New Galloylated Flavanonols from the Australian Plant, *Glochidion sumatranum*

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Abstract

Bioassay-guided fractionation of the CH₂Cl₂/MeOH extract from the Australian plant *Glochidion sumatranum* resulted in the isolation of four new galloylated flavanonols, (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate (**1**), (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate (**2**), (2*R*, 3*R*)-dihydromyricetin-4'-*O*-gallate (**3**), and (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**), along with the known compound, (2*R*, 3*R*)-dihydromyricetin (**5**). The structures of **1–5** were determined by NMR and MS analysis and their absolute configuration was elucidated by comparison of the CD data with literature values. Compounds **1/2** and **3/4** are two pairs of structural isomers that were shown to interconvert by transesterification during NMR and LC-MS studies. This process involved the intra-molecular migration of the galloyl moieties between C-3' and C-4' of the flavanonol skeleton. Compounds **1** and **3** were identified as the more stable isomers. Compounds **1**, **3**, and **5** showed weak activity against the Gram-negative bacterium *Pseudomonas aeruginosa* and the Gram-positive bacterium *Staphylococcus aureus*.

Key words

Glochidion sumatranum (Euphorbiaceae), galloylated flavanonols, structural isomer, antibacterial activity

Introduction

The genus *Glochidion* (Euphorbiaceae) is comprised of approximately 300 species that are mainly distributed in tropical Asia and Australia [1,2]. Previous chemical investigations on this genus have led to the isolation of several triterpenoids [1-5], triterpenoid saponins [6,7], and lignan glucosides [8,9]. *Glochidion sumatranum* Miq., also known as the umbrella cheese tree, is a native plant growing in the tropical and sub-tropical regions of Australia. No chemical investigations have been previously reported on this species.

As a part of a research collaboration aimed at the discovery of new natural product leads against *Pseudomonas aeruginosa*, we undertook high-throughput screening (HTS) of a pre-fractionated natural product extract library (Eskitis Institute, Australia) using a *P. aeruginosa in vitro* viability assay. *P. aeruginosa* is an opportunistic pathogen that causes nosocomial infections in hospitalised patients. Treatment is made difficult by resistance to a number of antibiotics, due to both outer membrane permeability and multidrug resistance efflux pumps [10,11]. The library was screened against an efflux pump knockout strain (PAO200) to potentially increase initial hits. Hits were confirmed by re-testing against a wild type strain (strain PAO1) and a Gram-positive bacterium *Staphylococcus aureus* (strain 01A1095) to estimate Gram-selectivity. Three fractions derived from *G. sumatranum* were found to display moderate PAO1 strain antibacterial activity. Bioassay-guided isolation on the large-scale organic extract of *G. sumatranum* resulted in the purification of four new galloylated flavanonols, (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate (**1**), (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate (**2**), (2*R*, 3*R*)-

dihydromyricetin-4'-*O*-gallate (**3**), and (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**), along with the known compound, (2*R*, 3*R*)-dihydromyricetin (**5**). The structures of **1–5** were determined by NMR and MS analysis and their absolute configuration was elucidated by comparison of the CD data with literature values. Compounds **1/2** and **3/4** are two pairs of structural isomers that were shown to interconvert by transesterification during NMR and LC-MS studies. This process involved the intramolecular migration of the galloyl moieties between C-3' and C-4' of the flavanonol skeleton. Compounds **1** and **3** were identified as the more stable isomers. Compounds **1**, **3**, and **5** showed weak activity against the gram-negative bacteria *Pseudomonas aeruginosa* and the gram-positive bacteria *Staphylococcus aureus*. Due to the instability observed of compounds **2** and **4**, these were not tested in the bacterial viability assays. In this paper we report the isolation and structure elucidation of compounds **1–5**, as well as the results of antibacterial screening of these secondary metabolites against *P. aeruginosa* strain PAO1 and *S. aureus* strain 01A1095.

Materials and Methods

General experimental procedures

NMR spectra were recorded at 30 °C on either a Varian 500 MHz or 600 MHz Unity INOVA spectrometer (Varian, Walnut Creek, CA, USA). The latter spectrometer was equipped with a triple resonance cold probe. The ¹H and ¹³C NMR chemical shifts were referenced to the solvent peak for DMSO-*d*₆ at δ_H 2.49 and δ_C 39.5. LRESIMS were recorded on a Waters ZQ mass spectrometer (Waters, Milford, MA, USA). HRESIMS were recorded on a Bruker Daltonics Apex III 4.7e Fourier-transform mass

spectrometer (Bruker, Bremen, Germany). IR and UV spectra were recorded on a Bruker Tensor 27 spectrometer (Bruker, Karlsruhe, Germany) and a Jasco V650 UV/Vis spectrophotometer (Jasco, Tokyo, Japan), respectively. CD spectra were collected on a Jasco 715 spectropolarimeter. Optical rotations were recorded on a Jasco P-1020 polarimeter. A BIOLINE orbital shaker (Edwards Instrument Company, Narellan, NSW, Australia) was used for the large-scale extraction of plant material. Alltech Davisil 40-60 μm 60 Å C₁₈ bonded silica was used for pre-adsorption work (Alltech, Deerfield, IL, USA). A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler (Waters, Milford, MA, USA) were used for HPLC. A ThermoElectron C₁₈ Betasil 5 μm 143 Å column (21.2 $\times$ 150 mm) (Thermo Scientific, Los Angeles, CA, USA) were used for semi-preparative HPLC separations. A Phenomenex C₁₈ Luna 3 μm 100 Å column (4.6 $\times$ 50 mm) (Phenomenex, Torrance, CA, USA), a Waters 2790 Separation Module equipped with a Waters 996 PDA detector, and a Waters Micromass ZQ were used for LC-MS. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade (RCI Lab-Scan, Bangkok, Thailand), and the H₂O was Millipore Milli-Q PF filtered (Millipore, Billerica, MA, USA).

Plant material

The leaves and twigs of *Glochidion sumatranum* Miq. were collected from State Forest 310 Gadgarra, Queensland, Australia, in November 1995. A voucher sample (602506) has been lodged at the Queensland Herbarium, Brisbane, Australia.

Extraction and Isolation

The air dried and ground plant material (10 g) was transferred to a conical flask (1 L), *n*-hexane (250 mL) was added and the flask was shaken at 200 rpm for 2 h. The *n*-hexane extract was filtered under gravity, then discarded. CH₂Cl₂ (250 mL) was added to the de-fatted plant material in the conical flask and shaken at 200 rpm for 2 h. The resulting extract was filtered under gravity, and set aside. MeOH (250 mL) was added and the MeOH/plant mixture was shaken for a further 2 h at 200 rpm. Following gravity filtration the plant material was extracted with another volume of MeOH (250 mL), while being shaken at 200 rpm for 16 h. All CH₂Cl₂/MeOH extractions were combined and dried under reduced pressure to yield a dark brown solid (2.9 g). This material was re-suspended in MeOH (150 mL) then loaded onto a MeOH conditioned polyamide gel (30 g) in a sintered glass column and washed by MeOH (300 mL) giving a dark crude 1.5g. A portion of this residue (0.75 g) was pre-adsorbed on C₁₈-bonded silica, then packed into a stainless steel cartridge (10 × 30 mm) that was subsequently attached to a C₁₈ Betasil HPLC column. Isocratic HPLC conditions of 90% H₂O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for the first 10 min, then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0. Fraction 25 yielded pure (2*R*, 3*R*)-dihydromyricetin (**5**, 28 mg, 0.56%). Fraction 35 was further purified by repeating the HPLC conditions described above and yielded **1** (10 mg, 0.20%) and **2** (8 mg, 0.16%) at 21 and 23 min, respectively. Fraction 32 was further purified by HPLC using the C₁₈ Betasil column, starting from 10% MeOH (0.1% TFA)/90% H₂O (0.1% TFA) to 80% MeOH (0.1% TFA)/20% H₂O (0.1% TFA) over

30 min at a flow rate of 9 mL/min. Compounds **3** (4 mg, 0.08%) and **4** (15 mg, 0.30%, *ca.* 80% purity for acquiring the NMR data) were eluted at 23 and 24 min, respectively.

***P. aeruginosa* optical density (OD₆₂₀) viability assay**

P. aeruginosa PAO1 or PAO200 strain (supplied by Pfizer Global Research and Development) cultures were prepared at 3.5×10^4 CFU/mL in cation-adjusted Mueller Hinton (caMH) broth (Difco, Detroit, MI, USA), from concentrated frozen stocks of culture. The final bacterial concentration in the assay was 1500 CFU/well. 45 μ L of diluted bacteria was added to a 384 well lidded, sterile clear plate (Becton Dickinson, Franklin Lakes, NJ, USA) containing controls/fractions by a Multidrop™ liquid handler (Thermo Scientific, Barrington, USA). Plates were incubated at 37 °C in a humidified incubator for 18 hours or until the wells reached an optical density (OD₆₂₀) of between 0.7-0.8, then allowed to cool for 30 minutes. Clear plate seals (Perkin Elmer, Meriden CT, USA) were placed over the plate surface before reading on a Multiskan Ascent reader (Thermo Scientific) at 620 nm.

Prior to addition of bacteria a 5 μ L addition of either test fractions, compounds or control samples were added to the assay plate. Samples were prepared by dilution of stock fractions/compounds/controls in 100% DMSO in to the assay plate with addition of 0.875 μ L of stock and 4.125 μ L of autoclaved Milli-Q filtered water with a Minitrak™ (Perkin Elmer, Meriden CT, USA) liquid handler. The final concentration of DMSO in the assay was 1.75%. Each assay plate contained both positive and negative controls in columns 23 and 24 respectively. The positive control, for uninhibited growth, consisted of 5 μ L of DMSO/milli-Q water to a final concentration of 1.75% and the negative control, or 100% cell death, was comprised of 5 μ L of the

broad spectrum antibiotic ciprofloxacin (Sigma, St Louis, MO, USA) at a final concentration of 5 µg/ml. Whole control plates were included for each assay run, which consisted of duplicate dose response curves of ciprofloxacin, each in triplicate.

***S. aureus* optical density (OD₆₂₀) viability assay**

The *S. aureus* assay was carried out as per *P.aeruginosa* assay, with the following modifications: the final bacterial concentration used was 1980 CFU/well and ciprofloxacin for the internal assay control wells was at 500 µg/mL. Incubation was for 19 hours, or until the OD₆₂₀ reached 0.45

(2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate (1): yellow powder (10 mg, 0.20%); $[\alpha]_D^{25} +29$ (*c* 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 213 (4.55), 288 (4.20) nm; CD (MeOH) $\Delta\epsilon_{325} +2.03$, $\Delta\epsilon_{293} -4.19$, $\Delta\epsilon_{270} +1.34$, $\Delta\epsilon_{252} +2.91$, $\Delta\epsilon_{226} +6.71$, $\Delta\epsilon_{206} +3.09$; IR (film) $\nu_{\max}$ 3200 (brs), 1708, 1639, 1516, 1462, 1347, 1176, 1023, 986 cm⁻¹; ¹H and ¹³C NMR, see Tables 1 and 2; ESI MS (pos. ion mode) *m/z* (rel. int.) 487 [C₂₃H₁₈O₁₂+H]⁺ (100), 509 [C₂₃H₁₈O₁₂+Na]⁺ (75), HR ESIMS *m/z* 509.0710 [M+Na]⁺ (calcd for C₂₃H₁₈O₁₂Na, 509.0690).

(2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate (2): yellow powder (8 mg, 0.16%); $[\alpha]_D^{25} +31$ (*c* 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 213 (4.57), 288 (4.22) nm; CD (MeOH) $\Delta\epsilon_{326} +2.23$, $\Delta\epsilon_{293} -5.28$, $\Delta\epsilon_{252} +2.43$, $\Delta\epsilon_{225} +6.23$, $\Delta\epsilon_{206} +3.58$; IR (film) $\nu_{\max}$ 3206 (brs), 1708, 1639, 1516, 1461, 1346, 1176, 1023, 986 cm⁻¹; ¹H and ¹³C NMR, see Tables 1 and 2; ESI MS (pos. ion mode) *m/z* (rel. int.) 487 [C₂₃H₁₈O₁₂+H]⁺ (100), 509 [C₂₃H₁₈O₁₂+Na]⁺ (40), HR ESIMS *m/z* 509.0695 [M+Na]⁺ (calcd for C₂₃H₁₈O₁₂Na, 509.0690).

(2R, 3R)-dihydromyricetin-4'-O-gallate (3): yellow powder (4 mg, 0.08%); $[\alpha]^{25}_D$ +26 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 214 (4.48), 288 (4.13) nm; CD (MeOH) $\Delta\epsilon_{326}$ +2.48, $\Delta\epsilon_{293}$ -5.08, $\Delta\epsilon_{252}$ +2.76, $\Delta\epsilon_{225}$ +6.93, $\Delta\epsilon_{206}$ +3.61; IR (film) $\nu_{\max}$ 3330, 1707, 1640, 1530, 1514, 1460, 1362, 1314, 1184, 1029, 985 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; ESI MS (pos. ion mode) *m/z* (rel. int.) 473 [$\text{C}_{22}\text{H}_{16}\text{O}_{12}+\text{H}$] $^+$ (100), 511 [$\text{C}_{22}\text{H}_{16}\text{O}_{12}+\text{K}$] $^+$ (52), HR ESIMS *m/z* 495.0537 [$\text{M}+\text{Na}$] $^+$ (calcd for $\text{C}_{22}\text{H}_{16}\text{O}_{12}\text{Na}$, 495.0534).

(2R, 3R)-dihydromyricetin-3'-O-gallate (4): yellow powder (15 mg, 0.30%); $[\alpha]^{25}_D$ +16 (*c* 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 213 (4.42), 289 (4.07) nm; IR (film) $\nu_{\max}$ 3220, 1730, 1641, 1449, 1356, 1316, 1184, 1163, 1022, 988 cm^{-1} ; ^1H and ^{13}C NMR, see Tables 1 and 2; ESI MS *m/z* (rel. int.) 473 [$\text{C}_{22}\text{H}_{16}\text{O}_{12}+\text{H}$] $^+$ (100), HR ESIMS *m/z* [$\text{M}+\text{Na}$] $^+$ 495.0512 (calcd for $\text{C}_{22}\text{H}_{16}\text{O}_{12}\text{Na}$, 495.0534).

Results and Discussion

The air-dried and ground plant material, *G. sumatranum*, was sequentially extracted with *n*-hexane, CH_2Cl_2 , and MeOH. The CH_2Cl_2 /MeOH extractions were combined, re-suspended in MeOH and passed through a polyamide gel column. The resulting crude was initially chromatographed by reversed-phase C_{18} HPLC (MeOH/ H_2O /0.1%TFA) to give 60 fractions. Fractions 25, 32, and 33 were further purified by several rounds of C_{18} HPLC (MeOH/ H_2O /0.1%TFA) to yield compounds **1–5**.

Compound **5** was the major metabolite isolated and was identified as (2*R*, 3*R*)-dihydromyricetin [(+)-ampelopsin] following comparison of the NMR data, optical rotation value, and CD spectrum with literature values [12,13]. Comparison of the NMR data of **1–4** with **5** showed that all compounds belonged to the flavanone structure class.

Compound **1** was obtained as a yellow powder. An $[M + Na]^+$ ion in the (+)-HRESIMS at m/z 509.0710 allowed the molecular formula $C_{23}H_{18}O_{12}$ to be assigned to **1**. The 1H NMR spectrum of **1** exhibited three exchangeable protons [δ_H 11.85 (1H, s), 10.82 (1H, s), and 9.54 (1H, s)], two *meta*-coupled aromatic doublets [δ_H 7.25 (1H, d, $J = 1.8$ Hz) and 7.14 (1H, d, $J = 1.8$ Hz)], a two-proton singlet from a symmetrical aromatic ring [δ_H 6.52 (2H, s)], two diagnostic doublets arising from a 5,7-dihydroxyl substituted flavanone A ring [δ_H 5.91 (1H, d, $J = 1.8$ Hz) and 5.89 (1H, d, $J = 1.8$ Hz)], an AB-type doublet of doublets due to the H-2 and H-3 protons of a flavanone skeleton [δ_H 5.01 (1H, d, $J = 10.8$ Hz), 4.43 (1H, d, $J = 10.8$ Hz)], together with an aromatic methoxy [δ_H 3.81 (3H, s)]. The ^{13}C NMR spectrum showed 23 resonances, which were assigned to one conjugated carbonyl (δ_C 197.3), one ester carbonyl (δ_C 163.7), 18 aromatic carbons (three aryl rings), two oxygenated sp^3 methines (δ_C 82.7 and 71.6) and one methoxy (δ_C 56.0). The aforementioned information implied **1** was a galloylated flavanone compound. Detailed analysis of the 2D NMR data (HSQC and HMBC) confirmed that **1** consisted of a dihydromyricetin core and a 3-*O*-methyl-gallate moiety (Fig. 2). However, 2D NMR experiments failed to connect these two parts together implying that the 3-*O*-methyl-gallate moiety could only be placed either at rings A or B of the dihydromyricetin core through an ester bond. Comparison of the NMR data of ring A in both **1** and **5** showed only minor differences ($\Delta\delta_H < 0.05$ ppm and $\Delta\delta_C < 0.2$ ppm), which ruled out the possibility of the esterification on this

ring. Significantly shifted carbon resonances were observed at C-1' ($\Delta\delta_{\text{C}} = 7.4$ ppm), C-3'/C-5' ($\Delta\delta_{\text{C}} = 4.3$ ppm), and C-4' ($\Delta\delta_{\text{C}} = 6.2$ ppm) indicating that the methoxylgalloyl esterification was on ring B. The symmetrical substituted pattern of ring B, which was implied by the NMR data, further required the 3-*O*-methyl-gallate moiety to be located at C-4'. The CD spectrum of **1** (Fig. 3) exhibited a positive Cotton effect at 325 nm and a negative Cotton effect at 293 nm due to the $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ transition, respectively, which is characteristic of flavanonols with a 2*R*-configuration [13,14]. Since the trans-orientation of H-2 and H-3 in **1** was assigned on the basis of their ^1H - ^1H coupling constant ($J = 10.8$ Hz), the absolute configuration at both C-2 and C-3 could be determined to be *R*. Thus **1** was assigned as (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate.

Compound **2** had a molecular formula of $\text{C}_{23}\text{H}_{18}\text{O}_{12}$ as determined by (+)-HRESIMS at m/z 509.0695 $[\text{M}+\text{Na}]^+$ (calcd 509.0690) indicating that **2** was an isomer of **1**. The ^1H NMR spectrum of **2** was very similar to that of **1** except that the two proton singlet arising from the symmetrical ring B in **1** (δ_{H} 6.52) was replaced by two meta-coupled doublets in **2** (δ_{H} 6.72 and 6.85). These data indicated that **2** had an unsymmetrical substitution pattern on ring B. Comparison of the ^{13}C NMR data of **2** and **5**, the resonances at C-2', C-4', and C-6' in **2** were shifted downfield by *ca* 5 ppm while the resonance at C-3' was shifted upfield by 6.6 ppm, indicating that the 3-*O*-methyl-gallate moiety located at C-3'. The stereochemistry of **2** was assigned as 2*R*, 3*R* following comparison of the ^1H - ^1H coupling constants and CD data of **2** with **1**. Thus compound **2** was assigned as (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate.

Compound **3** was obtained as an optically active yellow gum. The molecular formula of $\text{C}_{22}\text{H}_{16}\text{O}_{12}$ was assigned by (+)-HRESIMS at m/z 495.0537 $[\text{M}+\text{Na}]^+$, which was

14 mass units less than **1**. The ^1H and ^{13}C NMR spectra of **3** were very similar to those of **1** except for the absence of the signals for the methoxy group at C-3'', and the replacement of the two *meta*-coupled doublets arising from H-2'' and H-6'' in **1** by a two proton singlet [δ_{H} 7.08, (2H, s)] in **3**. This indicated the methoxy group at C-3'' in **1** was replaced by a hydroxy group in **3**, which yielded the symmetrical galloyl moiety. Detailed 2D NMR data analysis further confirmed the planar structure of **3**. In the similar manner to **1** and **2**, compound **3** was assigned 2*R*, 3*R* absolute configuration following ^1H - ^1H coupling constant and CD data analysis.

Compound **4** was an isomer of **3**. The ^1H and ^{13}C NMR spectra of **4** were very similar to **2** except for the replacement of the 3''-*O*-methyl-gallate in **2** by a gallate in **4**. This structural difference was supported by the absence of the methoxy signal in **2** and the appearance of symmetrical gallate signals in the NMR spectra of **4**. Detailed 2D analysis further confirmed the structure of **4** as (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate.

The initial ^1H NMR analysis of **1** and **2** showed > 90% and >80% purity, respectively. However, upon standing at room temperature for *ca* 4 hours, each compound underwent transesterification to form a **1/2** mixture (*ca* 3:2). Several efforts of HPLC purification only resulted in obtaining a small amount of pure **1**, indicating **1** appeared to be the more stable form. A similar phenomenon was encountered during the purification process of **3** and **4**. Since only two isomers were detected during the process, as determined by ^1H NMR and LC-MS time studies (Fig. 4), a mechanism involving the intra-molecular migration of the galloyl moiety between the *m*- and *p*-hydroxyl group was proposed (Scheme 1). The fact that the *p*-galloyl isomer existed as the relatively more stable and abundant form suggested that the *p*-hydroxyl was more reactive or acidic than *m*-hydroxyl group, as *p*-hydroxyl

groups in **2** or **4** are more likely to attack C-7'' to drive the equilibrium towards compounds **1** or **3**, respectively. Indeed, the stronger acidity of *p*-hydroxyl group has been reported in literature 15. Methylation of a mixture of *p*- and *m*-digallates with diazomethane in acetone only yielded penta-*O*-methyl-*m*-digallates as the sole product (Scheme 2).

Freshly purified compounds **1**, **3** and **5** were tested for antibacterial activity against the gram-negative bacteria, *P. aeruginosa* (strain PAO1) and the gram-positive bacterium, *S. aureus* (strain 01A1095), reported in Table 3. Since the structural isomers of compounds **1** and **3** (compounds **2** and **4**) were found to be more unstable by comparison, these were not assessed for their antibacterial activity. Due to the time period associated with the assay it is assumed that compounds **1** and **3** would, however, contain a proportion of their isomers and activity reported here is therefore more likely due to the isomer mixtures. In the antibacterial assay up to 175 μ M all compounds displayed only minor activity against both bacterial strains.

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Supporting information

¹H and ¹³C NMR spectra for compounds **1-4** are available as Supporting Information.

References

- ¹ Hui WH, Lee WK, Ng KK, Chan CK. The occurrence of triterpenoids and steroids in three *Glochidion* species. *Phytochemistry* 1970; 9: 1099-1102
- ² Talapatra B, Dutta S, Maiti BC, Pradhan DK, Talapatra SK. Terpenoid constituents of Indian *Glochidion* species: *Glochidion accuminatum* and *Glochidion thomsoni*. Partial synthesis of glochidone. *Aust J Chem* 1974; 27: 2711-2714
- ³ Hui WH, Li MM. Lupene Triterpenoids from *Glochidion eriocarpum*. *Phytochemistry* 1976; 15: 561-562
- ⁴ Hui WH, Li MM. Triterpenoids from *Glochidion macrophyllum* and *G. puberum*. *Phytochemistry* 1978; 17: 156-157
- ⁵ Srivastava R, Kulshreshtha DK. Triterpenoids from *Glochidion heyneanum*. *Phytochemistry* 1988; 27: 3575-3578
- ⁶ Zhang Z, Gao ZL, Fang X, Wang YH, Xiao H, Hao XJ, Liu GM, He HP. Two new triterpenoid saponins from *Glochidion puberum*. *J Asian Nat Prod Res* 2008; 10: 1029-1034
- ⁷ Kiem PV, Thua VK, Yen PH, Nhiem NX, Tung NH, Cuong NX, Minh CV, Huong HT, Hyun JH, Kang HK, Kim YH. New triterpenoid saponins from *Glochidion eriocarpum* and their cytotoxic activity. *Chem Pharm Bull* 2009; 57: 102-105

- ⁸ Otsuka H, Hirata E, Shinzato T, Takeda Y. Isolation of lignan glucosides and neolignan sulfate from the leaves of *Glochidion zeylanicum* (Gaertn) A. Juss. Chem Pharm Bull 2000; 48: 1084-1086
- ⁹ Cai WH, Matsunami K, Otsuka H, Shinzato T, Takeda Y. Lignan and neolignan glucosides, and tachioside 2'-O-4''-O-methylgallate from the leaves of *Glochidion rubrum*. J Nat Med 2009; 63: 408-414
- ¹⁰ Nikaido H. Multidrug efflux pumps of gram-negative bacteria. J Bacteriol 1996; 178: 5853-5859
- ¹¹ Li XZ, Nikaido H, Poole K. Role of mexA-mexB-oprM in antibiotic efflux in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1995; 39: 1948-1953
- ¹² Zhang Y, Que S, Yang X, Wang B, Qiao L, Zhao Y. Isolation and identification of metabolites from dihydromyricetin. Magn Reson Chem 2007; 45: 909-916
- ¹³ Onda M, Li SS, Li XA, Harigaya Y, Takahashi H, Kawase H, Kagawa, H. Heterocycles, XXIV. Synthesis of optically pure 2,3-*trans*-5,7,3',4',5'-pentahydroxyflavan-3,4-diols and comparison with naturally-occurring leucodelphinidins. J Nat Prod 1989; 52: 1100-1106
- ¹⁴ Nonaka GI, Muta M, Nishioka I. Myricatin, a galloyl flavanonol sulfate and prodelphinidin gallates from *Myrica rubra*. Phytochemistry 1983; 22: 237-241
- ¹⁵ Nishizawa M, Yamagishi T, Nonaka G, Nishioka I. Tannins and related compounds. Part 5. Isolation and characterization of polygalloylglucoses from Chinese gallotannin. J Chem Soc Perkin Trans I 1982; 2963-2968

Legends for Figures

Table 1: ^1H -NMR spectroscopic data for **1–4**.^a

Table 2: ^{13}C -NMR spectroscopic data for **1–4**.^a

Table 3: Antibacterial activities for compounds **1**, **3**, and **5**

Fig. **1** Structures of compounds **1–5**.

Fig. **2** The selected HMBC correlations ($\rightarrow$) of **1**.

Fig. **3** CD spectra of compounds **1–3** and **5**.

Fig. **4** LC-MS study on the transesterification process of **1** and **2** (UV=254 nm).

(a) The freshly purified **1**. (b) The freshly purified **2**. (c) The final equilibrium mixture of **1/2** (*ca* 3:2) after *ca* 4 hours or longer.

Scheme. **1** Proposed transesterification mechanism of **1–4**.

Scheme. **2** Methylation of the equilibrium mixture of *p*- and *m*-digallates [15].

Table 1

No	1	2	3	4
2	5.01 (1H, d, 10.8)	5.01 (1H, d, 11.1)	5.01 (1H, d, 10.0)	5.01 (1H, d, 10.0)
3	4.43 (1H, d, 10.8)	4.52 (1H, d, 11.1)	4.43 (1H, d, 10.0)	4.53 (1H, d, 10.0)
6	5.91 (1H, d, 1.8)	5.91 (1H, d, 1.8)	5.91 (1H, d, 1.8)	5.91 (1H, d, 1.8)
8	5.89 (1H, d, 1.8)	5.88 (1H, d, 1.8)	5.89 (1H, d, 1.8)	5.88 (1H, d, 1.8)
2'	6.52 (1H, s)	6.72 (1H, d, 1.8)	6.51 (1H, s)	6.71 (1H, d, 1.5)
6'	6.52 (1H, s)	6.85 (1H, d, 1.8)	6.51 (1H, s)	6.85 (1H, d, 1.5)
2''	7.14 (1H, d, 1.8)	7.18 (1H, d, 1.8)	7.08 (1H, s)	7.09 (1H, s)
6''	7.25 (1H, d, 1.8)	7.25 (1H, d, 1.8)	7.08 (1H, s)	7.09 (1H, s)
3''-OMe	3.81 (3H, s)	3.82 (3H, s)		
5-OH	11.85 (1H, brs)	11.87 (1H, brs)	11.85 (1H, brs)	11.87 (1H, brs)
-OH ^b	10.82 (1H, s)	10.83 (1H, s)	10.83 (1H, s)	
	9.54 (1H, s)		9.53 (1H, s)	

^a Recorded at 600 MHz in DMSO-*d*₆, δ_{H} in ppm, *J* in Hz. ^b unassigned exchangeable protons.

Table 2

No	1	2	3	4
2	82.7	82.6	82.7	82.7
3	71.6	71.4	71.6	71.5
4	197.3	197.7	197.3	197.7
5	163.3	163.3	163.3	163.3
6	96.1	96.1	96.1	96.1
7	166.8	166.8	166.8	166.8
8	95.0	95.0	95.0	95.0
4a	100.4	100.5	100.4	100.5
8a	162.3	162.4	162.3	162.5
1'	134.5	127.2	134.4	127.2
2'	106.8	113.5	106.7	113.6
3'	150.0	139.1	150.0	139.1
4'	127.2	138.1	127.2	138.1
5'	150.0	146.3	150.0	146.3
6'	106.8	112.8	106.7	112.7
1''	118.9	118.6	118.9	118.7
2''	105.4	105.4	109.2	109.2
3''	147.9	148.0	145.5	145.7
4''	139.7	139.9	138.7	139.0
5''	145.2	145.4	145.5	145.7
6''	111.3	111.3	109.2	109.2
7''	163.7	163.9	163.8	164.1
3''-OMe	56.0	56.0		

^a Recorded at 150 MHz in DMSO-*d*₆.

Table 3

compound	Bacterial growth inhibition % ($\pm$ SD)	
	<i>P. aeruginosa</i> (PAO1)	<i>S. aureus</i> (01A1095)
1	71% $\pm$ 1 ^a	49% $\pm$ 3 ^a
3	56% $\pm$ 2 ^b	70% $\pm$ 8 ^c
5	52% $\pm$ 2 ^a	NT

Ciprofloxacin (purity > 98%) was used as control: IC₅₀ = 0.038 $\pm$ 0.01 μ M (PAO1), IC₅₀ = 124.65 $\pm$ 19.78 μ M (01A1095); NT: not tested; ^a 175 μ M; ^b 17.5 μ M; ^c 350 μ M

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Scheme. 1

Scheme. 2

Supporting Information

New Galloylated Flavanonols from the Australian Plant, *Glochidion sumatranum*

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- S-1** ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate
(**1**) in DMSO- d_6
- S-2** ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate
(**1**) in DMSO- d_6
- S-3** ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate
(**2**) in DMSO- d_6
- S-4** ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate
(**2**) in DMSO- d_6
- S-5** ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-gallate (**3**) in DMSO- d_6
- S-6** ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-gallate (**3**) in DMSO- d_6
- S-7** ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**) in DMSO- d_6
- S-8** ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**) in DMSO- d_6

S-1 ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate (**1**) in $\text{DMSO}-d_6$

S-2 ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-(3''-*O*-methyl)-gallate (**1**) in $\text{DMSO-}d_6$

S-3 ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate (**2**) in $\text{DMSO}-d_6$

S-4 ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-(3''-*O*-methyl)-gallate (**2**) in $\text{DMSO}-d_6$

S-5 ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-gallate (**3**) in DMSO- d_6

S-6 ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-4'-*O*-gallate (**3**) in $\text{DMSO-}d_6$

S-7 ^1H NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**) in DMSO-*d*₆

S-8 ^{13}C NMR spectrum of (2*R*, 3*R*)-dihydromyricetin-3'-*O*-gallate (**4**) in $\text{DMSO}-d_6$