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Minor *C*-geranylated flavanones from *Paulownia tomentosa* fruits with MRSA antibacterial activity

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ABSTRACT

Exhaustive chromatographic separation of the chloroform portion of the ethanolic extract obtained from *Paulownia tomentosa* (Thunb). Steud. (Paulowniaceae) fruits has led to isolation of ten *C*-6 geranylated flavanones tomentodiplacone C–I and mimulone C–E, featured by 3′-methoxy and 4′-hydroxy or 4′-hydroxy substitution of the B-ring of the flavonoid, respectively. The structures of these compounds were determined by using mass spectrometry (including HRMS) and 1D and 2D NMR spectroscopy. The absolute configurations of the compounds at *C*-2 were determined using circular dichroism. The obtained compounds showed the presence of a geranyl moiety functionalized by a carbonyl, hydroxyl or methoxyl group, or by formation of tetrahydrofuran or fused-pyrane ring, respectively. All of the flavanones described were isolated for the first time from a natural source. The antibacterial activities of selected compounds isolated along with the previously isolated geranylated flavanones were evaluated against a common panel of microbes and MRSA strains. The selected isolated compounds were tested for their ability to affect eukaryotic translation initiation *via* dual-luciferase reporter assay (firefly and renilla).

1. Introduction

Prenylation plays an important role in the diversification of aromatic natural products, contributing to the description of more than 1000 prenylated polyphenols obtained from plants. The occurrence of prenylated flavonoids is rather limited in plant families such as *Asteraceae*, *Berberidaceae*, *Cannabaceae*, *Capparaceae*, *Euphorbiaceae*, *Fabaceae*, *Guttiferae*, *Moraceae*, *Myrsinaceae*, *Rutaceae* or *Umbellifereae* (Yazaki et al., 2009). The most frequent type of prenyl substitution of flavonoids is a 3,3-dimethylallyl side chain. Compounds with a C_5 (isopentenyl) or C_{10} (geranyl) side chain are quite abundant compared to those with a C_{15} (farnesyl) or with a further modified (e.g., by oxidation, reduction, dehydration, cyclization, or hydroxylation) prenyl moiety which are not common in nature (Barron and Ibrahim, 1996; Epifano et al., 2007; Yazaki et al., 2009). The addition of an isoprenoid chain renders the derivate molecule more effective than the parent compound from a pharmacological point of view, probably because a prenyl group increases the lipophilicity and confers on the molecule a strong affinity for biological membranes (Botta et al., 2005; Epifano et al., 2007).

Paulownia tomentosa (Thunb.) Steud. (Paulowniaceae) is a deciduous tree, about 10–20 m tall, native to China. The leaves are large and heart-shaped; the flowers are pale violet, blossoming before leaves appear. The fruits are dry, reddish-brown capsules approximately 3–4 cm long, containing numerous tiny winged

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seeds that are dispersed by wind and water (Erbar and Gülden, 2011) Previous publications have reported the content of phenolic glycosides, acylglycerols, furanquinones, naphthoquinones, iridoids, lignans, and phytosterols in MeOH and EtOH extracts obtained from *P. tomentosa* (Damtoft and Jensen, 1999; Franzyk et al., 1999; Babula et al., 2006; Kobayashi et al., 2008; Si et al., 2008; Asai et al., 2009). Furthermore, *P. tomentosa* (Thunb). Steud. (Paulowniaceae) is a rich source of prenylated flavonoids. More than 20, mostly novel compounds with a prenyl or geranyl side chain at C-6 of the flavonoid skeleton have been isolated from the flowers, fruits and leaves of *P. tomentosa* (Jiang et al., 2004; Šmejkal et al., 2007, 2008b; Asai et al., 2008; Kobayashi et al., 2008; Schneiderová et al., 2012). Only two compounds showed the presence of a five carbon side chain (Šmejkal et al., 2007; Asai et al., 2008); for the others a 10-carbon side chain was typical. Further, only a few compounds showed a geranyl moiety modified by hydroxylation at *C*-6″ or *C*-7″ (Šmejkal et al., 2007, 2008b; Asai et al., 2008; Schneiderová et al., 2012).

The antioxidant, antibacterial, antiphlogistic and cytotoxic activities of *P. tomentosa* geranyl flavonoids have been described recently (Šmejkal et al., 2007, 2008a,b, 2010; Asai et al., 2008; Hošek et al., 2010; Kollár et al., 2011). It has also been discovered that the glandular hairs on its young reproductive organs contain flavonoids at concentrations over 1000 times greater than those on the surfaces of its young leaves (Kobayashi et al., 2008). Some seasonal variations in the concentrations of *C*-geranyl flavonoids have also been described (Holubová and Šmejkal, 2011).

In this paper we report the isolation and structural elucidation of 10 new *C*-geranylated flavanones (**1**–**10**) (Fig. 1) substituted at position *C*-6 of the flavanone skeleton using 1D and 2D NMR experiments as well as MS, UV, IR, and CD. All of the isolated compounds showed a geranyl modified by formation of heterocyclic moiety (**6**, **8**, **9**), carbonyl (**1**–**3**, **7**, **10**), hydroxyl (**1**, **5**, **6**, **8**–**10**), or methoxyl (**1**, **2**, **4**, **5**, **10**) groups, respectively. We suppose that these compounds are not isolation artifacts.

The antimicrobial activity of some of the newly isolated compounds together with some previously obtained geranylated flavanones was tested against several Gram-negative and Gram-positive bacteria species, including methicillin-resistant *Staphylococcus aureus* strains, and showed varying degrees of activity.

Furthermore, some of the isolated compounds were tested to determine their ability to affect eukaryotic translation initiation *via* dual-luciferase reporter assay. The compounds tested showed little such ability.

2. Results and discussion

Compounds **1**–**10** were isolated as amorphous yellowish solids by the extensive chromatographic separation of the chloroform portion of an ethanolic extract of *P. tomentosa* fruits. The MeOH-soluble portion of the $CHCl_3$ extract was chromatographed on silica gel. On the basis of TLC and HPLC analysis, similar fractions were combined to make up 20 fractions marked alphabetically from A to T. Fractions C, D, and F were further separated using column chromatography, preparative RP-HPLC, prep. TLC or some combination of these techniques (Fig. 2).

The basic characteristics of the structures of compounds isolated were deduced by analyzing the UV and IR spectra. Compounds **1**–**7** and **10** showed similar maxima at ~230 (sh) nm, ~290 nm and ~340 (sh) nm; compounds **8** and **9** showed a different course of spectra with maxima at ~230 (sh) nm, ~275 (sh) nm, ~295 nm, ~315 (sh) nm and ~360 (sh) nm. Generally, the spectra corresponded to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ electronic transitions of the

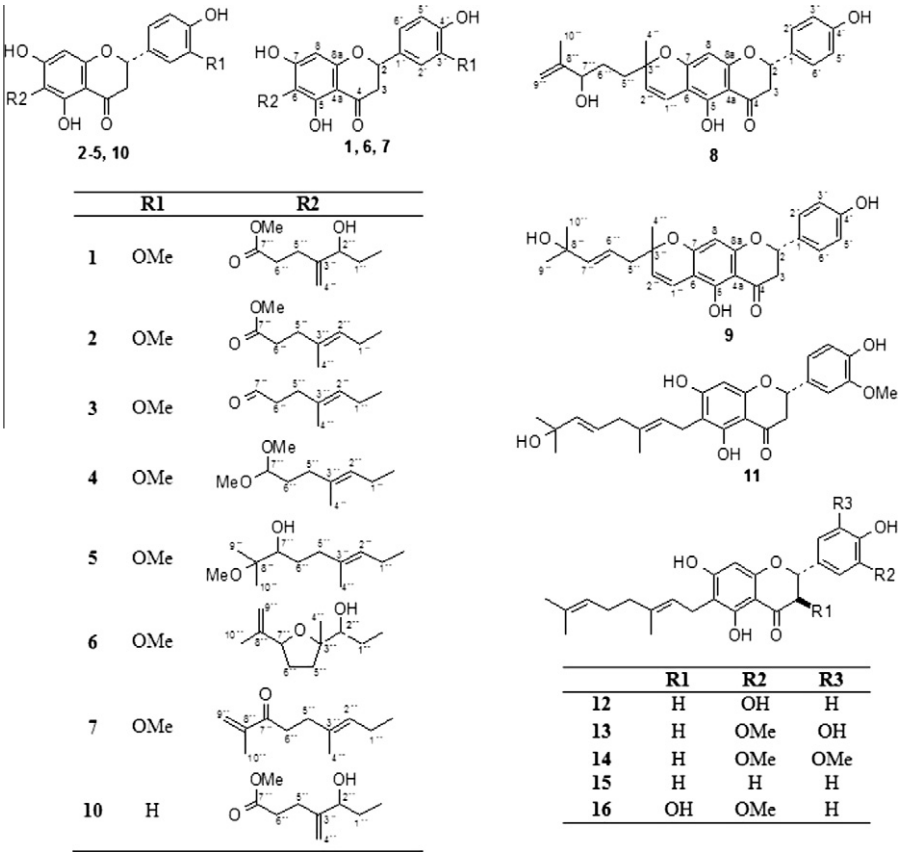

Fig. 1. Structures of compounds isolated.