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Hepatoprotective triterpenes from traditional Tibetan medicine *Potentilla anserina*

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ABSTRACT

A methanol extract from the tuberous roots of *Potentilla anserina* (Rosaceae) exhibited hepatoprotective effects against D-galactosamine (D-GalN)/lipopolysaccharide-induced liver injuries in mice. Six triterpene 28-*O*-monoglucopyranosyl esters, potentillanosides A–F, were isolated from the extract along with 32 known compounds, including 15 triterpenes. The structures of potentillanosides A–F were determined on the basis of spectroscopic properties and chemical evidence. Four ursane-type triterpene 28-*O*-monoglycosyl esters, potentillanoside A (IC_{50} = 46.7 μM), 28-*O*-β-D-glucopyranosyl pomolic acid (IC_{50} = 9.5 - μM), rosamutin (IC_{50} = 35.5 μM), and kaji-ichigoside F1 (IC_{50} = 14.1 μM), inhibited D-GalN-induced cytotoxicity in primary cultured mouse hepatocytes. Among these four triterpenes, potentillanoside A, rosamutin, and kaji-ichigoside F1 exhibited *in vivo* hepatoprotective effects at doses of 50–100 mg/kg, p.o. The mode of action was ascribable to the reduction in cytotoxicity caused by D-GalN.

Introduction

The plant *Potentilla anserina* L. (Rosaceae) is widely distributed in the western areas of China, particularly in the Qinghai-Tibetan Plateau (Wang et al., 2010; Xia and You, 2011). In traditional Tibetan medicine, roots of this plant have been used to treat malnutrition, anemia, diarrhea, and haemorrhage (Chen et al., 2010). Several chemical constituents of this plant, such as tannins (Schimmer and Lindenbaum, 1995), flavan-3-ols and flavonoids (Kombal and Glasl, 1995), triterpenes (Chu et al., 2008; Li et al., 2003), triterpene glycosides (Zhao et al., 2008), polysaccharides (Chen et al., 2010; Wang et al., 2010), and amino acids (Xia and You, 2011) have been reported. In addition, biological activities such as antimutagenic (Schimmer and Lindenbaum, 1995), anti-hepatitis B virus (Zhao et al., 2008), and immunomodulatory activities (Chen et al., 2010) of the extracts and/or constituents have been reported. During our studies on medicinal herbs in Tibet and Xinjiang autonomous regions in China, such as *Cistanche tubulosa* (Morikawa et al., 2010a,b; Pan et al., 2010; Xie et al., 2006; Yoshikawa et al., 2006), *Punica granatum* (Xie et al., 2008), and *Poacynum hendersonii* (Morikawa et al., 2012), a methanol extract of the tuberous roots of *P. anserina* was found to have a protective effect against liver injuries induced by D-galactosamine (D-GalN)/lipopolysaccharide (LPS) in mice. From this methanol extract, we have isolated six new triterpene 28-*O*-monoglucopyranosyl esters named potentillanosides A–F (**1**–**6**) along with 32 compounds, including 15 triterpenes (**7**–**21**). This study deals with the isolation and structural elucidation of these new triterpenenes (**1**–**6**) and their hepatoprotective effects and their possible mode of action.

Results and discussion

Effects of P. anserina methanol extract and its fractions on D-GalN/LPS-induced liver injuries in mice

Dried tuberous roots of *P. anserina* were extracted with methanol under conditions of reflux to yield a methanol extract (23.0% from dried material). The methanol extract at a dose of 500 mg/kg, p.o. in mice showed inhibitory effects against an increase in serum levels of aspartate aminotransaminase (sAST) and alanine transaminase (sALT), which are the markers of liver injuries induced by D-GalN/LPS (Table 1). Following this, the methanol extract was partitioned into ethyl acetate (EtOAc)–H_2O mixture (1:1, v/v) to furnish an EtOAc-soluble fraction (0.58%) and an aqueous phase. The latter was subjected to Diaion HP-20 column chromatography (H_2O → MeOH) to yield H_2O- and MeOH-eluted fractions (21.5% and 0.73%, respectively). A bioassay-guided fractionation established that the EtOAc-soluble and MeOH-eluted fractions were active (percentage inhibition at 250 mg/kg, p.o., 95.8% and 85.1%, respectively, for sAST and 97.0% and 99.1%,

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Table 1
Inhibitory effects of the methanol extract from the tuberous roots of *P. anserina* and its fractions on D-GalN/LPS-induced liver injuries in mice.

Treatment	Dose (mg/kg, p.o.)	*n*	sAST		sALT	
			(Karmen unit)	Inhibition (%)	(Karmen unit)	Inhibition (%)
Normal (vehicle)	–	8	107 ± 9[b]	–	20 ± 2[b]	–
Control (D-GalN/LPS)	–	12	5572 ± 768	–	3703 ± 515	–
MeOH extract	250	6	4231 ± 994	24.5	2649 ± 765	28.6
	500	8	2029 ± 673[a]	64.8	1158 ± 336[b]	69.1
Control (D-GalN/LPS)	–	12	5344 ± 1100	–	3600 ± 881	–
EtOAc-soluble fraction	250	7	328 ± 71[b]	95.8	129 ± 47[b]	97.0
MeOH-eluted fraction	250	6	886 ± 426[b]	85.1	394 ± 225[b]	89.6
H_2O-eluted fraction	500	6	2906 ± 1396	46.6	2445 ± 1428	32.3
Normal (vehicle)	–	5	95 ± 5[b]	–	19 ± 1[b]	–
Control (D-GalN/LPS)	–	8	9126 ± 1477	–	9830 ± 1650	–
Hydrocortisone[c]	10	7	627 ± 262[b]	94.2	247 ± 123[b]	97.7

Each value represents the mean ± S.E.M.
Significantly different from the control.
[a] $p < 0.05$.
[b] $p < 0.01$.
[c] Commercial hydrocortisone was purchased from Sigma–Aldrich Chemical (St. Louis, MO, USA) (Morikawa et al., 2010a).

respectively, for sALT), whereas the H_2O-eluted fraction showed no notable activity (Table 1).

Isolation

The active fractions, the EtOAc-soluble and MeOH-eluted fractions, were subjected to normal-phase silica gel and reversed-phase ODS column chromatographic purification steps, and finally by HPLC to furnish potentillanosides A (**1**, 0.013%), B (**2**, 0.00067%), C (**3**, 0.00005%), D (**4**, 0.00046%), E (**5**, 0.0015%), and F (**6**, 0.00047%), respectively. Additionally, 15 triterpenes, i.e., pomolic acid (**7**, 0.0027%) (Amimoto et al., 1992; Kuang et al., 1989), 28-*O*-*β*-D-glucopyranosyl pomolic acid (**8**, 0.00033%) (Amimoto et al., 1992), tormentic acid (**9**, 0.0074%) (Kuang et al., 1989; Taniguchi et al., 2002), rosamutin (**10**, 0.063%) (Jia et al., 1993), euscaphic acid (**11**, 0.00037%) (Guang-Yi et al., 1989; Kuang et al., 1989), kaji-ichigoside F1 (**12**, 0.0085%) (Guang-Yi et al., 1989; Seto et al., 1984), 2*α*,19*α*-dihydroxy-3-oxours-12-en-28-oic acid (**13**, 0.00088%) (Taniguchi et al., 2002), 2-oxopomolic acid (**14**, 0.00006%) (D'Abrosca et al., 2005), 2-oxopomolic acid 28-*O*-*β*-D-glucopyranosyl ester (**15**, 0.00049%) (Jia et al., 1993), 2*α*-hydroxyursolic acid (**16**, 0.00020%) (Kuang et al., 1989; Taniguchi et al., 2002), cecropiacic acid (**17**, 0.00047%) (Lontsi et al., 1987), maslinic acid (**18**, 0.00029%) (Kuang et al., 1989; Taniguchi et al., 2002), 24-deoxy-sericoside (**19**, 0.011%) (Zhou et al., 1992), arjunglucoside I (**20**, 0.00010%) (Abe and Yamauchi, 1987), and alphitolic acid (**21**, 0.00004%) (Kuang et al., 1989; Yagi et al., 1978), were isolated (Fig. 1) together with gallic acid (0.00032%) (Nawwar et al., 1982), gallic acid methyl ester (0.00017%) (Khalid et al., 1989), ellagic acid (0.00081%) (Khac et al., 1990; Nawwar et al., 1994), ellagic acid 4-*O*-*α*-L-arabinofuranoside (0.0011%) (Zafrilla et al., 2001), ducheside B (0.0010%) (Ye and Yang, 1996), (+)-catechin (0.00058%) (Davis et al., 1996; Jia et al., 1993; Khalid et al., 1989), (+)-gallocatechin (0.0029%) (Davis et al., 1996), (+)-catechin 7-*O*-*β*-D-glucopyranoside (0.0015%) (Kashiwada et al., 1986), quercetin 3-*O*-*β*-D-glucuropyranosiduronic acid (0.00018%) (Möhle et al., 1985), quercetin 3-*O*-sambubioside (0.00015%) (Webby, 1991), quercetin 3-*O*-*β*-D-xylopyranosyl-(1 → 2)-*β*-D-glucopyranoside-3′-*O*-*β*-D-glucopyranoside (0.00014%) (Hübner et al., 1999), 6-*O*-*p*-coumaroylsucrose (0.00067%) (Gouda et al., 2006), 6-*O*-feruloylsucrose (0.00011%) (Bokern et al., 1991), and L-tryptophan (0.016%) (Fig. S1).

*Structures of potentillanosides A–F (**1**–**6**)*

Potentillanoside A (**1**) was obtained as an amorphous powder and had a positive optical rotation ($[\alpha]_D^{26}$ + 20.4 in MeOH). Its IR

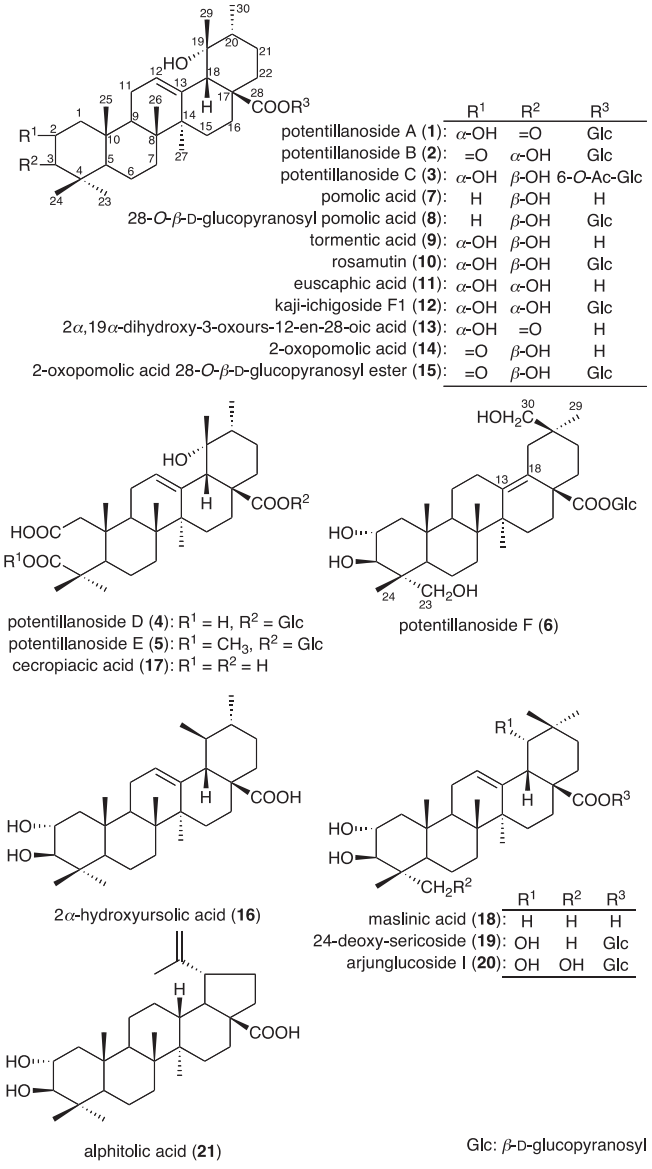

Fig. 1. Triterpene constituents (**1**–**21**) from the tuberous roots of *P. anserina*.