **2a**, **2a** and **3a** were obtained in the amounts of 21, 7, 40 and 15 mg, respectively. The ^{1}H-NMR data were identical with those of authentic samples.

Hydrolysis of 2b Using a Cell-Free System of *Streptomyces cinereocrocatus* The incubation and separation were carried out essentially as described in the previous paper[9] 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 (active III) afforded **2b** (7 mg), the monoacetate of **2a** (0.6 mg) and (±)-**2a** (2.3 mg), (±)-**2a** $[\alpha]_D^{24}$ 0° (c=0.08, $CHCl_3$).

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 washed with water and concentrated *in vacuo* (49 mg). The crude product in benzene was chromatographed on neutral alumina (activity III, 10 g). From the methylene chloride eluate, the starting material (**2b**), the monoacetate of **2a**, and (±)-**2a** were obtained in the amounts of 20, 2, and 25 mg, respectively, (±)-**2a** $[\alpha]_D^{24}$ 0° (c=0.12, $CHCl_3$).

***trans*-Diethylstilbestrol Oxide Dicamphanate (4a and 4b)** A solution of *trans*-diethylstilbestrol oxide (4.5 g, 16 mmol) and (−)-camphanic acid chloride (12 g, 57 mmol) in anhydrous pyridine was stirred for 3.5 d at room temperature. The reaction mixture was diluted with ice-water and then the precipitate was collected by filtration. The crystals were extracted with ethyl acetate, and the solution was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to give a residue (8.9 g) (**4a** and **4b**). *Anal.* Calcd for $C_{38}H_{44}O_9$: C, 70.79; H, 6.88. Found: C, 71.22; H, 6.80. MS *m/z*: 645 (M^+), 514, 463, 125, 97, 43 (base peak). ^{1}H-NMR δ (ppm): 0.71 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.09 (6H, s, 16,16′-CH_3), 1.13 (6H, s, 17,17′-CH_3), 1.15 (2H, m, 8-, or 8′-CH_2), 1.22 (6H, s, 18 or 18′-CH_3), 1.70 (2H, m, 8-, or 8′-CH_2), 1.82 (2H, m, 14α- or 14′α-H), 2.01 (2H, ddd, J=13.6, 10.9, 4.6 Hz, 14β- or 14′β-H), 2.21 (2H, ddd, J=13.8, 9.3, 4.6 Hz, 13α- or 13′α-H), 2.68 (2H, ddd, J=13.5, 10.6, 4.3 Hz, 13β-,13′β-H), 7.29 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.56 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H).

Separation of 4a and 4b 1) Recrystallization from acetone of a mixture of **4a** and **4b** gave **4a** as colorless needles, mp 259.5—261.5 °C $[\alpha]_D^{23}$ −22.2° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{44}O_9$: C, 70.79; H, 6.88. Found: C, 69.99; H, 6.70. ^{1}H-NMR δ (ppm): 0.72 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.13 (6H, s, 16 or 16′-CH_3), 1.16 (2H, m, 8- or 8′-CH_2), 1.17 (6H, s, 17,17′-CH_3), 1.18 (6H, s, 18,18′-CH_3), 1.71 (2H, m, 8,8′-CH_2), 1.71 (2H, m, 8,8′-CH_2), 1.79 (2H, m, 14α,14′α-H), 2.01 (2H, ddd, J=13.2, 10.7, 4.6 Hz, 14β,14′β-H), 2.21 (2H, ddd, J=13.5, 9.3, 4.6 Hz, 13α,13′α-H), 2.58 (2H, ddd, J=13.5, 10.6, 4.3 Hz, 13β,13′β-H), 7.17 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.42 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 8.9 (tq, J=126.5, 4.1 Hz, C-9, 9′), 9.8 (dq, J=127.4, 1.9 Hz, C-18, 18′), 16.9 (br dq, J=126.5, 4.6 Hz, C-16, 16′,17,17′), 27.7 (tq, J=127.4, 4.6 Hz, C-8, 8′), 29.0 (t, J=135.6 Hz, C-14, 14′), 30.8 (t, J=135.6 Hz, C-13, 13′), 54.7 (m, C-15, 15′), 54.9 (m, C-10, 10′), 72.4 (br s, C-1, 1′), 90.8 (br s, C-12, 12′), 121.0 (dd, J=158.4, 4.6 Hz, C-4, 4′, 6, 6′), 128.2 (dd, J=162.2, 7.3 Hz, C-3, 3′, 7, 7′), 137.4 (br dd, J=11.0, 7.3 Hz, C-2, 2′), 149.1 (br dd, J=7.2, 3.6 Hz, C-5, 5′), 166.1 (s, C-19, 19′), 177.8 (m, C-11, 11′). CD (c=1.0 mg/ml) [θ]: −10400 (240) (negative maximum), 0 (256), +2470 (272) (positive maximum), 0 (282).

2) The residue of the mother liquid from the first recrystallization of a mixture of **4a** and **4b** was recrystallized from benzene to give **4b** as colorless needles, mp 245—248 °C, $[\alpha]_D^{23}$ −14.6° (c=0.10, $CHCl_3$). *Anal.* Calcd for $C_{38}H_{44}O_9$: C, 70.79; H, 6.88. Found: C, 70.85; H, 6.79. ^{1}H-NMR δ (ppm): 0.72 (6H, t, J=7.3 Hz, 9.9′-CH_3), 1.16 (2H, m, 8- or 8′-CH_2), 1.13 (6H, s, 16,16′-CH_3), 1.17 (6H, s, 17,17′-CH_3), 1.18 (6H, s, 18,18′-CH_3), 1.71 (2H, m, 8,8′-CH_2), 1.79 (2H, m, 14α,14′α-H), 2.01 (2H, ddd, J=13.2, 10.7, 4.6 Hz, 14β,14′β-H), 2.21 (2H, ddd, J=13.5, 9.3, 4.6 Hz, 13α,13′α-H), 2.58 (2H, ddd, J=13.5, 10.6, 4.3 Hz, 13β, 13′β-H), 7.17 (4H, br d, J=8.6 Hz, 4,4′,6,6′-H), 7.42 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 8.9 (tq, J=126.5, 4.1 Hz, C-9, 9′), 9.8 (dq, J=127.4, 1.9 Hz, C-18, 18′), 16.9 (br dq, J=126.5, 4.6 Hz, C-16, 16′, 17, 17′), C-16, 16′, 17, 17′), 27.7 (tq, J=127.4, 4.6 Hz, C-8, 8′), 29.0 (t, J=135.6 Hz, C-14, 14′), 30.8 (t, J=135.6 Hz, C-13, 13′), 54.7 (m, C-15, 15′), 54.9 (m, C-10, 10′), 72.4 (br s, C-1, 1′), 90.8 (br s, C-12, 12′), 121.0 (dd, J=158.4, 4.6 Hz, C-4, 4′, 6, 6′), 128.2 (dd, J=162.2, 7.3 Hz, C-3, 3′, 7, 7′), 137.4 (br dd, J=11.0, 7.3 Hz, C-2, 2′), 149.1 (br dd, J=7.2, 3.6 Hz, C-5, 5′), 166.1 (s, C-19, 19′), 177.8 (m, C-11, 11′). CD (c=1.0 mg/ml) [θ]: −5400 (240) (negative maximum), 0 (254), +1260 (272) (positive maximum), 0 (280).

Treatment of a Mixture of 4a and 4b with Ammonium Hydroxide in MeOH A mixture (50 mg) of **4a** and **4b** was dissolved in a mixture (0.003—0.04% aqueous NH_3) of various concentrations of aqueous NH_3

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 × 3). The methylene chloride extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo*. The ratios of the starting material (**4a** and **4b**) and reaction products (**2a** and **3a**) were determined by ^{1}H-NMR analysis. The reaction products in benezene were chromatograhed on neutral alumina (activity III). Elution with benzene afforded a product, which was recrystallized from benzene–*n*-hexane to afford methyl camphanate (**5**) as colorless needles, mp 109.5 °C. *Anal.* Calcd for $C_{11}H_{16}O_4$; C, 72.25; H, 7.60. Found: C, 72.48; H, 7.44. ^{1}H-NMR δ (ppm): 0.96 (3H, s, 7-CH_3), 1.06 (3H, s, 8-CH_3), 1.12 (3H, s, 9-CH_3), 1.70 (1H, ddd, J=13.12, 9.1, 4.3 Hz, 4α-H), 1.92 (1H, ddd, J=13.4, 10.8, 4.6 Hz, 4β-H), 2.03 (1H, ddd, J=13.7, 9.2, 4.6 Hz, 5α-H), 2.43 (1H, ddd, J=13.4, 10.6, 4.3 Hz, 5β-H), 3.84 (3H, s, 1-OCH_3). ^{13}C-NMR δ (ppm): 9.70 (dq, J=127.4 Hz, C-9), 16.7 (tq, J=126.4, 56.8 Hz, C-7, 6), 29.0 (tt, J=135.6 Hz, C-4), 30.7 (tt, J=135.6 Hz, C-6), 91.1. (br s, C-3), 168.0 (br s, C-10), 178.0 (br s, C-2).

1) The ratio of starting material: **2a**:**3a** was 40:16:35 with 0.04% aqueous NH_3 in MeOH/20 min. 2) The ratio of starting material: **2a**:**3a** was 56:15:7 under 0.01% NH_3aq in MeOH/1 h. 3) The ratio of starting material: **2a**:**3a** was 43:43:3 with 0.01% aqueous NH_3 in MeOH/5 h. 4) The ratio of starting material:**2a**:**3a** was 77:13:2 with 0.003% aqueous NH_3 in MeOH/4 h. 5) The ration of starting material:**2a**:**3a** was 80:7:0 with 0.003% aqueous NH_3 in MeOH/2 h.

Treatment of 4a with Ammonium Hydroxide in MeOH The reaction products (**2a** and **3a**) from **4a** with 0.003% aqueous NH_3 in MeOH for 4—8 h were determined by ^{1}H-NMR analysis. 1) The ratio of **4a**:**2a**:**3a** was 100:0:0 with 0.003% aqueous NH_3 in MeOH/4 h. 2) The ratio of **4a**:**2a**:**3a** was 70:18:6 with 0.003% aqueous NH_3 in MeOH/7 h. 3) The ratio of **4a**:**2a**:**3a** was 54:27:9 with 0.003% aqueous NH_3 in MeOH/8 h.

***trans*- and *cis*-Dimethyl Ethers (1d and 6b) of 1a** Compounds **1d** and **6b** of **1a** were prepared from **1a** (2g) by methylation[10] with dimethyl sulfate in MeOH except that the reaction temperature was 85 °C. The reaction mixture was diluted with ice-water and then extracted with ethyl acetate. The ethyl acetate solution was washed with water, dried (Na_2SO_4) and 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 (**1d**) of **1a** as colorless needles, mp 124—125 °C. MS *m/z*: 296 (M^+) (base peak), 267, 159, 121. The ^{1}H-NMR spectrum was identical with that reported previously.[16] 2) Elution with benzene and recrystallization of the product from methanol gave the *trans*-monomethyl ether (**1c**) of **1a**. The MS and ^{1}H-NMR data were identical with those of the authentic sample (**1c**). 3) The mother liquid of the above recrystallization from methanol was concentrated *in vacuo* and the residue was chromatographed on silica gel (59 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. ^{1}H-NMR δ (ppm): 0.93 (6H, t, J=7.3 Hz, 9,9′-CH_3), 2.54 (4H, br d, J=7.3 Hz, 8,8′-CH_2), 3.68 (6H, s, 5,5′-OCH_3), 6.64 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 6.89 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H). HRMS Calcd for $C_{20}H_{24}O_2$: 296.1698. Found: 296.1776.

***cis*-Diethylstilbestrol (6a)** Compound **6a** was prepared by the method of White and Ludwig.[8] The proportions of *cis*- and *trans*-diethylstilbestrol were determined by ^{1}H-NMR analyses, to be 93% and 7%, respectively. **6a**: ^{1}H NMR δ (ppm): 0.93 (6H, t, J=7.3 Hz, 9,9′-CH_3), 2.52 (4H, q, J=7.3 Hz, 8,8′-CH_2), 6.55 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 6.79 (4H, br d, J=8.6 Hz, 3,3′,7,7′-H), 8.00 (2H, s, 5,5′-OH). ^{13}C-NMR δ (ppm): 13.6 (tq, J=126.5, 5.0 Hz, C-9, 9′), 27.9 (tq, J=126.5, 4.1 Hz, C-8, 8′), 115.1 (dd, J=158.6, 4.6 Hz, C-4, 4′, 6, 6′), 131.5 (dd, J=158.0, 7.8 Hz, C-3, 3′, 7, 7′), 135.2 (m, C-1, 1′), 138.9 (m, C-2, 2′), 155.9 (br ddd, J=9.2, 9.2, 2.7 Hz, C-5, 5′).

Monomethyl Ether (2c) of (±)-2a Compound **2c** of **2a** was prepared by epoxidation with *m*-chloroperbenzoic acid.[7] Recrystallization from *n*-hexene–benzene gave **2c** as colorless needles, mp 109—111 °C. *Anal.* Calcd for $C_{19}H_{22}O_3$: C, 76.48; H, 7.43. Found: C, 76.58; H, 7.37. MS *m/z*: 298 (M^+), 241, 148 (base peak), 133. ^{1}H-NMR δ (ppm): 0.67 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.13 (2H, q, J=7.3 Hz, 8 or 8′-H), 1.72 (2H, q, J=7.6 Hz, 8 or 8′-CH_2), 3.82 (3H, s, 5-OCH_3), 6.87 (2H, br d, J=8.5 Hz, 4′,6′-H), 6.95 (2H, br d, J=8.6 Hz, 4′,6′-H), 7.25 (2H, br d, J=8.6 Hz, 3,7-H), 7.34 (2H, br d, J=8.6 Hz, 3′,7′-H), 8.35 (1H, br s, 5′-OH). ^{13}C-NMR δ (ppm): 9.3 (tq, J=125.6, 4.1 Hz, C-9, 9′), 28.4 (tq, J=129.2, 4.1 Hz, C-8, 8′), 55.5 (q, J=143.9 Hz, 4′-OCH_3), 72.8 (br s, C-1, 1′), 114.2 (br d, J=159.0, 5.1 Hz, C-4, 6), 115.6 (br d, J=157.6, 4.6 Hz, C-4′, 6′), 129.0 (dd, J=160.4, 7.8 Hz, 3,3′,7,7′), 131.2 (br dd, J=9.2, 6.4 Hz, C-2′), 132.5 (br dd, J=9.2, 4.6 Hz, C-2), 157.3 (br ddd, J=9.2, 9.2, 2.8 Hz, C-5), 159.7 (m, C-5).

The Dimethyl Ether (2d) of (±)-2a Compound **2d** of (±)-**2a** was prepared by the method of Jellinck and Bowen[7] except that **1d** (100 mg) was used as a starting material. Recrystallization from acetone gave **2d** as colorless needles, mp 115—117 °C. *Anal.* Calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.29; H, 7.18. MS *m/z*: 312 (M^+), 148 (base peak), 133, 77. ^{1}H-NMR δ (ppm): 0.67 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.13 (2H, q, J=7.6 Hz, 8-, or 8′-CH_2) 1.73 (2H, q, J=7.6 Hz, 8, or 8′-CH_2), 3.82 (6H, s, 5,5′-OCH_3), 6.96 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 7.34 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 9.3 (tq, J=126.5, 4.1 Hz, 9,9′-CH_3), 28.4 (tq, J=127.4, 4.6 Hz, C-8, 8′), 55.5 (q, J=143.9 Hz, 5,5′-OCH_3), 72.8 (br s, C-1, 1′), 114.2 (dd, J=158.4, 4.6 Hz, C-4, 4′, 6, 6′), 129.0 (dd, J=157.6, 7.3 Hz, C-3, 3′, 7, 7′), 132.4 (m, C-2, 2′), 159.7 (m, C-5, 5′).

The Dimethyl Ether (7) of *cis*-Diethylstilbestrol Oxide Compound **7** of *cis*-diethylstilbestrol oxide was prepared by the method of Jellinck and Bowen[7] except that **6b** was used as the starting material. The product was obtained as an oil. MS *m/z*: 312 (M^+), 255, 148 (base peak), 117. ^{1}H-NMR δ (ppm): 0.86 (6H, t, J=7.3 Hz, 9,9′-CH_3), 1.93 (2H, dq, J=7.3 Hz, 8,8′-CH_2), 2.21 (2H, dq, J=7.3 Hz, 8,8′-CH_2), 3.65 (6H, s, 5,5′-OCH_3), 6.62 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 7.03 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 9.8 (tq, J=126.5, 4.6 Hz, C-9, 9′), 27.5 (tq, J=126.0, 4.6 Hz, C-8, 8′), 55.2 (q, J=143.9 Hz, 5,5′-OCH_3), 72.8 (br s, C=1, 1′), 113.4 (dd, J=158.6, 4.6 Hz, C-4, 4′, 6, 6′), 129.0 (dd, J=157.6, 7.8 Hz, C-3, 3′, 7, 7′), 132.5 (m, C-2, 2′), 158.6 (m, C-5, 5′). HRMS Calcd for $C_{20}H_{24}O_2$: 312.1734. Found: 312.1725.

The Mono- and Dimethyl Ether (3b and 3c) of 3a Compounds **3b** and **3c** of **3a** (200 mg) were prepared by methylation with dimethyl sulfate in MeOH except that the reaction temperature was 85 °C. The reaction mixture (230 mg) in benzene was chromatographed on silica gel. 1) Elution with benzene afforded the dimethyl ether of **3a**. MS *m/z*: 256 (M^+) (base peak), 240, 147, 121, 91. ^{1}H-NMR δ (ppm): 0.64 (3H, t, J=7.3 Hz, 9-CH_3), 0.82 (3H, t, J=7.3 Hz, 9′-CH_3), 2.28 (2H, q, J=7.3 Hz, 8,8′-CH_2), 2.32 (2H, q, J=7.3 Hz, 8,8′-CH_2), 3.80 (6H, s, 5,5′-OCH_3), 6.91 (4H, br d, J=8.9 Hz, 4,4′,6,6′-H), 7.16 (4H, br d, J=8.9 Hz, 3,3′,7,7′-H). ^{13}C-NMR δ (ppm): 9.4 (tq, J=127.4, 4.1 Hz, C-9′), 9.9 (tq, J=125.6, 4.1 Hz, C-9), 30.7 (tq, J=129.8, 4.6 Hz, C-8), 32.5 (br s, C-1), 114.3 (dd, J=160.8, 5.0 Hz, C-4, 4′, 6, 6′), 131.2 (dd, J=159.5, 7.3 Hz, C-3, 3′, 7, 7′), 134.7 (m, C-2, 2′), 159.3 (m, C-5, 5′), 211.1 (m, C-1′). 2) Elution with methyl chloride afforded the monomethyl ester of **3a**. MS *m/z*: 241 (M^+) (base peak), 147, 133. ^{1}H-NMR δ (ppm): 0.64 (3H, t, J=7.3 Hz, 9-CH_3), 0.82 (3H, t, J=7.3 Hz, 9′-CH_3), 2.27 (2H, q, J=7.3 Hz, 8,8′-CH_2), 2.31 (2H, q, J=7.3 Hz, 8,8′-CH_2), 3.80 (3H, s, 5-OCH_3), 6.83 (2H, br d, J=9.2 Hz, 4′,6′-H), 7.16 (2H, br d, J=9.2 Hz, 3,7-H), 8.43 (1H, s, 5′-OH). ^{13}C-NMR δ (ppm): 9.4 (tq, J=127.4, 4.1 Hz, C-9′), 9.9 (tq, J=125.6, 4.1 Hz, C-9), 30.8 (tq, J=128.3, 4.6 Hz, C-8). 32.5 (tq, J=126.0, 4.1 Hz, C-8′), 55.4 (q, J=143.9, 5-OCH_3), 65.8 (br s, C-1), 114.2 (dd, J=161.3, 4.6 Hz, C-4, 6), 115.7 (dd, J=160.4, 4.1 Hz, C-4′, 6′), 131.2 (dd, J=158.1, 7.3 Hz, C-3, 3′, 7, 7′), 133.4 (m, C-2′), 134.7 (m, C-2), 156.9 (br ddd, J=9.2, 9.2, 2.8 Hz, C-5′), 159.2 (m, C-5), 211.3 (m, C-1′).

Treatments of 2a, 2b, and 2d under Acidic and Basic Conditions The reaction products formed from **2a**, **2c**, and **2d** under acidic and basic conditions were determined by ^{1}H-NMR analysis. The results and conditions sre described in Table I.

The Monomethyl Ether (2c) of (+)- and (−)-2a Chromatographic separation of the monomethyl ether (**2c**) of (±)-**2a** was achieved by using an HPLC Chiralcel OJ column (Daicel Chemical Co.). The chromatographic profile and conditions are shown in Fig. 1. The first elution gave (−)-**2c** as an oil, $[\alpha]_D^{20}$ −12.6° (c=0.10, EtOH). CD (c= 1.03 mg/ml EtOH) (θ): −29310 (222) (negative maximum), −3040 (230), −15770 (236), +8620 (284) (positive maximum). The second elution gave (+)-**2c** as an oil, $[\alpha]_D^{20}$ +13.7° (c=0.10, EtOH). CD (c=1.02 mg/ml, EtOH) [θ]: +24830 (222) (positive maximum), +3070 (230), +15920 (236), −9230 (283) (negative maximum). The ^{1}H-NMR spectra of the enantiomers were identical with that of (±)-**2c**.

The Mono (4′-bromobenzoate) of (−)-2c The monomethyl ether (−)-**2c** (47 mg) was treated with 4-bromobenzoic acid chloride (110 mg) for 2 h at room temperature (24 °C). The reaction product was diluted with ice-water, and extracted with benzene, and the extract was washed with 10% HCl, 5% Na_2CO_3, and water, then dried (Na_2SO_4) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (eluent, *n*-hexane–benzene 50:50). Recrystallization from EtOH gave the mono (4-bromobenzoate) of (−)-**2c** as colorless needles, mp 119.5—120.5 °C. ^{1}H-NMR δ (ppm): 0.72 (3H, t, J=7.3 Hz, 9- or 9′-CH_3) 0.74 (3H, t, J=7.3 Hz, 9- or 9′-CH_3) 1.21 (2H, m, 8- or 8′-CH_2) 1.73 (2H, m, 8- or 8′-CH_2), 3.84 (3H, s, 5-OCH_3), 6.93 (2H, br d, J=8.6 Hz, 4,6-H), 7.23 (2H, br d, J=8.6 Hz, 4′,6′-H), 7.30 (2H, br d, J=8.6 Hz, 3,7-H), 7.45 (2H, br d, J=8.6 Hz, 3′,7′-H), 7.67 (2H, br d, J=8.6 Hz, aromatic-H), 8.07

(2H, br d, $J=8.6$ Hz, aromatic-H).

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