Cylindol A, a Novel Biphenyl Ether with 5-Lipoxygenase Inhibitory Activity, and a Related Compound from Imperata cylindrica

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CYLINDOL A, A NOVEL BIPHENYL ETHER WITH 5-LIPOXYGENASE INHIBITORY ACTIVITY, AND A RELATED COMPOUND FROM *IMPERATA CYLINDRICA*

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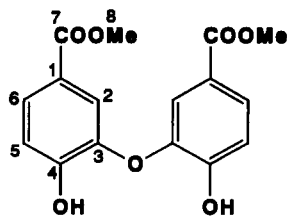
ABSTRACT.—Cylindol A [**1**] and B [**2**], two novel substances, have been isolated from *Imperata cylindrica*, and their structures have been elucidated on the basis of their spectral data coupled with chemical evidence and total synthesis. Cylindol A [**1**] showed 5-lipoxygenase inhibitory activity.

In the course of our investigations of pharmacologically active substances from medicinal plants, we have devoted our attention to the occurrence of natural products having 5-lipoxygenase inhibitory activity since these compounds may be useful as anti-inflammatory drugs in therapeutic applications.

The rhizomes of *Imperata cylindrica* Beauvois (Gramineae) (Japanese name "Chigaya") have been used in Chinese medicine as diuretic and anti-inflammatory agents (1–3). However, only a few studies concerning the constituents of this plant have been conducted and the presence of migrated hopane and arborane triterpenoids was reported by Nishimoto *et al.* (4–7). In this paper, we describe the isolation of the new biphenyl ether compounds, cylindols A [**1**] and B [**2**], from *I. cylindrica*, and the total synthesis and 5-lipoxygenase inhibitory activity of cylindol A [**1**].

An EtOAc-soluble extract of the rhizomes of *I. cylindrica* was repeatedly chromatographed over Si gel to afford cylindols A [**1**] and B [**2**]. Cylindol A [**1**] showed a molecular ion at *m/z* 318 in the eims. The ir spectrum of **1** displayed absorption bands at 3360 and 1695 cm^{-1}, indicating the presence of OH and ester functionalities in the molecule. The presence of a 1,2,4-tri-substituted phenyl ring was elucidated based on the ^{13}C-nmr data and the coupling pattern of aromatic protons in the ^{1}H-nmr spectrum. From an analysis of the eims and ^{13}C-nmr spectra, the structure was indicated as a symmetrical biphenyl ether. Cylindol B [**2**] was considered to be an isomer of cylindol A [**1**] from its ir and nmr spectra. In the ms spectrum, however, a molecular ion typical of a dimeric structure was not detected but only a monomer ion was observed at *m/z* 168. When cylindol B [**1**] was acetylated by treatment with Ac_2O and pyridine, acetylcylindol B [**3**] showed a molecular ion at *m/z* 402. The chemical shifts and splitting patterns in



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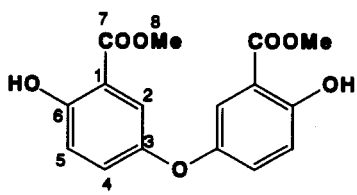

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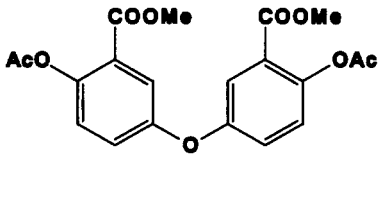

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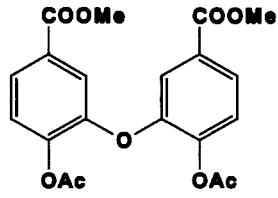

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the ^{1}H-nmr spectrum of acetylcylindol B [**3**] pointed to the presence of a 1,2,4-tri-substituted phenyl ring. Therefore, cylindol B [**2**] was considered to be a symmetrical 1,2,4-tri-substituted biphenyl ether compound like cylindol A [**1**]. Cylindol A [**1**] was also acetylated, and acetylcylindol A [**4**] showed a molecular ion at *m/z* 402. In these two acetates, the acetylation shifts (ca. 0.35 ppm at H-5 in cylindol A, 0.28 ppm in cylindol B) indicated two possible relative locations of the functionalities in each molecule. The tentative structures **1** and **2** were proposed for cylindol A and B. However, it was not unambiguously determined which structure corresponded to which compound. Therefore, the structure of **1** was confirmed by total synthesis. As shown in Figure 1, compounds **5** and **6** were coupled to give a biphenyl ether, and then oxidation, demethylation, and esterification reactions were performed to yield the target compound. The spectral data of the synthetic compound were completely identical with those of cylindol A [**1**]. In this manner, the structures of cylindol A and B were determined as **1** and **2**, respectively.

5-Lipoxygenase was inhibited by cylindol A [**1**] (64% at 30 μM; known standard, phenidone, 50% at 15 μM), but not by cylindol B [**2**]. It is possible, therefore, that cylindol A [**1**] is one of the anti-inflammatory principles of *I. cylindrica*. Further detailed studies on the biological activity are now underway.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The

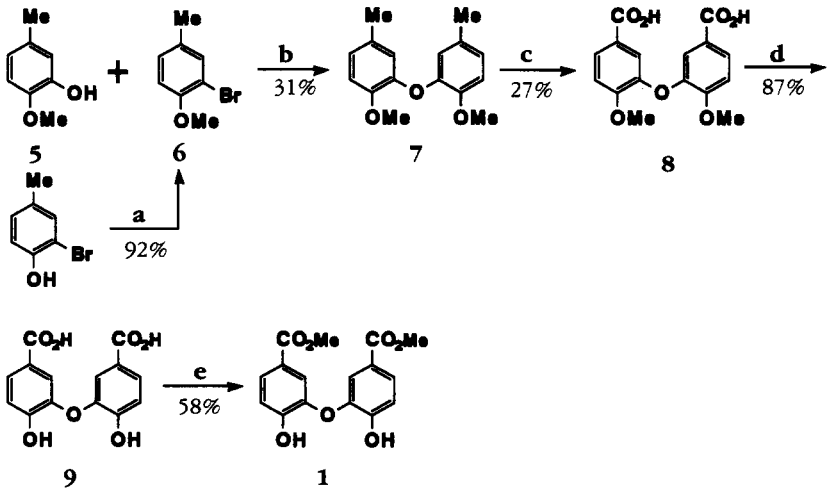

FIGURE 1. Total Synthesis of Cylindol A [**1**]. **a**) Me_2SO_4/NaOH/EtOH/110°. **b**) K_2CO_3/KI/Cu/DMSO/160°. **c**) $KMnO_4$/KOH/H_2O/reflux. **d**) BBr_3/CH_2Cl_2/0°~room temperature. **e**) concentrated H_2SO_4/MeOH/reflux.

ir spectra were taken on a Shimadzu IR-408 spectrometer. Mps were determined on a Yamaco micro-melting point apparatus and are uncorrected. Uv spectra were taken on a Hitachi U-2000 spectrometer. Eims were obtained on a JEOL JMS DX-303 or a JEOL JMS D-100 mass spectrometer. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL JNM GX-500, Hitachi R-3000, and JEOL JNM EX-270 nmr spectrometers using TMS as an internal standard. ^{13}C-Nmr multiplicities were determined by off-resonance decoupling experiments. Synthetic starting materials were supplied by Tokyo Kasei Co., Ltd.

PLANT MATERIAL.—The rhizomes of *I. cylindrica* were supplied by Nippon Hunmatsu Yakuhin, Ltd., Osaka, Japan. A voucher specimen has been deposited in the herbarium of the Pharmaceutical Institute of Tohoku University.

EXTRACTION AND ISOLATION.—The rhizomes of *I. cylindrica* (2 kg) were cut and extracted with 5 liters of boiling MeOH and 5 liters of boiling H_2O. The residues from the MeOH and H_2O extracts (280 g and 70 g) were combined, then partitioned between EtOAc and H_2O. The EtOAc extract was concentrated *in vacuo*.

The EtOAc extract (35 g) was directly chromatographed on Si gel. After elution of higher fatty acids and their esters with $CHCl_3$-MeOH (100:1), a pale yellow oil (110 mg) was eluted by a solution of $CHCl_3$-MeOH (20:1), which was repeatedly chromatographed by Si gel prep. tlc using solutions of $CHCl_3$-MeOH (15:1) and EtOAc-MeOH (60:1) as eluates, to afford cylindols A [**1**] (7 mg) and B [**2**] (5 mg), in 0.00035% and 0.00025% yield, respectively.

Cylindol A [**1**] was isolated as a colorless amorphous powder; mp 217–219°; ir (film) ν max 3360, 1695 cm^{-1}; uv (MeOH) λ max (log ϵ) 220 (4.55), 257 (4.39), 288 (4.02) nm; ^{1}H nmr (CD_3OD, 500 MHz) δ 3.90 (6H, s, 2×CH_3-8), 7.09 (2H, d, J=7.9 Hz, 2×H-5), 7.52 (2H, d, J=1.8 Hz, 2×H-2), 7.80 (2H, dd, J=7.9 and 1.8 Hz, 2×H-6); ^{13}C nmr (CD_3OD, 125 MHz) δ 52.5 (q, C-8), 118 (d, C-5), 121.5 (d, C-2), 123 (s, C-1), 128 (d, C-6), 145.5 (s, C-3), 154.8 (s, C-4), 168 (s, C-7), eims *m/z* [M]$^+$ 318 (60), 286 (100), 269 (45), 255 (37), 128 (30); hreims: found 318.0742 (calcd for $C_{16}H_{14}O_7$ 318.0740).

Cylindol B [**2**] was isolated as a colorless amorphous powder; mp 187–190°; ir (film) ν max 3540, 1695 cm^{-1}; uv (MeOH) λ max (log ϵ) 220 (4.55), 257 (4.39), 288 (4.02) nm; ^{1}H nmr (CD_3OD, 300 MHz) δ 3.98 (6H, s, 2×CH_3-8), 6.93 (2H, d, J=9.6 Hz, 2×H-5), 7.64 (4H, complex, 2×H-2, 2×H-4); ^{13}C nmr (CD_3OD, 75 MHz) δ 56.4 (q, C-8), 114 (d, C-5), 116 (d, C-2), 123 (s, C-1), 125 (d, C-4), 149 (s, C-3), 153 (s, C-6), 170 (s, C-7).

Acetylation of cylindol A.—To a solution of 1 mg of cylindol A [**1**] in 1 ml of pyridine, 0.5 ml of Ac_2O was added at room temperature. After 3 h, the reaction mixture was azeotroped with toluene to give acetylcylindol A [**4**]; ir (film) ν max 1770, 1725 cm^{-1}; eims *m/z* [M]$^+$ 402 (45), 318 (30), 168 (100); ^{1}H nmr (CD_3OD, 500 MHz) δ 2.32 (6H, s, 2×OAc), 3.96 (6H, s, 2×CH_3-8), 7.44 (2H, d, J=7.9 Hz, 2×H-5), 7.65 (2H, d, J=1.8 Hz, 2×H-2), 7.97 (2H, dd, J=7.9 and 1.8 Hz, 2×H-6).

Acetylation of cylindol B.—To a solution of 1 mg of cylindol B [**2**] in 0.5 ml of pyridine, 0.5 ml of Ac_2O was added at room temperature. After stirring 3 h, the reaction mixture was azeotroped with toluene and purified by Si gel tlc using $CHCl_3$-MeOH (30:1) as eluate: ir (film) ν max 1765, 1685 cm^{-1}; eims *m/z* [M]$^+$ 402 (10), 318 (30), 168 (100), 153 (35); ^{1}H nmr (CD_3OD, 300 MHz) δ 2.36 (6H, s 2×OAc), 3.95 (6H, s, 2×CH_3-8), 7.21 (2H, d, J=8.1 Hz, 2×H-5), 7.72 (2H, dd, J=8.1 and 2.3 Hz, 2×H-4), 7.78 (2H, d, J=2.3 Hz, 2×H-2).

*Methylation of 2-bromo-*p*-cresol.*—A solution of 25.8 g (138 mmol) of 2-bromo-*p*-cresol in 50 ml of EtOH and 21.1 g (167 mmol) of Me_2SO_4 was treated with aqueous NaOH (8.6 g in 15 ml of H_2O) at 0°. The reaction mixture was heated to 110°. After 4 h, the reaction mixture was concentrated *in vacuo* and partitioned between Et_2O and 5% HCl. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo* to give 25.4 g (92%) of **6**; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 1.56 (3H, s), 3.85 (3H, s), 6.78 (1H, d, J=8.3 Hz), 7.05 (1H, dd, J=8.3 and 2.0 Hz), 7.35 (1H, d, J=2.0 Hz); ^{13}C nmr ($CDCl_3$, 67.8 MHz) δ 20.1, 56.3, 111.3, 111.8, 128.9, 131.4, 133.7, 153.7.

Coupling reaction of 2-methoxy-5-methyl phenol [**5**] *and* **6**.—To a solution of 5 g (36.2 mmol) of 2-methoxy-5-methyl phenol [**5**] and 8.73 g (43.4 mmol) of **6** in 10 ml of DMSO were added 7.5 g (54.3 mmol) of K_2CO_3, 1.2 g (7.2 mmol) of KI, and 280 mg of Cu powder. The reaction mixture was heated to 150°. After 3 h, the reaction mixture was diluted with Et_2O, washed with H_2O, 5% HCl, and brine. The organic layer was dried over $MgSO_4$, concentrated *in vacuo* and chromatographed on Si gel (C_6H_{14}-EtOAc, 10:1) to give 2.9 g (31%) of **7**; ^{1}H nmr ($CDCl_3$, 270 MHz) δ 2.49 (6H, s), 3.82 (6H, s), 6.63 (2H, br s), 6.85 (4H, br s); ^{13}C nmr ($CDCl_3$, 67.8 MHz) δ 20.7, 56.2, 112.6, 119.6, 123.9, 130.6, 145.8, 148.4.

Oxidation of **7**.—To a suspension of 2.2 g (8.55 mmol) of **7** in 200 ml of H_2O were added 13.5 g (85.5 mmol) of $KMnO_4$ and 7.18 g (128 mmol) of KOH. The reaction mixture was refluxed for 10 h, filtered, cooled to 0°, and acidified with concentrated HCl to give 745 mg (27%) of **8**; ^{1}H nmr (DMSO-d_6, 270 MHz) δ 3.96 (6H, s), 7.35 (2H, d, J=8.6 Hz), 7.36 (2H, d, J=2.0 Hz), 7.86 (2H, dd, J=8.6 and 2.0 Hz), 12.9 (2H, br s); ^{13}C

nmr (DMSO-d_6, 67.8 MHz) δ 55.9, 112.7, 118.7, 123.0, 126.4, 144.4, 153.8, 166.3.

Demethylation of **8**.—To a stirred suspension of 400 mg (1.26 mmol) of **8** in 3 ml of CH_2Cl_2 was added 0.6 ml (6.3 mmol) of BBr_3 at 0°. After 3 h, the reaction mixture was poured into ice-cooled H_2O and the precipitate was filtered to give 316 mg (86%) of **9**; ^{1}H nmr (DMSO-d_6, 270 MHz) δ 7.11 (2H, d, J=8.3 Hz), 7.35 (2H, d, J=2.0 Hz), 7.70 (2H, dd, J=8.3 and 2.0 Hz), 10.5 (2H, br s).

Esterification of **9**.—To a solution of 300 mg (1.03 mmol) of **9** in 30 ml of anhydrous MeOH was added 0.1 ml of concentrated H_2SO_4. The reaction mixture was refluxed for 5 h, concentrated *in vacuo*, and partitioned between Et_2O and H_2O. An organic layer was washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. Purification by Si gel cc ($CHCl_3$-MeOH, 50:1) afforded 192 mg (58%) of cylindol A [**1**] as an amorphous powder; mmp with the natural compound, 217–219°.

5-Lipoxygenase inhibitory activity of cylindol A [**1**].—The 5-lipoxygenase assay was run using a crude enzyme preparation from rat basophilic leukemia cells (RBL-1). Cylindol A was pre-incubated with the enzyme for 5 min at room temperature and the reaction was initiated by addition of arachidonic acid. Following 8 min incubation at room temperature, the reaction was terminated by addition of 2 ml of 1 N NaOH, and absorbance read at 234 nm to determine levels of 5-HETE (8,9).

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