Anti-inflammatory Neolignans from *Piper kadsura*

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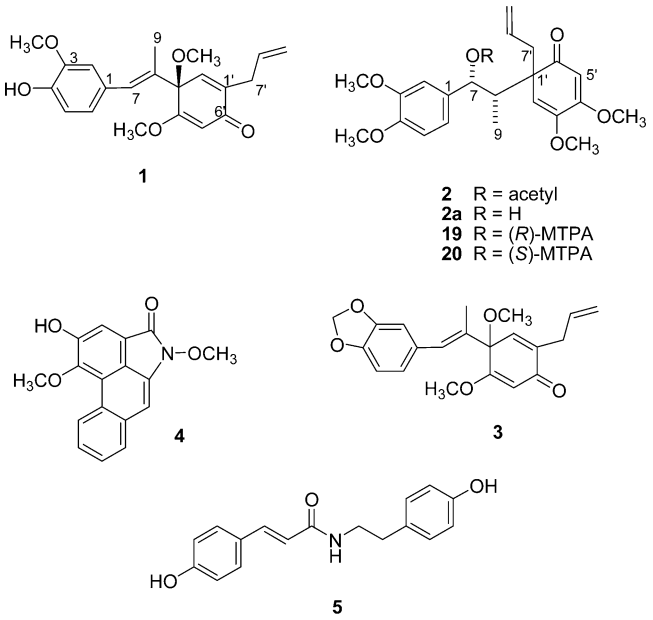
Two new neolignans, piperkadsin A (**1**) and piperkadsin B (**2**), as well as 11 known neolignans, three known alkaloids, the highly oxygenated compound (+)-crotepoxide, and stigmasterol were isolated from the stems of *Piper kadsura*. The anti-inflammatory activities of these compounds were evaluated. Compounds **1**, **2**, futoquinol (**3**), piperlactam S (**4**), and *N*-*p*-coumaroyl tyramine (**5**) showed potent inhibition of PMA-induced ROS production in human polymorphonuclear neutrophils with IC_{50} 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 H_2O, $CHCl_3$, and *n*-BuOH. With repeated column chromatography the $CHCl_3$ 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 $C_{21}H_{24}O_5$ 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 ^{13}C 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 ^{13}C 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 $-OCH_3$ (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 $C_{24}H_{30}O_7$ 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-CH_2 protons.[7] In addition, the ^{1}H 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 AMX_3-type signal of a $CH_3-CH-CH-(O)-$ unit. The presence of the veratryl ring system, allyl group, and $CH_3-CH-CH-(O)-$ unit was further confirmed by the ^{1}H−^{1}H COSY spectrum. Along with these moieties, the ^{1}H 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 ^{13}C 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($-OCH_3$), and 56.3 ($-OCH_3$), 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′-OCH_3 groups were reconfirmed by NOE experiments. Irradiation of 3′-OCH_3 gave 7.9% enhancement on H-2′, and irradiation of 4′-OCH_3 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 esters[9] **19** and **20**, respectively. The $\Delta\delta$ ($\delta_{S-MTPA\ ester} - \delta_{R-MTPA\ ester}$) values of the C(9)$H_3$, C(7′)$H_2$, 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-Δ-$^{2',4',8'}$-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] licarin D (**7**), [12] stigmasterol (**8**), kadsurin B (**9**),[13] kadsurenone (**10**),[13] galgravin (**11**),[14] (+)-crotepoxide (**12**),[15] futoenone (**13**),[16] liliflone (**14**),[17] (7*R*,8*R*,3′*R*)-7-acetoxy-3′,4′-dimethoxy-3,4-methylenedioxy-6′-oxo-Δ-$^{1',4',8'}$-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 μM of isolated compounds showed that **1**−**5** potently diminished PMA-induced ROS production in a concentration-dependent manner. The IC_{50} values of compounds **1**−**5** were 4.3 ± 1.0, 12.2 ± 3.2, 13.1 ± 5.3, 7.0 ± 1.9, and 8.4 ±1.3 μM, 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. ^{1}H, ^{13}C, 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 μm, Eurochrom, Knauer) or Cosmosil 140 C_{18} OPN (Nacalai). Silica gel 60F_{254} (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 × 3) under reflux. The MeOH extract were evaporated to dryness and partitioned successively between H_2O and $CHCl_3$, followed by *n*-BuOH (each 1.5 L × 3). The $CHCl_3$ fraction (350 g) was subjected to column chromatography on silica gel (10 × 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 μm) 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 C_{18} OPN column (60% MeOH/H_2O) 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 recrystalized from MeOH to give **11** (3.5 g). In the same manner, precipitates were separated from fractions 9 and 14 and recrystalized 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 μm) 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; $[\alpha]^{24}_D$ −130.3 (*c* 0.43, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 238 (4.22), 287 (4.13) nm; IR (neat) ν_{max} 3423, 1660, 1605, 1513 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.65 (3H, s, H-9), 3.11 (2H, m, H-7′), 3.21 (3H, s, 3′-OCH_3), 3.75 (3H, s, 4′-OCH_3), 3.81 (3H, s, 3-OCH_3), 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′), 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); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 13.8 (C-9), 32.5 (C-7′), 52.2 (3′-OCH_3), 55.5 (3-OCH_3), 55.9 (4′-OCH_3), 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 $C_{21}H_{24}O_5$, 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; $[\alpha]^{24}_D$ 34.7 (*c* 0.98, $CHCl_3$); 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}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 0.78 (3H, d, J = 6.5 Hz, H-9), 2.10 (3H, s, $-COCH_3$), 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, $-OCH_3$), 3.77 (3H,s, $-OCH_3$), 3.81 (3H,s, $-OCH_3$), 3.85 (3H,s, $-OCH_3$), 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); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 8.6 (C-9), 21.1 ($-COCH_3$), 43.1 (C-7′), 48.4 (C-8), 55.1 (3′-OCH_3), 55.8/55.9 (3, 4-OCH_3), 56.3 (4′-OCH_3), 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 ($-COCH_3$), 201.5(C-6′); APCIMS *m*/*z* 371 $[M - CH_3COO]^+$; HRFABMS *m*/*z* 430.1983 ($[M]^+$, calcd for $C_{24}H_{30}O_7$, 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, $[\theta]_{242}$ +793, $[\theta]_{248}$ +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 H_2O and extracted with Et_2O. The Et_2O layer was washed with H_2O, dried with Na_2SO_4, and evaporated in vacuo. The residue was purified by preparative TLC (benzene/EtOAc/acetone, 4:2:0.5) to yield **2a**: oil; $[\alpha]^{24}_D$ 67.4 (*c* 0.42, $CHCl_3$); UV (MeOH) λ max (log ϵ) 234 (4.32), 276 (3.93), 316 (3.52) nm; ^{1}H NMR ($CDCl_3$, 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, $-OCH_3$), 3.81 (3H,s, $-OCH_3$), 3.87 (3H,s, $-OCH_3$), 3.89 (3H,s, $-OCH_3$), 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); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 7.7 (C-9), 43.0 (C-7′), 48.9 (C-8), 55.4 ($-OCH_3$), 55.9 ($-OCH_3$ × 2), 56.4($-OCH_3$), 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 $[M - H_2O + H]^+$.

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 CH_2Cl_2, (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**: ^{1}H NMR ($CDCl_3$, 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-OCH_3), 3.55 (3H,s, $-OCH_3$), 3.77 (3H,s, $-OCH_3$), 3.80 (3H,s, $-OCH_3$), 3.86 (3H,s, $-OCH_3$), 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 CH_2Cl_2, (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**: ^{1}H NMR ($CDCl_3$, 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-OCH_3), 3.60 (3H,s, $-OCH_3$), 3.70 (3H, s, $-OCH_3$), 3.78 (3H,s, $-OCH_3$), 3.85 (3H, s, $-OCH_3$), 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).

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