Identification of Bitter Components from *Artemisia princeps* Pamp.

Hee-Jung Park, Jin-Gyeong Cho, Yoon-Su Baek¹, Kyeong-Hwa Seo, Su-Yeon Kim, Myung-Sook Choi²,
Kyung-Tae Lee³, Tae-Sook Jeong⁴, Hae-Gon Chung⁵, Eun-Gyeong Kang⁵, and Nam-In Baek*

Graduate School of Biotechnology and Institute of Life Science & Resources, Kyung Hee University, Yongin, Gyeonggi 17104, Korea

¹Floriculture Research Division, National Institute of Horticultural and Herbal Science, RDA, Wanju, Jeonbuk 55365, Korea

²Department of Food Science & Nutrition, Kyungpook National University, Daegu 41966, Korea

³College of Pharmacy, Kyung Hee University, Seoul 02447, Korea

⁴National Research Laboratory of Lipid Metabolism & Atherosclerosis, KRIBB, Daejeon 34141, Korea

⁵Gangwha Agricultural R&D Center, Incheon 23038, Korea

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*Corresponding Author
Tel: +82-31-201-2661
Fax: +82-31-204-8116
E-mail: nibaek@khu.ac.kr

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Abstract Identification of bitter components from the aerial parts of *Artemisia princeps* Pamp. was performed to search for a method to eliminate the bitter taste from *A. princeps* products. The aerial parts of *A. princeps* were extracted in an aqueous EtOH solution, and the obtained extracts were partitioned into essential-oil, flavonoid-rich, *n*-BuOH, and aqueous fractions. Two purified bitter sesquiterpenoids were identified through repeated column chromatography of the bitterest fraction, the flavonoid-rich fraction, through an activity-guided fractionation method. The compounds were identified to be 1 α ,6 α ,8 α -trihydroxy-5 α ,7 β H-guaia-3,9,11(13)-trien-12-oic acid and artecadin, respectively, based on the interpretation of NMR, MS, and IR spectroscopic data. Both compounds were 50 times bitterer than caffeine and had similar bitterness to quinine HCl. Neither eupatilin nor jaceosidin, the major active components of *A. princeps*, showed any bitterness.

Keywords: artecadin, *Artemisia princeps*, bitterness, 1 α ,6 α ,8 α -trihydroxy-5 α ,7 β H-guaia-3,9,11(13)-trien-12-oic acid

Introduction

The genus *Artemisia*, a member of the Compositae family, has over 400 species and includes individuals distributed throughout Asia, Europe, and North America. About 30 species grow wild in Korea; *A. princeps* Pamp., *A. annua* L., *A. capillaries* Thunberg, and *A. iwayomogi* Kitamura are used for edible and medicinal purposes. *A. princeps* Pamp. grows abundantly in Ganghwa island, Incheon, Korea. This species is unique in that it has short white hairs covering the stems and back sides of its leaves. Light purple flowers blossom between July and September. After the drying process, stems and leaves turn grey-green, and its unique aroma gets stronger. The content of the flavonoids, the major active components, is influenced by the region of cultivation. The climate of Ganghwa Island, with a low mean temperature, a large range of daily temperatures, and extended periods of sunshine with little precipitation, is advantageous for the accumulation of flavonoids. Other reported components include terpenoids (1), flavonoids (2,3), and phenylpropanoids (4). Extracts of *A. princeps* Pamp. have been used to treat metabolic diseases such as obesity (5), arteriosclerosis (6), cancer (7), and hepatotoxic disease (8). The flavonoid compounds eupatilin and jaceosidin are representative active compounds. Eupatilin functions against gastric ulcers (9),

inhibits endometrial cancer cells (10), oxidizes LDL, and has anti-inflammatory (11) and anti-diabetic effects (2). Jaceosidin shows anti-diabetic (2), antioxidant, and anti-inflammatory (12) activities and eliminates peroxynitrite (13). Based on these desirable properties of *A. princeps* Pamp. extracts, various products have been developed, including a medicine, functional foods, and some cosmetics. However, the bitter taste of the extracts decreases consumer preference for such food products. The bitter taste can be eliminated in several ways. One reported method uses an absorbent such as activated charcoal powder. While this method shows the strongest effect, the active components of *A. princeps* Pamp. may also possibly be absorbed. Another method uses the produced cyclodextrin (CD) polymers. CD is an annular molecule with a hydrophobic structure. Gel permeation chromatography of the CD polymer indicates a high potential with small loss of active compounds. The high cost and complicated process to produce CD polymers is undesirable to manufacturers. Thus, in order to allow profitable manufacture of *A. princeps* Pamp., a method to neutralize the bitterness must be determined.

Our present study was initiated to identify the bitter compounds from the aerial parts of *A. princeps* Pamp. The aerial parts of *A. princeps* Pamp. were extracted in an aqueous EtOH solution, and the

obtained extracts were partitioned using solvents with different polarities. The solvent fractions were evaluated for bitter taste; repeated column chromatography to identify the bitter fraction through an activity-guided fractionation method afforded two purified bitter compounds. The compounds were identified as sesquiterpenoids through the interpretation of NMR, MS, and IR spectroscopic data and were evaluated for bitterness.

Materials and Methods

Plant materials The aerial parts of *A. princeps* Pamp. were provided by the Ganghwa Agricultural Technology Service Center and identified by Dr. Hae-Gon Jung of Ganghwa Agricultural R&D Center, Incheon, Korea. A voucher specimen (KHU-20090901) was reserved at the Laboratory of Natural Products Chemistry, Kyung Hee University, Yongin, Korea.

Experimental materials and apparatus Caffeine and quinine HCl were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan) and Wako Pure Chemical Industries Ltd. (Osaka, Japan), respectively. All other reagents were purchased from Sigma-Aldrich Co., Ltd. (St. Louis, MO, USA). The silica gel (SiO₂) and octadecyl silicagel (ODS) used for column chromatography (c.c.) were Kiesel gel 60 (Merck, Darmstadt, Germany) and RP-18 F_{254S} (Merck), respectively. Flash column chromatography was carried out using the SNAP Cartridge KP-Sil (Biotage, Uppsala, Sweden). TLC analysis was carried out using Kiesel gel 60 F₂₅₄ and RP-18 F_{254S} (Merck) and was detected using a UV lamp, Spectroline Model ENF-240 C/F (Spectronics Corporation, New York, NY, USA). The optical rotation was measured on a JASCO P-1010 digital polarimeter (JASCO, Tokyo, Japan). The IR spectrum was obtained using a Perkin Elmer Spectrum One FT-IR spectrometer (PerkinElmer, Waltham, MA, USA). The FAB-MS was recorded on a JEOL JMSAX-700 (JEOL, Tokyo, Japan). The ¹H-NMR (400 MHz) and ¹³C-NMR (100 MHz) spectra were recorded using a Varian Unity Inova AS-400 FT-NMR spectrometer (Varian Medical Systems, Palo Alto, CA, USA).

Extraction and solvent fractionation of *A. princeps* Pamp.

Components The dried aerial parts of *A. princeps* (3 kg) were extracted with 80% EtOH (18 L) at room temperature for 12 h. The extracts were subsequently filtered through filter paper, and the filtrate was evaporated *in vacuo*, yielding a dark-green residue (75 g). The EtOH extract was suspended in water (3 L) and then successively partitioned with *n*-hexane-EtOAc (12:1, 3 Lx2) to obtain the essential oil fraction (APE, 27 g) and with *n*-hexane-EtOAc (1:1, 3 Lx3) to obtain the flavonoid-rich fraction (APF, 10 g); subsequently, the residue was partitioned with *n*-BuOH (2.5 Lx2).

Isolation of bitter compounds from the flavonoid-rich fraction

The flavonoid-rich fraction (APF, 5 g) was subjected to flash column

c.c. (SNAP Cartridge KP-Sil, 100 g) and eluted with a gradient solution (*n*-hexane-EtOAc=1:1, 3.5 L→CHCl₃-MeOH-H₂O=5:50:1→1:30:1→80% EtOH, 1 L of each). The eluted solutions were monitored by TLC (CHCl₃-MeOH=30:1, 15:1) to produce 17 fractions (APF-1 to APF-17). All fractions went subject to bitterness testing. Fraction APF-7 [480 mg, elution volume/total volume (V_e/V_t) 0.08-0.11] was subjected to SiO₂ c.c. (4×15 cm) and eluted with *n*-hexane-CHCl₃-EtOAc (15:10:1→10:10:1, 2 L of both), yielding 13 fractions (APF-7-1 to APF-7-13). Fraction APF-7-5 (50 mg, V_e/V_t 0.35-0.42) was subjected to ODS c.c. (2×6 cm) and eluted with acetone-H₂O (1:4, 0.5L) to yield 13 fractions (APF-7-5-1 to APF-7-5-13) along with purified compound **1** [APF-7-5-9, 8 mg, V_e/V_t 0.65-0.68, TLC (RP-18 F_{254S}) R_f 0.40, acetone-H₂O=1:1]. APF-8 (139 mg, V_e/V_t 0.11-0.13) was subjected to ODS c.c. (2×11 cm) and eluted with MeOH-H₂O (3:1, 0.8 L), yielding 11 fractions (APF-8-1 to APF-8-11). Fraction APF-8-5 (27 mg, V_e/V_t 0.11-0.13) was subjected to SiO₂ c.c. (2×11 cm) and eluted with CHCl₃-MeOH (20:1, 1 L) to yield seven fractions (APF-8-5-1 to APF-8-5-7), along with purified compound **2** [APF-8-5-5, 5 mg, V_e/V_t 0.65-0.68, TLC (Kieselgel 60 F₂₅₄) R_f 0.33, CHCl₃-MeOH=12:1]. The major flavonoids of *A. princeps* Pamp., eupatilin and jaceosidin, were isolated from the flavonoid-rich fraction using a previously reported method (14).

Table 1. ¹H- (400 MHz) and ¹³C-NMR (100 MHz) data of compounds **1** and **2** (CDCl₃)¹⁾

No	1		2	
	δ _H , coupling pattern, J in Hz	δ _C	δ _H , coupling pattern, J in Hz	δ _C
1	-	83.7	3.58, dd, 10.4, 6.8	76.6
2	2.56, br s	47.2	2.61, ddd, 15.6, 6.8 2.55, dd, 15.6, 10.4	47.4
3	5.46, br s	124.4	-	210.8
4	-	142.6	2.60, dq, 6.8, 11.2	45.4
5	2.69, d, 11.2	66.1	1.56, dd, 11.2, 11.2	51.6
6	3.95, dd, 11.2, 8.8	80.6	4.09, dd, 11.2, 11.2	84.7
7	3.08, dd, 11.2, 8.8	51.1	2.59, brdd, 11.2, 11.2	51.7
8	4.10, br d, 11.2	71.6	2.08, m 1.61, m	22.3
9	5.45, br s	128.5	2.09, m 1.34, ddd, 11.6, 11.6, 2.8	37.4
10	-	138.6	-	42.2
11	-	139.7	-	140.5
12	-	171.8	-	172.2
13	6.21, br d, 3.6 6.20, br d, 3.6	123.9	6.01, d, 3.2 5.51, d, 3.2	117.5
14	1.90, s	18.2	1.09, s	12.2
15	1.90, s	24.6	1.18, d, 6.8	14.1

¹⁾Compound **1** Pale yellow powder (CHCl₃); [α]_D²⁵ +9.0° (c 0.14, MeOH); negative FAB/MS *m/z* 297 [M-H]⁻, 263 [M-(OH)-(H₂O)]⁻, 245 [M-(OH)-2(H₂O)]⁻, 227 [M-(OH)-3(H₂O)]⁻; IR (KBr, ν, cm⁻¹) 3399, 2853, 1748, 1652, 1456; ¹H-NMR (400 MHz, CDCl₃, δ_H) and ¹³C-NMR (100 MHz, CDCl₃, δ_C) Compound **2** White amorphous powder (CHCl₃); [α]_D²⁵ +47.0° (c 0.11, MeOH); negative FAB/MS *m/z* 263 [M-H]⁻, 247 [M-OH]⁻; IR (KBr, ν, cm⁻¹) 3446, 2850, 1666, 1518; ¹H-NMR (400 MHz, CDCl₃, δ_H) and ¹³C-NMR (100 MHz, CDCl₃, δ_C)

Preparation of samples and standard solutions for bitterness evaluation

The solvent fractions were diluted using distilled water with a minimum amount of EtOH to achieve complete dissolution. The concentrations of the *n*-BuOH and H₂O fractions were adjusted to 0.4 mg/mL, and those of essential oil and flavonoid-rich fractions were adjusted to 0.2 mg/mL. Standard solutions for the comparison of bitter taste were prepared using caffeine and quinine HCl aqueous solutions: caffeine: 0.014, 0.027, 0.054, 0.108, 0.216, 0.432 mg/mL; quinine HCl: 1.25, 2.5, 5.0, 7.50, 10.0, 12.50 mg/mL, and 5 mL of each sample was put in a 10 mL-plastic cup for tasting. The concentrations of the sub-fractions obtained from column chromatography, eupatilin, jaceosidin, and isolated compounds, were adjusted to 1 mg/mL. Finally, 0.2 mL of each sample solution was placed on a 3-cm-diameter plastic spoon and dried for tasting.

Panelists For the bitterness evaluation of the solvent fractions, 20 panelists compared the taste of the extract to a caffeine solution and 22 compared the taste of the extract to a quinine HCl solution (without sex distinction, aged 21-30 years). None of the taste panelists had any history of a known taste disorder. The evaluation experiment was performed in one room at 20-25°C. The panelists were told to hold the samples in their mouths for 5 s and to determine the taste concentration similar to that of the standard solvent. For bitterness evaluation of the sub-fractions and isolated compounds, 5 panelists each placed a sample-containing spoon on the inner part of the tongue and assigned a bitterness score from 0 (no sense) to 10 (extremely bitter). These 5 panelists discussed bitterness levels prior to sample testing using the standard solutions of caffeine and quinine HCl.

Results and Discussion

The EtOH extracts of *A. princeps* Pamp. were successively partitioned

into essential oil, flavonoid-rich, *n*-BuOH, and H₂O fractions, and all fractions were evaluated for bitterness. The two bitterest fractions were the essential oil fraction and the flavonoid-rich fraction. The essential oil fraction was 32 and 0.27 times bitterer than the caffeine standard and quinine HCl, respectively (Table 2). The flavonoid-rich fraction was rated as the bitterest; it was more than 50 times bitterer than the caffeine standard and had a similar bitterness to the quinine HCl standard. Column chromatographic separation using activity-guided fractionation was performed on the flavonoid-rich fraction, and the obtained sub-fractions and compounds were evaluated for bitterness using an alternative bitterness tasting experiment. Since APF-7 and APF-8 showed the strongest bitter taste (Fig. 1), the activity-guided isolation procedure was performed to ultimately obtain two purified bitter compounds: compound **1** and compound **2**.

Compound **1** was a pale yellow powder and showed a pseudomolecular ion peak [M-H]⁻ at *m/z* 279 in the negative FAB/MS spectrum, indicating a molecular weight of 280. The IR spectrum (KBr) showed absorbance bands due to hydroxyl (3399 cm⁻¹) and carboxyl (1748 cm⁻¹) groups, as well as a double bond (1652 cm⁻¹). In the ¹H-NMR spectrum, 2 olefin methine proton signals [δ_{H} 5.46 (br s, H-3), 5.45 (br s, H-9)], 1 exo-methylene proton signal [δ_{H} 6.21 (d, *J*=3.6 Hz, H-13a), 6.20 (d, *J*=3.6 Hz, H-13b)], and 2 oxygenated methine proton signals [δ_{H} 4.10 (br d, *J*=11.2 Hz, H-8), 3.95 (dd, *J*=11.2, 8.8 Hz, H-6)] were observed in the downfield region. In the upfield region, 2 methine proton signals [δ_{H} 3.08 (dd, *J*=11.2, 8.8 Hz, H-7), 2.69 (d, *J*=11.2 Hz, H-5)], 1 methylene proton signal (δ_{H} 2.56, br s, H-2), and 2 allylic methyl proton signals (δ_{H} 1.90, 6H, s, H-14, 15) were observed (Table 1). In the ¹³C-NMR spectrum, 15 signals were observed, indicating that compound **1** was a sesquiterpenoid. The data indicated the presence of a carboxylic carbon signal δ_{C} 171.8, C-12), 3 olefin quaternary carbon signals δ_{C} 142.6 (C-4), 139.7 (C-11), 138.6 (C-10), and 2 olefin methine carbon signals δ_{C} 128.5 (C-9), 124.4 (C-3). Through a DEPT experiment, the peak observed at δ_{C} 123.9 was

Table 2. Comparison of bitterness of the solvent fractions from *Artemisia princeps* Pamp. extract with those of quinine HCl and caffeine standard solutions

		H ₂ O fr. (0.4 mg/mL)	<i>n</i> -BuOH fr. (0.4 mg/mL)	essential-oil fr. (0.2 mg/mL)	flavonoid-rich fr. (0.2 mg/mL)
Quinine HCl	Not bitter (1.25 mg/mL)	19	-	-	-
	Little bitter (2.50 mg/mL)	3	9	1	-
	A little bitter (5.00 mg/mL)	-	12	8	1
	Bitter (7.50 mg/mL)	-	1	12	2
	Quite bitter (10.00 mg/mL)	-	-	1	12
	Extremely bitter (12.50 mg/mL)	-	-	-	7
Caffeine	Not bitter (0.014 mg/mL)	11	-	-	-
	Little bitter (0.027 mg/mL)	9	7	6	-
	Bitter (0.054 mg/mL)	-	9	7	2
	Quite bitter (0.108 mg/mL)	-	4	7	4
	Extremely bitter (0.216 mg/mL)	-	-	-	11
	Unendurable (0.432 mg/mL)	-	-	-	3

The number in each cell indicates the number of panelists who evaluated the equivalence between the concentration of the standard solution and that of sample.

Fig. 1. Isolation procedure for bitter compounds from the flavonoid-rich fraction of *Artemisia princeps* Pamp. through the activity-guided fractionation method. Samples were prepared at 1 mg/mL, and 0.2 mL of each sample solution was placed on a 3-cm-diameter plastic spoon and dried for tasting. Five panelists were told to indicate the bitterness level from 0 (not bitter) to 10 (extremely bitter). The panelists discussed the standard bitterness level prior to sample rating using the standard solutions of caffeine and quinine HCl. Results shown in the brackets are the mean value of the five panelists.

determined to be an exomethylene carbon signal. One oxygenated quaternary carbon signal δ_C 83.7 (C-1), 2 oxygenated methine carbon signals δ_C 80.6 (C-6), 71.6 (C-8), 2 methine carbon signals δ_C 66.1 (C-5), 51.1 (C-7), 1 methylene carbon signal δ_C 47.2, (C-2), and 2 methyl carbon signals δ_C 24.6, (C-15), 18.2 (C-14) were detected (Table 1). The combination of the NMR and MS data identified the molecular formula of compound **1** as $C_{15}H_{22}O_6$ with an unsaturation degree of 6. Therefore, compound **1** was suggested to be a guaiane acid sesquiterpenoid with 3 double bonds and 3 hydroxyl groups. To confirm the position of each of the functional groups, correlation spectroscopy (COSY) and heteronuclear multiple bond connectivity spectroscopy (HMBC) experiments were performed. The olefin methine proton signal at δ_H 5.46 (H-3) was correlated with the methylene proton signal at δ_H 2.56 (H-2), and the oxygenated methine proton signal at δ_H 3.95 (H-6) correlated with the methine proton signals at δ_H 2.69 (H-5) and 3.08 (H-7). The oxygenated methine proton signal at δ_H 4.10 (H-8) correlated with H-7 and the olefin methine proton signal at δ_H 5.45 (H-9) in the COSY spectrum (Fig. 2). In the HMBC spectrum, correlations were identified between H-2 (δ_H 2.56) and the olefin quaternary carbon signal C-4 (δ_C 142.6), H-3 (δ_H 5.46) and the oxygenated quaternary carbon signal C-1 (δ_C 83.7), the olefin methine carbon C-5 signal (δ_C 66.1), and the methyl carbon signal C-15 (δ_C 24.6). Correlations were also seen between H-5 (δ_H 2.69) and both C-2 (δ_C 47.2) and C-7 (δ_C 51.1) and between H-9 (δ_H 5.45) and C-1, C-7, the olefin quaternary carbon signal C-10 (δ_C 138.6), and the methyl carbon signal C-14 (δ_C 18.2). The exomethylene proton signals H-13 (δ_H 6.21 and 6.20) correlated to C-

7, the olefin quaternary carbon signal C-11 (δ_C 139.7), and the carboxyl carbon signal C-12 (δ_C 171.8), which confirmed the presence of methyl groups at C-14 and C-15, hydroxyl groups at C-1, C-6, and C-8, 3 double bonds between C-3 and C-4, C-9 and C-10, and C-11 and C-13, and carboxylic carbon at C-12 (Fig. 2). Taken together, the data above identified compound **1** to be 1,6,8-trihydroxy-5,7-guaia-3,9,11(13)-trien-12-oic acid, a guaiane sesquiterpenoid. The stereo structure of chiral carbons was determined by comparison of the NMR data and the specific rotation value ($[\alpha]_D^{25} +9.0^\circ$, c 0.14, MeOH) with those of the same compound previously isolated from *Achillea coarctata* (15). Finally, compound **1** was identified to be 1 α ,6 α ,8 α -trihydroxy-5 α ,7 β H-guaia-3,9,11(13)-trien-12-oic acid.

Compound **2** was a white amorphous powder and showed a green-blue spot on TLC after spraying with 10% H_2SO_4 and heating. The negative FAB-MS showed a pseudomolecular ion peak $[M-H]^-$ at m/z 263, thus indicating a molecular weight of 264. The IR spectrum (KBr) showed absorbance bands due to hydroxyl (3446 cm^{-1}) and ketone (1666 cm^{-1}) groups and double bonds (1518 cm^{-1}). In the 1H -NMR spectrum, a typical exocyclic methylene group proton signal [δ_H 6.01 (d, $J=3.2$ Hz, H-13a), 5.51 (d, $J=3.2$ Hz, H-13b)] and 2 oxygenated methine proton signals [δ_H 3.58 (dd, $J=10.4, 6.8$ Hz, H-1), 4.09, (dd, $J=11.2, 11.2$ Hz, H-6)] were detected. In the high magnetic field, the methine and methylene proton signals between δ_H 1.34-2.61 and 2 methyl proton signals [δ_H 1.18 (d, $J=6.8$ Hz, H-15), 1.09 (s, H-14)] were observed (Table 1). A series of 15 signals in the ^{13}C -NMR spectrum indicated that compound **2** was also a sesquiterpenoid. One ketone carbon signal at δ_C 210.8 (C-1), one lactone carbon signal

Fig. 2. Chemical structures of bitter sesquiterpenoids isolated from the aerial parts of *A. princeps* Pamp., and key correlations in the ^1H - ^1H COSY and HMBC spectra. COSY correlations are represented by bold lines, and HMBC correlations are represented by single-headed arrows from H to C.

at δ_{C} 172.2 (C-12), one olefin quaternary carbon signal at δ_{C} 140.5 (C-11), and one exomethylene carbon signal at δ_{C} 117.5 (C-13) were observed. Two oxygenated methine carbon signals at δ_{C} 84.7 (C-6) and 76.6 (C-1) in the oxygenated region; 3 methine carbon signals at δ_{C} 51.7 (C-7), 51.6 (C-5), and 45.4 (C-4); 3 methylene carbon signals at δ_{C} 47.4 (C-2), 37.4 (C-9), and 22.3 (C-8); one quaternary carbon signal at δ_{C} 42.2 (C-10); and 2 methyl carbon signals at δ_{C} 14.1 (C-15) and 12.2 (C-14) were observed in the high magnetic field (Table 1). The combination of the NMR and MS data indicated the molecular formula of compound 2 as $\text{C}_{15}\text{H}_{20}\text{O}_4$ with an unsaturation degree of 5. Therefore, compound 2 was suggested to be an eudesmanolide sesquiterpenoid, including 1 double bond, 1 ketone, 1 hydroxyl, and 1 α -exomethylene- γ -lactone group. A partial structure from C-1 to C-2 and from C-15 to C-4, C-5, C-6, C-7, C-8, and C-9 was confirmed from the correlations observed in sequence in the ^1H - ^1H COSY spectrum (Fig. 2). In the HMBC spectrum, the ketone carbon signal (δ_{C} 210.8, C-3) showed correlations with the methine proton signal (δ_{H} 2.60, H-4), the methyl proton signal (δ_{H} 1.18, H-15), and the methylene proton signal (δ_{H} 2.61, 2.55, H-2). The H-15 methyl proton signal was correlated to 2 methine carbon signals [δ_{C} 51.6 (C-5), 45.4 (C-4)]. Another methyl proton signal (δ_{H} 1.10, H-14) showed correlation with the quaternary (δ_{C} 42.2, C-10) and the methylene carbon signals at (δ_{C} 37.4, C-9), the oxygenated methine carbon signal (δ_{C} 76.6, C-1), and C-5. The lactone carbon signal (δ_{C} 172.2, C-12) was correlated with the exomethylene proton signals (δ_{H} 6.01, 5.51, H-13) (Fig. 2). Therefore, compound 2 was identified as 1,6-dihydroxy-3-oxoeudesm-11(13)-en-12-oic acid-12,6-olide. The high values of vicinal coupling constants ($J=11.2$ Hz) among methine protons, H-4, H-5, H-6, and H-7, suggest that they all are in *trans*-axial positions. Other stereo-chemistry characteristics were confirmed as shown in Fig. 2 by comparison of coupling patterns and chemical shifts in the NMR data, as well as the specific rotation value ($[\alpha]_{\text{D}}^{25}+47.0^\circ$, c 0.11, MeOH) of those reported in the literature (16,17). Finally, compound 2 was identified to be 1 β ,6 α -dihydroxy-3-oxoeudesm-11(13)-en-12-oic acid-12,6 α -olide, more commonly known as artemcalin.

The bitterness of the 2 isolated compounds was evaluated. Both

compounds were determined to be extremely bitter. The basic tastes, sour, salty, sweet, bitter, and umami, are recognized by taste receptor proteins in the oral cavity. The gustatory system consists of taste receptor cells on the papillae of the taste buds. The TAS2R proteins function as bitter taste receptors (18), and 43 human TAS2R genes have been reported (19). Individual hTAS2Rs are activated by bitter compounds, demonstrating selectivity for chemical groups and even stereo-selectivity (20). The structural basis for the unique ability of the hTAS2Rs to recognize numerous chemically diverse and low-affinity agonists is not fully understood. More than 600 chemicals have been reported as bitter compounds; the list includes many sesquiterpenoids (21). Both of the isolated compounds, 1 and 2, turned out to be 50 times bitterer than caffeine and have a bitter taste similar to that of quinine HCl. Conversely, neither eupatilin nor jaceosidin, the major active components of *A. princeps* Pamp., provoked bitter responses in study participants. TLC spraying with 10 % H_2SO_4 and heating shows sesquiterpenoids as dark green or green-blue and flavonoid compounds as yellow. The R_f values of compounds 1 and 2 were quite different from those of eupatilin and jaceosidin, which suggests that elimination of the sesquiterpenoids from *A. princeps* extracts will not be difficult. The bitter compounds have different characteristic such as molecular polarity from those of active principles of this plant, eupatilin or jaceosidin. Therefore, the bitter compounds can be removed by relatively simple methods such as solvent fractionation or resin adsorption. However, the practical application to remove the bitter compounds needs further study.

After establishing a method to remove the sesquiterpenoids from *A. princeps* extracts, it may be possible to develop eupatilin and jaceosidin into useful, active flavonoid products without the bitter taste. A greater demand for *A. princeps* Pamp. products may encourage agricultural development of *Artemisia*.

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