Preparation of Monomethyl Ether Derivatives of (+)-, (−)- and (±)-Indenestrols A and B

Taiko ODA and Yoshihiro SATO*

Kyoritsu College of Pharmacy, Shibakoen 1-chome, Minato-ku, Tokyo 105, Japan. Received March 4, 1993

In order to evaluate the contribution of the two phenolic hydroxyl groups to the biological activities of indenestrols A (IA) and B (IB), monomethyl ether derivatives of IA and IB were prepared and each enantiomer was separated to >99% purity by high-pressure liquid chromatography using a chiral column. The structures of the enantiomers were confirmed by proton and carbon-13 nuclear magnetic resonance and circular dichroism spectroscopic studies.

Keywords optical resolution; indenestrol A monomethyl ether; indenestrol B monomethyl ether; circular dichroism

Diethylstilbestrol, a synthetic non-steroidal estrogen, exhibits biological activities similar to those of the endogenous potent steroidal hormone, estradiol.[1] Its pharmacological, toxicological and carcinogenic properties have been reviewed.[2]

In the preceding paper[3] of our studies,[4] we reported the synthesis of optically active indenestrols A (IA) and B (IB).[5] Furthermore, the effects of these derivatives on the polymerization of microtubule proteins *in vitro*, and on the relative plating efficiency, chromosome number, and cellular microtubule architecture of Chinese hamster V79 cells were elucidated.[6]

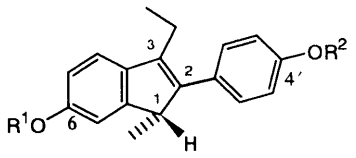
In order to clarify the importance of the two phenolic hydroxyl groups for the biological activities of IA and IB, we synthesized the optically active monomethyl ethers (eight isomers) of IA and IB and their racemic compounds (four derivatives) (Chart 1). Structural confirmation was performed by physico-chemical methods such as proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) and circular dichroism (CD) spectroscopic examinations.

Results and Discussion

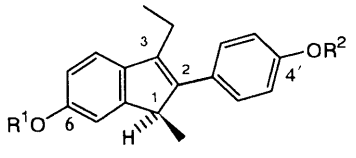
Syntheses of (±)-4′- and 6-O-Methyl Ether of Indenestrol A In the preceding paper,[3] we reported that (±)-IA was obtained from *E,E*-dienestrol by treatment with dilute sulfuric acid in methanol. Therefore, we attempted the synthesis of monomethyl ether derivatives of IA under the same conditions except that the monomethyl ether derivative (monoMe-DIEN) of *E,E*-dienestrol was used as a starting material, resulting in the formation of a mixture of (±)-4′- and 6-O-methyl ether derivatives (4′MeIA and 6MeIA) of IA in 97% yield.

The four enantiomeric components of the mixture of 4′- and 6-MeIA were separated on a chiral column, under the conditions given in the legend to Fig. 1. The methyl ether positions in the four products were determined by comparison of the ^{1}H- and ^{13}C-NMR data with those of IA (Tables I and II). Since the ^{1}H- and ^{13}C-NMR spectra of the products from the first and third fractions were identical, as were those of the products from the second and fourth fractions, and also on the basis of ^{1}H-NMR analysis, the O-methyl ether group in the compounds of the first and third fractions was determined to be at the 6-O position, and that in the compounds of the second and fourth fractions was determined to be at the 4′-O position.

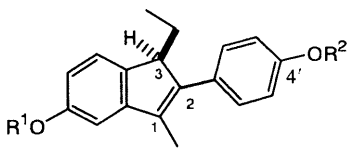
Next, optical rotations of the products from the first, second, third and fourth fractions were determined as $[\alpha]_D^{24}$ −265.9°, −312.6°, +258.6°, and +281.6°, respectively. The CD spectra of the (+)-4′MeIA and (+)-6MeIA derivatives showed positive Cotton effects, whereas the (−)-isomers had negative ones. Since the CD spectral patterns of (+)- and (−)-4′MeIA and 6MeIA were similar to those of



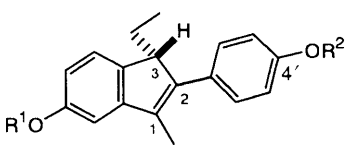
[(+)-1*R*]IA : $R^1=R^2=H$
[(+)-1*R*]6MeIA : $R^1=Me$; $R^2=H$
[(+)-1*R*]4′MeIA : $R^1=H$; $R^2=Me$



[(−)-1*S*]IA : $R^1=R^2=H$
[(−)-1*S*]6MeIA : $R^1=Me$; $R^2=H$
[(−)-1*S*]4′MeIA : $R^1=H$; $R^2=Me$



[(+)-3*R*]IB : $R^1=R^2=H$
[(+)-3*R*]6MeIB : $R^1=Me$; $R^2=H$
[(+)-3*R*]4′MeIB : $R^1=H$; $R^2=Me$



[(−)-3*S*]IB : $R^1=R^2=H$
[(−)-3*S*]6MeIB : $R^1=Me$; $R^2=H$
[(−)-3*S*]4′MeIB : $R^1=H$; $R^2=Me$

Chart 1

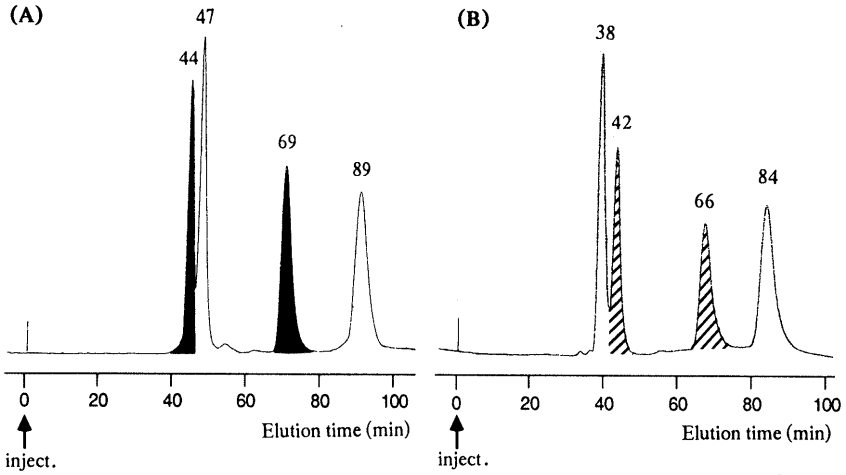


Fig. 1. HPLC Chromatograms of Monomethyl Ether Derivatives of (±)-IA (A) and IB (B)

A 100-μl sample (5 mg) of monomethyl ether derivatives of (±)-IA and IB was injected onto a Chiral OJ column. The sample was eluted with a 20% 2-propanol/*n*-hexane solution at a flow rate of 2 ml/min. Sample detection was monitored by measuring UV absorption at 254 nm. (A): ■, (−)- and (+)-6MeIA; □, (−)- and (+)-4′MeIA. (B): □, (−)- and (+)-4′MeIB; ▨, (−)- and (+)-6MeIB.

TABLE I. ^{1}H-NMR Spectral Data for Indenestrols and Their Methyl Ether Derivatives

	Chemical shift (ppm)					
	(+)-IA	(−)-IA	(+)-6MeIA [F-3]	(−)-6MeIA [F-1]	(+)-4′MeIA [F-4]	(−)-4′MeIA [F-2]
8-Me	1.11	1.11	1.14	1.14	1.11	1.11
10-Me	1.24	1.24	1.24	1.24	1.24	1.24
9-CH_2	2.62	2.62	2.63	2.63	2.62	2.62
1-H	3.75	3.75				
6-OMe			3.82	3.82		
4′-OMe					3.83	3.83
5-H	6.78	6.78	6.87	6.86	6.79	6.78
3′,5′-H	6.91	6.91	6.92	6.92	7.00	7.00
7-H	6.97	6.97	7.09	7.09	6.98	6.98
4-H	7.17	7.17	7.27	7.27	7.20	7.19
2′,6′-H	7.21	7.21	7.23	7.23	7.30	7.30
6-OH	8.09	8.10			8.11	8.11
4′-OH	8.37	8.36	8.40	8.39		

	Chemical shift (ppm)					
	(+)-IB	(−)-IB	(+)-6MeIB [F-3]	(−)-6MeIB [F-2]	(+)-4′MeIB [F-4]	(−)-4′MeIB [F-1]
10-Me	0.40	0.40	0.39	0.39	0.39	0.39
9-CH_2	1.58	1.58	1.60	1.61	1.59	1.59
9-CH_2	1.94	1.94	1.94	1.94	1.91	1.90
8-Me	2.15	2.15	2.19	2.19	2.16	2.16
6- or 4′OMe			3.84	3.84	3.84	3.84
1-H	3.84	3.84	3.86	3.84	3.86	3.87
5-H	6.68	6.68	6.75	6.75	6.69	6.69
7-H	6.81	6.81	6.92	6.92	6.83	6.80
3′,5′-H	6.93	6.93	6.93	6.94	7.02	7.02
4-H	7.22	7.22	7.28	7.28	7.24	7.24
2′,6′-H	7.27	7.27	7.33	7.33	7.36	7.36
6-OH	8.07	8.07			8.10	8.09
4′-OH	8.41	8.41	8.44	8.44		

IA, the absolute structures of these methyl ethers of the IA enantiomers were concluded to be (+)-1*R* and (−)-1*S* respectively. Thus, the structures of the first, second, third and fourth elution products were determined as (−)-6MeIA, (−)-4′MeIA, (+)-6MeIA, and (+)-4′MeIA, respectively.

Synthesis of 4′- and 6-O-Methyl Ether Derivatives of (±)-Indenestrol B First, we thought that (±)-4′- or 6-MeIB could be prepared by the method used in the previous study,[3] but with monoMe-DIEN as the starting material. However, analysis of the reaction products by ^{1}H- and ^{13}C-NMR indicated that the products were composed of many kinds of isomers. Therefore, we per-

Table II. ^{13}C-NMR Spectral Data for Monomethyl Ether Derivatives of IA and IB

Carbon	Chemical shifts (ppm)			
	4′MeIA	6MeIA	4′MeIB	6MeIB
C–1	46.2	46.3	133.6	133.2
C–2	144.0	145.1	146.4	147.1
C–3	138.5	137.9	51.0	51.0
C–4	120.4	120.2	123.8	123.8
C–5	114.1	112.6	112.2	110.7
C–6	156.4	158.9	157.6	160.3
C–7	111.5	110.2	106.9	105.4
C–8	17.2	17.2	11.7	11.7
C–9	19.8	19.8	24.0	24.0
C–10	14.2	14.2	8.5	8.5
C–3a	137.6	137.6	137.8	138.9
C–7a	151.4	151.2	149.3	149.5
C–1′	131.0	127.8	129.9	120.9
C–2′,6′	130.5	130.7	130.9	131.1
C–3′,5′	114.6	116.1	114.6	116.4
C–4′	159.3	157.1	159.5	157.3
C–4′OMe	55.5		55.5	
C–6OMe		55.7		55.6

formed thermal isomerization of the (±)-monomethyl ether derivatives (4′- and 6MeIA) of (±)-IA, and monomethyl ether derivatives of (±)-IB were separated by use of a chiral column, as described previously.[3] Furthermore, chromatographic separation of the individual monomethyl ether derivatives (4′- and 6MeIB) of IB enantiomers was achieved as described above; the chromatographic profile and conditions are shown in Fig. 1.

The chromatogram showed four peaks, and the four elution products were identified by ^{1}H- and ^{13}C-NMR analyses (Tables I and II). The products from the first and fourth fractions gave identical ^{1}H- and ^{13}C-NMR spectra, confirming them to be the 4′-methyl ether derivatives of IB. On the other hand, the products from the second and third fractions were determined to be the 6-methyl ether derivatives of IB.

Next, the optical rotations of the products from the first, second, third and fourth fractions were determined to be $[\alpha]_D^{22}$ −332°, −279°, +262°, and +340°, respectively. Since the CD spectral patterns of (+)- and (−)-4′MeIB and 6MeIB were similar to those of IB, the absolute structures of the 4′MeIB and 6MeIB enantiomers were concluded to be (+)-3*R* and (−)-3*S*, respectively.

We are planning to use these optically active methyl ether derivatives of indenestrols A and B and their racemic compounds for biological activity studies.

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 ($\delta=0$). The ^{13}C-NMR spectra were determined at 67.8 MHz using a JEOL JNM-GX 270 FT NMR spectrometer with 32 k data points for acquisition of free induction decays. For measurement of carbon–proton coupling constants, the coupling information was retained using a gated decoupling facility, which permitted retention of the NOE. Abbreviations used: s = singlet, d = doublet, t = triplet, br = broad, m = multiplet, dd = doublet of doublets, q = quartet. MS was 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 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 using 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.

Materials *E,E*-Dienestrol was obtained from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan. All other reagents were obtained from Wako Pure Chemical Industries, Ltd.

Monomethyl Ether of *E,E*-Dienestrol The monomethyl ether of *E,E*-dienestrol was prepared as described previously.[7]

Synthesis of a Mixture of (±)-4′MeIA and (±)-6MeIA A mixture of (±)-4′MeIA and 6MeIA was prepared as described previously,[3] except that the monomethyl ether of *E,E*-dienestrol was used as the starting material. The reaction mixture was poured into ice-water, and extracted with ethyl acetate, then the extract was washed with water, dried (Na_2SO_4) and concentrated *in vacuo* to afford a brown residue, which contained the product (97%) and a by-product (3%) as revealed by ^{1}H-NMR analyses. The *n*-hexane–benzene extract of the residue was purified by silica gel column chromatography (eluent, *n*-hexane–benzene (50 : 50)) to provide a mixture of (±)-4′MeIA or 6MeIA, $[\alpha]_D^{24}$ 0 ($c=0.32$, EtOH). MS *m/z*: 280 (M^+) (base peak), 251, 135.

(+)- and (−)-4′MeIA and (+)- and (−)-6MeIA Chromatographic separation of the individual 4′MeIA and 6MeIA enantiomers 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 product was (−)-6MeIA as an oil, $[\alpha]_D^{24}$ −265.9° ($c=0.13$, EtOH). CD ($c=0.65$ mg/ml, EtOH) [θ] (nm): −29090 (239), −7810 (253), −32990 (301) (negative maximum). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II. The second elution product was (−)-4′MeIA as an oil, $[\alpha]_D^{24}$ −312.6° ($c=0.12$, EtOH). CD ($c=0.62$ mg/ml, EtOH) [θ] (nm): −35340 (240), −9970 (257), −36020 (301) (negative maximum). MS *m/z*: 280 (M^+) (base peak), 251, 135. ^{1}H- and ^{13}C-NMR data are shown in Tables I and II. The third elution product was (+)-6MeIA as an oil, $[\alpha]_D^{24}$ +258.6° ($c=0.14$, EtOH). CD ($c=0.71$ mg/ml, EtOH) [θ] (nm): 28730 (239), 5750 (254), 34280 (301) (positive maximum). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II. The fourth elution product was (+)-4′MeIA as an oil, $[\alpha]_D^{24}$ +281.6° ($c=0.11$, EtOH). CD ($c=0.76$ mg/ml, EtOH) [θ] (nm): 32970 (240), 9950 (257), 33640 (301) (positive maximum). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II.

Synthesis of a Mixture of (±)-4′MeIB and (±)-6MeIB A mixture of (±)-4′MeIB and 6MeIB was prepared by thermal isomerization.[3] After cooling, the brown solid (505 mg) was extracted with acetone and the extract was concentrated *in vacuo*. The ratio (50 : 50) of (±)-4′- and 6MeIA and (±)-4′- and 6MeIB was determined by ^{1}H-NMR analysis and the products were purified by silica gel (100 g) column chromatography. A mixture of the four products was eluted with methylene chloride–benzene (20 : 80), and then (±)-4′- and 6MeIB were separated through these acetates. A mixture of (±)-4′MeIB and (±)-6MeIB was obtained, $[\alpha]_D^{24}$ 0 ($c=0.32$, EtOH). MS *m/z*: 280 (M^+), 251 (base peak).

(+)- and (−)-4′MeIB and (+)- and (−)-6MeIB Chromatographic separation of the individual 4′MeIB and 6MeIB enantiomers 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 product was (−)-4MeIB as an oil, $[\alpha]_D^{24}$ −332° ($c=0.11$, EtOH). CD ($c=1.05$ mg/ml, EtOH) [θ] (nm): −6910 (244) (negative maximum), −4050 (264), −6760 (287). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II. The second elution product was (−)-6′MeIB as an oil. $[\alpha]_D^{24}$ −279° ($c=0.07$, EtOH). CD ($c=0.75$ mg/ml, EtOH) [θ] (nm): −5600 (239), −2980 (261), −5800 (290) (negative maximum). MS *m/z*: 280 (M^+), 251 (base peak). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II. The third elution product was (+)-6MeIB as an oil, $[\alpha]_D^{24}$ +262° ($c=0.10$, EtOH). CD ($c=0.98$ mg/ml, EtOH) [θ] (nm); 5660 (239), 3010 (261), 5930 (290) (positive maximum). ^{1}H- and ^{13}C-NMR data are shown in Table II. The fourth elution product was (+)-4′MeIB as an oil, $[\alpha]_D^{24}$ +340° ($c=0.12$, EtOH). CD ($c=1.05$ mg/ml, EtOH) [θ] (nm): 6480 (244) (positive maximum), 3920 (264), 5350 (287). ^{1}H- and ^{13}C-NMR data are shown in Tables I and II.

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