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Polyphenolic Constituents of *Cynomorium songaricum* Rupr. and Antibacterial Effect of Polymeric Proanthocyanidin on Methicillin-Resistant *Staphylococcus aureus*

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ABSTRACT: Oligomeric and polymeric flavan-3-ols were obtained by chromatographic fractionation of extracts from *Cynomorium songaricum* Rupr. The structure of the polymeric constituent, cynomoriitannin, was characterized using spectral and chemical data. Results from acid-catalyzed degradation indicated that cynomoriitannin is a polymeric proanthocyanidin predominantly composed of epicatechin, together with low proportions of epicatechin-3-*O*-gallate and catechin as extension units. The terminal unit was chiefly composed of catechin, with an admixture of epicatechin. Size exclusion chromatographic analysis demonstrated a mean polymerization degree of 14. Two new phloroglucinol adducts (cynomoriitannin-phloroglucinol adducts A and B) obtained by acid-catalyzed degradation of cynomoriitannin in the presence of phloroglucinol were characterized using spectral analyses. Six oligomeric flavan-3-ols were also identified as follows: procyanidin B3, catechin-(6′-8)-catechin, catechin-(6′-6)-catechin, epicatechin-(4β-8)- epicatechin-(4β-8)-catechin, epicatechin-(4β-6)-epicatechin-(4β-8)-catechin, and arecatannin A1, respectively. These flavan-3-ols were isolated from *C. songaricum*. This is the first time that this procedure has been described. The antibacterial activity of the fractions and constituents was tested against methicillin-resistant *Staphylococcus aureus* (MRSA). The crude acetone−water (7:3) extract had moderate activity against MRSA. Cynomoriitannin was the most effective of the plant constituents against MRSA.

KEYWORDS: *proanthocyanidin, Cynomorium songaricum Rupr., antibacterial effect, methicillin-resistant Staphylococcus aureus, phloroglucinol adducts*

INTRODUCTION

Proanthocyanidins (PAs) are oligomers and polymers of flavans that are widely distributed in the plant kingdom. Previous studies have demonstrated the antibacterial and other pharmacological effects of PA oligomers and their gallate esters.[1] Epigallocatechin gallate and its oxidative products have been shown to have antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), and they markedly decrease the minimum inhibitory concentrations (MICs) of oxacillin and other antibiotics.[2−4] Condensed procyanidins isolated from the fruit peel of *Zanthoxylum piperitum* have also been shown to be synergistic with β-lactams against MRSA.[5]

Cynomorium songaricum Rupr. is an obligate root parasitic plant that mainly grows in Northwestern China and Central Asia. It is a source of healthy food and nutrients[6] and has been widely used in traditional Chinese medicine. A previous report indicated that procyanidins obtained from the methanol extract of this plant possessed potent superoxide (SOD)-like and α-glucosidase inhibitory activity. These properties have been shown to increase with increasing degrees of polymerization.[7] Polymeric procyanidin, which is mainly composed of epicatechin, was reported to have the most potent activities among them. These procyanidins have also been reported to act as inhibitory properties against HIV-1 protease in water extracts of *C. songaricum*.[8] However, to date, the oligomeric and polymeric flavan-3-ols from *C. songaricum* have not been adequately characterized. In this study, we identified six oligomeric flavan-3-ols and described the structural characterization of polymeric PA. We also investigated the antibacterial activity of these extracts against MRSA.

MATERIALS AND METHODS

General Procedures. Electrospray ionozation (ESI)-MS spectra were recorded using a Bruker amaZon ETD spectrophotometer in the negative-ion mode. Circular dichroism (CD) spectra were measured on a JASCO J-720 W spectrophotometer. ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian INOVA AS 600 instrument (600 MHz for ^{1}H and 150 MHz for ^{13}C) at 27 °C, in acetone-d_6 or acetone-d_6 containing 10% D_2O (v/v), unless mentioned otherwise. Chemical shifts were expressed as δ (ppm) relative to tetramethylsilane, based on solvent signals (acetone-d_6: δ_H 2.04 and δ_C 29.8).

Diaion HP-20 (Mitsubishi Chemicals, Tokyo, Japan), MCI gel CHP-20P (Mitsubishi Chemicals), and Toyopearl HW-40 (Tosoh, Tokyo, Japan) were used for column chromatography. Normal-phase high-performance liquid chromatography (HPLC) was performed at room temperature on a YMC-Pack SIL A003 (YMC, Kyoto, Japan) column (4.6 mm i.d. × 250 mm) with *n*-hexane−MeOH−tetrahydrofuran−formic acid (55:33:11:1, v/v/v/v) containing 450 mg/L oxalic acid as the solvent.

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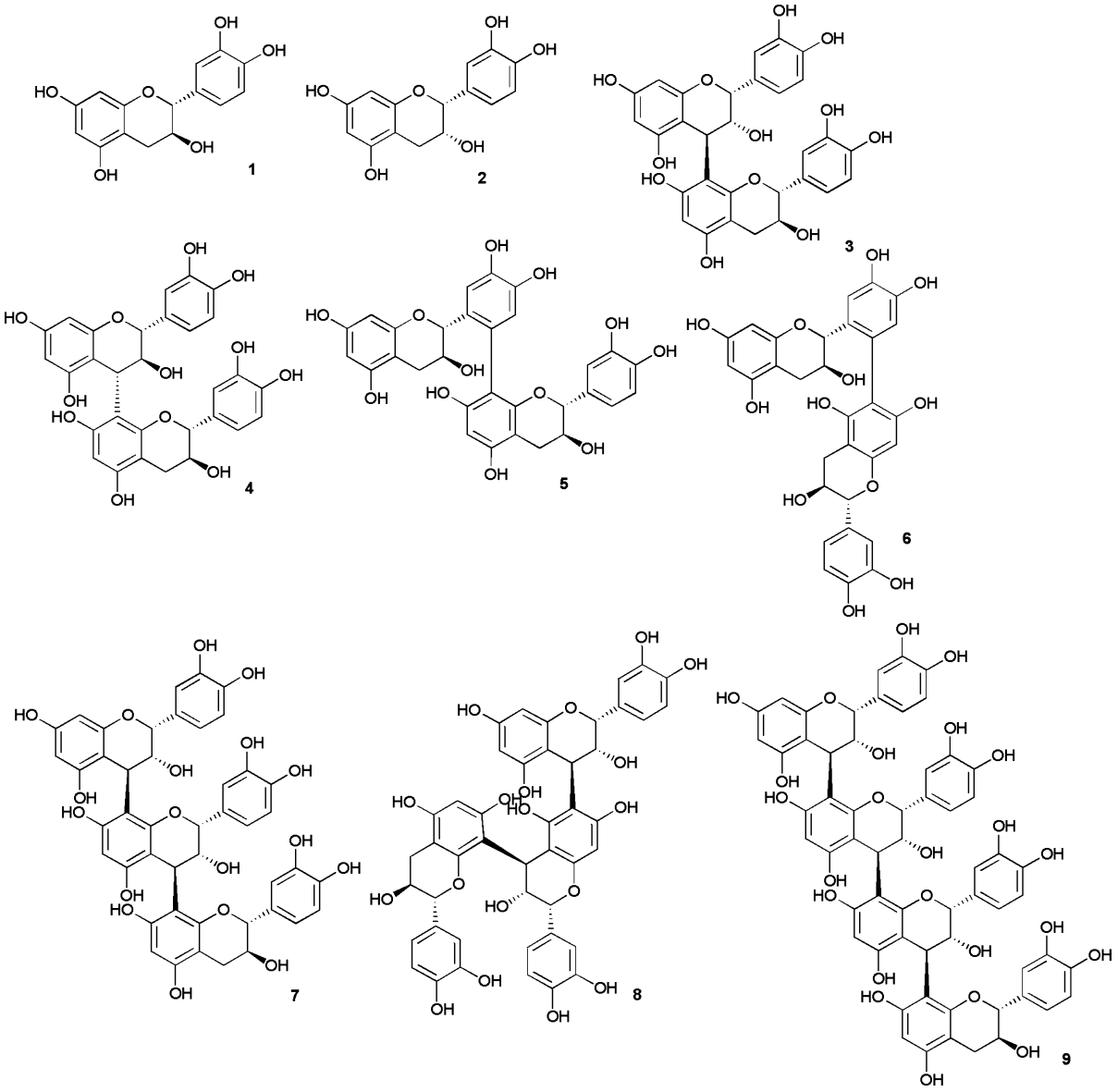

Figure 1. Structures of compounds isolated from *C. songaricum*.

Analytical reversed-phase HPLC was carried out on a YMC-Pack ODS A302 (4.6 i.d. × 150 mm, YMC) column eluted with 0.01 M H_3PO_4–0.01 M KH_2PO_4–MeOH (9:9:2, v/v/v) at 40 °C unless mentioned otherwise. Preparative reversed-phase HPLC was performed on a YMC-Pack ODS A324 (10 mm i.d. × 300 mm, YMC) column. HPLC-UV analyses detection was undertaken at 280 nm.

Materials. Fresh stems of *C. songaricum* were collected in a marginal zone of Hobq Desert, Inner Mongolia, China (39.83 N, 108.7 E), on May 7, 2008. The voucher specimen was kept in the Herbarium of Inner Mongolia University. The plants were dried at room temperature in a dark space.

The MRSA strain (OM 623) used in this study was a clinical isolate from Okayama University Hospital (Okayama, Japan). Catechin, epicatechin, and phloroglucinol were purchased from Sigma (St. Louis, MO). Solvents used for HPLC were purchased from Merck (Darmstadt, Germany), and those for LC-MS were from Sigma. Oxacillin, used in the bacterial experiments, was purchased from Sigma.

Purification of Oligomeric and Polymeric Flavan-3-ols from *C. songaricum*. The stems of *C. songaricum* (100 g) were homogenized in 70% aqueous acetone (v/v, 3 L) at room temperature. The filtered homogenate was concentrated to 500 mL at 40 °C under reduced pressure. The concentrated solution was applied to a Diaion HP-20 (5 cm i.d. × 30 cm) column, and adsorbed compounds were successively eluted with H_2O, 20, 40, 60, and 100% aqueous MeOH, and 70% aqueous acetone to give the corresponding H_2O (28.8 g), 20 (1.53 g), 40 (5.49 g), 60 (3.22 g), and 100% (0.11 g) aqueous MeOH, and 70% aqueous acetone (47 mg) fractions, respectively.

Separation of each of the fractions was monitored with normal- and reversed-phase HPLC. The 40% aqueous MeOH fraction was subjected to column chromatography over Toyopearl HW-40 (2.2 cm i.d. × 35 cm) with 70% aqueous EtOH and then with 70% aqueous acetone to give fractions I–V. Fraction II (112.4 mg) was chromatographed on MCI gel CHP-20P (1.1 cm i.d. × 30 cm) with 0% → 10% → 20% → 30% → 100% MeOH in water, to give (+)-catechin (compound **1**, 19.3 mg) and (−)-epicatechin (compound **2**, 2.3 mg) (Figure 1). Fraction III (239.3 mg) was fractionated by column chromatography on MCI gel CHP-20P (1.1 cm i.d. × 30 cm) with 0% → 10% → 15% → 20% → 30% → 100% MeOH in water, to give procyanidin B1 (compound **3**, 7.2 mg), procyanidin B3 (compound **4**, 8.4 mg), catechin-(6′-8)-catechin (compound **5**, 1.7 mg), and catechin-(6′-6)-catechin (compound **6**, 1.4 mg). Fraction IV (224.1 mg) was chromatographed on a MCI gel CHP-20P (1.1 cm i.d. × 30 cm) with 0% → 10% → 15% → 20% → 30% → 100% MeOH in water, to give epicatechin-(4β-8)-epicatechin- (4β-8)-catechin (com-

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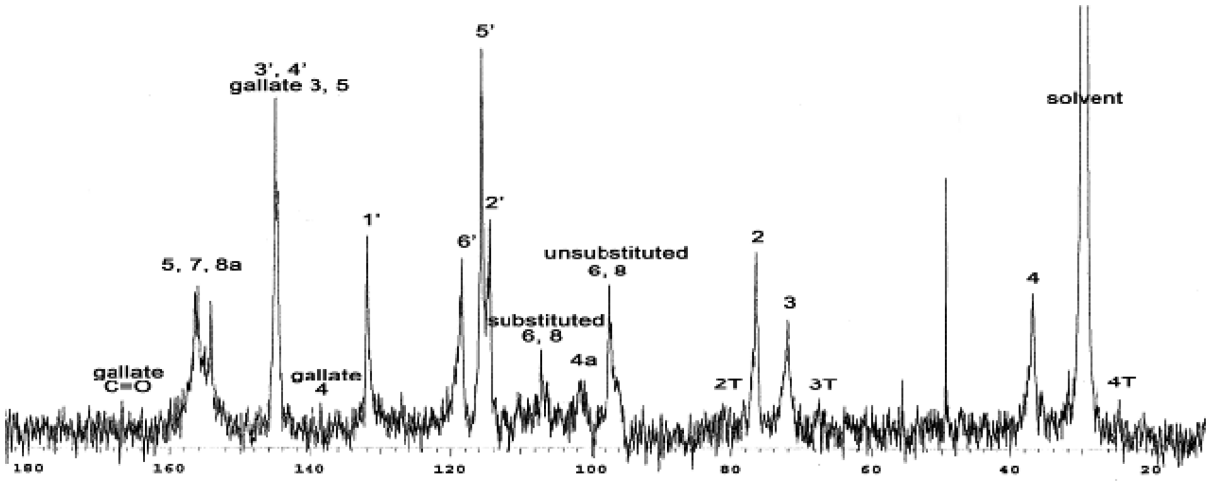

Figure 2. ^{13}C NMR spectra of cynomoriitannin (**10**). The signals indicated by 2T, 3T, and 4T are of the terminal unit. The spectrum also suggested the presence of gallate structure in **10**. The signal at δ 49.8 is due to the residual MeOH in the sample.

pound **7**, 8.6 mg). Fraction V (224.1 mg) was subjected to a MCI gel CHP-20P (1.1 cm i.d. × 30 cm) column with 0% → 10% → 15% → 20% → 30% → 100% aqueous MeOH, and factions were further purified by preparative HPLC to give epicatechin-(4β-6)-epicatechin-(4β-8)-catechin (compound **8**, 3.4 mg) and a tetrameric procyanidin, arecatannin A1 (compound **9**, 5.3 mg). The eluate in 70% acetone from the Toyopearl HW-40 column (fraction V) was a polymeric PA, cynomoriitannin (compound **10**, 2.46 g).

*Procyanidin B3 (**4**).* Compound **4** was an off-white amorphous powder, $[\alpha]_D^{20}$ −192.8° (MeOH, *c* 1.0). ^{1}H NMR: Major rotamer, δ 6.77 (d, *J* = 1.8 Hz, H-2$_U$′), 6.76 (d, *J* = 7.8 Hz, H-5$_U$′), 6.65 (d, *J* = 9 Hz, H-2$_L$′), 6.62 (d, *J* = 2.4 Hz, H-2$_L$′), 6.47 (dd, *J* = 1.8, 7.8 Hz, H-6$_U$′), 6.22 (dd, *J* = 1.8, 7.8 Hz, H-6$_L$′), 6.15 (brs, H-6$_L$), 5.90 (d, *J* = 2.4 Hz, H-6$_U$), 5.78 (d, *J* = 2.4 Hz, H-8$_U$), 4.58 (d, *J* = 7.2 Hz, H-2$_L$), 4.39 (m, 2H, H-3$_U$, 4$_U$), 4.24 (m, H-2$_U$), 3.80(m, H-3$_L$), 2.69 (dd, *J* = 6, 15.6 Hz, H-4$_{La}$), 2.49 (dd, *J* = 7.8, 16.2 Hz, H-4$_{Lb}$). Minor rotamer, δ 7.01 (d, *J* = 1.8 Hz, H-2$_L$′), 6.95 (d, *J* = 1.8 Hz, H-2$_U$′), 6.83 (dd, *J* = 1.8, 8.4 Hz, H-6$_L$′), 6.79 (dd, *J* = 1.8, 7.8 Hz, H-6$_U$′), 6.75 (d, *J* = 7.8 Hz, H-5$_L$′), 6.67 (d, *J* = 7.8 Hz, H-5$_U$′), 6.03 (s, H-6$_L$), 5.99 (d, *J* = 2.4 Hz, H-6$_U$), 5.83 (d, *J* = 2.4 Hz, H-8$_U$), 4.68 (d, *J* = 7.8 Hz, H-2$_L$), 4.51 (m, 2H, H-3$_U$, 4$_U$), 4.34 (m, H-2$_U$), 4.01 (m, H-3$_L$), 2.86 (dd, *J* = 5.4, 16.2 Hz, H-4$_{La}$), 2.56 (dd, *J* = 7.8, 16.2 Hz, H-4$_{Lb}$).

*Catechin-(6′-8)-catechin (**5**).* Compound **5** was an off-white amorphous powder, $[\alpha]_D^{20}$ −204.8° (MeOH, *c* 1.0). ESI-MS *m*/*z*: 577 [M − H]$^-$. ^{1}H NMR (CD_3OD): δ 6.81 (brs, H-2$_U$′), 6.70 (d, *J* = 1.8 Hz, H-2$_L$′), 6.68 (d, *J* = 7.8 Hz, H-5$_L$′), 6.63 (brs, H-5$_U$′), 6.58 (dd, *J* = 1.8, 7.8 Hz, H-6$_L$′), 6.10 (brs, H-6$_L$), 5.88 (d, *J* = 2.4 Hz, H-8$_U$), 5.80 (d, *J* = 1.8 Hz, H-6$_U$), 4.75 (d, *J* = 6.6 Hz, H-2$_U$), 4.70 (d, *J* = 6.0 Hz, H-2$_L$), 3.98 (m, H-3$_L$), 3.95 (m, H-3$_U$), 2.72 (dd, *J* = 5.4, 16.8 Hz, H-4$_{Lb}$), 2.66 (dd, *J* = 4.7, 16.2 Hz, H-4$_{Ub}$), 2.58 (dd, *J* = 5.4, 16.2 Hz, H-4$_{La}$), 2.40 (dd, *J* = 6.6, 16.2 Hz, H-4$_{Ua}$).

*Catechin-(6′-6)-catechin (**6**).* Compound **6** was an off-white amorphous powder, $[\alpha]_D^{20}$ −30.4° (MeOH, *c* 1.0). ESI-MS *m*/*z*: 577 [M − H]$^-$. ^{1}H NMR (CD_3OD): δ 6.81 (br s, H-2$_U$′), 6.74 (d, *J* = 2.4 Hz, H-2$_L$′), 6.70 (d, *J* = 8.4 Hz, H-5$_L$′), 6.63 (dd, *J* = 2.4, 8.4 Hz, H-6$_L$′), 6.55 (br s, H-5$_U$′), 6.08 (br s, H-6$_L$), 5.90 (d, *J* = 2.4 Hz, H-8$_U$), 5.88 (d, *J* = 2.4 Hz, H-6$_U$), 4.85 (d, *J* = 4.8 Hz, H-2$_U$), 4.52 (d, *J* = 6.6 Hz, H-2$_L$), 4.11 (m, H-3$_U$), 3.99 (m, H-3$_L$), 2.93 (dd, *J* = 6.0, 16.2 Hz, H-4$_{Lb}$), 2.58 (2H, m, H-4$_{Ub}$, 4$_{La}$), 2.48 (dd, *J* = 6.0, 16.2 Hz, H-4$_{Ua}$).

*Epicatechin-(4β-8)-epicatechin-(4β-8)-catechin (**7**).* Compound **7** was an off-white amorphous powder, $[\alpha]_D^{20}$ +63.8° (MeOH, *c* 1.0). ESI-MS *m*/*z*: 865 [M − H]$^-$. ^{1}H NMR: δ 6.98−6.66 (9H, br), 6.02−5.90 (4H, br), 5.06−4.71 (5H, br), 4.10−3.94 (3H, br), 2.69 (br s, H-4$_{La}$), 2.58 (dd, *J* = 7.2, 16.8 Hz, H-4$_{Lb}$). ^{13}C NMR: δ 157.4−155.2 (9C), 145.4, 145.2 (3C), 145.0, 144.8, 132.1 (3C), 119.1−118.7 (3C), 115.8, 115.6, 115.5 (2C), 114.9, 114.5, 109.6, 107.3, 105.2, 100.3, 97.0 (2C), 96.4, 95.8 (2C), 81.7, 76.7 (2C), 72.8, 71.5, 67.6, 36.8 (2C).

*Epicatechin-(4β-6)-epicatechin-(4β-8)-catechin (**8**).* Compound **8** was an off-white amorphous powder, $[\alpha]_D^{20}$ +117.8° (MeOH, *c* 1.0). ESI-MS *m*/*z*: 865 [M − H]$^-$. ^{1}H NMR: δ 6.96−6.58 (9H, br), 6.08−5.92 (4H, br), 4.89−4.72 (3H, br), 4.55−4.45 (2H, br), 4.01−3.87 (3H, br), 2.63 (br), 2.50 (br). ^{13}C NMR: δ 159.0−155.2 (9C), 145.4−144.8 (6C), 131.9−130.6 (3C), 119.4−119.0 (3C), 115.8−114.7 (6C), 107.8−106.8 (2C), 101.3−100.4 (3C), 97.0−95.4 (4C), 81.8, 77.0−76.2 (2C), 72.1−71.2 (2C), 67.3, 37.1 (2C), 27.7.

*Arecatannin A1 (**9**).* Compound **9** was an off-white amorphous powder, $[\alpha]_D^{20}$ +127.2° (MeOH, *c* 1.0). ESI-MS *m*/*z*: 1153 [M − H]$^-$. ^{1}H NMR: δ 6.98−6.65 (12H, br), 6.01−5.93 (5H, br), 5.14−4.86 (4H, br), 4.65−4.56 (3H, br), 4.07−3.88 (4H, br), 2.68−2.66 (1H, br), 2.56 (dd, *J* = 5.4, 16.2 Hz). ^{13}C NMR: δ 158.5−154.3 (12C), 145.9−144.8 (8 C), 133.1−130.2 (4C), 120.1−118.7 (4C), 115.9−114.4 (8C), 107.3−106.0 (3C), 100.7 (3C), 98.6, 96.8−95.0 (5C), 81.4, 76.9−76.4 (3C), 72.7−71.1 (3C), 67.4, 37.1−36.7 (3C), 27.5.

*Cynomoriitannin (**10**).* Compound **10** was a light-brown amorphous powder. ^{13}C NMR: δ 29−37 (C-4), 70−81 (C-3, C-2), 95−110 (C-4a, C-6, C-8), 113−120 (C-2′, C-5′, C-6′), 130−132 (C-1′), 143−147 (C-3′, C-4′), 152−159 (C-5, C-7, C-8a) (Figure 2).

Size Exclusion Chromatography (SEC). The molecular weight distribution of cynomoriitannin as the polymeric flavan-3-ol fraction was characterized by SEC[9] using TSK gel Super AW3000 column (6.0 mm i.d. × 150 mm; Tosoh, Japan) with *N*,*N*-dimethylformamide (DMF) containing 0.5% (v/v) 3 M $HCOONH_4$ as a mobile phase at 40 °C. The flow rate was maintained at 0.5 mL/min, and the elution was monitored at 280 nm. (+)-Catechin (**1**) [retention time (t_R), 4.95 min), procyanidin B1 (**3**) (t_R 4.67 min), epicatechin-(4β-8)-epicatechin-(4β-8)-catechin (**7**) (t_R 4.43 min), and arecatannin A1 (**9**) (t_R 4.23 min) were used as molecular weight markers. The equation (Log M = −0.863 × t_R + 6.6269, R^2 = 0.985) was obtained to describe that relationship between M and t_R. The number average molecular weight (M_n) and weight average molecular weight (M_w) of cynomoriitannin were calculated from its SEC profile, based on this equation.

Acid-Catalyzed Degradation of Cynomoriitannin in the Presence of Phloroglucinol. Cynomoriitannin (1 mg) was treated with 1% HCl in EtOH (0.2 mL) in the presence of phloroglucinol (1 mg) overnight at room temperature as described previously.[9] LC-MS on an ODS column (YMC-Triart C18, 100 mm × 2.0 mm, 3 μm) was performed for the identification of degradation products. Elution was undertaken with two solvents, A (1% aqueous formic acid) and B (acetonitrile−water−formic acid, 50:49:1, v/v/v) using the following gradient elution program: initially 5% B, 5−7% B over 10 min, 7% B for 5 min, then 7−35% B over 35 min, and 35−40% B over 10 min. Eluted materials were monitored with UV at 280 nm and with ESI-MS detector in the negative-ion mode. The formation of the catechin-phloroglucinol adduct ([M − H]$^-$, *m*/*z*: 413), the epicatechin−

phloroglucinol adduct ([M − H]$^-$, *m/z*: 413), the (epi)catechin dimer−phloroglucinol adduct ([M − H]$^-$, *m/z*: 701), catechin ([M − H]$^-$, *m/z*: 289), and the (epi)catechin trimer−phloroglucinol adduct ([M-H]$^-$, *m/z*: 989) were demonstrated by their molecular weights and also by HPLC comparisons with isolated products.

The HPLC profile of the products (Figure 3) indicated the presence of the following compounds in the reaction mixture: catechin-(4*α*-2)-phloroglucinol (**15**, 20.7 min), epicatechin-(4*β*-2)-phloroglucinol (**11**, 22.0 min), (+)-catechin (**1**, 30.4 min), 3-*O*-galloylepicatechin-(4*β*-2)-phloroglucinol (**12**, 36.2 min), and (−)-epicatechin (**2**, 39.6 min), which were confirmed by comparisons with the authentic samples. The molar ratios of the products were calculated based on the peak area relative to that of (+)-catechin.

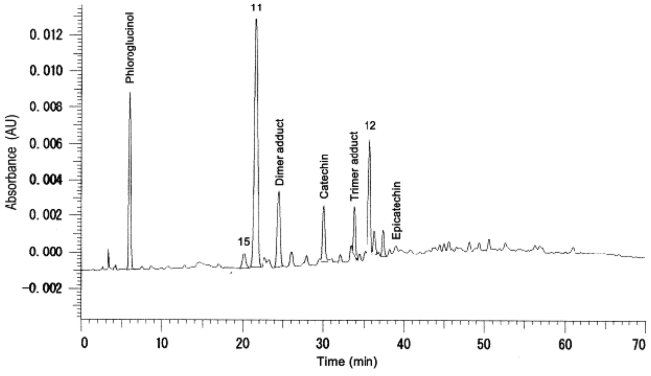

Figure 3. HPLC profile of degradation mixture of cynomoriitannin at 280 nm.

Isolation and Characterization of Phloroglucinol Adducts. Cynomoriitannin (100 mg) was treated with phloroglucinol (100 mg) in 1% HCl-EtOH (20 mL) overnight at room temperature. After evaporation of the solution to dryness under reduced pressure, the residue was subjected to column chromatography on Toyopearl HW-40 (30 cm × 1.1 cm) with 70% ethanol, and the eluent was monitored with RP-HPLC. Fractions were purified by preparative HPLC. In another experiment, 500 mg of the sample was treated in an analogous way, to give the following compounds.

*Epicatechin-(4β-2)-phloroglucinol (**11**).* Compound **11** was an off-white amorphous powder, $[\alpha]_D^{20}$ +112.4° (MeOH, *c* 1.0). ESI-MS *m/z*: 413 [M − H]$^-$, CD (MeOH) [*θ*] (nm): −6.3 × 10^5 (203), +9.1 × 10^5 (214), +4.6 × 10^4 (236), −1.7 × 10^4 (270). ^{1}H NMR: *δ* 6.98 (d, *J* = 1.2 Hz, H-2′), 6.76 (d, *J* = 8.4 Hz, H-5′), 6.73 (dd, *J* = 1.8, 8.4 Hz, H-6′), 6.04 (d, *J* = 1.8 Hz, H-8), 6.03 (d, *J* = 2.4 Hz, H-6), 5.92 (brs, H-3″, 5″), 5.06 (brs, H-2), 4.60 (d, *J* = 1.2 Hz, H-4), 4.04 (brs, H-3). ^{13}C NMR: *δ*158.7−157.8 (6C, C-5, 7, 8a, 2″, 4″, 6″). 145.4 (C-4′ or 3′), 145.2 (C-3′ or 4′), 132.4 (C-1′), 119.2 (C-6′), 115.5 (C-2′ or 5′), 115.2 (C-5′ or 2′), 106.9 (C-1″), 100.6 (C-4a), 96.4−95.9 (4C, C6, 8, 3″, 5″), 77.0 (C-2), 72.5 (C-3), 36.9 (C-3).

*Epicatechin-3-O-gallate-(4β-2)-phloroglucinol (**12**).* Compound **12** was an off-white amorphous powder, $[\alpha]_D^{20}$ +54.4° (MeOH, *c* 1.0). ESI-MS *m/z*: 565 [M − H]$^-$, CD (MeOH) [*θ*] (nm): −4.4 × 10^5 (205), +4.9 × 10^5 (213), +3.1 × 10^5 (236), −7.4 × 10^4 (289). ^{1}H NMR: *δ* 7.01 (2H, s), 6.96 (d, *J* = 1.8 Hz, H-2′), 6.73 (dd, *J* = 1.8, 8.4 Hz, H-6′), 6.71 (d, *J* = 7.8 Hz, H-5′), 6.07 (d, *J* = 1.8 Hz, H-8), 5.98 (d, *J* = 2.4 Hz, H-6), 5.95 (2H, br s, H3‴, 5‴), 5.42 (s, H-2), 5.19 (t, *J* = 1.8 Hz, H-3), 4.58 (d, *J* = 1.6 Hz, H-4). ^{13}C NMR: *δ* 167.2 (C═O), 158.2−157.2 (6C, C-5, 7, 8a, 2‴, 4‴, 6‴), 145.9 (2C, C-3″, 5″), 145.4−145.3 (2C, C-3′, 4′), 139.1 (C-4″), 131.1 (C-1′), 118.8 (C-6′), 115.5 (C-2′ or 5′), 114.7 (C-2′ or 5′), 109.9 (2C, C-2″, 6″), 105.8 (C-1‴), 100.5 (C-4a), 96.5−95.3 (4C, C-6, 8, 3‴, 5‴), 75.4 (C-2), 75.0 (C-3), 34.0 (C-4).

*Cynomoriitannin-phloroglucinol A (**13**).* Compound **13** was an off-white amorphous powder, $[\alpha]_D^{20}$ +102.6° (MeOH, *c* 1.0). ESI-MS *m/z*: 521 [M − H]$^-$, CD (MeOH) [*θ*] (nm): +6.3 × 10^5 (212), +4.5 × 10^5 (234), −3.7 × 10^4 (279). ^{1}H NMR (acetone-d_6-D_2O, 9:1): *δ* 6.98 (d, *J* = 2.4 Hz, H-2′), 6.73 (d, *J* = 7.8 Hz, H-5′), 6.66 (dd, *J* = 2.4, 7.8 Hz, H-6′), 6.38 (d, *J* = 1.2 Hz, H-6), 6.37 (d, *J* = 1.8 Hz, H-8), 5.96 (2H, s, H-3″, 5″), 5.92 (2H, brs, H-3‴, 5‴), 5.18 (s, H-2), 4.61 (d, *J* = 1.2 Hz, H-4), 3.95 (t, *J* = 1.2 Hz, H-3). ^{13}C NMR: *δ* 158.2 (C-4‴), 157.6 (C-4″), 157.4 (2C, C-2‴, 6‴), 157.1 (C-5), 156.6 (C-8a), 156.3 (2C, C-2″, 6″), 145.2−144.9 (2C, C-3′, 4′), 133.9 (C-7), 132.3 (C-1′), 118.8 (C-6′), 115.4−115.0 (2C, C-2′, 5′), 110.8 (C-8), 110.3 (C-6), 109.2 (C-1″), 109.0 (C-1‴), 107.0 (C-4a), 96.0 (C-3‴, 5‴), 95.3 (C-3″, 5″), 76.4 (C-2), 72.5 (C-3), 36.8 (C-4).

*Cynomoriitannin-phloroglucinol B (**14**).* Compound **14** was a light-brown amorphous powder, $[\alpha]_D^{20}$ +16.8° (MeOH, *c* 1.0). ESI-MS *m/z*: 685 [M − H]$^-$, CD (MeOH) [*θ*] (nm): −6.6 × 10^4 (202), +3.8 × 10^3 (217), −2.9 × 10^4 (222), −2.8 × 10^4 (226), −1.3 × 10^4 (239), +1.4 × 10^4 (287). ^{1}H NMR: *δ* 6.98 (d, *J* = 2.4 Hz, H-2$_U$′), 6.73 (d, *J* = 8.4 Hz, H-5$_U$′), 6.65 (dd, *J* = 2.4, 7.8 Hz, H-6$_U$′), 6.51 (d, *J* = 8.4 Hz, H-5$_L$′), 6.24 (br s, H-2$_L$′), 6.05 (d, *J* = 7.8 Hz, H-6$_L$′), 5.93 (d, *J* = 2.4 Hz, H-8$_U$), 5.92 (br s, H-6$_L$), 5.90 (d, *J* = 2.4 Hz, H-6$_U$), 5.89 (br s, H-3″, 5″), 5.11 (br s, H-2$_U$), 4.57 (d, br, *J* = 8.4 Hz, H-*β*), 4.41 (d, br, *J* = 7.8 Hz, H-4$_U$), 4.13 (br s, H-*α*), 3.93 (br s, H-3$_U$), 2.76 (t, br, *J* = 12 Hz, H-γ_a), 2.46 (br, H-γ_b). ^{13}C NMR: *δ* 160.9 (C-7$_L$), 158.6 (C-4″), 157.9 (2C, C-2″, 6″), 157.4 (C-5$_U$), 157.2 (2C, C-7$_U$, 8a$_U$), 156.7 (C-8a$_L$), 153.6 (C-5$_L$), 145.2 (2C, C-4$_U$′, 4$_L$′), 144.9 (C-3$_U$′), 143.4 (C-3$_L$′), 137.9 (C-1$_L$′), 132.4 (C-1$_U$′), 119.0 (C-6$_U$′), 118.9 (C-6$_L$′), 115.6 (C-5$_L$′), 115.4 (C-5$_U$′), 115.1 (C-2$_U$′), 115.0 (C-2$_U$′), 108.6 (C-1″), 104.3 (2C, C-4a$_L$, 8$_L$), 102.0 (C-4a$_U$), 96.3 (C-6$_L$), 95.7 (2C, C-8$_U$), 95.1 (3C, C-6$_U$, 3″, 5″), 91.6 (C-*β*), 76.4 (C-2$_U$), 72.7 (C-3$_U$), 48.4 (C-*α*), 36.8 (C-4$_U$), 32.3 (C-*γ*).

Estimation of MICs. The antibacterial effects of the isolated procyanidins against MRSA were estimated using a liquid microdilution method.[10] Briefly, MRSA strains were cultured overnight at 32 °C in cation-supplemented Mueller−Hinton broth (CSMHB; Difco, Detroit, MI) containing $CaCl_2$ (50 *μ*g/mL) and $MgCl_2$ (25 *μ*g/mL). They were then diluted with 0.85% NaCl and plated at 10^4 CFU well^{-1} on 96-well plates. The cell suspensions in the wells were incubated at 32 °C for 24 h in the absence or presence of serially diluted test compounds. The MICs of the compounds were defined as the lowest concentrations at which the culture lacked turbidity after incubation.

RESULTS AND DISCUSSION

Purification of Oligomeric and Polymeric Flavans from *C. songaricum* and Structure of Cynomoriitannin. The 70% acetone extract of *C. songaricum* was subjected to column (Diaion HP-20, 40% MeOH) fractionation and was further purified by column chromatography (Toyopearl HW-40C and MCI gel CHP-20P) and by preparative HPLC to yield compounds **1**−**9** and a polymeric PA named cynomoriitannin (**10**). This is the first time that compounds **4**−**9** have been isolated from *C. songaricum*.

Compounds **1**−**9** were identified using NMR, MS spectra, and comparisons with the literature.[11−15] The structure of Cynomoriitannin (**10**) was characterized on the basis of ^{13}C NMR, SEC, and acid-catalyzed degradation analyses. The ^{13}C NMR spectrum showed signals assignable to A-ring (C-4a, -5, -6, -7, -8, and -8a), C-ring (C-2, -3, and -4), and B-ring (C-1′−C-6′) carbons (see Figure 2), indicating that compound **10** is a PA polymer mainly composed of (−)-epicatechin, as described previously.[16]

PAs can be depolymerized under acidic conditions, releasing terminal subunits as flavan monomers and extension subunits as flavanyl cation intermediates. The cation intermediates are trapped by a nucleophilic reagent to form stable adducts.[17] Thus, the constituent monomeric units of cynomoriitannin can be investigated by acid-catalyzed degradation in the presence of an excess amount of phloroglucinol, which is odorless, environmentally friendly, and regarded to be a better trapping reagent than benzyl mercaptan.[18]

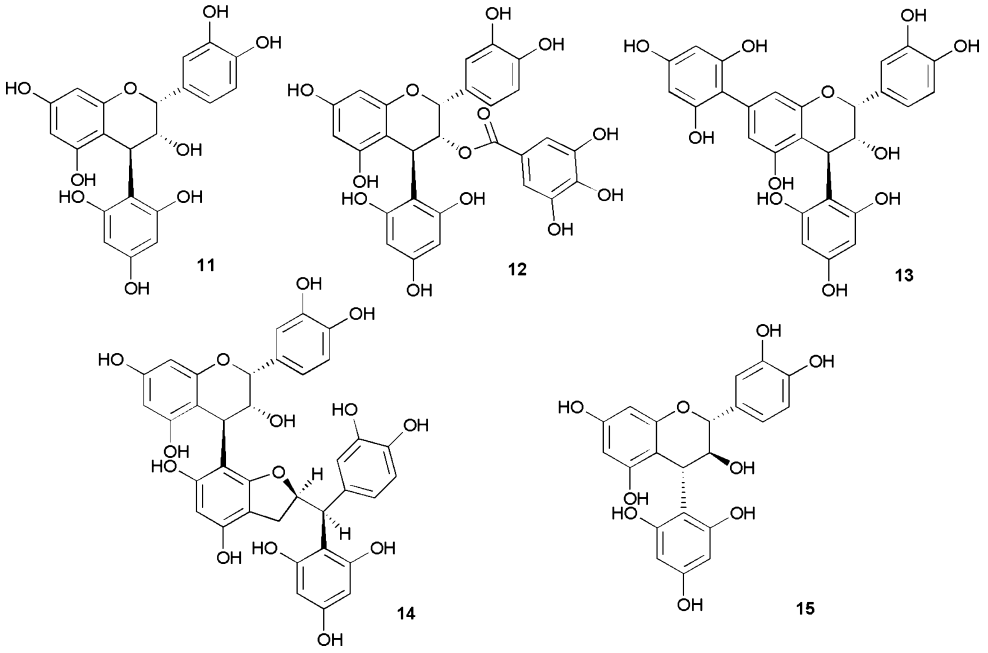

Figure 4. Structures of degradation products from cynomoriitannin.

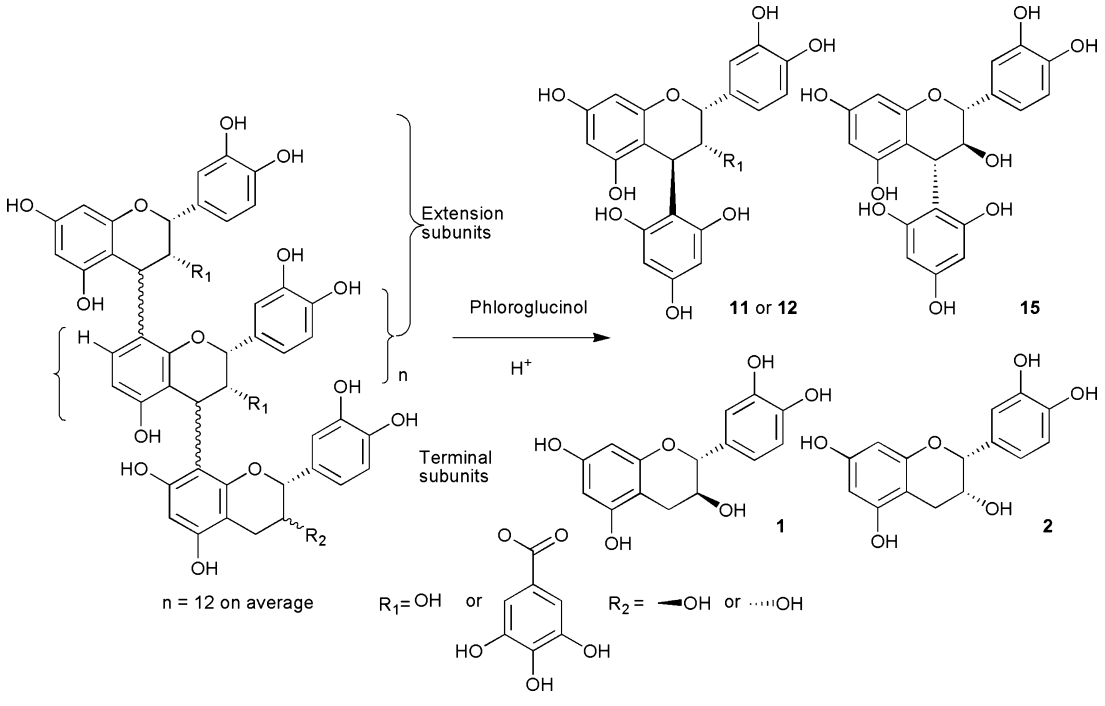

Figure 5. Degradation of cynomoriitannin (**10**) in the presence of phloroglucinol.

Three phloroglucinol adducts were identified based on LC-MS data and by comparing of HPLC retention time peaks observed with authentic samples. These were epicatechin-(4β-2)-phloroglucinol (**11**), epicatechin-3-*O*-gallate-(4β-2)-phloroglucinol (**12**), and catechin-(4α-2)-phloroglucinol (**15**) (Figure 4). These adducts were derived from the extension units in the PA structure, and the relative molar ratio was estimated as 10.2:0.98:0.94 on the basis of the HPLC analysis. HPLC analysis of the degradation mixture also exhibited the presence of (+)-catechin (**1**) and (−)-epicatechin (**2**) in the proportion 4.5:1. The extension units were mainly composed of (−)-epicatechin together with trace amounts of epicatechin-3-*O*-gallate and (+)-catechin. The terminal units were composed of (+)-catechin and smaller amounts of (−)-epicatechin.

SEC techniques have been used by others to the determine molecular weight distribution of polymeric compounds, which are routinely summarized as number average (M_n) and weight average (M_w) molecular weights. SEC analysis of PAs has traditionally been carried out on methylated or acetylated derivatives to reduce interactions between the hydroxyl groups of PA and the SEC gels.[19] However, this method of derivatization is time-consuming and often results in poor recovery due to side reactions.

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In the present study, SEC analysis of cynomoriitannin was performed on the free phenolic form, using DMF containing 0.5% (v/v) of 3 M $HCOONH_4$ as the mobile phase. The molecular weight distribution analysis based on the elution profile of cynomoriitannin indicated that the number (M_n) and weight (M_w) average molecular weight were 4.4×10^3 and 6.6×10^3, respectively. This M_n value corresponds to PA 14-mer structure taking into account the constituent proportion. The structure of cynomoriitannin is shown in Figure 5.

Identification of New Degradation Products from Cynomoriitannin. Cynomoriitannin-phloroglucinol A (**13**) was isolated as an off-white amorphous powder. The $[M - H]^-$ ion peak at m/z 521 in ESI-MS indicated the molecular formula $C_{27}H_{22}O_{11}$. The ^1H NMR spectrum of **13** showed a set of ABX spin systems (δ6.98, d, J = 1.8 Hz; 6.73, d, J = 7.8 Hz and 6.67, dd, J = 1.8, 8.4 Hz) and a pair of meta-coupled proton signals (δ6.38 and 6.37, each d, J = 1.5 Hz), as well as two 2H singlets at δ5.97 and 5.92, indicating the presence of two phloroglucinol groups in its structure (Table 1). The signal pattern of the aliphatic protons was similar to those of compound **11**. However, the ^{13}C NMR spectrum showed a downfield shift of C-4a (δ 107.0) relative to that of compound **11**, indicating a difference in the A-ring structure. Compound **13** had an additional phloroglucinol group on the A-ring of epicatechin-(4β-2)-phloroglucinol (**11**). In the HMBC spectrum, H-4 (δ 4.61), H-6 (δ 6.38), and H-8 (δ 6.37) were correlated with C-4a (δ 107.0), and in turn, H-6, H-8, and the phloroglucinol 2H singlet at δ 5.96 were correlated with C-1 of the other phloroglucinol structure (δ 109.2) (Figure 6). The CD spectrum (Figure 7) of compound **13** exhibited a positive Cotton effect at 220−240 nm with a noticeable amplitude, indicating the *R* configuration at epicatechin C-4. The assigned structure suggested that compound **13** was a side reaction product from acid-catalyzed degradation.

Table 1. ^1H and ^{13}C NMR Assignments and HMBC Correlations for Compound 13

	compd **13** (acetine-d_6, 27 °C)		
carbon no.	carbon δ (151 Hz)	proton δ (600 Hz)	HMBC
2	76.4	5.18 (s)	3, 1′, 2′, 6′
3	72.5	3.95 (m, J < 1 Hz)	2, 4, 4a, 1‴
4	36.8	4.61 (d, J = 1.2 Hz)	2, 3, 4a, 1‴, 2‴, 6‴
4a	108.9		
5	157.2		
6	110.3	6.37 (d, J = 1.8 Hz)	5, 8, 1″
7	133.9		
8	110.9	6.38 (d, J = 1.8 Hz)	6, 8a, 1″
8a	156.7		
1′	132.3		
2′	115.4	6.98 (d, J = 1.8 Hz)	2, 3′, 4′, 6′
3′	144.9 or 145.2		
4′	144.9 or 145.2		
5′	115.0	6.73 (d, J = 7.8 Hz)	3′, 4′
6′	118.8	6.67 (dd, J = 8.4, 1.8 Hz)	5′
1″	109.2		
2″	156.3		
3″	95.3	5.97 (brs)	1″, 2″, 4″
4″	157.4		
5″	95.3	5.97 (brs)	1″, 4″, 6″
6″	156.3		
1‴	106.9		
2‴	157.6		
3‴	96.0	5.92 (brs)	
4‴	158.2		
5‴	96.0	5.92 (brs)	
6‴	157.6		

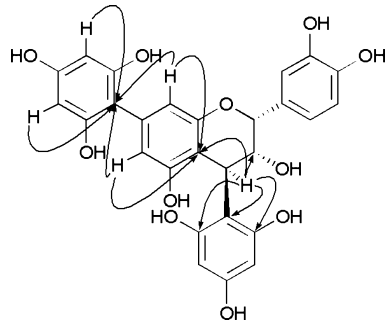

Figure 6. Important HMBC correlations of compound **13**.

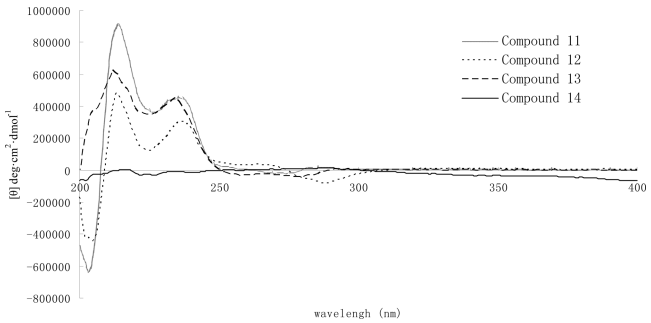

Figure 7. CD spectrum of degradation products of cynomoriitannin.

Cynomoriitannin-phloroglucinol B (**14**) was obtained as a light-brown amorphous powder. The $[M - H]^-$ ion peak at m/z 685 in ESI-MS indicated the molecular formula $C_{36}H_{30}O_{14}$. The ^1H NMR spectrum (Figure 6 and Table 2) of compound **14** showed a set of signals forming a typical ABX spin system (δ 6.98, d, J = 2.0 Hz; 6.73, d, J = 8.4 Hz; 6.65, dd, J = 2.0, 8.4 Hz), two *ortho*-coupled doublets (δ 6.65, d, J = 8.0 Hz; 6.05, d, J = 8.0 Hz), and a broad singlet at δ 6.24 forming another ABX system. These two ABX systems of two flavan-3-ol B-rings in compound **14** were also indicated by the $^1H-^1H$ COSY spectrum. The ^{13}C NMR spectrum showed aliphatic carbon signals at δ 91.6 and 48.4. These chemical shifts were markedly different from those of the ordinary flavan-3-ols and procyanidins, and the presence of a 2-benzyl-2,3-dihydrobenzofuran structure in compound **14** was assumed based on comparison with previous data.[20] As shown in Table 2, an epicatechin structure was proposed on the basis of ^{13}C signals from the HSQC spectrum and the structure of compound **14**.

The HMBC spectrum showed correlations of H-4_U (δ 4.41) with C-$4a_U$ (δ 102.0), C-7_L (δ 160.9), and C-$8a_L$ (δ 156.7), indicating that the dihydrobenzofuran and epicatechin structures were connected by a C−C linkage at C-$4a_U$. On the other hand, a HMBC correlation of one of the *meta*-coupled doublets at δ 5.90 with C-$4a_U$ and an aromatic singlet at δ 5.92 with C-5_L (δ153.6), shown by the arrows in Figure 8, substantiated that the dihydrobenzofuran unit was connected to C-4 of the upper epicatechin unit. On the basis of the composition of the polymeric PA structure of compound **10**, it

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Table 2. ^{1}H and ^{13}C NMR Assignments, COSY, and HMBC Correlations for Compound 14

		compd **14** (acetone-d_6, 27 °C)			
	carbon no.	carbon δ (151 Hz) (150 Hz)	proton δ (600 Hz)	COSY	HMBC
upper unit	2	76.4	5.11 (brs)	3_U, 4_U	C-$1'_U$, $2'_U$, $6'_U$,
	3	72.7	3.93 (brs)	2_U, 4_U	
	4	36.8	4.41 (d, J = 7.8 Hz)	2_U, 3_U	C-2_U, 4a, 5_U, 7_L, 8_L, $8a_L$,
	4a	102.0			
	5	157.4			
	6	95.1	5.90 (d, J = 2.4 Hz)	8_U	C-4a, 5_U
	7	157.2			
	8	95.7	5.93 (d, J = 2.4 Hz)	6_U	C-7_U, $8a_U$
	8a	157.2			
	1′	132.4			
	2′	115.1	6.98 (d, J = 2.4 Hz)	$6'_U$	C-$3'_U$, $6'_U$
	3′	144.9			
	4′	145.2			
	5′	115.4	6.73 (d, J = 8.4 Hz)	$6'_U$	C-$3'_U$, $4'_U$
	6′	119.0	6.65 (dd, J = 2.4, 7.8 Hz)	$2'_U$, $5'_U$	
	α	48.4	4.13 (brs)	β	C-2″, 6″
	β	91.6	4.41 (d, J = 7.8 Hz)	α,γa	
	γ	32.3	2.76(t, br, J = 12 Hz)	α, γb	
			2.46 (br)	γa	
lower unit	4a	104.3			
	5	153.6			
	6	96.3	5.92 (brs)		
	7	160.9			
	8	104.3			
	8a	156.7			
	1′	137.9			
	2′	115.0	6.24 (brs)		
	3′	143.8			
	4′	145.2			
	5′	115.6	6.51 (d, J = 8.4 Hz)		C-$3'_L$, $4'_L$
	6′	118.9	6.05 (d, J = 7.8 Hz)		
phloroglucinol unit	1″	108.6			
	2″	157.9			
	3″	95.1	5.89 (brs)		
	4″	158.6			
	5″	95.1	5.89 (brs)		
	6″	157.9			

was proposed that the dihydrobenzofuran unit was formed from an epicatechin unit. The configuration at C-β of the dihydrobenzofuran unit and the *R* configuration at C-4_U (rather than the *S* configuration) were thus inferred as shown in Figure 8.[20] On the other hand, the absence of a noticeable Cotton effect in the 220−240 nm of the CD suggests the existence of an *S* configuration at C-α.

The structure of cynomoriitannin-phloroglucinol B may be present in the polymeric PA, and it can also be formed from

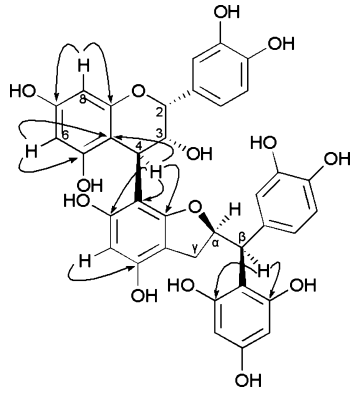

Figure 8. Important HMBC correlations of compound **14**.

two continuous epicatechin extension units during the degradation process. Compounds with similar structures have also been isolated from hot water extracts of *Unicaria gambir* and are thought to be derivatives of catechin.[20−22]

Antibacterial Activity of Extracts and Oligomeric and Polymeric Flavan-3-ols Obtained from *C. songaricum*. The antibacterial effects of the crude extract, Diaion fractions, and cynomoriitannin on MRSA are shown in Table 3. The

Table 3. Inhibition Activity (MIC and Inhibition Concentration: μg/mL) against MRSA for Extracts, Diaion Fractions, and Cynomoriitannin from *C. songaricum*

extracts	MIC (μg/mL)	effective concn[a] (μg/mL)
crude acetone extract	512	64
deposit[b]	512	64
H_2O fraction[c]	>1024	512
20% MeOH fraction[c]	256	8
40% MeOH fraction[c]	128	16
60% MeOH fraction[c]	128	16
100% MeOH fraction[c]	256	64
70% acetone fraction[c]	256	128
cynomoriitannin	64	8

[a]Concentration at which an inhibitory effect on the proliferation of the bacterial strain was observed. [b]Precipitate obtained by concentration of the filtrate from the plant homogenate. [c]These fractions were obtained by column chromatography of crude acetone extract with the solvents indicated.

crude aqueous acetone extract exhibited moderate antibacterial activity against MRSA OM 623 strain (MIC 512 μg/mL). Among the Diaion fractions, 40 and 60% MeOH fractions had the lowest MICs (128 μg/mL). The monomeric and oligomeric flavans obtained from the 40% MeOH fraction had MICs > 1024 μg/mL and exhibited limited inhibitory effects on the growth of MRSA at lower concentrations (data not shown).

Previous studies have reported that procyanidin dimers generally have weak antibacterial effects on MRSA.[23] However, cynomoriitannin was found to have a MIC of 64 μg/mL and exhibited higher antibacterial activity than a procyanidin polymer from *Z. piperitum*.[5] It also reduced the MIC of oxacillin against MRSA strains by 64−512-fold or more. Further investigation of antibacterial effects of cynomoriitannin is now in progress.

These findings suggest that PA polymers are effective against MRSA, acting either directly or by restoring the antibacterial effects of coadministered antibiotics. Thus, PA polymers may

form the basis of developing anti-MRSA drugs and alternative therapies. PA itself is a widely used food additive but is not microbiologically active. Other derivatives of PAs are found in grapes, apples, and cacao.[24−26] The relationships between structure and antimicrobial activity of PAs against MRSA may be an important area for further research.

In summary, six oligomeric procyanidins, procyanidin B3 (**4**), catechin-(6′-8)-catechin (**5**), catechin-(6′-6)-catechin (**6**), epicatechin-(4β-8)-epicatechin-(4β-8)-catechin (**7**), epicatechin-(4β-6)-epicatechin-(4β-8)-catechin (**8**), and arecatannin A1 (**9**), were isolated from *C. songaricum*. The polymeric PA, cynomoriitannin, was shown to be composed mainly of epicatechin together with low proportions of epicatechin-3-*O*-gallate and catechin in the extension units. The terminal unit consists chiefly of catechin, with an admixture of epicatechin. The mean degree of polymerization was 14. Two new phloroglucinol adducts from the degradation mixture of cynomoriitannin were isolated and identified as cynomoriitannin-phloroglucinol A (**13**) and cynomoriitannin-phloroglucinol B (**14**) based on spectral analyses. Preliminary investigation of the antibacterial activity indicated that the crude extract had moderate effect on MRSA. Cynomoriitannin obtained from the extract showed the most effective inhibition against MRSA (MIC 128 μg/mL) among the purified compounds.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Mayer, R.; Stecher, G.; Wuerzner, R.; Silva, R. C.; Sultana, T.; Trojer, L.; Feuerstein, I.; Krieg, C.; Abel, G.; Popp, M.; Bobleter, O.; Bonn, G. K. Proanthocyanidins: Target compounds as antibacterial agents. *J. Agric. Food Chem.* **2008**, *56*, 6959−6966.

(2) Stapleton, P. D.; Shah, S.; Anderson, J. C.; Hara, Y.; Hamilton-Miller, J. M. T.; Taylor, P. W. Modulation of β-lactam resistance in *Staphylococcus aureus* by catechins and gallates. *Int. J. Antimicrob. Agents* **2004**, *23*, 462−467.

(3) Hatano, T.; Kusuda, M.; Hori, M.; Shiota, S.; Tsuchiya, T.; Yoshida, T. Theasinensin A, a tea polyphenol formed from (−)-epigallocatechin gallate, suppresses antibiotic resistance of methicillin-resistant *Staphylococcus aureus*. *Planta Med.* **2003**, *69*, 984−989.

(4) Hatano, T.; Hori, M.; Kusuda, M.; Ohyabu, T.; Ito, H.; Yoshida, T. Characterization of the oxidation products of (−)-epigallocatechin gallate, a bioactive tea polyphenol, on incubation in neutral solution. *Heterocycles* **2004**, *63*, 1547−1554.

(5) Kusuda, M.; Inada, K.; Ogawa, T. O.; Yoshida, T.; Shiota, S.; Tsuchiya, T.; Hatano, T. Polyphenolic constituent structures of *Zanthoxylum piperitum* fruit and the antibacterial effects of its polymeric procyanidin on methicillin-resistant *Staphylococcus aureus*. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 1423−1431.

(6) Ma, L. J.; Chen, G. L.; Jin, S. W.; Wang, C. X. The anti-aging effect and the chemical consttuents of *Cynomorium songaricum* Rupr. *Acta Hortic.* **2006**, *765*, 23−30.

(7) Ma, C. M.; Sato, N.; Li, X. Y.; Nakamura, N.; Hattori, M. Flavan-3-ol contents, anti-oxidative and α-glucosidase inhibitory activities of *Cynomorium songaricu*m. *Food Chem.* **2010**, *118*, 116−119.

(8) Ma, C. M.; Nakamura, N.; Miyashiro, H.; Hattori, M.; Shimotohno, K. Inhibitory effects of constituents from *Cynomorium songaricum* and related triterpene derivatives on HIV-1 protease. *Chem. Pharm. Bull.* **1999**, *47*, 141−145.

(9) Kimura, Y.; Ito, H.; Kawaji, M.; Ikami, T.; Hatano, T. Characterization and antioxidative properties of oligomeric proanthocyanidin from prunes, dried fruit of *Prunus domestica* L. *Biosci., Biotechnol., Biochem.* **2008**, *72*, 1615−1618.

(10) Shiota, S.; Shimizu, M.; Mizushima, T.; Ito, H.; Hatano, T.; Yoshida, T.; Tsuchiya, T. Marked reduction in the minimum inhibitory concentration (MIC) of beta-lactams in methicillin-resistant *Staphylococcus aureus* produced by epicatechin gallate, an ingredient of green tea (*Camellia sinensis*). *Biol. Pharm. Bull.* **1999**, *22*, 1388−1390.

(11) Hatano, T.; Hemingway, R. W. Conformational isomerism of phenolic procyanidins: Preferred conformations in organic solvents and water. *J. Chem. Soc. Perkin Trans 2* **1997**, 1035−1043.

(12) Sang, S. M.; Cheng, X. F.; Stark, R. E.; Rosen, R. T.; Yang, C. S.; Ho, C. T. Chemical studies on antioxidant mechanism of tea catechins: analysis of radical reaction products of catechin and epicatechin with 2,2-diphenyl-1-picrylhydrazyl. *Bioorg. Med. Chem.* **2002**, *10*, 2233−2237.

(13) Saito, A.; Doi, Y.; Tanaka, A.; Matsuura, N.; Ubukata, M.; Nakajima, N. Systematic synthesis of four epicatechin series procyanidin trimers and their inhibitory activity on the Maillard reaction and antioxidant activity. *Bioorg. Med. Chem.* **2004**, *12*, 4783−4790.

(14) Shoji, T.; Mutsuga, M.; Nakamura, T.; Kanda, T.; Akiyama, H.; Goda, Y. Isolation and structural elucidation of some procyanidins from apple by low-temperature nuclear magnetic resonance. *J. Agric. Food Chem.* **2003**, *51*, 3806−3813.

(15) Saito, A.; Mizushina, Y.; Tanaka, A.; Nakajima, N. Versatile synthesis of epicatechin series procyanidin oligomers, and their antioxidant and DNA polymerase inhibitory activity. *Tetrahedron* **2009**, *65*, 7422−7428.

(16) Czochanska, Z.; Foo, L. Y.; Newman, R. H.; Porter, L. J. Polymeric proanthocyanidins: Stereochemistry, structural units, and molecular weight. *J. Chem. Soc. Perkin Trans. 1* **1980**, 2278−2286.

(17) Hemingway, R. W.; McGraw, G. W. Kinetics of acid-catalyzed cleavage of procyanidins. *J. Wood Chem. Technol.* **1983**, *3*, 421−425.

(18) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740−1746.

(19) Kennedy, J. A.; Taylor, A. W. Analysis of Proanthocyanidins by High-Performance Gel Permeation Chromatography. *J. Chromatogr., A* **2003**, *995*, 99−107.

(20) Taniguchi, S.; Kuroda, K.; Yoshikado, N.; Doi, K.; Tanabe, M.; Shibata, T.; Yoshida, T.; Hatano, T. New dimeric flavans from gambir, an extract of *Uncaria gambir*. *Heterocycles* **2007**, *74*, 595−605.

(21) Taniguchi, S.; Kuroda, K.; Doi, K.; Tanabe, M.; Shibata, T.; Yoshida, T.; Hatano, T. Revised structures of gambirins A1, A2, B1, and B2, chalcane-flavan dimers from gambir (*Uncaria gambir* extract). *Chem. Pharm. Bull.* **2007**, *55*, 268−272.

(22) Nonaka, G.; Nishika, I. Novel Biflavonoids, chlcan-flavan dimmers from gambir. *Chem. Pharm. Bull.* **1980**, *28*, 3145−3149.

(23) Hatano, T.; Kusuda, M.; Inada, K.; Ogawa, T.; Shita, S.; Tsuchiya, T.; Yoshida, T. Effects of tannins and related polyphenols on

methicillin-resistant *Staphylococcus aureus*. *Phytochemistry* **2005**, *66*, 2047−2055.
(24) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.
(25) Foo, L. Y.; Lu, Y. R. Isolation and identification of procyanidins in apple pomace. *Food Chem.* **1999**, *64*, 511−518.
(26) Gu, L. W.; House, S. E.; Wu, X. L.; Ou, B. X.; Prior, R. L. Procyanidin and catechin contents and antioxidant capacity of cocoa and chocolate products. *J. Agric. Food Chem.* **2006**, *54*, 4057−4061.