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Short communication

Propolins and glyasperin A from stingless bee nests

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ABSTRACT

Chemical investigation of the dichloromethane extracts of the propolis collected from bee (*Tetragonula biroi* Friese) hives in San Roque, Sorsogon, Philippines afforded propolin A (**1**), propolin E (**2**), propolin H (**3**), glyasperin A (**4**), squalene, a mixture of lupeol, α-amyrin and β-amyrin, and another mixture of urs-12-en-3-one, olean-12-en-3-one and lup-12-en-3-one. The structures of **2–4** were elucidated by extensive 1D and 2D (COSY, HSQC, and HMBC) NMR spectroscopy, while **1** was identified by comparison of its NMR data with **2**.

Introduction

Propolis has been reported to contain flavonoids and phenolic compounds that have shown pharmacological activities (Toreti et al., 2013). Propolis is known to exhibit numerous medicinal properties, including cytotoxic (Matsuno et al., 1997; Kimoto et al., 2001), free radical scavenging (Basnet et al., 1997), anti-HIV (Ito et al., 2001), antimicrobial (Park et al., 1998), and antiherpes (Vynograd et al., 2000). Reviews on the chemical constituents and biological activities of propolis have been provided (Toreti et al., 2013; Huang et al., 2007; Bankova et al., 2000).

A recent study on Philippine propolis identified by LC–MS artepillin C and pinobanksin-3-*O*-hexanoate as possible phenolic compounds in propolis of stingless bees, *Tetragonula biroi* and exudates from *Persea americana* Mill, *Artocarpus heterophyllus* Lam, *Mangifera indica* L., *Canarium ovatum* Engl, and *Nephelium lappaceum* L. that are utilized by *T. biroi* as propolis source (Belina-Aldamita et al., 2013). In another study, the flavonoids and phenolic compounds found in propolis and *M. indica*, *A. heterophyllus*, *C. ovatum*, *Chrysophyllum cainito* and *Achras sapota* were identified as pinobanksin-5,7-dimethyl ether, artepillin C, luteolin-5-methyl ether, pinobanksin-3-*O*-(butyrate or isobutyrate) and kaempferide by matching the retention time and the positive and negative ion chromatograms of the samples to the literature data of known flavonoid and phenolic compounds from the study of Gardana et al. (2007) (Alvarez et al., 2013). The ethanol extract of Philippine propolis was also reported as a potential inhibitor of QS-mediated violacein production in *Chromobacterium violaceum* (Lamberte et al., 2011).

We report herein the isolation of propolin A (**1**), propolin E (**2**), propolin H (**3**), glyasperin A (**4**), and squalene from the dichloromethane extracts of the propolis collected from bee (*T. biroi* Friese) hives in San Roque, Sorsogon, Philippines. A mixture of lupeol, α-amyrin and β-amyrin, and another mixture of urs-12-en-3-one, olean-12-en-3-one and lup-12-en-3-one were also obtained from the dichloromethane extracts of the propolis. To the best of our knowledge this is the first report on the isolation of

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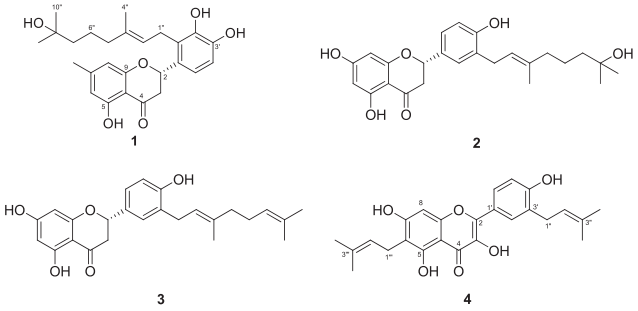
these compounds from Philippine propolis and the first report on the isolation of **4** from propolis.



Materials and methods

NMR spectra were recorded on a Varian VNMRS spectrometer in $CDCl_3$ at 600 MHz for ^1H NMR and 150 MHz for ^{13}C NMR spectra. The 2D NMR experiments (COSY, HSQC, and HMBC) for propolin E, propolin H, and glyasperin A were performed by using Varian gCOSY, gHSQCAD and gHMBCAD pulse programs. CD spectra were recorded by a JASCO J-715 spectropolarimeter. Column chromatography was performed with silica gel 60 (70–230 mesh). Thin layer chromatography was performed with plastic backed plates coated with silica gel F_{254} and the plates were visualized by spraying with vanillin/H_2SO_4 solution followed by warming.

The propolis used in this study was collected from bee (*Tetragonula biroi* Friese) hives in San Roque, Sorsogon, Philippines in May 2012.

In the first isolation, propolis (50 g) was pulverized by mortar and pestle, soaked in 200 ml of CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford the crude extract (27.5 g) which was chromatographed using increasing proportions of acetone in CH_2Cl_2 at 10% increment as eluents. The CH_2Cl_2 fraction from the chromatography of the crude extract was rechromatographed (4×) in petroleum ether to afford squalene (10 mg). The 60% acetone in CH_2Cl_2 fraction from the chromatography of the crude extract was combined and rechromatographed in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume). The less polar fractions were rechromatographed (3×) in CH_3CN:Et_2O:CH_2Cl_2 (0.5:0.5:9 by volume) to afford **2** (8 mg) after washing and trituration with petroleum ether. The more polar fractions were rechromatographed (4×) in CH_3CN:Et_2O:CH_2Cl_2 (1:1:8 by volume) to afford **1** (4 mg) after washing and trituration with petroleum ether.

In the second isolation, dried propolis (100 g) was pulverized with mortar and pestle, soaked in 400 ml CH_2Cl_2 for three days and then filtered. The filtrate was concentrated under vacuum to afford the crude extract (71 g). The crude extract was fractionated by silica gel chromatography using increasing proportions of acetone in CH_2Cl_2 (10% increment) as eluents. The 10% acetone in CH_2Cl_2 fraction was rechromatographed (2×) using 5% EtOAc in petroleum ether as eluent to afford a mixture of urs-12-en-3-one, olean-12-en-3-one, and lup-12-en-3-one (18 mg) after washing with petroleum ether. The 20% acetone in CH_2Cl_2 fraction was rechromatographed (3×) using 10% EtOAc in petroleum ether as eluent to afford a mixture of lupeol, α-amyrin, and β-amyrin (22 mg) after washing with petroleum ether. The 50% acetone in CH_2Cl_2 fraction was triturated in petroleum ether and then rechromatographed using CH_2Cl_2, followed by Et_2O:CH_3CN:CH_2Cl_2 (1:1:8 by volume) as eluents. The fractions eluted with CH_2Cl_2 were combined and rechromatographed (5×) using Et_2O:CH_3CN:CH_2Cl_2 (1:1:8 by volume) as eluent to afford **3** (15 mg) after trituration with petroleum ether. The fractions eluted with Et_2O:CH_3CN:CH_2Cl_2 (1:1:8 by volume) from the rechromatography of the 50% acetone in CH_2Cl_2 fraction were rechromatographed (6×) in the same solvent to afford **4** (12 mg) after trituration with petroleum ether. Compounds **1**, **2** and squalene were also detected in the second isolation, but were no longer purified.

Results and discussion

The structures of **2**–**4** were elucidated by extensive 1D and 2D NMR spectroscopy, while **1** was identified by comparison of its ^1H NMR data with those of **2**. Propolin A (**1**) (Chen et al., 2003, 2008), propolin E (**2**) (Chen et al., 2008; Weng et al., 2007) and propolin H (**3**) (Weng et al., 2007) were reported as constituents of Taiwanese propolis. Glyasperin A (**4**) was previously isolated from the roots of *Glycyrrhiza aspera* (Zeng et al., 1992).

Propolins with diverse biological activities have been isolated from propolis. Propolins A–F isolated from Taiwanese propolis could effectively induce human melanoma cell apoptosis and were strong antioxidant agents (Chen et al., 2004). Propolin A exhibited cytotoxic properties against human melanoma, C6 glioma, and HL-60 with IC_{50} values of 6.0, 3.5, and 7.5 μg/ml, respectively (Chen et al., 2003). Propolins A and B induced cytotoxicity effect in human melanoma A2058 cells and were strong antioxidants (Chen et al., 2007a). The anti-methicillin resistant *Staphylococcus aureus* (MRSA) activity of propolin D (MIC 8–16 mg/l) and propolin C (MIC 8–32 mg/l) was reported (Raghukumar et al., 2010). On the other hand, propolin G was reported as a potent caspase-dependent inducer of apoptosis in brain cancer cells. With Taiwanese propolis extract, propolin G demonstrated a protective effect against oxidative stress in rat cortical neurons (Huang et al., 2007). Another study reported that propolin H inhibited the proliferation of human lung carcinoma cell lines in MTT assay (Weng et al., 2007).

Authors' contributions

CYR conducted the experiments, elucidated the structures of the compounds and wrote the manuscript. C-C Shen obtained the NMR, CD and HR-MS data and reviewed the manuscript. RFG and VE conducted the experiments. RA and CC collected the propolis and contributed to the introduction. All the authors approved the final manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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