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Acylated sucroses and acylated quinic acids analogs from the flower buds of *Prunus mume* and their inhibitory effect on melanogenesis ☆

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ABSTRACT

The methanolic extract from the flower buds of *Prunus mume*, cultivated in Zhejiang Province, China, showed an inhibitory effect on melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells. From the methanolic extract, five acylated sucroses, mumeoses A–E, and three acylated quinic acid analogs, 5-*O*-(*E*)-*p*-coumaroylquinic acid ethyl ester, and mumeic acid-A and its methyl ester, were isolated together with 13 known compounds. The chemical structures of the compounds were elucidated on the basis of chemical and physicochemical evidence. Inhibitory effects of the isolated compounds on melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells were also investigated. Acylated quinic acid analogs substantially inhibited melanogenesis. In particular, 5-*O*-(*E*)-feruloylquinic acid methyl ester exhibited a potent inhibitory effect [inhibition (%): 21.5 ± 1.0 ($P < 0.01$) at 0.1 μM]. Moreover, its biological effect was much stronger than that of the reference compound, arbutin [inhibition (%): 10.6 ± 0.6 ($P < 0.01$) at 10 μM]. Interestingly, the obtained acylated quinic acid analogs displaying melanogenesis inhibitory activity showed no cytotoxicity [cell viability >97% at 10 μM]. It is concluded that acylated quinic acid analogs are promising therapeutic agents for the treatment of skin disorders.

1. Introduction

Prunus (P.) *mume* SIEB. et ZUCC. (Rosaceae) has been widely cultivated as an ornamental plant in Japan, Taiwan, China, and Korea, and its fruit has been used as a food garnish (pickled ume) and drink (ume brandy). In addition, the flowers, immature fruit, leaves, branches, seeds, and roots of *P. mume* have been exploited as traditional Chinese medicines. In particular, the flowers have been prescribed for treatment of skin disorders and eye pain and for detoxification, stomachic, expectorant, and sedative purposes. Previous studies have focused on the constituents from *P. mume* and their associated bioactivities [e.g., anticancer activity (Jeong et al., 2006), effect on adrenocorticotropic hormone and catecholamine levels in plasma (Ina et al., 2004), radical scavenging activity (Matsuda et al., 2003), inhibitory activity on squalene synthase (Choi et al., 2007) and inhibitory activity on aldose reductase and platelet aggregation (Yoshikawa et al., 2002)].

Identifying inhibitors of melanin production derived from natural medicines is of interest (Fujimoto et al., 2012; Matsuda et al., 2009; Nakamura et al., 2010, 2012a,b; Nakashima et al., 2010). As a continuation of our studies on inhibitors of melanogenesis derived from medicinal flowers, it was found that a methanolic (MeOH) extract from flower buds of Chinese *P. mume* showed inhibitory effects on melanogenesis. From the MeOH extract, five new acylated sucroses, mumeoses A (**1**), B (**2**), C (**3**), D (**4**), and E (**5**), and three new acylated quinic acid analogs, 5-*O*-(*E*)-*p*-coumaroylquinic acid ethyl ester (**6**), mumeic acid-A (**7**), and mumeic acid-A methyl ester (**8**) were isolated, together with 13 known compounds (Fig. 1). In this paper, the isolation and structural elucidation of **1**–**8** are described, as well as inhibitory effects of acylated quinic acid analogs on melanogenesis in theophylline-stimulated B16 melanoma 4A5 cells.

2. Results and discussion

2.1. Isolation of compounds from the flower buds of P. mume

A MeOH extract of the dried flower buds (30.4%) of *P. mume* (cultivated in Zhejiang Province, China) showed melanogenesis inhibitory activity [inhibition (%): 31.1 ± 1.1 ($P < 0.01$) at 100 μg/mL]. The MeOH extract was partitioned into an EtOAc–H_2O (1:1, v/v) mixture to furnish an EtOAc-soluble fraction (6.6%) and an aqueous layer. The latter was further extracted with 1-butanol to give 1-butanol- (7.5%) and H_2O- (13.0%) soluble fractions. The

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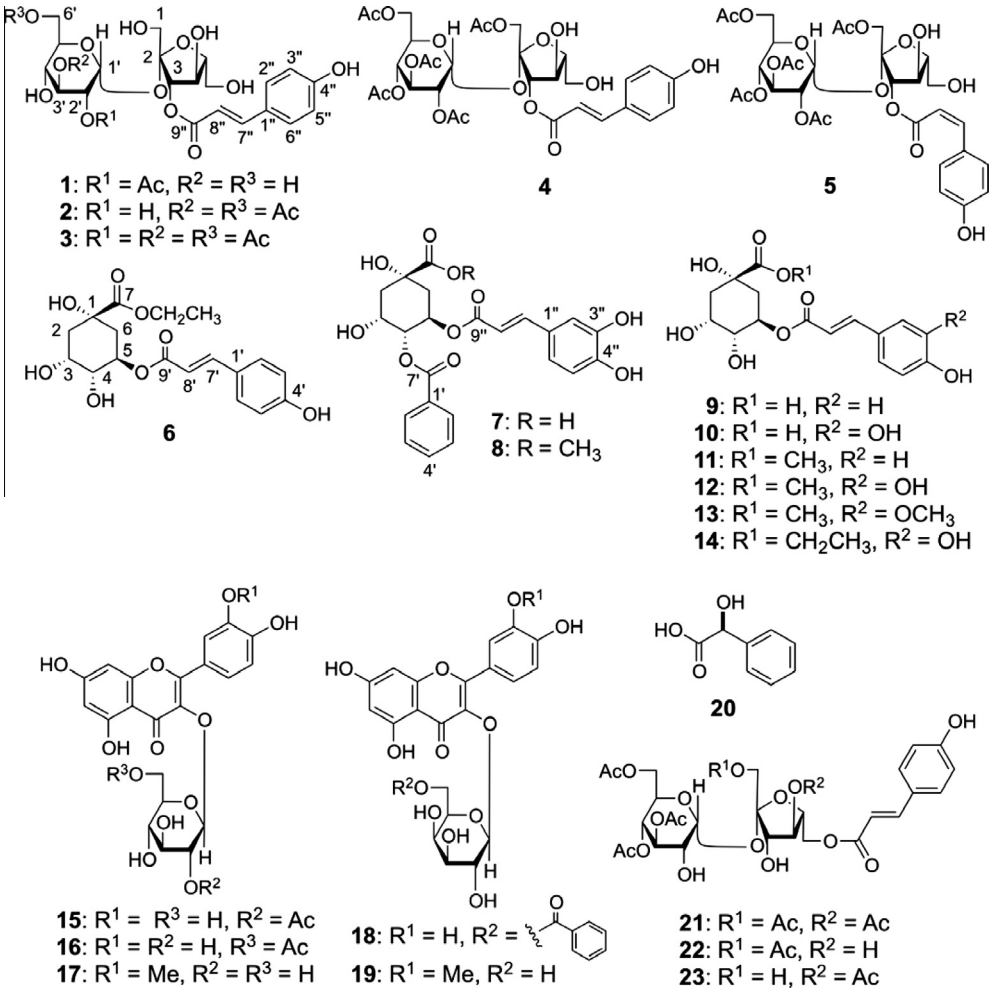

Fig. 1. Structures of compounds isolated from the flower buds of *P. mume*.

1-butanol- and the EtOAc-soluble fractions were found to have significant inhibitory effects on melanogenesis [inhibition (%): 27.5 ± 6.8 ($P < 0.01$), 41.5 ± 1.9 ($P < 0.01$), respectively at 100 μg/mL], but the H_2O-soluble fraction showed no detectable effect even at 100 μg/mL. The 1-butanol- and the EtOAc-soluble fraction were then subjected to normal- and reversed-phase silica-gel column chromatography and repeated HPLC. From the 1-butanol-soluble fraction, three new acylated sucroses, mumeose A (**1**, 0.00032%), B (**2**, 0.0010%), and C (**3**, 0.00050%), and a new acylated quinic acid analog, 5-*O*-(*E*)-*p*-coumaroylquinic acid ethyl ester (**6**, 0.0031%), were isolated together with eight known compounds, 5-*O*-(*E*)-*p*-coumaroyl quinic acid (**9**, 0.015%) (Parejo et al., 2004), chlorogenic acid (**10**, 0.11%) (Yoshikawa et al., 1999), 5-*O*-(*E*)-*p*-coumaroyl quinic acid methyl ester (**11**, 0.0013%) (Jaiswal and Kuhnert, 2011), chlorogenic acid methyl ester (**12**, 0.11%) (Zhu et al., 2005), 5-*O*-(*E*)-feruloylquinic acid methyl ester (**13**, 0.0013%) (Smarrito et al., 2008), chlorogenic acid ethyl ester (**14**, 0.038%) (Abe and Marumo, 1972), quercetin 3′-*O*-(2″-*O*-acetyl)-β-D-glucopyranoside (**15**, 0.0011%) (Machida et al., 2009), and D-mandelic acid (**20**, 0.047%). From the EtOAc-soluble fraction, two new acylated sucroses, mumeoses D (**4**, 0.00047%) and E (**5**, 0.00023%) were isolated, as well as two new acylated quinic acid analogs, mumeic acid-A (**7**, 0.0039%) and mumeic acid-A methyl ester (**8**, 0.0034%), together with seven known compounds, 5-*O*-(*E*)-*p*-coumaroylquinic acid methyl ester (**11**, 0.0014%), chlorogenic acid methyl ester (**12**, 0.016%), chlorogenic acid ethyl ester (**14**, 0.0027%), quercetin 3′-*O*-(2″-*O*-acetyl)-β-D-glucopyranoside (**15**, 0.0016%), quercetin 3-*O*-(6″-*O*-acetyl)-β-D-glucopyranoside (**16**, 0.0010%) (Wang et al., 2008), isorhamnetin 3-*O*-β-D-glucopyranoside (**17**, 0.017%) (Beck and Häberlein, 1999), quercetin 3-*O*-(6″-*O*-benzoyl)-β-D-galactopyranoside (**18**, 0.00059%) (Singh et al., 2009), isorhamnetin 3-*O*-β-D-galactopyranoside (**19**, 0.0006%) (Hsich et al., 2004), and quercetin (0.0068%). In this case, acylated sucroses, prunoses I (**21**), II (**22**), III (**23**), which were isolated from the flower buds of Japanese *P. mume* (Yoshikawa et al., 2002; Matsuda et al., 2003), were not detected in the flower buds of Chinese *P. mume.*

*2.2. Structures of mumeoses A–E (**1**–**5**), 5-O-(E)-p-coumaroylquinic acid ethyl ester (**6**), mumeic acid-A (**7**), and mumeic acid-A methyl ester (**8**)*

Mumeose A (**1**) was isolated as a white amorphous powder with positive optical rotation (**1**: $[\alpha]_D^{15}$ +114.8, in MeOH). Its IR spectrum showed absorption bands at 3400, 1730, 1697, 1603, 1515, and 1033 cm^{-1} due to hydroxy, ester, α,β-unsaturated ester, aromatic ring, and ether functions. FABMS in the positive-ion mode gave a quasimolecular ion peak ($[M+Na]^+$) at *m/z* 553, from which the molecular formula $C_{23}H_{30}O_{14}$ was determined by high-resolution (HR) MS. Treatment of **1** with a 10% aqueous KOH-1,4-dioxane (1:1, v/v) mixture yielded D-sucrose, which was identified by comparison of the retention time and optical rotation (t_R: 19.8 min with positive rotation) with that of an authentic sample on