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Sepicanin A- A new geranyl flavanone from *Artocarpus sepicanus* with activity against methicillin-resistant *Staphylococcus aureus* (MRSA)

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Abstract

Bioassay-guided fractionation of the EtOH extract of *Artocarpus sepicanus* Diels leaves has led to the isolation of a new geranyl flavanone (**1**), along with the known compounds, afzelechin-3-*O*- α -L-rhamnopyranoside and β -sitosterol glucoside. The structure of the new compound was established by UV, IR, HRESIMS, 1D and 2D NMR experiments. Antimicrobial testing of the three compounds indicated that **1** displayed a significant selective antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) with IC₅₀ and MIC values of 1.4 and 2.9 μ M, respectively.

Graphical Abstract

Keywords

Flavanone; *Artocarpus sepicanus*; antibacterial; Methicillin-resistant *Staphylococcus aureus*

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1. Introduction

The genus *Artocarpus* (Moraceae) consists of approximately 50 species of large evergreen trees (Kijjoa et al., 1996). Many prenylated stilbenes (Hakim et al., 2002; Likhitwitayawuid et al., 2001; Puntumchai et al., 2004; Su et al., 2002), benzofurans (Hakim et al., 2002a, 2002b; Soekamto et al., 2003), flavonoids (Hakim et al. 2002a, Kijjoa et al. 1996; Ko et al. 2005; Suhartati et al. 2001) and chalcones (Jayasinghe et al. 2004; Shimizu et al. 2000) have been reported from various *Artocarpus* species. Several prenylated stilbenes and flavonoids showed cytotoxic (Hakim et al. 2002, Kijjoa et al. 1996, Ko et al. 2005, Suhartati et al. 2001), anti-mycobacterial (Puntumchai et al., 2004), and anti-inflammatory (Su et al. 2002; Wei et al. 2005) activities. As part of our ongoing research to investigate the antimicrobial activity of higher plants (Galal et al. 2001; Radwan et al. 2008; Ross et al. 2005, 2008) the ethanol extract of *Artocarpus sepicanus* leaves was evaluated for antimicrobial activities. Because this species has not been evaluated phytochemically, we decided to undertake a biologically-guided fractionation, which led to the isolation of a new geranyl flavanone (**1**), with an IC₅₀ value of 1.4 μ M against methicillin-resistant *Staphylococcus aureus* (MRSA), along with two known compounds, afzelechin-3-*O*-rhamnopyranoside and β -sitosterol glucoside.

2. Results and Discussions

Compound **1** was isolated as a yellow powder. The HRESIMS (positive ion mode) showed $[M+H]^+$ at m/z 425.1931 and $[M+Na]^+$ at m/z 447.1750 compatible with a molecular formula of C₂₅H₂₈O₆. The IR spectrum showed that **1** contained hydroxyl ($\nu_{\max}$ 3336 cm⁻¹) and conjugated ketone ($\nu_{\max}$ 1635 cm⁻¹) functional groups. The UV absorption bands at $\lambda_{\max}$ 213 and 290 nm were suggestive of a flavanone skeleton (Mabry et al. 1970). A bathochromic shift (36 nm) produced in the UV spectrum (methanol) after the addition of NaOAc indicated a free hydroxyl group at C-7, while its bathochromic shift on the addition of AlCl₃ and AlCl₃/HCl (20 nm) suggested the presence of an hydroxyl group at C-5 which was supported by the presence of a hydrogen bonded proton at δ 12.1 in ¹H NMR spectrum (Mabry et al. 1970). The ¹H NMR spectroscopic data of **1** (Table 1) displayed a 1,2,4-trisubstituted benzene ring B [δ 7.36 (1H, d, $J=8.4$ Hz, H-6'), δ 6.43 (1H, dd, $J=8.4, 2.0$ Hz, H-5') and δ 6.49 (1H, d, $J=2.0$, H-3')], with two phenol groups at C-2' (δ_C 158.5) and C-4' (δ_C 155.3) along with one proton singlet at δ_H 6.06 which was assigned to H-8 and correlated to δ_C 95.5 in the HMQC spectrum. The ¹³C NMR, DEPT and HMQC NMR spectroscopic data of **1** displayed 25 signals, including three methyl, four methylene, seven methine and eleven quaternary carbons. Two of these signals resonated at δ_C 74.5 and 41.9 ppm characteristic for C-2 and C-3 of the flavanone skeleton (Agrawal et al. 1989). The absolute configuration at C-2 was determined to be *S* on the basis of the strong negative cotton effect at 293 nm observed in the CD spectrum of **1** (Gaffield, W. 1970) (Supplementary data). The ¹H NMR also showed the presence of three methyl singlets at δ_H 1.56, 1.62 and 1.65, three methylenes at δ_H 1.93, 2.06 and 3.27 in addition to two olefinic protons at δ 5.07 (t, H-6''), 5.26 (t, H-2''), attributed to a geranyl substituent (Radwan et al. 2008). The geranyl moiety was located at C-6 of ring A based on HMBC correlations of H-1'' at δ 3.27 with C-6 (δ 107.5), C-5 (δ_C 164.4), and C-7 (δ_C 160.6) (Figure 1). The ¹³C NMR data further established the hydroxylation at C-5 and C-7 of ring A and at C-2' and

C-4' of ring B. Therefore, compound **1** was assigned as 2(S)-6-geranyl-5,7,2',4'-tetrahydroxy-flavanone. In fact, the structure of compound **1** has been reported by Sakamoto et al. (1989) as a copying error from Ohmoto et al. (1986). The later reported the isolation of 2(S)-5'-geranyl-5,7,2',4'-tetrahydroxy-flavanone (Ohmoto et al. 1986) in which the geranyl is located at C-5' and not at C-6. Based on the above evidence **1** is a new flavanone named Sepicanin A, and this explains the discrepancy in the literature (Ohmoto et al. 1986; Sakamoto et al. 1989).

Afzelechin-3-*O*- α -L-rhamnopyranoside and β -sitosterol glucoside were identified by comparing their spectroscopic data with literature values (Drewes et al. 1992; Koizumi et al. 1979).

The three isolated compounds were evaluated *in vitro* for antimicrobial activity against the fungi *Candida albicans*, *Aspergillus fumigatus*, *Cryptococcus neoformans*, and the bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Mycobacterium intracellulare*, and MRSA. Compound **1** showed a selective and significant antibacterial activity against MRSA with an IC₅₀ value of 1.4 μ M and MIC value of 2.9 μ M. While it did show weak antifungal activity against *Candida albicans* with IC₅₀ and MIC values of 15.3 and 47.1 μ M, respectively, it was completely inactive against the other test organisms at test concentrations of 118 μ M. Moreover, compound **1** was potentially bactericidal having a minimum bactericidal concentration (MBC) of 2.9 μ g/mL (Table 2). The remaining two compounds were inactive against all test microbes. Additionally, none of the compounds showed toxicity against Vero cells (Monkey kidney fibroblast) at concentrations up to 10.0 μ g/mL.

3. Experimental

3.1. General

Optical rotation was measured on an Autoplot IV automatic polarimeter. CD spectrum was recorded on a Jasco DIP-370 spectropolarimeter. ¹H, ¹³C, and 2D-NMR spectra were recorded using the residual solvent signal as an internal standard on a Varian AS 400 spectrometer. IR spectra were taken on a Bruker Tensor 27. UV spectra were recorded on a Hewlett-Packard 8435 spectrometer. High resolution mass spectra were measured using a Bruker BioApex FT mass spectrometer. TLC was performed on sheets precoated with silica gel 60 F₂₅₄ (Merck, Germany) using CHCl₃/MeOH (8:2). Compounds were detected by spraying with 1% vanillin in H₂SO₄/EtOH (1:9), followed by heating.

3.2. Plant material

The plant material was collected and identified by Dr. Topul Rali in Papua New Guinea in 1998 and a voucher specimen is deposited at the School of Natural and Physical Sciences, University of Papua New Guinea.

3.3. Extraction, isolation and characterization

Air-dried leaves of *A. sepicanus* (2 kg) were extracted by maceration in 95% EtOH (4 days) with frequent sonication. The ethanol extract was concentrated under vacuum to yield a residue (24 g). Twenty grams of the residue were subjected to fractionation by vacuum

liquid chromatography over a silica gel column (300 g, 160–200 mesh) eluting with *n*-hexane-EtOAc [90:10, 80:20, 60:40, 40:60, 20:80, 10:90, 100:0, (500 mL of each solvent mixture)] followed by EtOAc-MeOH [80:20, 60:40, 40:60, 20:80, (500 mL of each solvent mixture)] yielding 11 fractions (A–K). All the fractions and the total extract, were evaluated for antimicrobial activity. Fractions F and I showed selective antimicrobial activity against MRSA with IC₅₀ values of 6.00 and 20.0 µg/mL, respectively. Fraction F (5.31 g, eluted with *n*-hexane-EtOAc, 10:90) was chromatographed over a silica gel column (250 g, 4×70 cm) eluted with CH₂Cl₂-MeOH (100:10 - 80:20) to afford 46 subfrs. (F 1–46, 100 mL each). Subfractions F33–35 were combined (784 mg, IC₅₀ = 1.5 µg/mL) and subjected to a silica gel column (40 g, 1.5 × 50 cm) eluted with CH₂Cl₂-MeOH (90:10) to afford **1** (6.3 mg, IC₅₀ = 0.60 µg/mL). Fraction I (2.15 g, eluted with EtOAc-MeOH, 60:40) was fractionated on a Si gel column (100 g, 5 × 120 cm) and eluted with CH₂Cl₂-MeOH (85:15) to afford 6 subfractions (I 1–6). Subfractions I1–3 (307 mg) were combined and were crystallized from MeOH to give **3** (93 mg). Subfraction I 5 (298 mg) was applied to a silica gel column (14 g, 4 × 70 cm) with CH₂Cl₂-MeOH (90:10) as an eluant to give **2** (30 mg).

3.3. 2(S)-6-geranyl-5, 7, 2', 4'-tetrahydroxy-flavanone (**Sepicanin A**) (**1**)

Pale yellow Powder; UV (MeOH): λ_{max} 213, 290 nm, UV (NaOAc): λ_{max} 213, 326 nm, UV (AlCl₃) 213, 310 nm, UV (AlCl₃/HCl) 213, 310 nm; IR ν_{max} (neat): 3336, 1635, 1602, 1292 and 1068 cm⁻¹; for ¹H and ¹³C NMR spectroscopic data see Table 1; HRESIMS *m/z* 425.1931 [M+H]⁺ (Calc. for C₂₅H₂₉O₆, 425.1964), 447.1750 [M+Na]⁺ (Calc. for C₂₅H₂₈O₆Na, 447.1784).

3.4. Antimicrobial assay

The antibacterial and antifungal activities of the isolated compounds were evaluated according to the previously reported method (Radwan et al 2007, 2008; Ross et al. 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.
Key HMBC correlations of **1**

Table 1¹H [400 MHz] and ¹³C NMR [100 MHz] data for compound **1** [Acetone- *d*₆]

Position	δ_{H}	δ_{C}
2	5.70dd (12.8, 2.8)	74.5
3	3.08 dd (16.8, 12.8)	41.9
	2.78 dd (16.8, 2.8)	
4	---	197.0
5	---	164.4
6	---	107.5
7	---	160.6
8	6.06 s	95.5
9	---	162.1
10	---	102.2
1'	---	117.0
2'	---	158.5
3'	6.49 d (2.0)	102.6
4'	---	155.3
5'	6.43 dd(8.4, 2)	106.9
6'	7.36 d (8.4)	127.7
1''	3.27 d (6.8)	21.3
2''	5.26 t (6.8)	122.9
3''	---	133.9
4''	1.93 t (8.0)	39.6
5''	2.06 m	26.5
6''	5.07 t (6.8)	124.3
7''	---	130.6
8''	1.65	24.9
9''	1.62	16.8
10''	1.56 s	15.3

^aAssignments confirmed by DEPT, gHMQC, gCOSY and gHMBC experiments

Table 2

In vitro Antimicrobial Activity of Compound **1** (all Values in μM)^{a-c}

Sample	MRSA			<i>C. albicans</i>		
	IC ₅₀ ^a	MIC ^b	MBC ^c	IC ₅₀ ^a	MIC ^b	MFC ^c
1	1.4	2.9	2.9	15.3	47.1	47.1
amphotericin B	nt ^e	nt ^e	nt ^e	0.3	0.7	1.3
ciprofloxacin	0.4	0.8	na ^d	nt ^e	nt ^e	nt ^e

^a IC₅₀ = the test concentration that affords 50% growth.

^b MIC (minimum inhibitory concentration) = the lowest test concentration that allows no detectable growth.

^c MBC/MFC (minimum bactericidal or fungicidal concentration) = the lowest test concentration that kills the organism.

^d na = not active at the highest test concentration.

^e nt = not tested.