

Anti-inflammatory Neolignans from *Piper kadsura*

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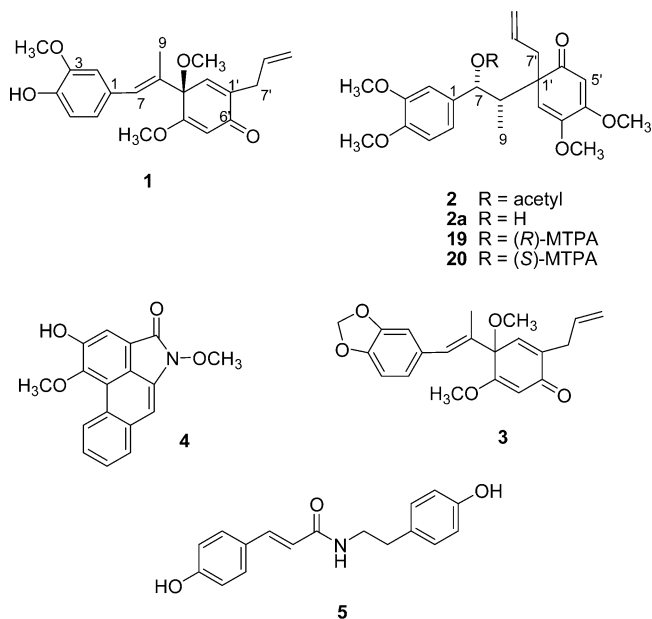
Received December 29, 2005

Two new neolignans, piperkadsin A (**1**) and piperkadsin B (**2**), as well as 11 known neolignans, three known alkaloids, the highly oxygenated compound (+)-crotopoxide, and stigmasterol were isolated from the stems of *Piper kadsura*. The anti-inflammatory activities of these compounds were evaluated. Compounds **1**, **2**, futoquinol (**3**), piperlactam **4**, and *N*-*p*-coumaroyl tyramine (**5**) showed potent inhibition of PMA-induced ROS production in human polymorphonuclear neutrophils with IC₅₀ values 4.3 ± 1.0, 12.2 ± 3.2, 13.1 ± 5.3, 7.0 ± 1.9, and 8.4 ± 1.3 μM, respectively.

Piper kadsura Ohwi (*P. futokadsura*) is a medicinal plant that grows in the forests of Taiwan.¹ The stems of *P. kadsura*, known as haifengteng, are widely used in the Chinese herbal medicinal prescriptions for the treatment of asthma and arthritic conditions. A variety of compounds have been found in this plant, including amides, lignans, neolignans, terpenes, and oxygenated cyclohexanes.² Some of these compounds are reported to have anti-platelet activating factor (PAF) activity. Among them, kadsurenone showed remarkable PAF antagonist characteristics.³ However, there has been relatively little information pertaining to its anti-inflammatory or immunomodulating activities. We reinvestigated the active principles of *P. kadsura* for their anti-inflammatory or immunomodulating potentials. This paper deals with the structural investigation of the new neolignans by spectroscopic means and their anti-inflammatory activities.

The stems of *P. kadsura* were extracted with MeOH and sequentially partitioned between H₂O, CHCl₃, and *n*-BuOH. With repeated column chromatography the CHCl₃ fraction gave two new neolignans, piperkadsin A (**1**) and piperkadsin B (**2**), and 16 known compounds.

Piperkadsin A (**1**) was obtained as a viscous oil. The IR (ν_{max} 1660 cm⁻¹) and UV (λ_{max} 287 nm) spectra indicated a dienone system. The molecular formula of **1** was consistent with C₂₁H₂₄O₅ from the HRFABMS spectrum, *m/z* 356.1629 [M]⁺. In the ¹H NMR spectrum of **1**, a set of three aromatic protons [δ 6.76 (d, *J* = 1.5 Hz, H-2), 6.78 (dd, *J* = 1.5, 7.5 Hz, H-6), 6.84 (d, *J* = 7.5 Hz, H-5)], an olefinic proton (δ 6.91, H-7), and an allylic methyl singlet (δ 1.65, H-9) suggested that a 3,4-dihydroxystyrene group was present. Other methylene protons at δ 3.11 (H-7') and olefinic protons at δ 5.82 (m, H-8'), 5.06 (1H, dd, *J* = 1.5, 5.5 Hz, H-9a'), and 5.08 (1H, dd, *J* = 12.0, 1.5 Hz, H-9b') were assigned to the allyl group. The singlets at δ 6.13 (s) and 5.80 (s) suggested two dienone protons at C-2' and C-5', respectively. As expected, the ¹³C NMR chemical shifts at δ 139.2 (C-1', s), 141.1 (C-2', d), 105.3 (C-5', d), 172.3 (C-4', s), and 186.9 (C-6', s) agreed with the dienone system. In addition, one aliphatic (δ 3.21) and two aromatic *O*-methyl groups (δ 3.75 and 3.81) were also observed. The aliphatic methoxy is attached to a tertiary carbon, since no other signal is found in the 3.5–5.0 ppm region except those of the



O-methyl groups. Comparison of the ¹H and ¹³C NMR spectra of **1** with those of futoquinol (**3**)^{4,5} indicated that **1** bears a 4-hydroxy-3-methoxyphenyl substituent instead of the piperonyl group in futoquinol. HMBC correlations from H-2, H-5, and -OCH₃ (δ 3.81) to C-3 and from H-2, H-5, and H-6 to C-4 also confirmed the partial structure of the 4-hydroxy-3-methoxyphenyl moiety. An NOE experiment performed on **1** showed an interaction between the methyl and the aryl groups, but no interaction between methyl and H-7, suggesting that the aryl and the methyl groups are *cis* to each other. The CD spectrum of **1** showed a strong positive couplet at 248 nm and a negative one at 319 nm, which were in agreement with those of lancifolin C,⁶ thus establishing the 3'*R* configuration of **1**. On the basis of these data we propose the structure of **1** as (3'*R*)-4-hydroxy-3,3',4'-trimethoxy-6'-oxo-Δ^{-1',4',7,8'}-8,3'-lignan.

Piperkadsin B (**2**) was also obtained as a viscous oil. IR (ν_{max} 1740, 1631 cm⁻¹) and UV (λ_{max} 235, 280 nm) spectra showed phenyl and α,β-unsaturated carbonyl absorption systems. The molecular formula of **2** was consistent with C₂₄H₃₀O₇ from the HRFABMS spectrum, *m/z* 430.1983 [M]⁺. The ¹H NMR spectrum of **2** revealed the presence of a veratryl ring system [three ABX aromatic protons at δ 6.65 (d, *J* = 1.5 Hz)/6.71 (dd, *J* = 1.5, 8.0 Hz)/6.77 (d, *J* = 8.0 Hz) and two *O*-methyl singlets at δ 3.81 and 3.84], an allyl group [methylene protons at δ 2.43 (dd, *J* = 7.0, 13.0 Hz)/2.70 (dd, *J* = 7.0, 13.0 Hz) and olefinic protons at δ 5.49

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(m)/4.92 (dd, $J = 1.5, 10.0$ Hz)/5.02 (dd, $J = 1.5, 17.0$ Hz)], and an acetoxy methyl at δ 2.10 (3H, s). Attachment of the allyl group to an sp^3 -hybridized carbon atom was indicated by the shielded chemical shift of the allylic-CH₂ protons.⁷ In addition, the ¹H NMR spectrum of **2** exhibited two doublets at δ 0.78 (3H, $J = 6.5$ Hz) and 6.10 (1H) and a doublet-quartet at δ 2.27 (1H, $J = 1.5, 6.5$ Hz), representing the typical AMX₃-type signal of a CH₃-CH-CH-(O)- unit. The presence of the veratryl ring system, allyl group, and CH₃-CH-CH-(O)- unit was further confirmed by the ¹H-¹H COSY spectrum. Along with these moieties, the ¹H NMR spectrum of **2** also revealed the presence of two methoxy groups at δ 3.70 and 3.77, indicating their attachment to sp^2 -hybridized carbon atoms, and two singlets at δ 5.22 and 5.44. This finding was consistent with the ¹³C NMR signals at δ 55.7 (s, C-1'), 110.5 (d, C-2'), 147.6 (s, C-3') 166.3 (s, C-4'), 102.5 (d, C-5'), 201.5 (s, C-6'), 55.1 (-OCH₃), and 56.3 (-OCH₃), corresponding to the 3,4-dimethoxy-6,6-disubstituted-cyclohexa-2,4-dienone group. These subunits were connected by HMBC data. Correlations observed in the HMBC spectrum from C-1 to H-8, H-7, H-2, and H-5; from C-1' to H-5', H-7', H-7, H-8, and H-9; from C-6' to H-2', H-5', and H-7'; and from acetyl carbonyl C- α to H-7 and H- β confirmed the proposed structure of **2**. The positions of the 3'- and 4'-OCH₃ groups were reconfirmed by NOE experiments. Irradiation of 3'-OCH₃ gave 7.9% enhancement on H-2', and irradiation of 4'-OCH₃ gave 8.8% enhancement on H-5'. The absolute configuration of C-7 in **2** was established by the modified Mosher's method.⁸ Hydrolysis of **2** with alcoholic KOH gave the alcohol **2a**, which was subsequently esterified by (S)- and (R)-MTPA chlorides to yield the (R)- and (S)-MTPA esters⁹ **19** and **20**, respectively. The $\Delta\delta$ ($\delta_{S-MTPA\ ester} - \delta_{R-MTPA\ ester}$) values of the C(9)H₃, C(7')H₂, C(2')H, and C(5')H were positive and the $\Delta\delta$ values of C(2)H, C(5)H, and C(6)H were negative, indicating that the configuration of C-7 in **2** was *R*. In addition, the absence of an observable coupling between H-8 and H-7 in **2** and **2a** indicates a preferred dihedral angle close to 90°. Therefore, a 7*R*, 8*S* absolute configuration was tentatively assigned on stable conformation considerations. On the basis of the above data, we propose the structure of **2** as (7*R*,8*S*)-7-acetoxy-3,3',4,4'-tetramethoxy-6'-oxo- $\Delta^{2',4',8'}$ -8.1'-lignan. The configuration of C1' remains unassigned.

In addition to **1** and **2**, futoquinol (**3**),^{4,5} piperlactam **S** (**4**),¹⁰ *N*-p-coumaroyl tyramine (**5**),¹¹ kadsurin **A** (**6**),⁵ liscarin **D** (**7**),¹² stigmasterol (**8**), kadsurin **B** (**9**),¹³ kadsurenone (**10**),¹³ galgravin (**11**),¹⁴ (+)-crotopoxide (**12**),¹⁵ futoenone (**13**),¹⁶ liliflone (**14**),¹⁷ (7*R*,8*R*,3'*R*)-7-acetoxy-3',4'-dimethoxy-3,4-methylenedioxy-6'-oxo- $\Delta^{1',4',8'}$ -8.3'-lignan (**15**),¹⁸ (7*S*,8*S*,1'*R*)- $\Delta^{8'-1'}$ -methoxy-3,4-methylenedioxy-1',6'-dihydro-6'-oxo-7-*O*-4',8,3'-neolignan (**16**),^{19,20} burchellin (**17**),²¹ and aristololactam AIIIa (**18**)²² were also isolated from the stems of *P. kadsura*. The structures of known compounds were established by comparison of physical properties with reported data.

The isolated compounds were evaluated for their anti-inflammatory and antioxidative properties. Phorbol-12-myristate-13-acetate (PMA)-activated human polymorphonuclear neutrophils (PMN) were used as target cells, and reactive oxygen species (ROS) production was determined by a lucigenin-amplified chemiluminescence.^{23,24} Our results showed that PMA (a PKC-dependent activator) could induce greatly elevated ROS production up to 10–20-fold higher than that of resting cells. Pretreatment with 1–50 μ M of isolated compounds showed that **1**–**5** potentially diminished PMA-induced ROS production in a concentration-dependent manner. The IC₅₀ values of compounds **1**–**5** were 4.3 $\pm$ 1.0, 12.2 $\pm$ 3.2, 13.1 $\pm$ 5.3, 7.0 $\pm$ 1.9, and 8.4 $\pm$ 1.3 μ M, respectively.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Nicolet Avatar 320 IR spectrometer. UV spectra were measured on a Hitachi U-3200 spectrophotometer in MeOH. Optical rotations were recorded on a Jasco-DIP-370 polarimeter. Circular dichroism spectra (CD) were recorded on Jasco J-715 spectrometer. ¹H, ¹³C, and 2D NMR

spectra were measured with a Varian Inova-500 spectrometer with deuterated solvents as internal standard. APCI-MS and HRFABMS were recorded on Finnigan LCQ and Finnigan/Thermo Quest MAT spectrometers, respectively. Column chromatography was performed on Sephadex LH-20 (Pharmacia) or silica gel 60 (70–230 or 230–400 mesh, Merck; or 12–26 μ m, Eurochrom, Knauer) or Cosmosil 140 C₁₈ OPN (Nacalai). Silica gel 60F₂₅₄ (Merck) was used for TLC (0.25 mm).

Plant Material. The stems of *Piper kadsura* (Choisy) Ohwi were collected in December 2003, in Taipei, Taiwan. Identification of plant materials was confirmed by comparing with a voucher specimen (No. 197740) that had been deposited in the Herbarium of the Department of Botany of National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The stems of *P. kadsura* (8.5 kg) were crushed and extracted with MeOH (60 L $\times$ 3) under reflux. The MeOH extract were evaporated to dryness and partitioned successively between H₂O and CHCl₃, followed by *n*-BuOH (each 1.5 L $\times$ 3). The CHCl₃ fraction (350 g) was subjected to column chromatography on silica gel (10 $\times$ 120 cm), with a gradient of EtOAc in *n*-hexane, and 14 fractions (1–14) were collected. Fraction 6 (75.4 g) was rechromatographed on a silica gel column using 15% EtOAc/*n*-hexane to give two main fractions. Fraction 6-1 (35.3 g) was repeatedly purified with silica gel (12–26 μ m) column chromatography with 10% EtOAc/*n*-hexane as eluent to give **6** (5.3 g), **7** (61 mg), and **8** (503 mg). Fraction 6-2 (20.9 g) was chromatographed on a Cosmosil 140 C₁₈ OPN column (60% MeOH/H₂O) and on a silica gel column with 10% EtOAc/*n*-hexane elution repeatedly to give **3** (5.5 g), **9** (476 mg), and **10** (1.2 g). A solid precipitate was separated from fraction 7 and recrystallized from MeOH to give **11** (3.5 g). In the same manner, precipitates were separated from fractions 9 and 14 and recrystallized from EtOAc/*n*-hexane and MeOH to give **12** (450 mg) and **13** (14.4 g), respectively. The filtrate of fraction 9 (13.5 g) was chromatographed on a Sephadex-LH-20 (EtOAc) and on a silica gel (12–26 μ m) column with 8% EtOAc/benzene elution to give **1** (191 mg), **4** (30 mg), **12** (3.5 g), and **14** (893 mg). Fraction 11 (11.3 g) gave **2** (470 mg), **15** (215 mg), **17** (124 mg), and **18** (406 mg) after repeated silica gel (25% EtOAc/*n*-hexane) and Sephadex LH-20 (EtOAc) column chromatography. Fraction 12 was chromatographed on Sephadex-LH-20 (acetone) to give **5** (32 mg) and **19** (25 mg).

Piperkadsin A (1): oil; $[\alpha]_D^{25} -130.3$ (c 0.43, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 238 (4.22), 287 (4.13) nm; IR (neat) ν_{max} 3423, 1660, 1605, 1513 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 1.65 (3H, s, H-9), 3.11 (2H, m, H-7'), 3.21 (3H, s, 3'-OCH₃), 3.75 (3H, s, 4'-OCH₃), 3.81 (3H, s, 3-OCH₃), 5.06 (1H, dd, $J = 1.5, 5.5$ Hz, H-9a'), 5.08 (1H, dd, $J = 12.0, 1.5$ Hz, H-9b'), 5.80 (1H, s, H-5'), 5.82 (1H, m, H-8'), 6.13 (1H, s, H-2'), 6.76 (1H, d, $J = 1.5$ Hz, H-2), 6.78 (1H, dd, $J = 1.5, 7.5$ Hz, H-6), 6.84 (1H, d, $J = 7.5$ Hz, H-5), 6.91 (1H, s, H-7); ¹³C NMR (CDCl₃, 125 MHz) δ 13.8 (C-9), 32.5 (C-7'), 52.2 (3'-OCH₃), 55.5 (3-OCH₃), 55.9 (4'-OCH₃), 79.6 (C-3'), 105.3 (C-5'), 111.8 (C-2), 114.0 (C-5), 116.8 (C-9'), 122.0 (C-6), 127.0 (C-7), 129.3 (C-1), 131.8 (C-8), 134.8 (C-8'), 139.2 (C-1'), 141.1 (C-2'), 144.4 (C-4), 146.0 (C-3), 172.3 (C-4'), 186.9 (C-6'); APCIMS m/z 357 [M + H]⁺; HRFABMS m/z 356.1629 [M]⁺ (calcd for C₂₁H₂₄O₅, 356.1624); CD (4.8 mg/100 mL MeOH, 216–400 nm) $[\theta]_{216}^{max} +14\ 826$, $[\theta]_{219}^0$, $[\theta]_{222}^{min} -11\ 829$, $[\theta]_{231}^0$, $[\theta]_{248}^{max} +3201$, $[\theta]_{271}^0$, $[\theta]_{274}^0$, $[\theta]_{319}^{min} -1683$, $[\theta]_{351}^0$, $[\theta]_{400}^0$.

Piperkadsin B (2): oil; $[\alpha]_D^{25} 34.7$ (c 0.98, CHCl₃); UV (MeOH) λ_{max} (log ϵ) 235 (4.22), 280 (3.75), 318 (3.57) nm; IR (neat) ν_{max} 3448, 1740, 1631, 1582, 1517 cm⁻¹; ¹H NMR (CDCl₃, 500 MHz) δ 0.78 (3H, d, $J = 6.5$ Hz, H-9), 2.10 (3H, s, -COCH₃), 2.27 (1H, dq, $J = 1.5, 7.0$ Hz, H-8), 2.43 (1H, dd, $J = 7.0, 13.0$ Hz, H-7'a), 2.70 (1H, dd, $J = 7.0, 13.0$ Hz, H-7'b), 3.70 (3H, s, -OCH₃), 3.77 (3H, s, -OCH₃), 3.81 (3H, s, -OCH₃), 3.85 (3H, s, -OCH₃), 4.92 (1H, dd, $J = 1.5, 10.0$ Hz, H-9'a), 5.02 (1H, dd, $J = 1.5, 17.0$ Hz, H-9'b), 5.22 (1H, s, H-2'), 5.44 (1H, s, H-5'), 5.49 (1H, m, H-8'), 6.10 (1H, s, H-7), 6.65 (1H, d, $J = 1.5$ Hz, H-2), 6.71 (1H, dd, $J = 1.5, 8.0$ Hz, H-6), 6.77 (1H, d, $J = 8.0$ Hz, H-5); ¹³C NMR (CDCl₃, 125 MHz) δ 8.6 (C-9), 21.1 (-COCH₃), 43.1 (C-7'), 48.4 (C-8), 55.1 (3'-OCH₃), 55.8/55.9 (3, 4-OCH₃), 56.3 (4'-OCH₃), 55.7 (C-1'), 74.0 (C-7), 102.5 (C-5'), 108.9 (C-2), 110.5 (C-2'), 110.9 (C-5), 117.6 (C-6), 118.2 (C-9'), 132.5 (C-8'), 132.8 (C-1), 147.6 (C-3'), 148.2 (C-4), 148.7 (C-3), 166.3 (C-4'), 169.6 (-COCH₃), 201.5 (C-6'); APCIMS m/z 371 [M - CH₃-COO]⁺; HRFABMS m/z 430.1983 ([M]⁺, calcd for C₂₄H₃₀O₇, 430.1992); CD (3.2 mg/100 mL MeOH, 209–400 nm) $[\theta]_{209}^{max}$

+15529, $[\theta]_{211}^0$, $[\theta]_{213}^{\min}$ -2582, $[\theta]_{218}^0$, $[\theta]_{223}^{\max}$ +2345, $[\theta]_{228}^0$, $[\theta]_{233}^{\min}$ -1710, $[\theta]_{236}^0$, $[\theta]_{242}^0$ +793, $[\theta]_{248}^0$ +427, $[\theta]_{281}^{\max}$ +7590, $[\theta]_{299}^0$, $[\theta]_{333}^{\min}$ -5492, $[\theta]_{376}^0$, $[\theta]_{400}^0$.

Hydrolysis of Piperkadsin B (2). To a solution of **2** (45 mg) in EtOH (5 mL) was added 4 N aqueous KOH (2 mL), and the solution was stirred at room temperature for 40 min. Then the solution was diluted with H₂O and extracted with Et₂O. The Et₂O layer was washed with H₂O, dried with Na₂SO₄, and evaporated in vacuo. The residue was purified by preparative TLC (benzene/EtOAc/acetone, 4:2:0.5) to yield **2a**: oil; $[\alpha]_{24}^{25}$ 67.4 (c 0.42, CHCl₃); UV (MeOH) λ max (log ϵ) 234 (4.32), 276 (3.93), 316 (3.52) nm; ¹H NMR (CDCl₃, 500 MHz) δ 0.67 (3H, d, J = 7.0 Hz, H-9), 2.25 (1H, q, J = 6.5 Hz, H-8), 2.62 (1H, dd, J = 7.0, 12.5 Hz, H-7'a), 2.78 (1H, dd, J = 7.0, 12.5 Hz, H-7'b), 3.76 (3H, s, -OCH₃), 3.81 (3H, s, -OCH₃), 3.87 (3H, s, -OCH₃), 3.89 (3H, s, -OCH₃), 4.97 (1H, d, J = 10.0 Hz, H-9'a), 5.06 (1H, d, J = 17.0 Hz, H-9'b), 5.22 (1H, s, H-7), 5.51 (1H, s, H-5'), 5.58 (1H, m, H-8'), 5.80 (1H, s, H-2'), 6.84 (3H, s, H-2, 5, 6); ¹³C NMR (CDCl₃, 125 MHz) δ 7.7 (C-9), 43.0 (C-7'), 48.9 (C-8), 55.4 (-OCH₃), 55.9 (-OCH₃ $\times$ 2), 56.4(-OCH₃), 56.6 (C-1'), 72.5 (C-7), 102.5 (C-5'), 108.9 (C-2), 110.9 (C-5), 113.0 (C-2'), 117.5 (C-6), 117.9 (C-9'), 133.0 (C-8'), 136.8 (C-1), 146.9 (C-3'), 147.9/148.8 (C-3, 4), 166.7 (C-4'), 203.1 (C-6'); APCIMS m/z 371 [M - H₂O + H]⁺.

Reaction of Alcohol 2a with (R)-MTPA.^{8,9} (R)-MTPA (25 mg) and thionyl chloride (1 mL) were refluxed for 3 h. After excess thionyl chloride was removed by vacuum evaporation, a solution of **2a** (9 mg) in 4 mL of dry CH₂Cl₂, (dimethylamino)pyridine (12.3 mg), and triethylamine (0.1 mL) were added and the solution was stirred for 16 h at room temperature. The reaction mixture was concentrated and purified by preparative TLC (benzene/EtOAc, 4:2) to yield the (R)-MTPA ester **19**: ¹H NMR (CDCl₃, 500 MHz) δ 0.78 (3H, d, J = 7.0 Hz, H-9), 2.30 (1H, dd, J = 2.0, 13.0 Hz, H-7'a), 2.49 (1H, dd, J = 3.0, 7.0 Hz, H-8), 2.63 (1H, dd, J = 3.0, 13.0 Hz, H-7'b), 3.54 (3H, s, MTPA-OCH₃), 3.55 (3H, s, -OCH₃), 3.77 (3H, s, -OCH₃), 3.80 (3H, s, -OCH₃), 3.86 (3H, s, -OCH₃), 4.90 (1H, d, J = 8.5 Hz, H-9'a), 4.93 (1H, d, J = 15.5 Hz, H-9'b), 5.19 (1H, s, H-2'), 5.41 (1H, m, H-8'), 5.47 (1H, s, H-5'), 6.29 (1H, d, J = 3.0 Hz, H-7), 6.81 (3H, s, H-2, 5, 6).

Reaction of Alcohol 2a with (S)-MTPA.^{8,9} (S)-MTPA (25 mg) and thionyl chloride (1 mL) were refluxed for 3 h. After excess thionyl chloride was removed by vacuum evaporation, a solution of **2a** (9 mg) in 4 mL of dry CH₂Cl₂, (dimethylamino)pyridine (12.3 mg), and triethylamine (0.1 mL) were added and the solution was stirred for 16 h at room temperature. The reaction mixture was concentrated and purified by preparative TLC (benzene/EtOAc, 4:2) to yield the (S)-MTPA ester **20**: ¹H NMR (CDCl₃, 500 MHz) δ 0.81 (3H, d, J = 7.0 Hz, H-9), 2.41 (1H, dd, J = 2.5, 13.0 Hz, H-7'a), 2.46 (1H, dd, J = 2.5, 7.0 Hz, H-8), 2.71 (1H, dd, J = 2.5, 13.0 Hz, H-7'b), 3.47 (3H, s, MTPA-OCH₃), 3.60 (3H, s, -OCH₃), 3.70 (3H, s, -OCH₃), 3.78 (3H, s, -OCH₃), 3.85 (3H, s, -OCH₃), 4.96 (1H, d, J = 10.5 Hz, H-9'a), 5.03 (1H, d, J = 17.0 Hz, H-9'b), 5.35 (1H, s, H-2'), 5.49 (1H, s, H-5'), 5.50 (1H, m, H-8'), 6.32 (1H, d, J = 2.5 Hz, H-7), 6.63 (1H, d, J = 2.0 Hz, H-2), 6.71 (1H, dd, J = 2.0, 8.0 Hz, H-6), 6.76 (1H, d, J = 8.0 Hz, H-5).

Acknowledgment. This study was supported by research grant NSC94-2113-M-077-003 from the National Science Council, Taiwan.

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NP0505521