Antioxidant Phenylpropanoid-Substituted Epicatechins from *Trichilia catigua*

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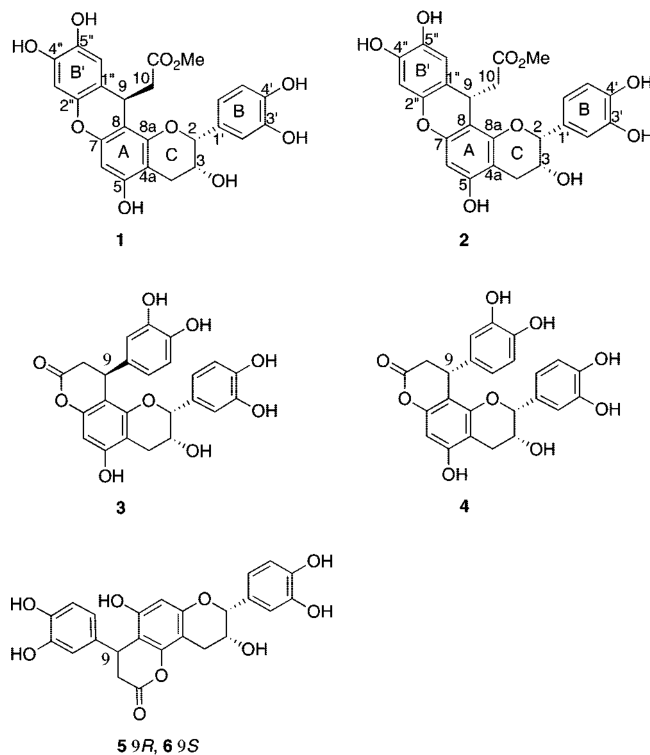
Two new phenylpropanoid-substituted epicatechins, namely, catiguanin A (**1**) and catiguanin B (**2**), were isolated from the bark of *Trichilia catigua* along with four known compounds, cinchonain Ia (**3**), cinchonain Ib (**4**), cinchonain Ic (**5**), and cinchonain Id (**6**). The structures of **1** and **2** were elucidated by analysis of spectroscopic data and by comparison of their NMR data with those of previously reported cinchonains. The isolated compounds exhibited potent antioxidant activity in the α,α-diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging test, with IC_{50} values in the 2.3–9.4 μM range.

Trichilia catigua J. (Meliaceae), a medium-sized flowering tree 3–5 m in height, is widely distributed in South American countries such as Brazil, Bolivia, Paraguay, and Argentina. It is known as "Catuaba" in Brazil and has been used in folk medicine for the treatment of fatigue, stress, and deficiency of memory.[1,2] In a related biological study, the MeOH extract of *T. catigua* bark was found to produce antidepressant-like effects in the forced swimming model of both mice and rats.[3] Flavalignans (phenylpropanoid-substituted epicatechins) such as cinchonains Ia and Ib,[4] sesquiterpenes,[5] some *γ*-lactones, and sterols[6] have been isolated from this plant. As part of our investigation of biologically active compounds in Brazilian medicinal plants,[7,8] we separated the constituents of the MeOH extract of the bark of *T. catigua*, which exhibited strong antioxidant activity (48% inhibition at 10 μg/mL) in the α,α-diphenyl-*β*-picrylhydrazyl (DPPH) radical scavenging test. Purification by HPLC resulted in the isolation of phenylpropanoid-substituted epicatechins, namely, catiguanins A (**1**) and B (**2**) along with the four known compounds cinchonain Ia (**3**), cinchonain Ib (**4**), cinchonain Ic (**5**), and cinchonain Id (**6**).[9–11] In this paper, we report the structure elucidation of **1** and **2** and the antioxidant activities of these compounds.

Catiguanin A (**1**) was isolated as a colorless, amorphous solid and has the molecular formula $C_{25}H_{22}O_{10}$, as deduced from the HRFABMS, which showed a quasimolecular ion peak at *m/z* 505 $[M + Na]^+$, indicating 15 degrees of unsaturation. The IR spectrum of **1** indicated the presence of hydroxy (3265 cm^{-1}), carbonyl (1708 cm^{-1}), and aromatic (1620 and 1452 cm^{-1}) groups. The NMR data (Table 1) showed the presence of a methyl ester group on the basis of the signals at δ 174.3 and 3.40 (3H, s). This group exhibited an HMBC correlation to the methylene of a $CH_2(10)CH(9)$ fragment derived from the COSY analysis, implying that the ester carbonyl group is bonded to C-10. The presence of a flavan-3-ol ring was deduced from the ^{13}C NMR resonances at δ 79.8 (C-2), 67.2 (C-3), and 29.6 (C-4) bearing protons at δ 4.92 (brs, H-2), 4.25 (brdd, $J = 4.6$, 1.9 Hz, H-3), and 2.80 (dd, $J = 17.0$, 1.9 Hz, H-4)/2.94 (dd, $J = 17.0$, 4.6 Hz, H-4), respectively, on the basis of HMQC. A C-2/C-3 *cis* relationship in the C ring was supported by negligible coupling between H-2 and H-3, because in the case of a C-2/C-3 *trans* relationship, a large coupling ($J = 8.0$ Hz) should be observed.[11] The ^1H NMR spectrum contained ABX-type aromatic resonances at δ 6.79 (d, $J = 8.2$ Hz), 6.85 (dd, $J = 8.2$, 1.9 Hz), and 7.04 (d, $J = 1.9$ Hz), typical of the B ring of epicatechin. The presence of an epicatechin moiety was confirmed by the HMBC correlations (Figure 1a) of H-2 with C-1′ (δ 132.2), C-2′ (δ 115.2), and C-6′ (δ 119.1). A singlet resonating at δ 6.11 suggested the A ring of the epicatechin unit in **1** is thus monosubstituted. In addition to the 15 resonances similar to those of epicatechin, the ^{13}C NMR



spectrum (Table 1) revealed the presence of a methine (δ 31.5, d), a methylene (δ 45.3, t), and an additional 1,2,4,5-tetrasubstituted benzene ring with two hydroxy groups [δ 116.2, s; 146.6, s; 104.3, d; 146.1, s; 142.3, s; 115.0, d]. This finding was further supported by two singlets appearing at δ 6.45 and 6.66 in the ^1H NMR spectrum. In the HMBC experiment shown in Figure 1a, H-9 correlated with C-1″, C-2″, C-6″, and C-8, whereas H-6 correlated with C-4a, C-5, C-7, and C-8. These results indicated that the C-9 of the $CH(9)CH_2(10)CO_2Me$ unit was linked to both C-8 in the A ring and C-1″ in the tetrasubstituted B′ ring. Thus, the above-mentioned spectroscopic data revealed that compound **1** was a phenylpropanoid-substituted epicatechin similar to the cinchonains.[9] The two aromatic proton singlets and the index of hydrogen deficiency (15 degrees of unsaturation) were accounted for by ring formation between C-2″ of the B′ ring and the OH group at C-7 of the A ring of the epicatechin moiety through an ether linkage. Additional diagnostic evidence for the location of this pyran ring was obtained by comparison of the ^{13}C NMR resonances originating from the A ring of **1** with those of cinchonains Ia (**3**) and Ic (**5**) isolated previously from *Cinchona succirubra.*[9] In **3**, which has a C_6-C_3 unit at the C-8 position, the resonances of C-4a and C-6 and that of C-8 were observed at lower [δ 96.2

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Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR Data of **1** and **2** in CD_3OD[a]

	1		**2**	
position	δ_C	δ_H	δ_C	δ_H
2	79.8	4.92 (brs)	80.1	4.92 (brs)
3	67.2	4.25 (brdd, 4.6, 1.9)	66.9	4.25 (brdd, 4.7, 1.9)
4	29.6	2.94 (dd, 17.0, 4.6) 2.80 (dd, 17.0, 1.9)	29.5	2.91 (dd, 16.8, 4.7) 2.83 (dd, 16.8, 1.9)
5	156.6		156.5	
6	95.9	6.11 (s)	95.8	6.11 (s)
7	152.9		152.9	
8	104.5		104.5	
4a	103.2		103.0	
8a	153.6		153.4	
1′	132.2		132.1	
2′	115.2	7.04 (d, 1.9)	115.1	6.99 (d, 1.6)
3′	146.0		146.1	
4′	145.8		146.0	
5′	116.0	6.79 (d, 8.2)	116.0	6.78 (d, 8.1)
6′	119.1	6.85 (dd, 8.2, 1.9)	119.2	6.86 (dd, 8.1, 1.6)
1″	116.2		115.9	
2″	146.6		146.4	
3″	104.3	6.45 (s)	104.4	6.46 (s)
4″	146.1		145.8	
5″	142.3		142.1	
6″	115.0	6.66 (s)	115.2	6.61 (s)
9	31.5	4.58 (dd, 7.1, 5.2)	31.2	4.49 (dd, 8.0, 3.6)
10	45.3	2.50 (dd, 14.0, 7.1)	44.8	2.50 (dd, 14.6, 8.0)
		2.60 (dd, 14.0, 5.2)		2.97 (dd, 14.6, 3.6)
−CO−	174.3		174.7	
$-OCH_3$	51.8	3.40 (s)	52.0	3.60 (s)

[a] All assignments were made by extensive analyses of 1D and 2D NMR (COSY, DEPT, HMQC, and HMBC).

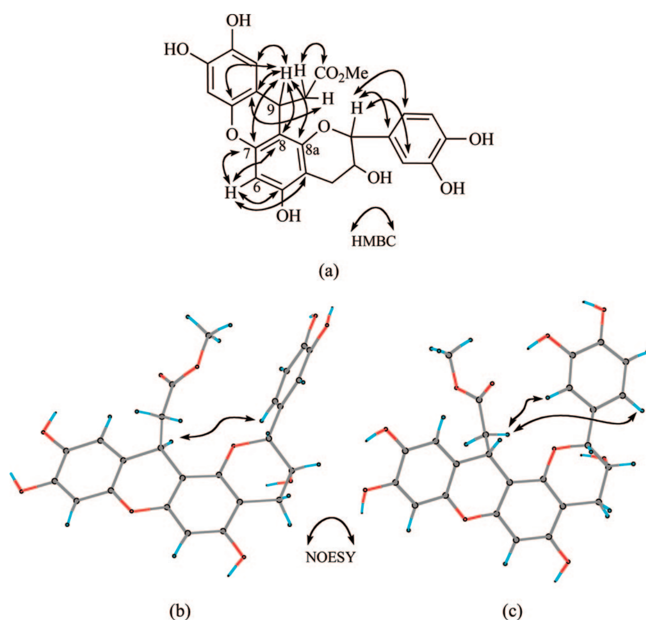

Figure 1. Representative HMBC (a) for **1** and **2** and selected NOESY for the most stable conformations of **1** (b) and **2** (c) calculated by Spartan.

(C-6) and 105.2 (C-4a)] and higher fields [δ 106.0 (C-8)] than those [δ 99.5 (C-8), 100.4 (C-4a), and 106.4 (C-6)] in **5**, which has a C_6-C_3 unit at the C-6 position. Compound **1** exhibited diagnostic resonances for the C-6, C-4a, and C-8 carbons at δ 95.9 (d), 103.2 (s), and 104.5 (s), consistent with those found in **3**. Thus, **1** was found to contain a dibenzopyran ring fused at the C-8 and C-1″ positions via C-9 and at the C-7 and C-2″ positions via an oxygen atom. The most stable structure of **1** calculated by the Spartan 06 program (MMFF) is shown in Figure 1b. This result was in accord with the NOESY correlations of **1** shown in Figure 1b, which revealed a cross-peak between H-9 and H-6′ in the B ring. This demonstrated that the CH_2CO_2Me group at C-9 is *trans*-oriented relative to the B ring. Thus, the relative configuration of catiguanin A (**1**) is 2*R**, 3*R**, 9*R**.

Catiguanin B (**2**) has the same molecular formula, $C_{25}H_{22}O_{10}$, as **1**, and its ^{1}H NMR data (Table 1) revealed the presence of the same structural units as **1**, such as a flavan-3-ol moiety with a C-2/C-3 *cis* (2*R**, 3*R**) relative configuration and a 3,4-dihydroxyphenylpropanoid unit. The ^{13}C NMR spectrum of **2** was also similar to that of **1**; in particular, it showed resonances for C-6, C-8, and C-4a at δ 95.8 (d), 104.5 (s), and 103.0 (s), respectively, thereby indicating that the phenylpropanoid unit was fused at the C-7 and C-8 positions of the A ring of epicatechin via a pyran ring.

This finding was supported by the HMBC correlations of H-9 with C-8, C-8a, C-1″, C-2″, and C-6″. Thus, **2** is thought to be a stereoisomer of **1** with respect to C-9. The most stable conformer of **2** was obtained by Spartan calculations shown in Figure 1c. In the NOESY correlations of **2** shown in Figure 1c, one of the two H-10 resonances appearing at δ 2.97 showed cross-peaks to the aromatic H-2′ and H-6′ resonances of the B ring, which matched the results of the Spartan calculation, indicating that C-9 may be assigned the *S** relative configuration. Thus, **2** is thought to be a stereoisomer of **1** with respect to C-9.

The absolute configuration at the chiral center C-9 in compounds **1** and **2** was established by comparing their circular dichroism (CD) spectra with those of cinchonains Ia (**3**) and Ib (**4**). Compounds **3** and **4** showed contrasting CD spectra, indicating that these Cotton effects ($\Delta\varepsilon$ (284) −19.7, $\Delta\varepsilon$ (257) +3.4 in **3** and $\Delta\varepsilon$ (286) +8.0, $\Delta\varepsilon$ (254) −14.1 in **4**) are mainly due to the stereogenicity (*S* for C-9 in **3**, *R* for C-9 in **4**) of C-9, which is largely unaffected by the C-2 and C-3 stereogenic centers of epicatechin.[9,11] Similarly, **1** exhibited positive and negative Cotton effects at 295 and 274 nm, respectively, whereas **2** exhibited negative and positive Cotton effects at 289 and 276 nm, respectively. The signs of the Cotton effects of **1** and **2** are in accord with those of **4** and **3**. Considering the co-occurrence of **3** and **4**, these similar Cotton effects led to the conclusion that the absolute configurations at C-9 in **1** and **2** are *R* and *S*, respectively, corresponding to those in **4** and **3**.

A plausible biosynthesis of **1** and **2** (Figure 2) from **4** and **3**, respectively, supports the absolute configuration of **1** and **2**

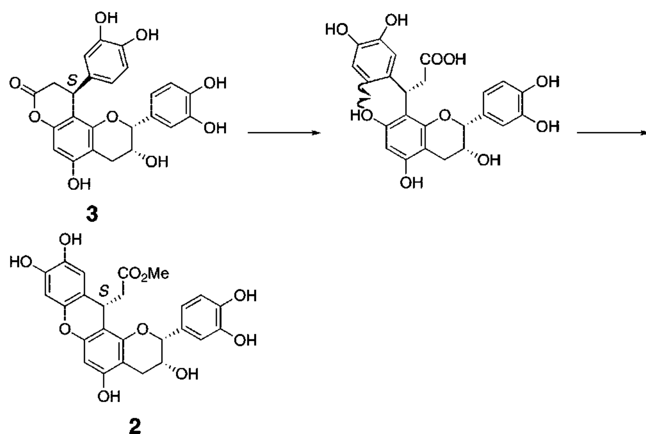

Figure 2. Plausible biosynthetic route to **2** from **3**.

Table 2. Antioxidant Activities of Compounds **1–6**

	1	**2**	**3**	**4**	**5**	**6**	catechin
IC_{50} (μM)[a]	7.1	6.7	9.4	5.1	2.5	2.3	10

[a] Inhibitory activity was expressed as the mean of 50% inhibitory concentration of triplicate determinations and was obtained by interpolation of concentration−inhibition curves.

elucidated by comparing their CD spectra with those of compounds **4** and **3**. Thus, the absolute configuration of catiguanins A (**1**) and B (**2**) may be defined as be (2*R*, 3*R*, 9*R*) and (2*R*, 3*R*, 9*S*), respectively.

The DPPH radical scavenging activities of compounds **1–6** are summarized in Table 2. Among the tested compounds, cinchonains Ic (**5**) and Id (**6**) showed the highest DPPH scavenging activities with IC_{50} values of 2.5 and 2.3 μM, respectively. The radical scavenging activities of compounds **1** and **2** as well as those of cinchonains Ia (**3**) and Id (**4**) are comparable with that of catechin used as a positive standard. In general, the DPPH radical can oxidize cysteine, ascorbic acid, α-tocopherol, polyhydroxy aromatic compounds, and aromatic amines.[12] The polyhydroxy aromatic structures are presumably responsible for antioxidant activities of **1**−**6**. As mentioned earlier, *T. catigua* has attracted a great deal of attention as a Brazilian medicinal plant for the treatment of some neurodegenerative diseases. Additionally, some antioxidants such as α-tocopherol,[13] 1,4-benzoxazines,[14] a bergenin derivative,[15] and flavonoid glycosides[16] exhibit neurotrophic activity, particularly neuroprotective activity. We examined the neurotrophic activities of compounds **1** and **3** by using PC12 cells;[17] however, both compounds had no effect on PC12 cells and NGF-mediated PC12 cells at 1–100 μM.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were recorded on a JASCO FT/IR-410 infrared and a Shimadzu UV-1650PC spectrophotometer, respectively. CD spectra were recorded on a JASCO J-725 spectropolarimeter. ^{1}H (600 MHz) and ^{13}C NMR (150 MHz) spectra were measured on a Varian Unity 600 instrument. NMR assignments were made using DEPT, H−H COSY, HMQC, and HMBC experiments. Chemical shifts are expressed as δ (ppm) with trimethylsilane (TMS) as the internal standard. The HRFABMS was recorded on a JEOL JMS-HX 110 instrument. Si gel column chromatography was performed on Kieselgel 60 (70–230 mesh and 230–400 mesh). HPLC was performed on a JASCO PU-1580 HPLC system equipped with a JASCO UV-1575 detector.

Plant Materials. The bark of *T. catigua* Adr. Juss. was purchased in Sao Paulo, Brazil, in 2005. Dr. G. Hashimoto (Centro de Pesquisas de Historia Natural) identified the plant species, and a voucher specimen (1761BK) has been deposited at the Institute of Pharmacognosy, Tokushima Bunri University.

Extraction and Isolation. The dried bark (5 kg) of *T. catigua* was powdered and extracted with MeOH at room temperature for 1 month. Evaporation of the solvent yielded 500 g of residue; 200 g of this residue was then chromatographed over Si gel (70–230 mesh) and eluted successively with CH_2Cl_2, CH_2Cl_2−EtOAc (9:1, 1:1), EtOAc, EtOAc−MeOH (7:3), and MeOH to yield 10 fractions (1–10). Fraction 6 was separated by column chromatography on silica gel and eluted with $CHCl_3$−MeOH−H_2O (7:3:0.5) to yield fractions 11–19. Fraction 13 was further separated by column chromatography on Sephadex LH-20 using MeOH, followed by separation on MCI gel CHP-20P (polystyrene gel) (37–75 μm) with a gradient solvent system of H_2O−MeOH (100% H_2O → 100% MeOH) to yield 6 fractions. The third fraction was purified by HPLC on a Cosmosil $5C_{18}$ AR-II column (10 × 250 mm) with H_2O−MeOH (2:3, at a flow rate of 2.0 mL/min) to yield catiguanin A (**1**, 4.5 mg). The fourth fraction was purified by HPLC in the same manner as the third to yield catiguanin B (**2**, 12.0 mg). The second fraction was purified by HPLC on a Cosmosil $5C_{18}$ AR-II column (10 × 250 mm) with MeOH−H_2O (11:9, at a flow rate of 2.0 mL/min) to yield compounds **3** (20.0 mg) and **4** (6.3 mg). The fifth fraction was purified by HPLC on a Cosmosil $5C_{18}$ AR-II column (10 × 250 mm) with MeOH−H_2O (3:2, at a flow rate of 2.0 mL/min) to yield compounds **5** (20.6 mg) and **6** (8.2 mg).

Catiguanin A (1): amorphous solid; $[\alpha]^{20}_D$ −100.6 (*c* 1.12, MeOH); UV (MeOH) λ_{max} (log ε) 282 (0.81) nm; CD (EtOH) $\Delta\varepsilon$ (295) +15.7, $\Delta\varepsilon$ (274) −20.0; IR υ_{max} 3265, 1708, 1620, 1452 cm^{-1}; FABMS *m/z* 505 [M + Na]$^+$ (6), 409 (17); HRFABMS *m/z* 505.1123 (calcd for $C_{25}H_{22}O_{10}Na$, 505.1111); ^{1}H and ^{13}C NMR, see Table 1.

Catiguanin B (2): amorphous solid; $[\alpha]^{20}_D$ −56.7 (*c* 2.50, MeOH); UV (MeOH) λ_{max} (log ε) 282 (2.64) nm; CD (EtOH) $\Delta\varepsilon$ (289) −32.7, $\Delta\varepsilon$ (276) +0.6, $\Delta\varepsilon$ (255) −37.8; IR υ_{max} 3275, 1703, 1618, 1449 cm^{-1}; FABMS *m/z* 505 [M + Na]$^+$ (4), 409 (12); HRFABMS *m/z* 505.1111 (calcd for $C_{25}H_{22}O_{10}Na$, 505.1111); ^{1}H and ^{13}C NMR, see Table 1.

Antioxidant Activity. The measurement of antioxidant activity was performed according to a procedure described previously.[12] Catechin was used as the standard antioxidant sample. DPPH and EtOH were used as stable free radical reagent and blank, respectively. The sample was dissolved in EtOH in the ratio of 1:1. The sample was diluted to achieve concentrations of 500, 100, 50, and 10 μg/mL, and 20 μL of each concentration was transferred to different vials in the same plate. DPPH (180 μL) was then immediately added to these vials. The absorbance at 517 nm was measured after 20 min The antioxidant activity was measured as the decrease in the absorbance of DPPH and expressed as percentage of the absorbance of a control DPPH solution without sample. The results are summarized in Table 2.

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