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ScienceDirect
Phytochemistry 69 (2008) 1609–1616
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Triterpenoid saponins from the fruits and galls of *Sapindus mukorossi*

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Received 1 December 2006; received in revised form 31 August 2007
Available online 10 March 2008

Abstract

Six saponins, sapinmusaponin K (**1**) [hederagenin-3-*O*-(3-*O*-acetyl-α-L-arabinopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], sapinmusaponin L (**2**) [hederagenin-3-*O*-(4-*O*-acetyl-α-L-arabinopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabino-pyranoside], sapinmusaponin M (**3**) [hederagenin-3-*O*-(2,3-*O*-diacetyl-β-D-xylopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], sapinmusaponin N (**4**) [hederagenin-3-*O*-(2,4-*O*-diacetyl-β-D-xylopyranosyl)-(1 → 3)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside], sapinmusaponin O (**5**) [3,7,20(*S*)-trihydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside], and sapinmusaponin P (**6**) [3,7,20(*R*)-trihydroxydammar-24-ene-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside], along with seven known saponins (**7**–**13**), were isolated from fruits and the galls of *Sapindus mukorossi*. Their structures were elucidated by 1D and 2D NMR spectroscopic techniques and acid hydrolysis. Biological evaluation indicated that saponins **1**–**4** and **7**–**13** showed moderate cytotoxicity against several human tumor cell lines.

Keywords: *Sapindus mukorossi*; Sapindaceae; Oleanane-type saponins; Dammarane-type saponins; Cytotoxic activity

1. Introduction

The fruit of *Sapindus mukorossi* Gaertn. (Sapindaceae), better known as soapnuts in tropical and sub-tropical regions of Asia (Nakayama et al., 1986), is generally used a commercial cleanser and has been shown to have medical applications based on its usages including antidermatophytic, antitussive, and antihelmintic activities (Waller and Yamasaki, 1996). In our work on the development of naturally-occurring bioactive agents, we have recently reported the isolation of several dammarane-type (sapinmusaponins A–E) (Kuo et al., 2005) and tirucallane-type (sapinmusaponins F–J) (Huang et al., 2006; Ni et al., 2006) saponins from the galls of *S. mukorossi*. A biological evaluation indicated that dammarane-type saponins had moderate cytotoxicity and that tirucallane-type saponins showed potent anti-platelet aggregation activity. The pericarps of *S. mukorossi* were also studied and several oleanane-type saponins that exhibited molluscicidal effects against *Pomacea canaliculata* and moderate cytotoxicity against human tumor cells (Huang et al., 2003) were isolated. Upon further investigation of the other parts of *S. mukorossi*, we have isolated and characterized four new oleanane-type saponins, sapinmusaponins K–N (**1**–**4**), and seven known saponins (**7**–**13**) from the EtOH extract of fruits of the title plant. In addition, further isolation of other active fractions from the previously collected galls of the title plant led to two dammarane-type saponins, sapinmusaponin O (**5**) and sapinmusaponin P (**6**). The structures of all newly isolated saponins (**1**–**6**) were

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doi:10.1016/j.phytochem.2007.10.033

established by spectroscopic analyses, mainly 2D NMR techniques, and chemical methods. Moreover, all of the isolated triterpenoid saponins (**1**–**13**), together with previously isolated **14** and **15** (Kuo et al., 2005), were evaluated for cytotoxicity against several human tumor cell lines.

2. Results and discussion

The EtOH extract of the fruit of *S. mukorossi* was extracted successively with *n*-hexane, $CHCl_3$ and *n*-BuOH. After evaporation of the $CHCl_3$ solvent, the residue was successively subjected to column chromatography on silica gel, Diaion HP-20 and Sephadex LH-20, and then separated by HPLC to give **1**–**4** and **7**–**13**. Compounds **5** and **6** were obtained as described in Section 3 by repeated chromatography on silica gel, Sephadex LH-20, and HPLC of the EtOH extract of the galls of the title plant.

The molecular formula of **1** was determined to be $C_{48}H_{76}O_{17}$ by HR-FAB-MS, which exhibited a quasi-molecular ion peak at *m/z* 947.4974 $[M+Na]^+$. The IR spectrum showed absorptions at 3398 (OH) and 1692 (C=O of COOH), 1458 (C=C), 1085 (C–O–C) cm^{-1}. The ^1H, ^{13}C NMR and DEPT spectra displayed six singlet methyls (δ_H 0.91, 0.93, 0.99, 1.01, 1.11 and 1.22; δ_C 14.1, 16.0, 17.4, 23.6, 26.1, and 33.2), an olefinic (δ_H 5.45; δ_C 122.5 and 144.7), and three anomeric signals (δ_H 5.00, 5.24 and 6.27; δ_C 101.1, 104.5 and 107.3), suggesting that **1** possessed a oleanane-type triterpene along with three sugar moieties (Table 1) (Abdulmagid Alabdul et al., 2006). Other resonances included an oxygenated methylene (δ_H 3.90, 4.26; δ_C 64.0), a carboxyl carbon (δ_C 180.2), and an acetate group (δ_C 21.0, δ_C 170.6; δ_H 2.01). Acid hydrolysis of **1** with 1 N HCl gave L-arabinose and L-rhamnose (2:1) as the component sugars, which were further treated with 1-(trimethylsilyl)imidazole and identified by comparison with authentic samples in a GC analysis. These findings indicated that saponin **1** possessed a hederagenin saponin with two L-arabinose units and an L-rhamnose unit, as well as an acetate group (Kanchanapoom et al., 2001). In the TOCSY spectrum of **1** (Fig. 1), the anomeric proton that was ascribed to L-arabinose [δ_H 5.00 (Ara-1′)] showed connectivity with three methines [δ_H 4.53 (Ara-2′), 3.95 (Ara-3′), and 4.09 (Ara-4′)] and two methylene protons [δ_H 4.20 (Ara-5a′), and 3.62 (Ara-5b′)]. The TOCSY spectrum also showed correlations between each glycosidic H-atom for the L-rhamnose and terminal L-arabinose. Moreover, the α-anomeric configurations of the L-arabinose ($J = 6.0$, 7.5 Hz) and L-rhamnose ($J = br\ s$) units were confirmed by their coupling constants (Lavaud et al., 2001). As to

Table 1
^{13}C and ^1H NMR spectroscopic data of the sugar moieties of sapinmusaponins K (**1**), L (**2**), M (**3**), and N (**4**)[a]

	1[b]		**2**[c]			**3**[d]		**4**[d]	
	^{13}C	^1H	^{13}C	^1H		^{13}C		^{13}C	^1H
Ara-					Ara-				
1′	104.5	5.00 *d* (6.0)	104.5	5.05 *d* (6.4)	1′	104.5	4.53 *d* (6.0)	104.0	4.52 *d* (6.0)
2′	75.1	4.53 *t* (7.5)	75.1	4.58 *t* (7.2)	2′	76.5	3.73 *t* (6.0)	76.3	3.69[e]
3′	75.1	3.95[e]	75.1	4.01 *dd* (8.4, 3.2)	3′	73.8	3.71[e]	73.8	3.68 *dd* (8.4, 3.6)
4′	69.5	4.09 *br s*	69.5	4.10 *br s*	4′	69.2	3.77 *br s*	69.3	3.74 *br s*
5′	66.2	4.20 *dd* (11.5, 2.5)	66.2	4.25[e]	5′	65.1	3.84 *br d* (12.4)	65.1	3.84 *br d* (12.0)
		3.62 *br d* (11.5)		3.68 *br d* (12.4)			3.52 *dd* (12.0, 2.0)		3.51 *dd* (11.6, 2.4)
Rha-					Rha-				
1″	101.0	6.27 *br s*	101.3	6.34 *br s*	1″	101.4	5.17 *d* (1.6)	101.3	5.16 *d* (1.6)
2″	71.9	4.84 *br s* ($W_{1/2}$ 3.0)	71.9	4.90 *br* ($W_{1/2}$ 2.8)	2″	71.6	4.06 *dd* (2.8, 2.0)	71.6	4.04 *dd* (3.6, 2.0)
3″	82.5	4.73 *dd* (9.0, 3.0)	82.6	4.78 *dd* (9.2, 2.8)	3″	81.5	3.83 *dd* (9.6, 2.8)	81.2	3.79 *dd* (9.6, 3.6)
4″	72.3	4.42 *t* (9.0)	72.3	4.43 *t* (9.2)	4″	72.5	3.45 *t* (9.6)	72.7	3.46 *t* (9.6)
5″	69.6	4.69 *dd* (9.0, 6.0)	69.5	4.73 *dd* (9.2, 6.0)	5″	70.5	3.86 *dd* (9.6, 6.0)	70.5	3.85 *dd* (9.6, 6.4)
6″	18.3	1.49 *d* (6.0)	18.3	1.55 *d* (6.0)	6″	17.9	1.23 *d* (6.0)	18.8	1.24 *d* (6.4)
Ara-					Xyl-				
1‴	107.3	5.24 *d* (7.5)	107.2	5.32 *d* (7.6)	1‴	104.0	4.77 *d* (7.6)	104.5	4.73 *d* (7.2)
2‴	70.0	4.69[e]	73.2	4.43 *t* (7.2)	2‴	73.5	4.81 *t* (9.2)	76.9	4.90 *t* (8.8)
3‴	77.1	5.30[e]	73.0	4.24 *dd* (7.2, 2.8)	3‴	76.9	4.99 *t* (9.2)	72.5	3.76 *t* (8.8)
4‴	67.1	5.46 *br s*	73.2	5.52 *br s*	4‴	69.2	3.79 *m*	72.4	4.74 *m*
5‴	66.9	4.13 *dd* (11.5, 2.5)	64.5	4.14 *dd* (12.4, 2.4)	5‴	66.7	3.96 *dd* (11.6, 6.0)	63.1	4.06 *dd* (12.4, 6.6)
		3.73 *br d* (11.5)		3.77 *br d* (12.4)			3.33[e]		3.33[e]
CH_3CO	170.6	2.01 *s*	170.6	2.02 *s*	CH_3CO	172.2	2.01 *s*, 2.02 *s*	172.3	2.07 *s*, 2.11 *s*
	21.0		21.0			172.1		172.0	
						20.8		20.7	
						20.8		21.0	

[a] *J* values (Hz) in parentheses.
[b] 125 MHz for ^{13}C in C_5D_5N.
[c] 100 MHz for ^{13}C in C_5D_5N.
[d] 100 MHz for ^{13}C in CD_3OD.
[e] Overlapping signals.