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Antimicrobial Activity of Stereoisomers of Morinols A and B, Tetrahydropyran Sesquineolignans

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The antimicrobial activity of all stereoisomers of morinols A and B was tested. All stereoisomers of morinols A and B showed antifungal activity against *Alternaria alternata*, especially (−)-morinol B which showed the strongest activity. The natural component, (+)-morinol A, and unnatural stereoisomer, (7*S*,7′*S*,8*R*,8′*R*)-morinol B, showed antibacterial activity against the gram-positive bacteria, *Bacillus subtilis* and *Listeria denitrificans*.

Key words: neolignan; tetrahydropyran neolignan; antimicrobial activity

Morinols A and B have been isolated from the Chinese medicinal herb, *Morina chinensis*,[1,2] as enantiomeric mixtures. The stereochemistry of morinols A and B was determined by an enantioselective synthetic study.[3] The biosynthesis of lignans and neolignans as enantiomeric mixtures has recently been reported.[1,2,4] This possibility means that an experiment on biological activity using isolated lignans and neolignans is not sufficient to clarify the biological activity of one stereoisomer, and also the plants do not biosynthesis the all stereoisomers. To examine the relationship between the stereochemistry of lignans and neolignans and their biological activity, all optically pure stereoisomers should be synthesized. Lignans and neolignans are large groups of natural products, because many types of bonding of the C6-C3 units have been found and each type has many kinds of oxidation. Although the many biological activities were have been reported for the lignans and neolignans,[5–8] the relationship between their stereochemistry and biological activity is not clear. Our efforts are continuing to collate a library for the structure and stereochemistry of lignans and neolignans.

The stereospecific antifungal and antibacterial activities of tetra-substituted tetrahydrofuran lignan[9,10] and 7,7′-oxo-matairesinol have already been reported.[11] Morinols A and B have a unique tetrahydropyran structure. The inhibition of cytokines has been reported by using enantiomeric mixtures,[2] although there is no report on the biological activity of optically pure morinols A and B. In our presented studies, the antimicrobial activity was tested by employing all the synthesized stereoisomers of morinols A and B (Table 1). This article compares the activity of all stereoisomers of the unique tetrahydropyran type of neolignan and shows the important stereochemistry for this activity.

Materials and Methods

Melting point (mp) data are uncorrected. NMR data were measured by a JNM-EX400 spectrometer, using TMS as a standard (0 ppm), MS data were measured with a JMS-MS700V spectrometer, and optical rotation values were evaluated with a Horiba SEPA-200 instrument. Elemental analysis was carried out with Yanako CHN MT-5 coder. The silica gel used was Wakogel C-300 (Wako, 200–300 mesh).

The NMR, MS data, and specific rotation values of **A-1**, **A-2**, **A-7**, **B-1**, **B-2**, and **B-5** have been presented in the previous work.[3]

*(2S,3R,5S)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**2**).* After a reaction solution of silyl ether **1**[3] (0.15 g, 0.26 mmol) and *n*-Bu_4NF (0.28 ml, 1 M in THF, 0.28 mmol) in THF (10 ml) was stirred at room temperature for 30 min, sat. aq. NH_4Cl solution and EtOAc were added. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 2/1) gave alcohol **2** (86 mg, 0.20 mmol, 77%) as a colorless oil, $[\alpha]^{20}_D$ +55 (*c* 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.54 (1H, ddd, J = 14.2, 8.3, 8.3 Hz, 4-*H*H), 1.64 (1H, ddd, J = 14.2, 5.4, 5.4 Hz, 4-H*H*), 1.86 (1H, m, CH_2=CHC*H*H), 1.96 (1H, m, CH_2=CHCH*H*), 2.02 (1H, m, CH), 2.10 (1H, m, CH), 2.22 (1H, br. s, OH), 3.86–3.88 (1H, overlapped, 6-*H*H), 3.86 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.88 (6H, s, OCH_3), 4.05 (1H, dd, J = 12.0, 6.6 Hz, 6-H*H*), 4.61 (1H, d, J = 8.8 Hz, ArC*H*OH), 4.66 (1H, d, J = 4.9 Hz, 2-H), 4.74 (1H, d, J = 17.1 Hz, *H*HC=C), 4.79 (1H, d, J = 10.3 Hz, H*H*C=C), 5.45 (1H, m, C*H*=CH_2), 6.81–6.85 (4H, m, ArH), 6.87–6.92 (2H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 27.9, 35.6, 38.7, 41.5, 55.75, 55.80, 65.4, 75.8, 79.2, 109.6, 110.5, 110.8, 111.6, 115.6, 119.2, 120.0, 132.4, 135.5, 137.0, 148.0, 148.56, 148.59, 149.0. Anal. Found: C, 69.83; H, 7.54. Calcd. for $C_{25}H_{32}O_6$: C, 70.12; H 7.53%. An enantiomer of **2**: $[\alpha]^{20}_D$ −55 (*c* 0.8, $CHCl_3$).

*(2S,3R,5S)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(triisopropylsilyloxy)methyl]tetrahydropyran (**3**).* Compound **3** was obtained from (*R*)-4-benzyl-3-{(*R*)-2-[(*R*)-(3,4-dimethoxyphenyl)(hydroxy)methyl]-5-hexenoyl}-2-oxazolidinone as a colorless oil by the same synthetic method as that described in the literature,[3] $[\alpha]^{20}_D$ +32 (*c* 1.2, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.94–1.02 (21H, m, *iso*-Pr), 1.55 (1H, m, 4-*H*H), 1.84 (1H, ddd, J = 12.8, 7.9, 7.9 Hz, 4-H*H*), 1.90–1.99 (2H, m), 2.13–2.25 (2H, m), 3.49 (1H, dd, J = 11.1, 11.1 Hz, 6-*H*H), 3.55 (1H, dd, J = 11.1, 4.6 Hz, 6-H*H*), 3.73 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.62 (1H, d, J = 5.8 Hz, 2-H), 4.67 (1H, d, J = 6.1 Hz, ArCHOSi), 4.85 (1H, dd, J = 17.1, 1.9 Hz, C*H*H=CH), 4.92 (1H, dd, J = 17.1,

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1.9 Hz, CH*H*=CH), 5.67 (1H, m, CH_2=C*H*), 6.73–6.80 (4H, m, ArH), 6.83–6.87 (2H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 12.5, 16.8, 18.0, 18.1, 27.9, 33.6, 37.5, 39.0, 44.7, 55.7, 55.8, 62.5, 77.1, 78.1, 110.0, 110.2, 110.5, 113.2, 116.0, 119.2, 122.0, 131.6, 135.5, 136.6, 148.2, 148.3, 148.5, 148.6. Anal. Found: C, 69.59; H, 8.95. Calcd. for $C_{34}H_{52}O_6Si$: C, 69.82; H, 8.96%. An enantiomer of **3**: $[\alpha]^{20}_D$ −32 (*c* 2.3, $CHCl_3$).

*(2S,3R,5S)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**4**).* Benzyl alcohol **4** was obtained from **3** by the same method as that described for **2** in 70% yield as a colorless oil, $[\alpha]^{20}_D$ −40 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.90–2.06 (3H, m), 2.06–2.20 (3H, m), 3.49 (1H, dd, J = 11.9, 4.1 Hz, 6-*H*H), 3.63 (1H, dd, J = 11.9, 5.0 Hz, 6-H*H*), 3.88 (9H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.66 (1H, d, J = 3.2 Hz, 2-H), 4.71 (1H, d, J = 8.7 Hz, ArC*H*OH), 4.93–4.97 (2H, m, CH_2=CH), 5.71 (1H, m, CH_2=C*H*), 6.82–6.85 (2H, m, ArH), 6.89–6.93 (4H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 26.5, 35.1, 38.6, 41.9, 55.8, 55.85, 55.88, 66.8, 75.5, 80.2, 109.3, 110.6, 110.8, 110.9, 115.9, 119.2, 119.4, 132.8, 135.9, 138.0, 148.60, 148.63, 149.1. Anal. Found: C, 69.80; H, 7.60. Calcd. for $C_{25}H_{32}O_6$: C, 70.12; H 7.53%. An enantiomer of **4**: $[\alpha]^{20}_D$ +40 (*c* 0.4, $CHCl_3$).

*(2R,3S,5S)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**6**).* Benzyl alcohol **6** was obtained from silyl ether **5**[3] by the same method as that described for **2** in 73% yield as a colorless oil, $[\alpha]^{20}_D$ +37 (*c* 1.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.33 (1H, ddd, J = 12.8, 12.8, 4.2 Hz, 4-*H*H), 1.47 (1H, m, 4-H*H*), 1.75 (1H, m, CH_2=CHC*H*H), 1.80–1.89 (1H, m, CH_2=CHCH*H*), 2.05 (1H, m, 3-H), 2.22 (1H, m, 5-H), 3.48 (1H, dd, J = 11.2, 11.2, 6-*H*H), 3.85 (3H, s, OCH_3), 3.87 (3H, s, OCH_3), 3.876 (3H, s, OCH_3), 3.883 (3H, s, OCH_3), 4.25 (1H, d, J = 8.5 Hz, ArC*H*OH), 4.50 (1H, d, J = 2.2 Hz, 2-H), 4.56 (1H, m, 6-H*H*), 4.77 (1H, d, J = 9.9 Hz, C*H*H=CH), 4.83 (1H, dd, J = 17.0 Hz, CH*H*=CH), 5.35 (1H, m, CH_2=C*H*), 6.76–6.86 (6H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 29.85, 29.92, 36.6, 38.5, 55.80, 55.82, 55.84, 55.9, 72.3, 81.0, 108.8, 109.2, 110.7, 110.8, 115.6, 117.3, 119.1, 134.1, 135.3, 137.3, 147.6, 148.6, 148.7, 149.1. Anal. Found: C, 69.81; H, 7.55. Calcd. for $C_{25}H_{32}O_6$: C, 70.12; H 7.53%. An enantiomer of **6**: $[\alpha]^{20}_D$ −37 (*c* 0.7, $CHCl_3$).

*(2S,3R,5R)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(triisopropylsilyloxy)methyl]tetrahydropyran (**7**).* Compound **7** was obtained as a colorless oil from (*S*)-4-benzyl-3-{(*S*)-2-[(*S*)-(3,4-dimethoxyphenyl)(hydroxy)methyl]-5-hexenoyl}-2-oxazolidinone by the same synthetic method as that described in the literatures,[3] $[\alpha]^{20}_D$ −62 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 0.97–1.03 (21H, m, *iso*-Pr), 1.45 (1H, ddd, J = 12.6, 12.6, 4.2 Hz, 4-*H*H), 1.78 (1H, m, 4-H*H*), 1.92 (1H, m, 3-H), 2.05 (1H, m, CH_2=CHC*H*H), 2.15 (1H, m, CH_2=CHCH*H*), 2.20 (1H, m, 5-H), 3.28 (1H, dd, J = 11.2, 11.2 Hz, 6-*H*H), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (6H, s, OCH_3), 4.01 (1H, br. d, J = 11.2 Hz, 6-H*H*), 4.39 (1H, d, J = 0.6 Hz, 2-H), 4.51 (1H, d, J = 5.7 Hz, ArCHOSi), 4.88–4.92 (2H, m, CH_2=CH), 5.53 (1H, m, CH_2=C*H*), 6.72–6.74 (2H, m, ArH), 6.78–6.82 (3H, m, ArH), 6.86 (1H, d, J = 1.8 Hz, ArH); ^{13}C NMR ($CDCl_3$) δ 12.4, 18.0, 18.1, 29.8, 30.0, 38.4, 38.8, 55.75, 55.80, 55.83, 70.9, 81.2, 108.7, 109.7, 110.1, 110.8, 115.7, 117.2, 118.9, 134.2, 135.3, 137.5, 147.5, 148.1, 148.5, 148.6. Anal. Found: C, 69.63; H, 8.92. Calcd. for $C_{34}H_{52}O_6Si$: C, 69.82; H, 8.96%. An enantiomer of **7**: $[\alpha]^{20}_D$ +61 (*c* 0.5, $CHCl_3$).

*(2S,3R,5R)-3-Allyl-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**8**).* The compound **8** was obtained from **7** by the same method as that described for **2** in 78% yield as a colorless oil, $[\alpha]^{20}_D$ −67 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.58 (1H, ddd, J = 12.6, 12.6, 4.4 Hz, 4-*H*H), 1.64 (1H, s, OH), 1.81 (1H, m, 4-H*H*), 1.96 (1H, m, 3-H), 2.07 (1H, m, CH_2=CHC*H*H), 2.18 (1H, m, CH_2=CHCH*H*), 2.25 (1H, m, 5-H), 3.33 (1H, dd, J = 11.3, 11.3 Hz, 6-*H*H), 3.82–3.90 (1H, overlapped, 6-H*H*), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.36 (1H, d, J = 7.0 Hz, ArC*H*OH), 4.50 (1H, d, J = 2.2 Hz, 2-H), 4.88–4.92 (2H, m, CH_2=CH), 5.54 (1H, m, CH_2=C*H*), 6.76 (1H, d, J = 8.4 Hz, ArH), 6.81–6.87 (5H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 29.5, 30.0, 37.0, 38.6, 55.79, 55.84, 55.90, 55.91, 71.2, 76.1, 76.7, 100.5, 108.7, 109.1, 110.8, 115.7, 117.3, 118.5, 134.0, 135.1, 137.5, 147.6, 148.6, 149.1. Anal. Found: C, 69.79; H, 7.57. Calcd. for $C_{25}H_{32}O_6$: C, 70.12; H 7.53%. An enantiomer of **8**: $[\alpha]^{20}_D$ +66 (*c* 0.4, $CHCl_3$).

*(2S,3R,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**A-3**).* Method A: A reaction mixture of alkene **2** (56 mg, 0.13 mmol), 1-bromo-3,4-dimethoxybenzene (56 mg, 0.26 mmol), Et_3N (56 µl, 0.40 mmol), and $PdCl_2(PPh_3)_2$ (9 mg, 0.013 mmol) in DMF (0.5 ml) was heated for 5 h at 90 °C under N_2 gas. After additions of H_2O and EtOAc, the organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 1/1) gave **A-3** (11 mg, 0.019 mmol, 15%) as a colorless oil. Method B: A reaction mixture of alkene **2** (40 mg, 0.093 mmol), 3,4-dimethoxyphenylboronic acid (21 mg, 0.12 mmol), $Cu(OAc)_2$ (35 mg, 0.19 mmol), LiOAc (19 mg, 0.29 mmol), and $Pd(OAc)_2$ (21 mg, 0.094 mmol) in DMF (0.5 ml) was heated at 100 °C for 2 h under N_2 gas. After additions of EtOAc and H_2O, the mixture was filtered. The organic solution was separated, washed with brine, and dried (Na_2SO_4). Concentration followed by silica gel column chromatography (EtOAc/hexane = 3/1) gave **A-3** (22 mg, 0.039 mmol, 42%) as a colorless oil, $[\alpha]^{20}_D$ +84 (*c* 0.55, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.55–1.70 (2H, m), 1.78 (1H, m), 1.90–2.17 (3H, m), 3.83–3.86 (1H, overlapped, *H*H-6 (*H*H-9′)), 3.83 (6H, s, OCH_3), 3.858 (6H, s, OCH_3), 3.864 (6H, s, OCH_3), 4.22 (1H, dd, J = 11.7, 5.4 Hz, H*H*-6 (H*H*-9′)), 4.68 (1H, d, J = 2.0 Hz, H-2 (H-7)), 4.74 (1H, d, J = 8.8 Hz, ArC*H*OH, H-7′), 5.59 (1H, m, ArCH=C*H* (H-8″)), 5.94 (1H, d, J = 16.1, ArC*H*=CH (H-7″)), 6.64–6.65 (2H, m, ArH), 6.73 (1H, d, J = 8.8 Hz, ArH), 6.78–6.93 (6H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 28.3, 34.3, 39.5, 41.1, 55.7, 55.8, 55.9, 66.3, 75.6, 79.9, 108.4, 109.8, 110.7, 111.0, 111.1, 111.2, 118.7, 119.6, 127.2, 130.4, 130.7, 132.9, 135.5, 148.1, 148.3, 148.8, 148.9, 149.2; EIMS m/z 564 (M^+, 84), 151 (100); HREIMS m/z 564.2723 (calcd for $C_{33}H_{40}O_8$, 564.2723).

*(2R,3S,5R)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**A-4**).* **A-4** was obtained from an enantiomer of **2**. The NMR data agreed with those for **A-3**. $[\alpha]^{20}_D$ −84 (*c* 0.34, $CHCl_3$).

*(2R,3R,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**A-5**).* **A-5** was synthesized by the same method as that described for **A-7** in the literature,[3] $[\alpha]^{20}_D$ +28 (*c* 0.22, $CHCl_3$). The NMR data agreed with those for **A-7**.[3] An enantiomer of **A-5** (**A-7**), $[\alpha]^{20}_D$ −28 (*c* 0.3, $CHCl_3$) in the literature.[3]

*(2S,3R,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**A-6**).* **A-6** was obtained from alkene **4** by employing method B described for the synthesis of **A-3** in 37% yield as a colorless oil, $[\alpha]^{20}_D$ −41 (*c* 0.24, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.95–2.09 (3H, m), 2.11–2.30 (4H, m), 3.51 (1H, dd, J = 11.8, 4.0 Hz, *H*H-6 (*H*H-9′)), 3.66 (1H, dd, J = 11.8, 4.7 Hz, H*H*-6 (H*H*-9′)), 3.85 (3H, s, OCH_3), 3.867 (3H, s, OCH_3), 3.873 (3H, s, OCH_3), 3.878 (3H, s, OCH_3), 3.884 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.70 (1H, d, J = 2.2 Hz, H-2 (H-7)), 4.77 (1H, d, J = 8.7 Hz, ArC*H*OH (H-7′)), 5.92 (1H, m, ArCH=C*H* (H-8″)), 6.18 (1H, d, J = 15.7 Hz, ArC*H*=CH (H-7″)), 6.75–6.96 (9H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 27.1, 34.3, 39.4, 41.8, 55.86, 55.90, 67.1, 75.6, 80.4, 108.5, 109.3, 110.7, 110.8, 110.9, 111.1, 118.8, 119.3, 119.4, 127.8, 130.7, 133.0, 136.0, 148.1, 148.7, 149.0, 149.2; EIMS m/z 564 (M^+, 30), 546 (84), 151 (100); HREIMS m/z 564.2723 (calcd. for $C_{33}H_{40}O_8$, 564.2723).

*(2R,3S,5R)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**A-8**).* **A-8** was obtained from an enantiomer of **4**. The NMR data agreed with those for **A-6**. $[\alpha]^{20}_D$ +41 (*c* 0.15, $CHCl_3$).

*(2R,3S,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**B-3**).* **B-3** was obtained from alkene **6** in 35% yield as a colorless oil by employing method B described for the synthesis of **A-3**, $[\alpha]^{20}_D$ −46

(c 0.13, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.38 (1H, ddd, J = 12.8, 12.8, 4.3 Hz, *H*H-4 (*H*H-9)), 1.55 (1H, m, H*H*-4 (H*H*-9)), 1.76 (1H, br. s, OH), 1.88–1.98 (2H, m, ArCH=CHC*H*$_2$, H$_2$-9″), 2.17 (1H, m, H-3 (H-8)), 2.29 (1H, m, H-5 (H-8′)), 3.52 (1H, dd, J = 11.1, 11.1 Hz, *H*H-6 (*H*H-9′)), 3.76 (3H, s, OCH_3), 3.84 (3H, s, OCH_3), 3.85 (6H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.90 (3H, s, OCH_3), 4.27 (1H, d, J = 8.6 Hz, ArC*H*OH (H-7′)), 4.55 (1H, s, H-2 (H-7)), 4.61 (1H, dd, J = 11.1, 2.9 Hz, H-6 (H*H*-9′)), 5.59 (1H, m, ArCH=C*H* (H-8″)), 6.10 (1H, d, J = 15.8 Hz, ArC*H*=CH (H-7″)), 6.61–6.67 (3H, m, ArH), 6.75–6.89 (6H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 29.2, 30.5, 37.0, 39.3, 55.7, 55.8, 55.9, 72.3, 77.2, 81.0, 108.4, 108.9, 109.6, 110.9, 111.1, 117.4, 118.7, 118.8, 127.4, 130.7, 130.8, 134.2, 135.2, 147.7, 148.2, 148.7, 148.8, 148.9, 149.0; FABMS m/z 565 [$(M + H)^+$, 1], 154 (100); HRFABMS m/z 565.2798 (calcd. for $C_{33}H_{41}O_8$, 565.2802).

*(2S,3R,5R)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**B-4**).* **B-4** was obtained from an enantiomer of **6**. The NMR data agreed with those for **B-3**. $[\alpha]^{20}_D$ +46 (c 0.22, $CHCl_3$).

*(2S,3R,5R)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(S)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**B-6**).* **B-6** was obtained from alkene **8** by employing method A (8% yield) and method B (52% yield) described for the synthesis of **A-3** as colorless crystals, mp 84–85 °C, $[\alpha]^{20}_D$ −60 (c 1.7, $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.64 (1H, ddd, J = 12.8, 12.8, 4.2 Hz, *H*H-4 (*H*H-9)), 1.80 (1H, s, OH), 1.90–2.08 (2H, m), 2.10–2.31 (3H, m), 3.38 (1H, dd, J = 11.2, 11.2 Hz, *H*H-6 (*H*H-9′)), 3.83–3.88 (1H, overlapped, H*H*-6 (H*H*-9′)), 3.83 (3H, s, OCH_3), 3.84 (6H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.86 (3H, s, OCH_3), 3.88 (3H, s, OCH_3), 4.39 (1H, d, J = 7.5 Hz, ArC*H*OH (H-7′)), 4.52 (1H, s, H-2 (H-7)), 5.73 (1H, m, ArCH=C*H* (H-8″)), 6.11 (1H, d, J = 15.7 Hz, ArC*H*=CH (H-7″)), 6.59–6.86 (9H, m, ArH); ^{13}C NMR ($CDCl_3$) δ 29.2, 29.5, 37.1, 39.4, 55.6, 55.7, 55.8, 71.2, 75.6, 81.1, 108.3, 108.7, 109.0, 110.7, 110.8, 111.0, 117.2, 118.3, 118.6, 127.5, 130.3, 130.8, 134.0, 135.1, 147.5, 148.0, 148.4, 148.6, 148.8, 148.9; EIMS m/z 564 (M^+, 100), 510 (88), 151 (75); HREIMS m/z 564.2723 (calcd. for $C_{33}H_{40}O_8$, 564.2723).

*(2S,3S,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**B-7**).* **B-7** was obtained by the same method as that described for **B-5**,[3] $[\alpha]^{20}_D$ −34 (c 0.88, $CHCl_3$). The NMR data agreed with those for **B-5**.[3] An enantiomer of **B-7** (**B-5**), $[\alpha]^{20}_D$ +34 (c 0.4, $CHCl_3$) in reference.[3]

*(2R,3S,5S)-3-(3,4-Dimethoxycinnamyl)-2-(3,4-dimethoxyphenyl)-5-[(R)-(3,4-dimethoxyphenyl)(hydroxy)methyl]tetrahydropyran (**B-8**).* **B-8** was obtained from an enantiomer of **8**, mp 84–85 °C, $[\alpha]^{20}_D$ +60 (c 1.1, $CHCl_3$). The NMR data agreed with those for **B-6**.

Organisms. Bacillus subtilis subsp. *subtiis* NBRC 13719^T, *Pseudomonas fluorescens* NBRC 14160^T, and *Staphylococcus aureus* subsp. *aureus* NBRC 14462 were purchased from the National Institute of Technology and Evaluation (NITE), Biological Resource Center, Japan. *Escherichia coli* JCM 1649, *Listeria denitrificans* JCM 11481, *Salmonella choleraesuis* subsp. *choleraesuis* JCM 6977 and *Yersinia intermedia* JCM 7579 were obtained from RIKEN, Japan. The phytopathogenic fungi, *Colletotrichum lagenarium*, *Bipolaris oryzae*, *Fusarium solani*, and *Alternaria alternata*, had been isolated from a farm at Ehime University and were kindly presented by Dr. Ohguchi. Each fungus was cultured on potato dextrose agar (PDA, Sigma-Aldrich, Canada).

Antibiotic spectra of morinols A and B and stereoisomers. The ability of each compound to inhibit the growth of a variety of Gram-positive and Gram-negative bacterial strains was assessed by the paper disc method (Advantec Toyo, Japan; 6-mm thin paper disc), and the minimum inhibitory concentration (MIC) was determined for those strains that showed sensitivity to the tested compounds. The paper disc test used agar plates containing Nissui nutrient broth (Nissui Pharmaceutical Co.). A hundred microliter of an exponential culture of each respective strain was made into molten agar containing the nutrient broth. After the agar had solidified, a paper disc containing 15 µl of 50 mM of the tested compound was put on to the agar plate. The plate was incubated for 24 h at 30 °C (*L. denitrificans* and *P. fluorescens*) or at 37 °C (*B. subtilis*, *S. aureus*, *E. coli*, *S. choleraesuis* and *Y. intermedia*), and the diameter of any halo of inhibition around the paper disc was measured. The MIC value was determined from a two-fold dilution series for any strain that showed a halo of inhibition.

Antifungal assay. The paper disc method was adopted for the first screening. Briefly, fungal mycelia were spotted on the center of the PDA plate (φ100-mm dish) and incubated at 28 °C until the colony diameter had became 4–5 cm. A paper disc soaked in dimethyl sulfoxide containing a test chemical was then placed at the edge of the colony. Growth inhibition was observed after culturing at 28 °C for 7–10 d. A further inhibition test was performed with the active compounds. Each compound was added to three aliquots each containing 3 ml of PDA at 50 °C, mixed rapidly and poured on to the PDA plates (φ50-mm dish). Dimethyl sulfoxide only served as the control. After incubating at 28 °C for 7–10 d, the area of the mycelial colony was measured by a caliper, the assays being triplicated.

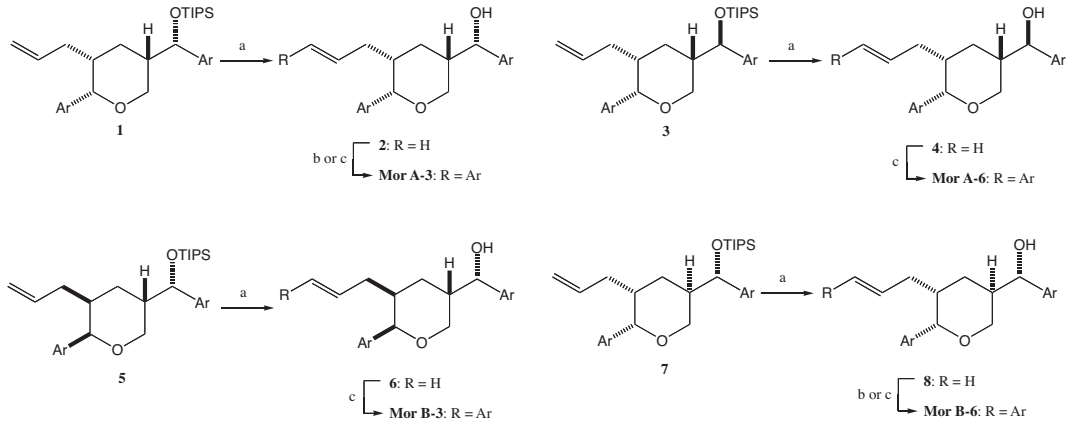
Results and Discussion

Preparation of the stereoisomers of morinols A and B

The syntheses of **A-1**, **A-2**, **A-7**, **B-1**, **B-2**, and **B-5** have already been reported.[3] **A-5** and **B-7** were synthesized by employing the synthetic method reported for **A-7** and **B-5**. In this project, two Heck-Mizorogi reaction conditions were tested to give the other stereoisomers. After desilylation of compounds **1**, **3**, **5**, and **7**, the resulting olefin was subjected to the Heck-Mizorogi reaction. Although the treatment of **2** and **8** with 1-bromo-3,4-dimethoxybenzene, triethylamine, and $PdCl_2(PPh_3)_2$ in DMF at 90 °C gave **A-3** and **B-6** in low yields (15% and 8%), respectively, the yield was improved by reaction with 3,4-dimethoxyphenylboronic acid, $Cu(OAc)_2$, LiOAc, and $Pd(OAc)_2$ in DMF[12] at 100 °C (**A-3**, 42% yield; **B-6**, 52% yield). **A-6** and **B-3** were obtained from **4** and **6** by employing 3,4-dimethoxyphenylboronic acid in 37% and 35% yields, respectively (Scheme 1). **A-4**, **A-8**, **B-4**, and **B-8** were respectively obtained from the enantiomers of **1**, **3**, **5**, and **7** by the same method. The syntheses of all 16 stereoisomers were achieved.

Antifungal activity of the stereoisomers of morinols A and B

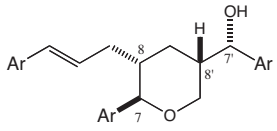
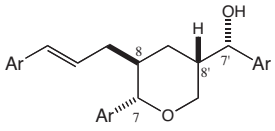
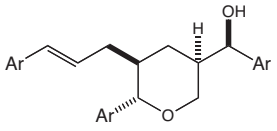
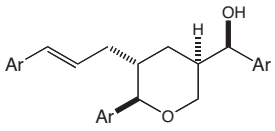
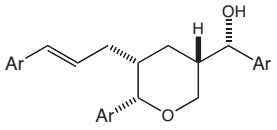
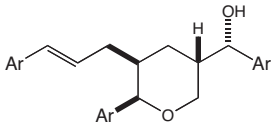
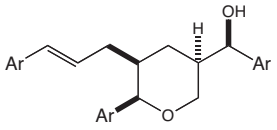
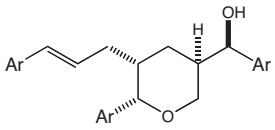
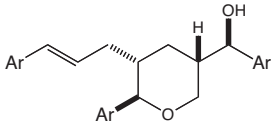
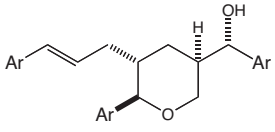
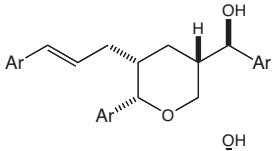
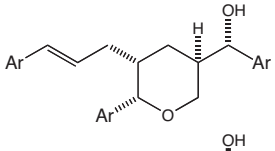
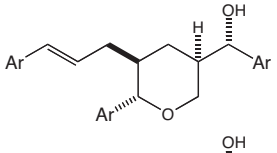
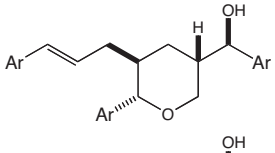
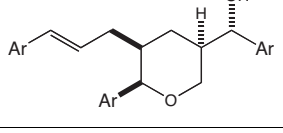
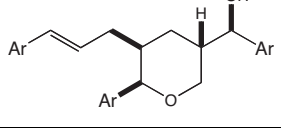
Since (−)-virgatusin, which is a tetra-substituted tetrahydrofuran lignan, and 3,4-dibenzoyltetrahydrofuran have respectively shown antifungal activity against *Colletotrichum lagenarium* and *Bipolaris oryzae*,[9,11] these fungal strains were included for this test. All stereoisomers showed antifungal activity against only *Alternaria alternata* at 0.5 mM. In particular, (−)-morinol B (**B-2**), which is one of the natural stereoisomers bearing a (7*R*, 8*R*, 7′*R*, 8′*R*) structure, showed the strongest activity (Table 1). The dependence on stereochemistry was weak, however, and specificity against fungal species was apparent in this test. This is the first report on the antifungal activity of morinols A and B. In our previous study,[9] the antifungal activity of tetra-substituted tetrahydrofuran lignans depended on the stereochemistry; in contrast, all stereoisomers of the tetrahydropyran sesquineolignan morinols showed activity. It is noteworthy that one of the natural stereoisomers had the strongest activity and the optimum stereochemistry for antifungal activity against *A. alternata*.



Scheme 1. Preparation of Morinols.

a) *n*-Bu_4NF, THF, r.t., 30 min (**2**, 77% yield; **4**, 70% yield; **6**, 73% yield; **8**, 78% yield); b) 1-Br-3,4-$(CH_3O)_2C_6H_3$, Et_3N, $PdCl_2(PPh_3)_2$, DMF, 90 °C, 5 h (**A-3**, 15% yield; **B-6**, 8% yield); c) 3,4-$(CH_3O)_2PhB(OH)_2$, $Cu(OAc)_2$, LiOAc, $Pd(OAc)_2$, DMF, 100 °C, 2 h (**A-3**, 42% yield; **A-6**, 37% yield; **B-3**, 35% yield; **B-6**, 52% yield). Ar, 3,4-dimethoxyphenyl

Table 1. Growth Rate (% ± σ, n = 3) of *Alternaria alternata* at 0.5 mM of the Morinol A and B Stereoisomers

Compound		Growth %	Compound		Growth %
No.	Structure		No.	Structure	
A-1	 (-)-morinol A	65.5 ± 3.29	**B-1**	 (-)-morinol B	56.6 ± 0.94
A-2	 (+)-morinol A	60.0 ± 0.90	**B-2**	 (+)-morinol B	43.0 ± 0.41
A-3		55.5 ± 2.77	**B-3**		71.5 ± 0.69
A-4		62.5 ± 4.43	**B-4**		66.5 ± 4.05
A-5		84.1 ± 6.67	**B-5**		72.5 ± 2.10
A-6		61.0 ± 2.96	**B-6**		54.3 ± 2.15
A-7		75.2 ± 1.29	**B-7**		66.1± 6.55
A-8		59.4 ± 1.97	**B-8**		78.3 ± 1.08

Ar, 3,4-dimethoxyphenyl; growth (%), (colony diameter of sample/colony diameter of control) × 100

Table 2. Antibacterial Activity of the Morinols (MIC, mM)

Species	**A-2**	**B-6**
Bacillus subtilis NBRC 13719	50	25
Staphylococcus aureus NBRC 14462	>50	>50
Listeria denitrificans JCM 11481	25	50

Antibacterial activity of the stereoisomers of morinols A and B

In the test of the antibacterial activity of natural component (−)-morinol A (**A-1**), (+)-morinol A (**A-2**), (+)-morinol B (**B-1**) and (−)-morinol B (**B-2**), no activity was shown against gram-negative bacteria, and only (+)-morinol A (**A-2**), which is a (7*S*,7′*R*,8*S*,8′*R*)-isomer, showed activity against the gram-positive bacteria, *B. subtilis* (MIC, 50 mM) and *L. denitrificans* (MIC, 25 mM). The growth of *S. aureus* was not inhibited by (+)-morinol A (**A-2**). To determine the important stereochemistry of morinols A and B for antibacterial activity, the activity of all other stereoisomers was tested by using synthesized compounds **A-3**–**A-8** and **B-3**–**B-8** (Table 2). The stereoisomers of morinol A, which is 8-8′ *cis* form, did not show activity, however, one of the stereoisomers of morinol B type bearing the 8-8′ *trans* form showed activity. Thus, the (7*S*,7′*S*,8*R*,8′*R*)-isomer (**B-6**) showed activity against *B. subtilis* (MIC, 25 mM) and *L. denitrificans* (MIC, 50 mM). The growth of *S. aureus* and gram-negative bacteria was not inhibited by **B-6**. Both **A-2** and **B-6** have the (7*S*,8′*R*) structure. Although **A-7** and **B-4** also have this (7*S*,8′*R*) structure, no antibacterial activity was observed. This fact means that the antibacterial activity of morinols A and B depends on the whole stereochemistry of these compounds. The antibacterial activity of the tetrahydropyran sesquineolignans, morinols A and B and their stereoisomers, was clarified for the first time. In our previous study, the tetra-substituted tetrahydrofuran lignan,[10] di-substituted tetrahydrofuran lignan, and di-substituted γ-butyrolactone lignan[11] showed antibacterial activity against gram-positive bacteria, but not against gram-negative bacteria. In this study of the tetrahydropyran sesquineolignan, the same result was apparent, showing activity against gram-positive bacteria. The bonding of the C6-C3 unit would be effective against gram-positive bacteria, but not against gram-negative bacteria.

The microbiological activity of tetrahydropyran sesquineolignan was shown for the first time. The stereochemistry was not particularly significant for antifungal activity, although the antibacterial activity was influenced by the stereochemistry.

Acknowledgments

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References

1) Su, B.-N., and Takaishi, Y., Morinol A and B, two tetrahydropyran sesquineolognans with a new carbon skeleton from *Morina chinensis*. *Chem. Lett.*, 1315–1316 (1999).
2) Su, B.-N., Takaishi, Y., and Kusumi, T., Morinols A-L, twelve novel sesquineolignans and neolignans with a new carbon skeleton from *Morina chinensis*. *Tetrahedron*, **55**, 14571–14586 (1999).
3) Yamauchi, S., Sugahara, T., Akiyama, K., Maruyama, M., and Kishida, T., Determination of the stereochemistry of the tetrahydropyran sesquineolignans morinol A and B. *J. Nat. Prod.*, **70**, 549–556 (2007).
4) Umezawa, T., Biosynthesis of lignans and related phenylpropanoid compounds. *Regul. Plant Growth Develop.*, **35**, 57–67 (2001).
5) MacRae, W. D., and Towers, G. H. N., Biological activities of lignans. *Phytochemistry*, **23**, 1207–1220 (1984).
6) Ayres, D. C., and Loike, J. D., "Lignans," Cambridge University Press, New York (1990).
7) Ward, S., Lignans, neolignans, and related compounds. *Nat. Prod. Rep.*, **16**, 75–96 (1999).
8) Saleem, M., Kim, H. J., Ali, M. S., and Lee, Y. S., An update on bioactive plant lignans. *Nat. Prod. Rep.*, **22**, 696–716 (2005).
9) Akiyama, K., Yamauchi, S., Nakato, T., Maruyama, M., Sugahara, T., and Kishida, T., Antifungal activity of tetra-substituted tetrahydrofuran lignan, (−)-virgatusin, and its structure-activity relationship. *Biosci. Biotechnol. Biochem.*, **71**, 1028–1035 (2007).
10) Maruyama, M., Yamauchi, S., Akiyama, K., Sugahara, T., Kishida, T., and Koba, Y., Antibacterial activity of a virgatusin-related compound. *Biosci. Biotechnol. Biochem.*, **71**, 677–680 (2007).
11) Akiyama, K., Maruyama, M., Yamauchi, S., Nakashima, Y., Nakato, T., Tago, R., Sugahara, T., Kishida, T., and Koba, Y., Antimicrobiological activity of lignan: effect of benzylic oxygen and stereochemistry of 2,3-dibenzyl-4-butanolide and 3,4-dibenzyltetrahydrofuran lignans on activity. *Biosci. Biotechnol. Biochem.*, **71**, 1745–1751 (2007).
12) Kishida, M., and Akita, H., Simple preparation of phenylpropanoid β-D-glucopyranoside congeners by Mizoroki-Heck type reaction using organoboron reagents. *Tetrahedron*, **61**, 10559–10568 (2005).