Research Article

Triterpenoid Saponins from the Pericarps of *Sapindus mukorossi*

Amita Sharma, Satish Chandra Sati, Om Parkash Sati,
Maneesha Dobhal Sati, and Sudhir Kumar Kothiyal

Department of Chemistry, Hemwati Nandan Bahuguna Garhwal University, Srinagar, Garhwal 246174, India

Correspondence should be addressed to Amita Sharma; amitasharma25fgh@gmail.com

Received 11 February 2012; Revised 5 April 2012; Accepted 5 April 2012

Academic Editor: Lian-Wen Qi

Copyright © 2013 Amita Sharma et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

A novel acetylated triterpene bisdesmoside saponin is elucidated as named Hederagenin 3-O- α -L-rhamnopyranosyl (3 $\rightarrow$ 1)-[2,4-O-diacetyl- α -L-arabinopyranosyl]-28-O- β -D-glucopyranosyl-(2 $\rightarrow$ 1)[3-O-acetyl- β -D-glucopyranosyl] ester (**1**) along with two known saponins, hederagenin 3-O-(α -L-arabinopyranoside-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside (**2**) and hederagenin 3-O-[β -D-xylopyranosyl-(1 $\rightarrow$ 3)- α -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- α -L-arabinopyranoside] (**3**), from the pericarps of *Sapindus mukorossi*. The structures of these saponins were characterized by means of chemical and spectral methods including advanced 2D NMR studies.

1. Introduction

Sapindus mukorossi Gaertn. of family *Sapindaceae* is well known as soap nut tree, which distributed in tropical and subtropical regions of Asia. Fruits of *Sapindus mukorossi* are popular ingredient in *Ayurvedic shampoos* and cleansers. The plant is an important remedy for relieving cough, detoxification, emetic, contraceptive, treatment of excessive salivation, epilepsy, and chlorosis [1–3]. Previous studies on the plants of this genus led to isolation and identification of triterpenoids, saponins [4–7], fatty acids [8], and flavonoids [9], from the pericarp, stem, and fruit of the plant. The present paper illustrates the isolation and structure revelation of a novel acetylated triterpene bisdesmoside saponin compound **1** (Figure 1) with the help of modern spectroscopic methods.

2. Result and Discussion

Compound **1** was obtained as an amorphous powder (85 mg), m.p. 278–280°C, positive to the Libermann-Burchard and Molish reagents. The IR spectrum showed absorptions at 3398 (OH), 1692 (C=O of COOH), and esteric band at 1719 cm^{-1} . The ESI-MS of **1** indicated the loss of masses of 43, 132, 146, and 162 due to loss of acetyl group, arabinose, rhamnose, and glucose from molecule, respectively (Figure 1).

These sugars were identified as Co-TLC comparison of the layer of the acid hydrolysate of **1** with authentic sugar samples. The pseudomolecular ion peak at m/z 1201.3467 $[\text{M}]^+$ indicated molecular formula $\text{C}_{59}\text{H}_{92}\text{O}_{25}$. The next ion peak at m/z 1085 showed the removal of two acetyl group, 985, 839, 634, and 473, respectively. The other ion peaks at m/z 985 showed removal of arabinose, at m/z 839 removal of rhamnose, and at m/z 634 and 473 removal of two glucose group, respectively. The amputation of water molecule at m/z 455 indicated the presence of aglycone hederagenin, molecular weight 456 and molecular formula $\text{C}_{30}\text{H}_{48}\text{O}_3$. The ^1H NMR (400 MHz, $\text{DMSO}-d_6$) spectra of protons of each of the six methyl group of aglycone appeared as singlets at δ_{H} 0.73, 0.77, 0.82, 1.70, 0.91, and 0.94 of 3H for each H-24, H-25, H-26, H-27, H-29, and H-30, respectively. The H-12 proton appeared as a triplet at δ 5.5 ($J = 3.5$ Hz) and H-3 as multiplet signal at 3.15. In ^1H NMR spectrum the four anomeric proton signals appeared as δ 4.43 (H-1'), 4.42 (H-1''), 4.73 (H-1'''), and 4.5 (H-1'''). The first three signals appeared as doublet while H-1'''' appeared as multiplet signal. The J values were calculated as $J_{1'-2'} = 7.5$, $J_{1''-2''} = 7.6$, $J_{1'''-2'''} = 7.2$, and $J_{1''''-2''''} = \text{multiplet}$, respectively. These coupling constants indicated the β -D pyranosyl configuration for glucose and α -L pyranosyl for arabinose and rhamnose, respectively. The methyl proton of

FIGURE 1: Mass fragmentation of compound **1**.

TABLE 1: ^{13}C , ^1H , and HMBC data of compound **1** in DMSO d_6 .

Position	Aglycone δ_{C} ppm	δ_{H} ppm	HMBC	Position	Glycone δ_{C} ppm	δ_{H} ppm	HMBC
(1)	38.05	1.36		Glc (Inner)			
(2)	26.10	—		1'	94.6	4.45 (d, 7.5)	C-28
(3)	81.20	2.73		2'	74.02	3.21 (dd, 7.2, 8.8)	
(4)	42.92	—		3'	79.05	3.84 (t, 8.8)	C-1''
(5)	15.55	—		4'	74.06	3.32 (m)	
(6)	26.13	—		5'	64.35	3.86 (d, 10.4)	
(7)	33.15	—				3.68 (dd, 10.4, 4.8)	
(8)	41.02	—		Glc (Terminal)			
(9)	47.05	—		1''	104.54	4.42 (d, 7.6)	
(10)	42.53	—		2''	73.09	3.26 (dd, 7.6, 8.4)	
(11)	27.82	—		3''	79.95	3.15 (t, 8.4)	
(12)	123.6	5.24	C-13	4''	69.53	3.23 (t, 8.4)	
(13)	145.02	—		5''	69.63	3.34 (m)	
(14)	45.48	—		6''	68.03	3.83 (d, 11.6)	
(15)	26.13	—				3.60 (dd, 11.6, 5.6)	
(16)	22.34	—		CH ₃ CO	170.02	2.02 (d, 7.6)	H-3''
(17)	46.23	—			23.05		
(18)	41.63	—		Rha			
(19)	45.52	—		1'''	102.05	4.73 (d, 7.2)	C-3
(20)	30.34	—		2'''	72.07	3.80 (dd, 3.2, 1.2)	
(21)	33.91	—		3'''	81.08	3.63 (dd, 9.6, 3.2)	C-1'''
(22)	35.31	—		4'''	73.00	3.36 (t, 9.6)	
(23)	64.24	—		5'''	70.07	3.66 (dd, 9.6, 6.0)	
(24)	12.83	0.82		CH ₃	17.52	1.25 (d, 6.0)	
(25)	16.77	0.73	C-9,1	Ara			
(26)	17.35	1.70		1''''	107.05	4.53 (m)	
(27)	25.72	0.91		2''''	71.05	3.69 (br, s)	
(28)	178.83	—		3''''	72.00	3.68 (m)	
(29)	32.92	0.94	C-19	4''''	68.94	3.75 (m)	
(30)	24.54	0.97	C-21	5''''	63.95	5.17 (d, 1.2)	
				CH ₃ CO	170.05	—	H-3''''
					21.32	2.12 s	
				CH ₃ CO	170.08	—	
					21.52	2.17 s	H-4''''

ribose resonated as a doublet at $\delta 1.25$ ($J = 6.0$). The study and the comparison of all the aforementioned data proved that aglycone of **1** was hederagenin (Table 1). The ^{13}C NMR (125 MHz, DMSO- d_6) of **1** showed that 30C out of 59 consisted of aglycone in the molecules. The DEPT pulse sequence showed the presence of ten methyl, thirteen methylene, twenty-five methine, and eleven quaternary carbon. The peaks identified as endocyclic double bond between C-12 and C-13 appeared at 123.03 (CH) and 145.21 (quaternary carbon), respectively [10]. A slight low frequency shift of C-28, $\delta 175.05$, and its IR absorption at 1740 cm^{-1} suggested that glycosidic linkages occurred. The attachment of polysaccharide chain at C-3 of aglycone was confirmed by 10 PPM high-frequency chemical shift at 81.07. Hence it was a 3, 28-bisdesmoside compound [11, 12]. The signal

for 6 methyl groups of aglycone appeared at $\delta 15.73$, 16.02, 17.63, 26.02, 36.00, and 26.10 for C-24, 25, 26, 27, 29, and 30, respectively. The remaining 29 carbon signals of 59 were assigned to four sugar moieties attached to the C-3 and C-28 carbons of aglycone. The two anomeric carbon signals of the sugars at C-3 carbons appeared at 102.05 (C-1''') and 107.05 (C-1'''). A 10 PPM low-frequency chemical shift $\delta 94.60$ (C-1') and 104.5 (C-1'') was observed for anomeric carbon of the two glucose moiety attached at the C-28 carbonyl carbon of aglycone as ester linkage. Most of the proton and carbons of each sugar unit were assigned on the basis of extensive NMR experiments such as COSY, TOCSY, HMBC, and HMQC spectra and compared to reported data [13, 14].

Three proton signals at $\delta 2.02$, 2.15, and 2.17 together with carbon signals at $\delta 23.05$, 21.32, 21.52, 170.02, 170.05, and

170.08 indicated the presence of three acetoxyl groups. The HMBC spectrum further confirmed three acetyl group from correlation among δ_{H} 3.6 (Ara-2), δ_{C} 170.05; δ_{H} 3.7 (Ara-4), δ_{C} 170.08; and δ_{H} 3.1 (Term Glc-3), δ_{C} 170.02 respectively. The HMBC and HMQC experiments confirm the position of double bond and sugars. The correlations were observed between δ_{H} 4.73 (H-1''' of Rha)/ δ_{C} 81.20 (C-3 of aglycone), δ_{H} 4.53 (H-1''' of Ara)/ δ_{C} 72.0 (C-2' of Rha), δ_{H} 4.4 (H-1' of Glc)/ δ_{C} 178.8 (C-28 of aglycone), and δ_{H} 4.4 (H-1'' of Glc terminal)/74.0 (C-2' of Glc). Due to long correlation between δ_{H} 3.6 (Ara-2''') and δ_{C} 172.05 (acetyl C=O); δ_{H} 3.7 (Ara-4''') and δ_{C} 170.08; and δ_{H} 3.1 (Glc-3'') and δ_{C} 170.02, the location of two acetate group was at C-2''' and C-4''' of arabinose and one at C-3'' of glucose terminal [15–17]. Based on all of the previous accumulated data, the structure of compound **1** has been determined as hederagenin 3-O- α -L-rhamnopyranosyl (3 $\rightarrow$ 1)-[2,4-O-diacetyl- α -L-arabinopyranosyl]-28-O- β -D-glucopyranosyl-(2 $\rightarrow$ 1) [3-O-acetyl-O- β -D-glucopyranosyl] ester.

3. Material and Methods

3.1. General Experimental Procedure. Melting point was recorded on the Perfit melting point apparatus. IR spectra were recorded on Perkin-Elmer, spectrum RX I FT-IR spectrometer (KBr discs). NMR spectra were obtained on Bruker, 400, Ultra shield NMR spectrometer (400 MHz for ^1H and 125 MHz for ^{13}C , DMSO- d_6 as solvent, TMS as internal standard). MS was recorded on LCMS Q-TOF micromass spectrometer and LCMS-LCQ, Finnigan, MAT mass spectrometer. Column chromatography was performed on silica gel (Merck 60–120 mesh, 15×100 cm). Thin-layer chromatography was carried out on silica gel (Merck 10–40 μm) and precoated plates were visualised by spraying with 7% H_2SO_4 as a universal spray reagent.

3.2. Plant Material. Fresh pericarps (6 kg) of *S. mukorossi* were collected from Durgadhar, District Rudrapryag (Uttarakhand) during March 2009 and identified by Taxonomical Laboratory, Department of Botany, H.N.B. Garhwal University Srinagar. A voucher specimen (GUH-8644) of the plant was deposited in Departmental Herbarium for future records.

3.3. Extraction and Isolation. Shade dried and powdered pericarps of *S. mukorossi* (4 kg) were extracted three times with 95% ethanol (5 L) at 35°C (15 hours) on a heating mantle. The extraction mixture was filtered off and concentrated under reduced pressure to yield black brown residue (380 g). This residue was fractionated with EtOAc (repeatedly 3–4 times) that yielded EtOAc soluble and insoluble fractions. EtOAc soluble layer (190 g) after evaporation of solvent under reduced pressure afforded (110 g) of crude extract. This crude extract (preadsorbed with silica gel) was column chromatographed using silica gel (Merck 60–120 mesh, 500 g) using solvent gradient system in order to increase polarity, for example, ethyl acetate:methanol (95: 5 $\rightarrow$

70:30). The fractions obtained were collected every 50 mL. The EtOAc: MeOH (88: 12) eluent afforded compound **1**. The elution with EtOAc: MeOH (85: 15) afforded compound **2** and (82: 18) afforded compound **3**.

3.4. Acid Hydrolysis. Compound **1** (3 mg) in 10% HCl-dioxane (1:1, 1 mL) was refluxed at 80°C for 4 h in a water bath, neutralized with aqueous Ag_2CO_3 , filtered, and then extracted with CHCl_3 (1 mL $\times$ 3). Aqueous layer (monosaccharide portion) was examined by TLC with *n*-BuOH-AcOH- H_2O (4:1:5, upper layer) and compared with authentic samples of D-glucose, L-arabinose, and L-rhamnose.

Hederagenin 3-O- $[\beta$ -D-Xylopyranosyl-(1 $\rightarrow$ 3)]- $[\alpha$ -L-Rhamnopyranosyl-(1 $\rightarrow$ 2)]- α -L-Arabinopyranoside (2). White amorphous powder (75 mg), IR ν_{max} (KBr) 3397, 2939, 1694 cm^{-1} . FABMS m/z : 989 $[\text{M} + \text{Na}]^+$, molecular formula $\text{C}_{58}\text{H}_{78}\text{O}_{18}$ (identical with the literature [18]).

Hederagenin 3-O- $[\beta$ -D-arabinopyranoside-(1 $\rightarrow$ 3)]- $[\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)]- α -L-arabinopyranoside (3). White amorphous powder (80 mg), IR ν_{max} (KBr) 3423, 2867, 1692 cm^{-1} (identical with the literature [19]).

References

- [1] R. D. Gaur, *Flora of Garhwal North West Himalaya*, Transmedia, Srinagar, India, 1999.
- [2] K. Nakayama, H. Fujino, R. Kasai, Y. Mitoma, N. Yata, and O. Tanaka, "Solubilizing properties of saponins from *S. mukorossi*, gaertn," *Chemical and Pharmaceutical Bulletin*, vol. 34, p. 3279, 1986.
- [3] Yunnan Institute of Botany, *Flora Yunnanica*, vol. 1, Science Press, Beijing, China, 1972.
- [4] H. C. Huang, M. D. Wu, W. J. Tsai et al., "Triterpenoid saponins from the fruits and galls of *Sapindus mukorossi*," *Phytochemistry*, vol. 69, no. 7, pp. 1609–1616, 2008.
- [5] Y.-H. Kuo, H.-C. Huang, L. I.-M. Y. Kuo et al., "New dammarane-type saponins from the galls of *Sapindus mukorossi*," *Journal of Agricultural and Food Chemistry*, vol. 53, no. 12, pp. 4722–4727, 2005.
- [6] T. Kanchanapoom, R. Kasai, and K. Yamasaki, "Acetylated triterpene saponins from the Thai medicinal plant, *Sapindus emarginatus*," *Chemical and Pharmaceutical Bulletin*, vol. 49, no. 9, pp. 1195–1197, 2001.
- [7] W. Ni, Y. Hua, H.-Y. Liu et al., "Tirucallane-type triterpenoid saponins from the roots of *Sapindus mukorossi*," *Chemical and Pharmaceutical Bulletin*, vol. 54, no. 10, pp. 1443–1446, 2006.
- [8] A. Sengupta, S. P. Basu, and S. Saha, "Triglyceride composition of *Sapindus mukorossi* seed oil," *Lipids*, vol. 10, no. 1, pp. 33–40, 1975.
- [9] S. C. Jain, "Isolation of flavonoids from soapnut, *Sapindus emarginatus* Vahl," *Indian Journal of Pharmaceutical*, vol. 38, no. 6, pp. 141–142, 1976.
- [10] W. Li, X. Li, J. Yang, L. H. Li, N. Li, and D. L. Meng, "Two new triterpenoids from the carpophore of *Xanthoceras sorbifolia* Bunge," *Pharmazie*, vol. 61, no. 9, pp. 810–811, 2006.
- [11] V. U. Ahmed, S. Bano, I. Fatima, and N. Bano, "Saponins from stem bark of *Guaiacum officinale*," *Journal of the Chemical Society of Pakistan*, vol. 10, pp. 247–251, 1988.

- [12] V. U. Ahmad, S. Perveen, and S. Bano, "Saponins from the leaves of *Guaiaacum officinale*," *Phytochemistry*, vol. 29, no. 10, pp. 3287–3290, 1990.
- [13] M. A. Ouyang, H. Q. Wang, Y. Q. Liu, and C. R. Yang, "Triterpenoid saponins from the leaves of *Ilex latifolia*," *Phytochemistry*, vol. 45, no. 7, pp. 1501–1505, 1997.
- [14] Y. Mimaki and S. Doi, *Natural Product Communications*, vol. 3, pp. 903–910, 2008.
- [15] R. Kasai, H. Fujino, T. Kuzuki et al., "Acyclic sesquiterpene oligoglycosides from pericarps of *Sapindus mukurossi*," *Phytochemistry*, vol. 25, no. 4, pp. 871–876, 1986.
- [16] H. C. Huang, S. C. Liao, F. R. Chang, Y. H. Kuo, and Y. C. Wu, "Molluscicidal saponins from *Sapindus mukorossi*, inhibitory agents of golden apple snails, *Pomacea canaliculata*," *Journal of Agricultural and Food Chemistry*, vol. 51, no. 17, pp. 4916–4919, 2003.
- [17] M. Nakatani, S. Hatanaka, H. Komura, T. Kubota, and T. Hase, "The structure of Rotugenoside, a new bitter triterpene glucoside from *Ilex Rotunda* fruits," *Bulletin of the Chemical Society of Japan*, vol. 62, pp. 469–473, 1989.
- [18] K. Nakayama, H. Fujino, R. Kasai, O. Tanaka, and J. Zhou, "Saponins of pericarps of Chinese *Sapindus delaVayi* (Pyishiautzu), a source of natural surfactants," *Chemical and Pharmaceutical Bulletin*, vol. 34, p. 2209, 1986.
- [19] M. Yoshikawa, H. K. Wang, V. Tosirisuk, and I. Kitagawa, "Chemical Modification of Oleanone-Oleagogycosides by Means of Anodic Oxidation," *Chemical Pharmaceutical Bulletin*, vol. 30, p. 3057, 1982.