Total Synthesis and Absolute Stereochemistry of the Natural Atropisomer of the Biflavone 4′,4‴,7,7″-Tetra-*O*-methylcupressuflavone

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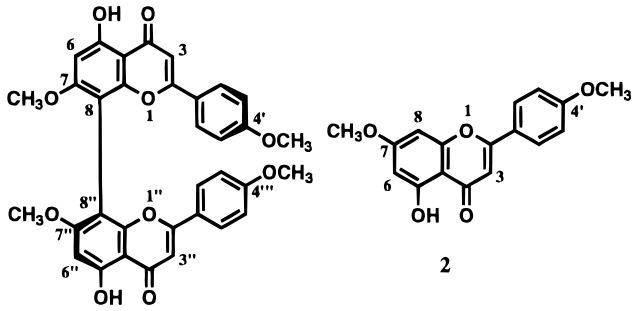
The total synthesis of a natural atropisomer, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone (**1**), has been achieved. The key intermediate, 3,3′-diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol (**5**), was synthesized by the solid state phenol coupling reaction, and the racemate **5** was enantioresolved as bis(camphanate) esters. The absolute configuration of bis(camphanate) ester (−)-**8b** was determined to be (a*R*) by X-ray analysis. The ester (a*R*)-(−)-**8b** was converted to the natural biflavone [CD(+)362.0]-(−)-**1**, leading to the (a*R*) absolute configuration of **1**. This conclusion is consistent with our previous theoretical determination of the absolute stereochemistry of biflavone **1** by the molecular orbital calculation of CD spectra.

Introduction

The CD exciton chirality method has been extensively used for determining the absolute configurations of natural products and synthetic chiral compounds.[1] On the other hand, to determine the absolute stereochemistry of chiral compounds having a twisted π-electron system, we have used a theoretical calculation to obtain the CD spectra by the π-electron self-consistent field/ configuration interaction/ dipole velocity molecular orbital method (SCF−CI−DV MO method).[2−4] For example, we have succeeded in determining the absolute configuration of dihydroazulene derivatives isolated from liverworts,[5] halenaquinols isolated from tropical marine sponges,[6,7] spiro troponoids,[8] cyanin dyes,[9] ternaphthalenes,[10] and unique chiral olefins.[11−13] The reliability of this theoretical CD method has been verified by synthetic studies of model compounds, by stereoselective total syntheses, and in some instances by X-ray crystallography.[5−13]

Chart 1[a]



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[CD(+)362.0]-(a*R*)-(−)-1

[a] The sign of optical rotation was taken from the $[\alpha]_D$ value of the natural sample in MeOH.

Previously, we have determined the absolute stereochemistry of a chiral biflavone isolated from plants, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone ((−)-**1**), by the molecular orbital calculation of CD spectra (Chart 1).[14] Chiral biflavone **1** is composed of two flavone units **2** connected at 8-8″ positions and can exist as an enantiomer devoid of centers of chirality, *i.e.*, a natural atropisomer.[15] The CD spectrum of **1** exhibits intense Cotton effects reflecting its twisted π-electron framework. The sign and intensity of CD Cotton effects of **1** were satisfactorily reproduced by the theoretical calculation, when the (a*R*)-enantiomer was chosen. Therefore, we reported that the naturally occurring 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone (**1**) has the (a*R*) absolute stereochemistry.[14] Recently, Lin *et al.* reported the determination of the absolute configuration of compound **1** by X-ray crystallography.[16] However, there is considerable confusion in their paper. Very recently, they reported

[⊗] Abstract published in *Advance ACS Abstracts,* October 1, 1997.

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(12) Harada, N.; Saito, A.; Koumura, N.; Roe, D. C.; Jager, W. F.; Zijlstra, R. W. J.; de Lange, B.; Feringa, B. L. *J. Am. Chem. Soc.* **1997**, *119*, 7249.
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Table 1. Solvent Dependence of $[\alpha]_D$ and CD Data of Biflavone 4′,4‴,7,7″-Tetra-*O*-methylcupressuflavone ([CD(+)362.0]-(a*R*)-(−)-1)[a]

solvent	$[\alpha]_D$	CD data
natural sample		
MeOH	−25.3° (*c* 0.3)[b]	
EtOH		λ_{ext} 362.0 nm ($\Delta\epsilon$ +25.6), 326.2 (−54.4), 267.5 (+21.3)[b]
synthetic sample		
MeOH	−11.3° (*c* 0.261)	λ_{ext} 362.6 nm ($\Delta\epsilon$ +22.0), 326.4 (−51.3), 268.6 (+19.4),
	(lit. +1° (*c* 0.2))[c]	226.8 (+19.4), 216.6 (−4.5), 207.4 (+11.6)
EtOH	+43.9° (*c* 0.259)	λ_{ext} 363.0 nm ($\Delta\epsilon$ +25.5), 327.0 (−55.3), 269.2 (+21.3),
		226.4 (+9.1), 216.8 (−2.6), 208.0 (+11.8)
	(lit. +77° (*c* 0.2))[c]	(lit. λ_{ext} 360 nm ($\Delta\epsilon$ +28.1), 324 (−66.4), 267 (+24.8))[c]
$CHCl_3$	+77.0° (*c* 0.257)	λ_{ext} 361.0 nm ($\Delta\epsilon$ +31.5), 326.2 (−64.0), 269.8 (+24.7)

[a] The sign of optical rotation was taken from the $[\alpha]_D$ value of the natural sample in MeOH. [b] From ref 14. [c] From ref 16.

the total synthesis of biflavone **1**[17] and came to the same absolute configuration as our theoretically determined one.

In this paper, we report the total synthesis of chiral biflavone **1**. Although there were several papers reporting the synthesis of racemic 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone (**1**),[18] the total synthesis of chiral **1** had not been reported when we began. We have succeeded in the total synthesis of natural biflavone and established its (a*R*) absolute stereochemistry.

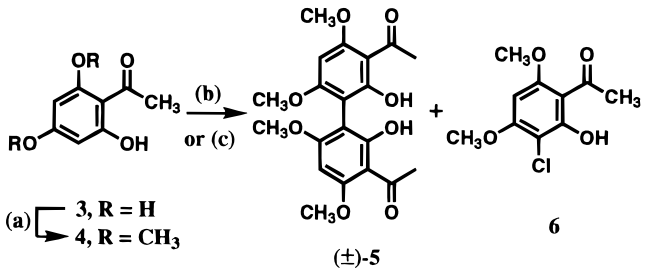
Results and Discussion

Designation of the Enantiomer 1 by Its CD Data. In general, enantiomers are designated by the sign of optical rotation, $[\alpha]_D$. For example, the designation (a*R*)-(−)-**1** means that the enantiomer **1** with negative rotation has the (a*R*) absolute configuration. However, it is also well-known that $[\alpha]_D$ values are dependent on the concentration and solvent used. In some extreme cases, the sign of $[\alpha]_D$ is inverted by changing solvents. As shown in Table 1, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone (**1**) falls in this extreme case. The $[\alpha]_D$ of the natural product **1** was first measured in methanol to show a negative value.[14] Later it was reported by Lin *et al.* that the $[\alpha]_D$ of **1** takes positive values when measured in MeOH and EtOH.[16] Therefore, the use of $[\alpha]_D$ value may cause confusion.

We have proposed to use CD data to designate enantiomers in addition to $[\alpha]_D$ values.[19] For example, the designation [CD(+)362.0]-(a*R*)-**1** means that the enantiomer **1** showing a positive CD Cotton effect at 362.0 nm has the (a*R*) absolute configuration. This method is very useful in the case of (−)-**1**, because the CD spectrum of **1** always exhibits a positive CD Cotton effect around 362.0 nm irrespective of the change of solvent as shown in Table 1.[20] Therefore, the natural biflavone is defined as [CD(+)362.0]-(a*R*)-**1**. To keep the consistency with the previous $[\alpha]_D$ data,[14] we adopt both designation methods in this paper; the natural product **1** is thus defined as [CD(+)362.0]-(−)-4′,4‴,7,7″-tetra-*O*-methylcupressuflavone.

It would be also useful to include *P* and *M* designations for these enantiomers. The (a*R*) enantiomer has *M* helicity and the (a*S*) isomer has *P* helicity.

Scheme 1[a]



[a] (a) $(CH_3)_2SO_4$, K_2CO_3/acetone; (b) $FeCl_3$, 50 °C, 12 h; (c) $FeCl_3$/silica gel, 43−45 °C, 6 d.

Synthesis of (±)-3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol (5) by the Solid State Phenol Coupling Reaction. As a chiral synthetic building block for the total synthesis of (−)-**1**, we selected 3,3′-diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol (**5**) (Scheme 1). To synthesize biphenyldiol **5**, phloroacetophenone **3**[21] was selectively methylated with dimethyl sulfate and K_2CO_3 in acetone giving 2,4-dimethylphloroacetophenone **4**[22] in 92% yield. Subsequently, the phenol coupling reaction of **4** using typical oxidants, *i.e.*, $FeCl_3$, or $(t\text{-Bu})_2O_2$, etc., in various solvents was completely unsuccessful.

We followed the solid state reaction conditions reported by Toda[23] and found that when a mixture of phenol **4** and $FeCl_3$ (1.0 equiv) was heated at 50 °C for 12 h, the desired coupling product **5** was obtained in 27% yield (Scheme 1). On the other hand, when phenol **4** was heated with excess $FeCl_3$ (5.2 equiv) at 50 °C for 5.5 h, the byproduct **6** was obtained in 33% yield, but no desired coupling product **5** was obtained. We explored the reaction condition further and found that the solid state phenol coupling reaction in the presence of $FeCl_3$/silica gel[24] brought the best yield of biphenyldiol **5**; a mixture of phenol **4** and 2.8 equiv of $FeCl_3$/silica gel (1:2) was heated at 43−45 °C for 6 d to give the desired coupling product (±)-**5** in 81% yield (mp 211−212 °C).

(16) Zhang, F.-J.; Lin, G.-Q.; Huang, Q.-C. *J. Org. Chem.* **1995**, *60*, 6427. See also: Additions and Corrections in *J. Org. Chem.* **1996**, *61*, 5700.

(17) Lin, G.-Q.; Zhong, M. *Tetrahedron Lett.* **1997**, *38*, 1087.

(18) (a) Murti, V. V. S.; Raman, P. V.; Seshadri, T. R. *Tetrahedron* **1967**, *23*, 397. (b) Ahmad, S.; Razaq, S. *Tetrahedron Lett.* **1971**, *48*, 4633. (c) Ahmad, S.; Razaq, S. *Tetrahedron* **1976**, *32*, 503. (d) Moriyama, S.; Okigawa, M.; Kawano, M. *J. Chem. Soc., Perkin Trans. 1* **1974**, 2132. (e) Parthasarathy, M. R.; Gupta, S. *Indian J. Chem.* **1984**, *23B*, 227.

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(20) See also the case where CD spectra can be quite solvent dependent: Cobb, D. I.; Lewis, D. A.; Armstrong, R. N. *J. Org. Chem.* **1983**, *48*, 4139.

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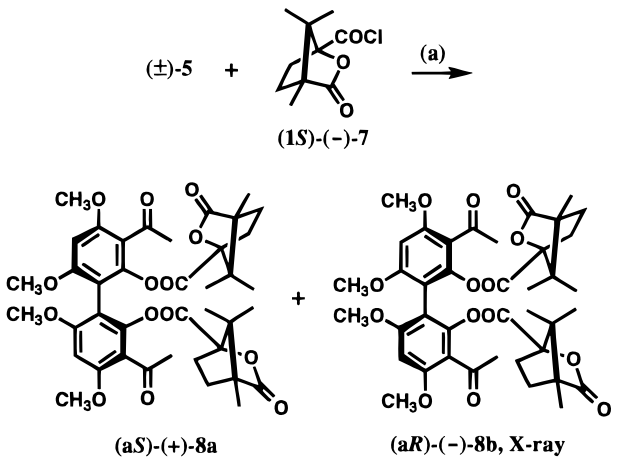
Scheme 2[a]



[a] (a) DMAP/pyridine, reflux.

Enantioresolution of (±)-3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol as Bis(camphanate) Esters 8 and X-ray Crystallographic Analysis.[25] Resolution of (±)-**5** using recently developed chiral dichlorophthalic acid method[10,26,27] was unsuccessful because of the steric hindrance around the phenol groups of **5**. However, application of the camphanate method to **5** (Scheme 2) using (1*S*)-(−)-camphanic acid chloride[28] and 4-(dimethylamino)pyridine in pyridine, refluxed for 3 days, yielded a diastereomeric mixture of esters **8a** and **8b**. The mixture was separated by HPLC on silica gel ($CH_3CN/CHCl_3$ 1:19): separation factor $\alpha = 1.18$; resolution factor $R_s = 1.62$. The diastereomeric mixture (ca. 700 mg) was separated to give the first eluted ester (+)-**8a** (42%, mp 255−257 °C, $[\alpha]^{22}_D$ +10.0° (*c* 0.78, $CHCl_3$)) followed by (−)-**8b** (46%, mp 208−209 °C, $[\alpha]^{22}_D$ −6.3° (*c* 1.02, $CHCl_3$)).

Recrystallization of diester **8b** from propyl acetate provided a colorless prism (0.35 × 0.33 × 0.32 mm) suitable for X-ray diffraction: monoclinic; space group $P2_1$. The absolute stereochemistry of the biphenyl moiety of diester (−)-**8b** was determined as (a*R*) by the internal reference method using the known absolute configuration of camphanate ester moiety (Figure 1).[30]

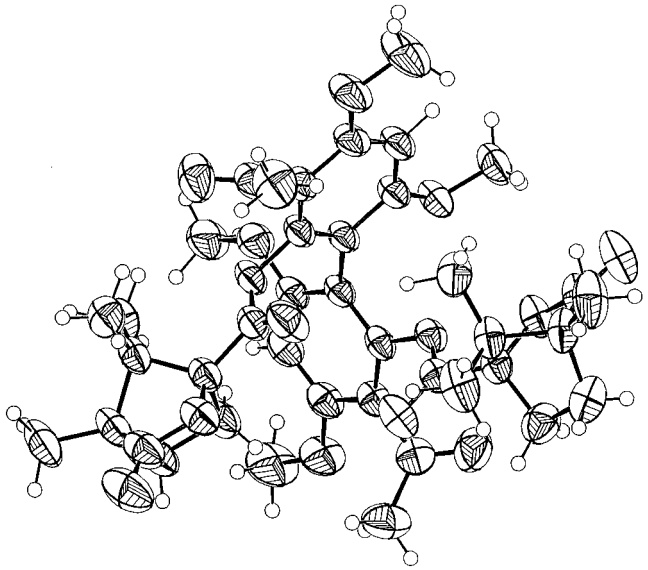
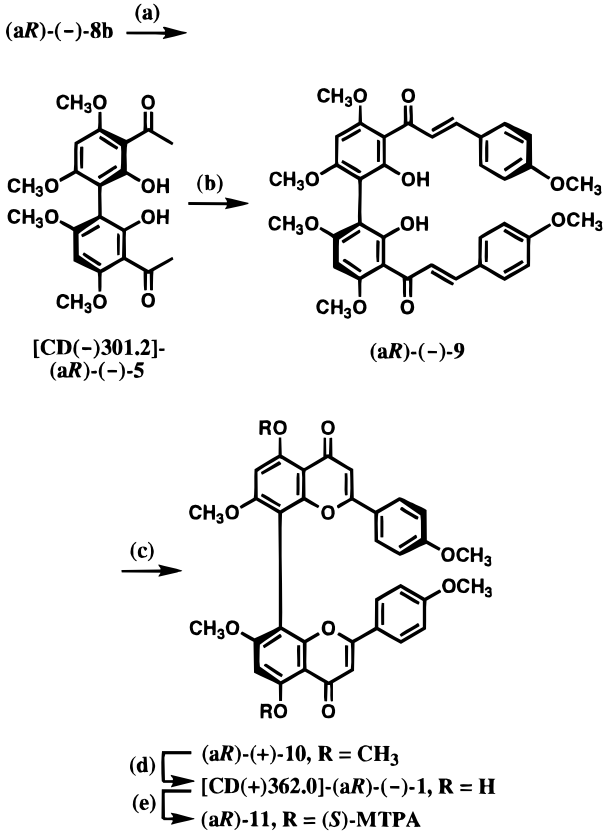


Figure 1. ORTEP drawing of diester (a*R*)-(−)-**8b**. The atoms are drawn as 50% probability.

Scheme 3[a]



[a] (a) 6 M HCl/EtOH, 78 °C, 18 h; (b) 4-anisaldehyde, KOH/EtOH; (c) I_2, H_2SO_4, DMSO, 80 °C, 20 h; (d) BCl_3/CH_2Cl_2, 0 °C; (e) (*S*)-(+)-MTPA chloride, $N(Et)_3$, CH_2Cl_2.

Total Synthesis of [CD(+)362.0]-(−)-4′,4‴,7,7″-Tetra-*O*-methylcupressuflavone (1). The total synthesis of the natural enantiomer of biflavone **1** started from chiral biphenyldiol (a*R*)-**5**. To recover chiral biphenyldiol (a*R*)-**5**, diester (−)-**8b** was hydrolyzed (Scheme 3). When diester **8b** was treated with aqueous LiOH in EtOH, deacetyl products were obtained instead of **5**. Therefore, diester **8b** was hydrolyzed under acidic conditions; a mixture of diester (−)-**8b**, aqueous 6 M HCl, and

(25) See the preliminary report of enantioresolution and X-ray analysis: Harada, N.; Li, H.-Y.; Nehira, T.; Hagiwara, M. *Enantiomer*, in press. The paper reports the X-ray analysis of ester (−)-**8b** using the crystal obtained by recrystallization from hexane/EtOAc. The final *R*-value, however, remained at a higher level because of the existence of EtOAc as crystalline solvent.

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(27) See also: (a) Harada, N.; Soutome, T.; Murai, S.; Uda, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1755. (b) Harada, N.; Hattori, T.; Suzuki, T.; Okamura, A.; Ono, H.; Miyano, S.; Uda, H. *Tetrahedron: Asymmetry* **1993**, *4*, 1789. (c) Harada, N.; Soutome, T.; Nehira, T.; Uda, H.; Oi, S.; Okamura, A.; Miyano, S. *J. Am. Chem. Soc.* **1993**, *115*, 7547. (d) Hattori, T.; Harada, N.; Oi, S.; Abe, H.; Miyano, S. *Tetrahedron: Asymmetry* **1995**, *6*, 1043. (e) Hagiwara, H.; Okamoto, T.; Harada, N.; Uda, H. *Tetrahedron* **1995**, *51*, 9891.

(28) Prepared from (−)-camphanic acid which was derived from (1*R*,4*R*)-(+)-camphor.[29] (−)-Camphanic acid: $[\alpha]^{22}_D$ −19.5° (*c* 1.0, 1,4-dioxane). Methyl ester of (−)-camphanic acid: $[\alpha]^{22}_D$ −22.4° (*c* 6.65, $CHCl_3$).

(29) Meyer, W. L.; Lobo, A. P.; McCarty, R. N. *J. Org. Chem.* **1967**, *32*, 1754.

(30) The authors have deposited atomic coordinates for this structure with the Cambridge Crystallographic Data Center. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Center, 12 Union Road, Cambridge, CB2 1EZ, U.K.

EtOH was heated at 78 °C for 18 h affording optically active biphenyldiol (a*R*)-**5** in 64% total yield, whose CD spectrum showed a negative CD Cotton effect at 301.2 nm. Although we had expected to obtain enantiopure biphenyldiol **5**, it was found that chiral biphenol **5** obtained partially racemized during the acid-catalyzed hydrolysis (79% ee). The enantiomeric excess of biphenyldiol **5** was determined by ^{1}H NMR spectroscopy using a chiral shift reagent, europium tris[3-((trifluoromethyl)hydroxymethylene)-(+)-camphorate], Eu(tfc)$_3$ as described in the Experimental Section.

Biphenyldiol (a*R*)-(−)-**5** was next converted to bichalcone (a*R*)-(−)-**9** (Scheme 3);[18c] a mixture of (a*R*)-(−)-**5**, 10% aqueous KOH, EtOH, and 4-anisaldehyde was heated at 70−75 °C overnight giving (a*R*)-(−)-**9** in 65% yield. Bichalcone (a*R*)-(−)-**9** was cyclized with iodine and catalytic amount of H_2SO_4 in DMSO[31] to afford 4′,4‴,5,5″,7,7″-hexa-*O*-methylcupressuflavone (a*R*)-(+)-**10** in 52% yield. Finally, hexa-*O*-methylcupressuflavone (a*R*)-(+)-**10** was selectively demethylated with BCl_3 in CH_2Cl_2 giving the desired natural product (a*R*)-(−)-**1** in 80% yield (78% ee). During the purification of the synthetic sample, we found that the racemic biflavone **1** is barely soluble in MeOH. Therefore, biflavone (a*R*)-**1** with 78% ee was recrystallized from methanol, providing optically pure biflavone (a*R*)-**1**. Its optical purity was confirmed by the ^{1}H NMR spectrum after conversion to (*S*)-MTPA ester (a*R*)-**11**, as described in Experimental Section. The physical data of 100% ee biflavone **1** are mp 147−149 °C (MeOH), $[\alpha]^{22}_D$ −11.3° (*c* 0.261, MeOH), and CD (EtOH) λ_{ext} 363.0 nm ($\Delta\epsilon$ +25.5).

CD Spectra and Absolute Stereochemistry of (−)-4′,4‴,7,7″-Tetra-*O*-methylcupressuflavone (1). The CD and UV spectra of synthetic biflavone (a*R*)-(−)-**1** are illustrated in Figure 2, and the CD curve is identical with that of the natural sample **1** shown in Figure 3. Therefore, the absolute stereochemistry of the natural atropisomer, [CD(+)362.0]-(−)-4′,4‴,7,7″-tetra-*O*-methylcupressuflavone (**1**), was determined to be (a*R*). This conclusion is consistent with our previous theoretical determination of the absolute stereochemistry of biflavone **1** by the molecular orbital calculation of CD spectra.

Experimental Section

General Procedures. Melting points are uncorrected. IR spectra were obtained as KBr disks. ^{1}H and ^{13}C NMR spectra were recorded on a 400 MHz/100 MHz or a 600 MHz/150 MHz spectrometer, equipped with the Nalorac inverse probe. All NMR data are reported in ppm (δ) downfield from TMS or residual signals ($CDCl_3$, $\delta_{H/C}$ 7.24/77.0) were used as internal standards. MS spectra were obtained by the electron ionization procedure (70 eV), unless otherwise noted. HPLC separation was carried out using a glass column (22 mm ϕ × 10 cm or 25 mm ϕ × 40 cm) of silica gel. The purities of the title compounds were shown to be ≥ 95% by ^{1}H NMR, TLC, HPLC, and/or elemental analysis.

3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol ((±)-5). The silica gel bound ferric chloride was made according to ref 12; silica gel (7.24 g) was dried in an oven at 150 °C for 11 h. To a mixture of $FeCl_3 \cdot 6H_2O$ (3.52 g) in dried diethyl ether (190 mL) and ethanol (10 mL) was added the dried silica gel. The solvents were removed to give a yellow solid, which was dried at 85 °C in vacuo (66−133 Pa). The silica gel bound ferric chloride (silica gel/$FeCl_3$ = 7:2) was obtained as a dark brown solid. The oxidative coupling reaction was carried out by adding silica gel bound ferric chloride (1.4 g) to a solution of 2,4-dimethylphloroacetophenone (**4**, 146 mg) in CH_2Cl_2 (10 mL). After well mixing by sonication and rotation, the solvent was removed at reduced pressure to give a dark solid, which was kept at 43−45 °C for 6 days. Water (1 mL) and CH_2Cl_2 (30 mL) were added, and the mixture was sonicated and filtered. The precipitate was washed with $CHCl_3$, and the combined organic layer was evaporated to afford a red crude product (213 mg). This was purified by silica

(31) Zhang, F.-J.; Li, Y.-L. *Chin. J. Chem.* **1994**, *12*, 73.

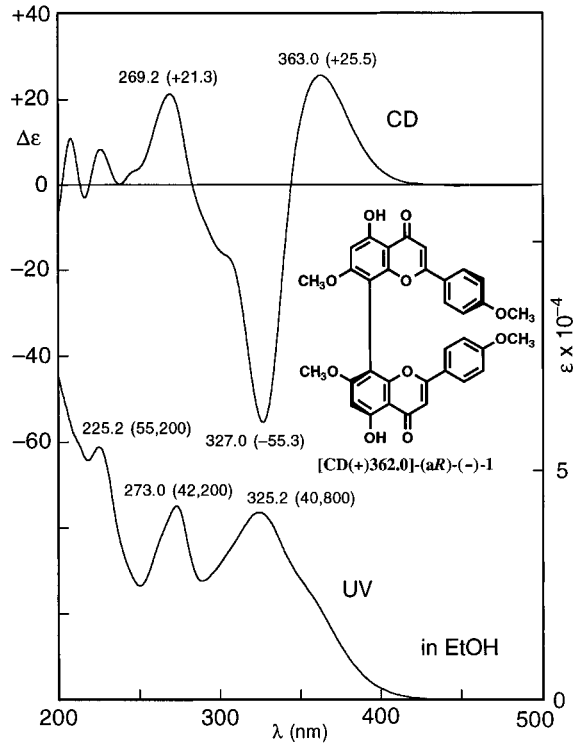

Figure 2. CD and UV spectra of the synthetic sample of biflavone, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone ([CD(+)-362.0]-(a*R*)-(−)-**1**) in EtOH.

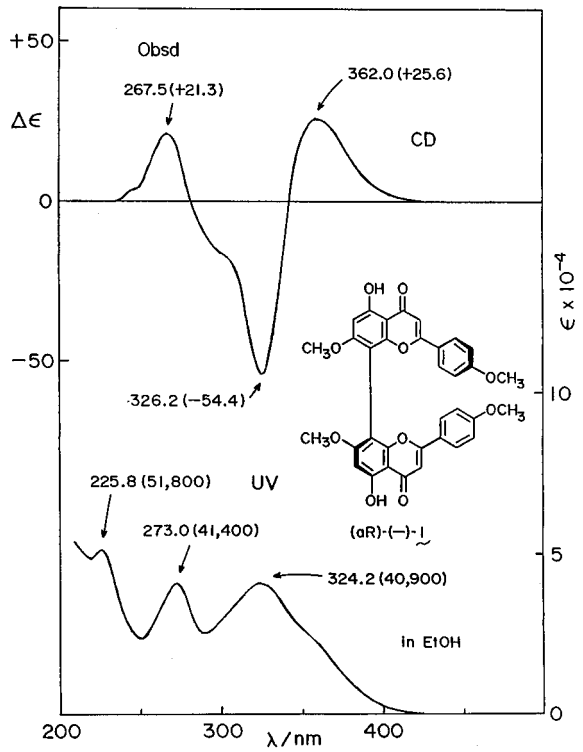

Figure 3. CD and UV spectra of the natural sample of biflavone, 4′,4‴,7,7″-tetra-*O*-methylcupressuflavone ([CD(+)-362.0]-(a*R*)-(−)-**1**) in EtOH.[14]

gel chromatography eluting with hexane/EtOAC (changing the ratio from 5:1, 3:1, 1:1, to 1:3) to yield biphenyldiol (±)-**5** (119 mg, 81%) as colorless small plates: mp 211−212 °C (H_2O/DMF); IR (KBr) ν_{max} 2949, 1618, 1590, 1405 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 2.61 (6 H, s), 3.80 (6 H, s), 3.92 (6 H, s), 6.06 (2 H, s), 13.98 (2 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 33.2, 55.1, 55.4, 86.4, 102.4, 106.1, 163.3, 163.9, 164.0, 203.3; MS *m*/*z* 390 (M^+, relative intensity 100), 375 (68), 333 (30); high-resolution mass spectrum (HRMS) calcd for $C_{20}H_{22}O_8$ 390.13146, found 390.13119. Anal. Calcd for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.63; H, 5.57.

The reaction yield for a larger scale (570 mg) was 74%.

3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol Bis(camphanate) Esters (8a and 8b) and HPLC Separation. To a mixture of biphenyldiol (±)-**5** (300 mg, 0.77 mmol) and (−)-camphanic acid chloride[28] (1.67 g, 6.42 mmol) in dry pyridine (20 mL) was added 4-(dimethylamino)pyridine (DMAP, 30 mg, 0.25 mmol). After being gently refluxed for 3 d under argon, the reaction mixture was extracted with EtOAc. The organic layer was washed with water, aqueous $CuSO_4$, water, and brine. The EtOAc layer was dried over $MgSO_4$ and evaporated in vacuo to give a crude product of bis(camphanate) esters (ca. 700 mg). To separate the diastereomeric esters, preparative HPLC was performed with a prepacked Kusano glass column of silica gel (25 mm $\phi \times$ 40 cm): UV/vis detector at 272 nm; RI detector; flow rate, 14 mL/min, 64 mg/each injection. The diastereomeric mixture was base-line separated eluting with MeCN/$CHCl_3$ (5:95): separation factor $\alpha = 1.18$, resolution factor $R_s = 1.62$. As the first-eluted fraction, camphanate ester (a*S*)-(+)-**8a** (242 mg, 42%, retention time 19.5 min) was obtained: mp 255−257 °C (fine needles from EtOH); IR (KBr) ν_{max} 1786, 1609, 1215, 1104 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.90 (6 H, s), 1.00 (6 H, s), 1.05 (6 H, s), 1.46 (2 H, ddd, J = 13.6, 9.2, 4.2 Hz), 1.52 (2 H, ddd, J = 13.2, 9.2, 3.7 Hz), 1.83 (2 H, ddd, J = 13.2, 10.8, 4.2 Hz), 2.22 (2 H, ddd, J = 13.6, 10.8, 3.7 Hz), 2.49 (6 H, s), 3.77 (6 H, s), 3.93 (6 H, s), 6.45 (2 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 9.6, 16.0, 16.1, 28.9, 30.3, 31.7, 54.5, 55.0, 56.1, 56.2, 91.0, 93.2, 107.9, 117.4, 146.6, 158.9, 160.4, 164.8, 178.1, 199.4; $[\alpha]^{22}_D$ +10.0° (*c* 0.78, $CHCl_3$); MS *m*/*z* 750 (M^+, relative intensity 100), 735 (57), 357 (28); HRMS calcd for $C_{40}H_{46}O_{14}$ 750.28870, found 750.28845.

From the second-eluted fraction, camphanate ester (a*R*)-(−)-**8b** (263 mg, 46%, retention time 23.4 min) was obtained: mp 208−209 °C (prisms from PrOAc); IR (KBr) ν_{max} 1785, 1604, 1215, 1103 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 0.55 (6 H, s), 0.94 (6 H, s), 1.02 (6 H, s), 1.61 (2 H, ddd, J = 13.0, 9.2, 4.4 Hz), 1.82 (2 H, ddd, J = 13.0, 10.6, 4.4 Hz), 2.09 (2 H, ddd, J = 13.6, 9.2, 4.4 Hz), 2.23 (2 H, ddd, J = 13.6, 10.6, 4.4 Hz), 2.47 (6 H, s), 3.83 (6 H, s), 3.91 (6 H, s), 6.44 (2 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 9.5, 15.2, 16.2, 28.8, 30.9, 31.8, 54.1, 54.9, 56.1, 56.2, 90.8, 93.2, 108.1, 116.5, 147.2, 159.5, 160.7, 164.9, 178.2, 198.7; $[\alpha]^{22}_D$ −6.3° (*c* 1.02, $CHCl_3$); UV (EtOH) λ_{max} 295.0 nm (ϵ 8700), 257.0 (16 900), 236.6 (29 800); CD (EtOH) λ_{ext} 315.0 nm ($\Delta\epsilon$ −6.0), 293.0 (+3.1), 274.4 (−7.8), 258.4 (+17.3), 240.8 (−11.5), 220.6 (−15.7); MS *m*/*z* 750 (M^+, relative intensity 100), 735 (51), 357 (31); HRMS calcd for $C_{40}H_{46}O_{14}$ 750.28870, found 750.28910.

X-ray Crystallography of 3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol Bis(camphanate) Ester ((−)-(8b)). Single crystals suitable for X-ray analysis were obtained as colorless prisms by recrystallization from PrOAc: mp 208−209 °C. A single crystal (dimension 0.35 × 0.33 × 0.32 mm) was selected for data collection and mounted on a Mac Science MXC18 automated four-circle diffractometer. The crystal was found to be monoclinic, and the unit cell parameters and orientation matrix were obtained. Data collection was carried out by using a $2\theta-\theta$ scan: formula, $C_{40}H_{46}O_{14}$; M_r = 750.28870; space group $P2_1$ (#4); a = 14.172 9 (3), b = 14.834 (3), c = 10.153 (2) Å, β = 110.24 (2)°; V = 2002.7 (7) Å^3; Z = 2; D_x = 1.245 g cm^{-3}; D_m = 1.241 g cm^{-3} by flotation using a CCl_4−hexane solution; radiation, Cu Kα (1.54178 Å); monochromator, graphite crystal; linear absorption coefficient, 7.01 cm^{-1}; temperature, 20 °C; scan speed, 14.0°/min; scan range, 1.64° + 0.2° tan θ; 2θ scan limits, 2−130°; standard reflections, 3 per 100 reflections; indices, (−9,−1,4), (9,1,−4), (7,−3,3); crystal stability, no indication of standard reflection decay during data collection; total reflections scanned, 3888; unique data $F_o > 3\sigma(F_o)$, 3552. The skeletal structure was solved by the direct method and successive Fourier syntheses. All hydrogen atoms were found by the difference Fourier syntheses. Absorption correction and full matrix least-squares refinement of positional and thermal parameters led to the final convergence with R = 0.0346 and R_w = 0.0409. The absolute stereochemistry of biphenyldiol ester (−)-**8b** was determined to be (a*R*) as shown in Figure 1 by the internal reference method using the known absolute configuration of the camphanate ester part.

3,3′-Diacetyl-4,4′,6,6′-tetramethoxy-2,2′-biphenyldiol ([CD(−)301.2]-(a*R*)-(−)-5). A mixture of bis(camphanate) ester (a*R*)-(−)-**8b** (135 mg, 0.18 mmol), aqueous HCl (6 M, 42 mL), and EtOH (49 mL) was heated under argon at 78 °C for 18 h. After the solvent and HCl were evaporated under reduced pressure, the residue was subjected to silica gel column chromatography eluting with hexane/EtOAc (changing the ratio from 3:1, 1:1, to 2:3) to afford optically active biphenyldiol (a*R*)-(−)-**5** (33.2 mg, 47%) and a mixture of the starting material and monocamphanate (57.8 mg). The recovery was recycled for the acid-catalyzed hydrolysis to give (a*R*)-(−)-**5** (12.1 mg, 17%: total yield, 64%): mp 218−220 °C (fine needles from $CHCl_3$); IR (KBr) ν_{max} 1618, 1591, 1281, 1133 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 2.61 (2 H, s), 3.81 (6 H, s), 3.92 (6 H, s), 6.06 (2 H, s), 13.97 (2 H, s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 31.1, 55.4, 55.8, 86.3, 102.4, 106.1, 163.3, 163.9, 164.0, 203.3; $[\alpha]^{22}_D$ −18.5° (*c* 2.45, $CHCl_3$); UV (EtOH) λ_{max} 338.0 nm (sh, ϵ 6300), 290.6 (34 200), 238.0 (23 100); CD (EtOH) λ_{ext} 301.4 nm ($\Delta\epsilon$ −15.2), 281.2 (+14.5), 247.8 (−7.6), 231.4 (−11.7), 211.6 (+44.9); HRMS calcd for $C_{20}H_{22}O_8$ 390.13144, found 390.13162. Anal. Calcd for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.24; H, 5.66.

The enantiomeric excess of biphenyldiol (a*R*)-(−)-**5** was determined to be 79% ee by the ^1H NMR spectrum of a mixture of (a*R*)-(−)-**5** (1.5 mg) and europium tris[3-((trifluoromethyl)-hydroxymethylene)-(+)-camphorate] ($Eu(tfc)_3$, 6.9 mg, 2 equiv) in $CDCl_3$: ^1H NMR (400 MHz, $CDCl_3$) δ 2.6714 ppm (0.625 H, s, corresponding to (a*S*)-enantiomer), 2.6836 (5.375 H, s, corresponding to (a*R*)-enantiomer), 3.84 (6 H, s), 3.96 (6 H, s), 6.10 (2 H, s), 14.12 (2 H, s).

Optically Active Bichalcone (a*R*)-(−)-9. To a solution of optically active biphenyldiol (a*R*)-(−)-**5** (79% ee, 33.2 mg, 0.0851 mmol) in a mixture of 10% aqueous KOH (3 mL) and EtOH (2 mL) was added a solution of 4-anisaldehyde (226 mg, 1.66 mmol) in EtOH (1 mL) under ice-cooling. The reaction mixture was kept under argon at 70 °C overnight and then at 75 °C for 3 h. After ice-cold 2 M HCl was added, the mixture was extracted with EtOAc for three times. The combined organic layer was washed with brine. Evaporation of the solvent and crystallization from MeOH gave optically active bichalcone (a*R*)-(−)-**9** (34.7 mg, 65%) as small red needles: mp 158−160 °C; IR (KBr) ν_{max} 1624, 1560, 1218 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 3.85 (12 H, s), 4.01 (6 H, s), 6.14 (2 H, s), 6.92 (4 H, d, J = 8.3 Hz), 7.56 (4 H, d, J = 8.3 Hz), 7.75 (2 H, d, J = 15.4 Hz), 7.80 (2 H, d, J = 15.4 Hz), 14.21 (2 H s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 55.4, 55.8, 55.9, 86.9, 102.9, 106.7, 114.3, 128.5, 128.7, 130.0, 141.9, 161.2, 162.8, 163.8, 164.6, 193.0; $[\alpha]^{22}_D$ −85.2° (*c* 0.23, $CHCl_3$); UV (EtOH) λ_{max} 370.2 nm (ϵ 37 000), 224.6 (49 000); CD (EtOH) λ_{ext} 397.0 nm ($\Delta\epsilon$ −4.8), 308.0 (+2.1), 233.0 (−9.6), 212.2 (+21.0); HRMS calcd for $C_{36}H_{34}O_{10}$ 626.21516, found 626.21520. Anal. Calcd for $C_{36}H_{34}O_{10}$: C, 69.00; H, 5.57. Found: C, 68.85; H, 5.91.

4′,4‴,5,5″,7,7″-Hexa-*O*-methylcupressuflavone ((a*R*)-(+)-10). To a solution of (a*R*)-(−)-**9** (25.6 mg, 0.0409 mmol) and iodine (3.6 mg, 0.0142 mmol) in DMSO (0.6 mL) with stirring was added concentrated H_2SO_4 (1 drop). After the reaction mixture was kept at 85 °C for 20 min, additional iodine (7.1 mg, 0.0279 mmol) and DMSO (0.4 mL) were added. The mixture was stirred at 80 °C overnight and poured into an ice-cold solution of $Na_2S_2O_3$ and KOH in water. The suspension was filtered, and the filtrate was subjected to preparative TLC on silica gel with developing system of EtOH/EtOAc (5:1) to give (a*R*)-(+)-**10** (13.2 mg, 52%) as a colorless solid: mp 155−156 °C (from $CHCl_3$); IR (KBr) ν_{max} 1638, 1339

cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.67 (6 H, s), 3.78 (6 H, s), 4.13 (6 H, s), 6.59 (4 H, s), 6.77 (4 H, br d, J = 9.0 Hz), 7.29 (4 H, br d, J = 9.0 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ 55.4, 56.1, 56.5, 91.6, 102.0, 106.7, 108.9, 114.3, 123.4, 127.1, 156.5, 160.6, 161.2, 161.7, 161.9, 178.2; $[\alpha]^{22}_D$ +23.4° (*c* 0.51, EtOH); UV (EtOH) λ_{max} 320.0 nm (ϵ 40 100), 268.0 (49 900), 224.0 (62 700); CD (EtOH) λ_{ext} 349.2 nm ($\Delta\epsilon$ +22.3), 312.4 (−42.1), 262.0 (+16.9); MS *m*/*z* 622 (M$^+$, relative intensity 100), 375 (68). Anal. Calcd for $C_{36}H_{30}O_{10}$: C, 69.45; H, 4.86. Found: C, 69.51; H, 5.06.

4′,4‴,7,7″-Tetra-*O*-methylcupressuflavone ([CD(+)362.0]-(a*R*)-(−)-1). To an ice-cold solution of (a*R*)-(+)-**10** (12.7 mg, 0.0204 mmol) in dry CH_2Cl_2 (1 mL) was added a solution of BCl_3 in CH_2Cl_2 (1 M, 50 mL, 0.05 mmol) under argon. The reaction mixture was stirred for 1 h, and then MeOH was added. After being stirred at 70 °C for 3 h, the mixture was evaporated in vacuo. The crude product obtained was subjected to preparative TLC developed with hexane/EtOAc (1:1) to yield the final biflavone (a*R*)-**1** in optically active form (9.7 mg, 80%, 78% ee): UV (EtOH) λ_{max} 324.4 nm (ϵ 40 000), 273.4 (40 600), 225.6 (51 400); CD (EtOH) λ_{ext} 362.8 nm ($\Delta\epsilon$ +19.8), 326.6 (−41.5), 301.6 (−12.6), 269.2 (+16.6), 225.8 (+6.7), 207.8 (+8.4). It was interesting to note that the racemate of biflavone **1** is hardly soluble in MeOH. Therefore when the optically active biflavone (a*R*)-**1** with 78% ee was recrystallized from methanol, optically pure biflavone (a*R*)-**1** was obtained from the mother liquor. Physical data of 100% ee biflavone [CD(+)362.0]-(a*R*)-(−)-**1**: mp 147−149 °C (MeOH); IR (KBr) ν_{max} 2925, 1652, 1590 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.80 (6 H, s), 3.82 (6 H, s), 6.59 (2 H, s), 6.60 (2 H, s), 6.86 (4 H, d, J = 9.0 Hz), 7.43 (4 H, br d, J = 9.0 Hz), 13.22 (2 H s); ^{13}C NMR (150 MHz, $CDCl_3$) δ 55.5, 56.2, 95.3, 103.5, 114.5, 127.6, 154.6, 162.57, 162.61, 163.3, 163.9, 182.9; $[\alpha]^{22}_D$ −11.3° (*c* 0.261, MeOH), see Table 1 for the solvent effect of $[\alpha]_D$ value; UV (EtOH) λ_{max} 359.0 nm (sh, ϵ 22 000), 325.2 (40 800), 273.0 (42 200), 225.2 (55 200); UV (MeOH) λ_{max} 325.0 nm (ϵ 37 100), 272.4 (37 900), 225.2 (47 400); UV ($CHCl_3$) λ_{max} 327.4 nm (ϵ 47 500), 274.4 (49 400); CD (EtOH) λ_{ext} 363.0 nm ($\Delta\epsilon$ +25.5), 327.0 (−55.3), 300.8 (sh, −15.8), 269.2 (+21.3), 226.4 (+9.1), 208.0 (+11.8), see Table 1 for the solvent dependence of CD data; MS *m*/*z* 594 (M$^+$, relative intensity 100), 595 (M$^+$ + 1, 49), 135 (28); HRMS calcd for $C_{34}H_{26}O_{10}$ 594.15258, found 594.15195.

The optical purity of biflavone (a*R*)-(−)-**1** obtained as crystals was 25% ee: CD ($CHCl_3$) λ_{ext} 362.6 nm ($\Delta\epsilon$ +6.6), 326.4 (−14.2), 276.0 (+5.0).

(*S*)-MTPA Esters 11 of Racemic Biflavone (±)-1. To a solution of racemic biflavone (±)-**1** (0.9 mg, 0.0015 mmol) in dry CH_2Cl_2 (0.5 mL) was added dry triethylamine (2 drops, distilled from KOH) under argon. After the solution was stirred for 10 min at room temperature, (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl, three drops) was added. The reaction was kept at room temperature overnight and then evaporated to dryness. The product was purified by preparative TLC on silica gel developed with hexane:EtOAc 2:1 to give (*S*)-MTPA diester of (±)-biflavone (**11**, 1.4 mg, 94%). The TLC confirmed that all biflavone (±)-**1** was converted to diester **11**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.77 (6 H, s), 3.80 (12 H, s), 3.81 (6 H, s), 3.90 (6 H, s), 3.91 (6 H, s), 6.57 (2 H, s), 6.59 (2 H, s), 6.69 (2 H, s), 6.72 (2 H, s), 6.86 (4 H, d, J = 9.1 Hz), 6.88 (4 H, d, J = 9.1 Hz), 7.28 (4 H, d, J = 9.1 Hz), 7.30 (4 H, d, J = 9.1 Hz), 7.42 (2 H, m), 7.52 (8 H, m), 7.57 (2 H, m), 7.91 (8 H, m).

(*S*)-MTPA Ester (a*R*)-11 of Optically Pure Biflavone [CD(+)362.0]-(a*R*)-(−)-1. Optically pure biflavone [CD(+)-362.0]-(a*R*)-(−)-**1** (0.6 mg, 0.001 mmol) was similarly converted to (*S*)-MTPA diester (a*R*)-**11** (0.9 mg, 91%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.80 (6 H, s), 3.90 (6 H, s), 6.59 (2 H, s), 6.69 (2 H, s), 6.88 (4 H, d, J = 9.1 Hz), 7.30 (4 H, d, J = 9.1 Hz), 7.57 (2 H, m), 7.91 (8 H, m). The appearance of a peak at δ 6.69 ppm and complete loss of a peak at δ 6.72 ppm clearly indicated that the biflavone (a*R*)-(−)-**1** synthesized here was enantiopure.

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Supporting Information Available: Experimental procedure for the synthesis of **4**, as well as ^{1}H and ^{13}C NMR spectra of key compounds (a*S*)-(+)-**8a**, (a*R*)-(−)-**8b**, and [CD(+)362.0]-(a*R*)-(−)-**1** (4 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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