

## A New Sesquiterpene Lactone from *Artemisia rubripes* Nakai

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The chromatographic separation of a methylene chloride extract of *Artemisia rubripes* led to the isolation of a new sesquiterpene lactone (**3**), together with four known compounds, a coumarin (**2**) and three terpenes (**1**, **4**, and **5**). Their structures were characterized to be 1 $\beta$ ,6 $\alpha$ -dihydroxy-4(15)-eudesmene (**1**), scopoletin (**2**), 1 $\alpha$ ,4 $\beta$ -dihydroxy-8 $\alpha$ -acetoxy-guaia-2,10(14), 11(13)-triene-6,12-olide (**3**), 1 $\alpha$ ,4 $\beta$ -dihydroxy-8 $\alpha$ -acetoxy-guaia-2,9,11(13)-triene-6,12-olide (**4**), and  $\beta$ -sitosterol-3-*O*- $\beta$ -D-glycoside (**5**) by spectroscopic means.

**Key words:** *Artemisia rubripes*, Sesquiterpene, Sesquiterpene lactone

### INTRODUCTION

Twenty *Artemisia* species are distributed in South Korea and rich in terpenoids. *Artemisia rubripes* (Compositae) has been used as a Korean traditional medicine for stomachache, vomiting, diarrhea, and hemostatic agent (Lee, 1979). The antimutagenic effect (Park *et al.*, 1996) and essential oils (Khanina *et al.*, 1991) of *Artemisia rubripes* were reported, but phytochemical study on this plant has not been fully investigated.

As part of our systematic study on the terpene constituents of the genus *Artemisia* (Kwak *et al.*, 1997; Kwon *et al.*, 2001; Kwon *et al.*, 2001), we have investigated *A. rubripes* collected at Dae-Kwan ryung, Gangwon Province in Aug. 1997. The chromatographic separation of a methylene chloride extract of *Artemisia rubripes* led to the isolation of a new sesquiterpene lactone (**3**), together with four known compounds, a coumarin (**2**) and three terpenes (**1**, **4**, and **5**).

### MATERIALS AND METHODS

#### General procedure

NMR: in CDCl<sub>3</sub>, Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl<sub>4</sub>, Nicolet model 205 FT-IR spectrophotometer. MS: VG70-VSEQ mass spectrometer.

Column chromatography : Silica gel 60 (Merck, 70230 mesh and 230400 mesh), Lichroprep. RP-18 (Merck) and Sephadex LH-20. TLC: Merck precoated Si gel F<sub>254</sub> plates and RP-18 F<sub>254s</sub> plates. LPLC: Merck Lichroprep Lobar<sup>®</sup>-A Si 60 (240×10 mm)

#### Plants material

The aerial parts of *A. rubripes* (Compositae) were collected at Dae-Gwan ryung, Gangwon Province in Aug. 1997. A voucher specimen (SKK-97-001) was deposited at the College of Pharmacy, Sungkyunkwan University.

#### Extraction and isolation

The aerial parts of *A. rubripes* (1 kg) were extracted with methylene chloride (MC) three times at room temperature. The resultant MC extract (60g) was chromatographed over silica gel using gradient solvent system of *n*-hexane: EtOAc:MeOH (4:1:0-10:10:1) to give seven fractions [Fr.W-1 (14 g), Fr.W-2 (10.5 g), Fr.W-3 (6 g), Fr.W-4 (3.9 g), Fr.W-5 (12 g), Fr.W-6 (6.1 g), Fr.W-7 (2.4 g)]. Fraction W-3 (6 g) was chromatographed on RP-flash column eluting with 80% MeOH to give four subfractions (W31-34). Subfraction W31 (800 mg) was purified with Lobar-A (CHCl<sub>3</sub>: Acetone=10:1) and HPLC (CHCl<sub>3</sub>: MeOH=30:1) to yield compound **1** (8 mg). Fraction W5 (12 g) was chromatographed with Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH=1:1) to give three subfractions (W51-W53). Subfraction W52 (6 g) was rechromatographed with Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>:MeOH=1:1) to give five subfractions (W521-W525). Subfraction W522 (3.3 g) was chromatographed with silica gel column (CHCl<sub>3</sub>:MeOH=20:1) to give five

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subfractions (W5221-W5225). Subfraction W5222 (700 mg) was recrystallized to yield a compound **2** (150 mg). Subfraction W5224 (320 mg) was chromatographed with HPLC (Hexane:EtOAc=1:1) and silica gel column (CHCl<sub>3</sub>:EtOAc=5:1) to afford compounds **3** (4 mg) and **4** (5 mg). Fraction W6 (6.2 g) was chromatographed with silica gel column (CHCl<sub>3</sub>:MeOH=20:1) to give four subfractions (W61-W64). Subfraction W64 (1.5 g) was chromatographed with Sephadex LH-20 (CH<sub>2</sub>Cl<sub>2</sub>: MeOH=1:1) and recrystallized (MeOH) to yield compound **5** (80 mg).

### 1β,6α-Dihydroxy-4(15)-eudesmene (1)

Colorless oil,  $[\alpha]_D^{24} +2.5^\circ$  (c 0.95, CHCl<sub>3</sub>); IR  $\nu_{\text{Max}}^{\text{NaCl}}$  (cm<sup>-1</sup>): 3019, 2400, 1215, 928, 757, 669; EI-MS  $m/z$  (rel. int.): 238 (M<sup>+</sup>, 15), 220 (75), 202 (27), 177 (44), 159 (47), 133 (32), 121 (60), 107 (75), 83 (100); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  0.71 (3H, s, H-14), 0.87 (3H, d,  $J = 7.0\text{Hz}$ , H-12), 0.95 (3H, d,  $J = 7.0\text{Hz}$ , H-13), 1.21 (1H, dd,  $J = 3.0, 10.0\text{Hz}$ , H-9a), 1.28 (1H, td,  $J = 4.0, 10.0\text{Hz}$ , H-9b), 1.55 (2H, m, H-3a, 8a), 1.75 (1H, d,  $J = 10.0\text{Hz}$ , H-5), 1.86 (2H, m, H-2), 1.92 (1H, ddd,  $J = 2.5, 3.0, 12.5\text{Hz}$ , H-8b), 2.07 (1H, td,  $J = 2.5, 5.5\text{Hz}$ , H-3b), 2.24 (1H, td,  $J = 2.5, 7.0\text{Hz}$ , H-11), 2.33 (1H, ddd,  $J = 2.5, 5.0, 13.0\text{Hz}$ , H-7), 3.42 (1H, dd,  $J = 4.5, 11.5\text{Hz}$ , H-1), 3.71 (1H, t,  $J = 10.0\text{Hz}$ , H-6), 4.74 (1H, d,  $J = 1.5\text{Hz}$ , H-15b), 5.02 (1H, d,  $J = 1.5\text{Hz}$ , H-15a); <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  11.82 (C-14), 16.44 (C-13), 18.43 (C-8), 21.32 (C-12), 26.26 (C-11), 32.17 (C-2), 35.34 (C-3), 36.55 (C-9), 41.93 (C-10), 49.59 (C-7), 56.15 (C-5), 67.25 (C-6), 79.28 (C-1), 108.03 (C-15), 146.48 (C-4).

### Scopoletin (2)

Pale-yellow needles, SI-MS  $m/z$ : 192 (M<sup>+</sup>) <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  3.97 (3H, s, -OCH<sub>3</sub>), 6.13 (1H, s, -OH), 6.29 (1H, d,  $J = 9.5\text{Hz}$ , H-3), 6.86 (1H, s, H-5), 6.94 (1H, s, H-8), 7.61 (1H, d,  $J = 9.5\text{Hz}$ , H-4) <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  56.65 (-OCH<sub>3</sub>), 103.44 (C-8), 107.70 (C-5), 111.74 (C-10), 113.69 (C-3), 143.52 (C-4), 144.22 (C-6), 149.91 (C-9\*), 150.51 (C-7\*), 161.67 (C-2) (\* exchangeable).

### 1α,4β-Dihydroxy-8α-acetoxy-guaia-2,10(14),11(13)-triene-6,12-olide (3)

Colorless gum,  $[\alpha]_D^{24} +6.6^\circ$  (c 0.95, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}^{\text{MeOH}}$  nm (log) 214; IR  $\nu_{\text{Max}}^{\text{NaCl}}$  (cm<sup>-1</sup>): 2933, 2861, 2360, 1746, 1256, 1043, 922, 751; HREI-MS:  $m/z$  320.1834 (calcd. 320.1234); EI-MS  $m/z$  (rel. int.): 320 (M<sup>+</sup>, 11), 305 (57), 263 (61), 242 (36), 230 (57), 199 (33), 171 (35), 135 (30), 121 (31), 98 (100); <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>): Table I <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>): Table I.

### 1α,4β-Dihydroxy-8α-acetoxy-guaia-2,9,11(13)-triene-6,12-olide (4)

Colorless gum,  $[\alpha]_D^{24} +12.4^\circ$  (c 0.95, CHCl<sub>3</sub>); UV  $\lambda_{\text{max}}^{\text{MeOH}}$

**Table I.** <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>) and <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>) spectral data of Compound **3**

Position	$\delta_c$	$\delta_H$ (J, Hz)	NOESY	HMBC (C→H)
1	87.00			2, 3, 5, 9, 14
2	135.93	5.71, d (5.5)	H-3, H-14a	3, 13
3	136.92	6.01, d (5.5)	H-2, H-15	2, 15
4	82.94			2, 3, 5, 15
5	63.55	2.23, d (11.0)	H-6, H-7, H-15, OAc	2, 3, 15
6	77.86	4.47, dd (9.0, 11.0)	H-5, H-8	5
7	46.08	3.58, dd (9.0, 10.5)	H-5, H-9a	5, 9, 13
8	74.92	4.93, td (6.0, 10.5)	H-6, H-9b	6, 9, 14
9a	36.67	2.86, d (10.0)	H-7, H-9b, OAc	14
9b		2.81, dd (6.0, 10.0)	H-8, H-9a, H-14b, OAc	
10	144.00			5, 9, 14
11	140.14			
12	169.69			8, 13, OAc
13a	125.22	6.35, dd (0.5, 3.5)	H-13b	
13b		5.87, dd (0.5, 3.5)	H-13a, OAc,	
14a	119.02	5.08, d (1.0)	H-2, H-9a, H-9b, H-14b9	
14b		5.28, d (1.0)	H-9a, H-9b, H-14a	
15	29.88	1.58, br.s	H-3, H-5, OAc	5
C=O	170.07			
OAc	21.45	2.17, s	H-5, H-9a, H-9b, H-13b, H-15	

nm (log) 216;  $\nu_{\text{Max}}^{\text{NaCl}}$  (cm<sup>-1</sup>): 2933, 2362, 1750, 1235, 1020, 751; EI-MS  $m/z$  (rel. int.): 320 (M<sup>+</sup>, 8), 305 (10), 260 (66), 217 (57), 200 (100), 171 (75), 165 (73), 121 (64) <sup>1</sup>H-NMR (500 MHz, CDCl<sub>3</sub>):  $\delta$  1.56 (3H, brs, H-15), 1.88 (3H, s, H-14), 2.16 (3H, s, OAc), 2.43 (1H, d,  $J = 10.0\text{Hz}$ , H-5), 4.14 (1H, td,  $J = 2.0, 6.5\text{Hz}$ , H-7), 4.46 (1H, t,  $J = 10.0\text{Hz}$ , H-6), 5.21 (1H, dd,  $J = 4.5, 9.5\text{Hz}$ , H-8), 5.67 (1H, brq,  $J = 1.5, 5.5\text{Hz}$ , H-9), 5.75 (1H, d,  $J = 3.5\text{Hz}$ , H-13b), 5.95 (1H, d,  $J = 5.5\text{Hz}$ , H-2), 5.99 (1H, d,  $J = 5.5\text{Hz}$ , H-3), 6.32 (1H, d,  $J = 3.5\text{Hz}$ , H-13a) <sup>13</sup>C-NMR (125 MHz, CDCl<sub>3</sub>):  $\delta$  21.52 (-C=O-CH<sub>3</sub>), 24.76 (C-15), 27.88 (C-14), 42.25 (C-7), 63.86 (C-5), 74.24 (C-8), 75.71 (C-6), 80.51 (C-4), 84.24 (C-1), 122.81 (C-13), 123.33 (C-9), 137.93 (C-2), 138.49 (C-3), 139.18 (C-11), 142.95 (C-10), 169.65 (C-12), 170.77 (O=C=O).

### β-Sitosterol-3-O-β-D-glycoside (5)

Colorless oil, ESI-S  $m/z$ : 576 (M<sup>+</sup>); <sup>1</sup>H-NMR (300 MHz, DMSO-d<sub>6</sub>):  $\delta$  0.71 (3H, s, H-18), 0.85-1.02 (12H, H-21, 26, 27, and 29), 1.05 (3H, s, H-19), 3.00-3.80 (H-2', 3', 4', 5', and 6'), 4.95 (1H, d,  $J = 5.0\text{Hz}$ , H-1'), and 5.41 (1H, m, H-6); <sup>13</sup>C-NMR (75 MHz, DMSO-d<sub>6</sub>):  $\delta$  11.6 (C-18), 11.7 (C-29), 18.6 (C-21), 18.9 (C-27), 19.1 (C-19), 19.7 (C-26), 20.6 (C-11), 22.6 (C-28), 23.8 (C-15), 25.4 (C-23), 27.7 (C-16), 28.7 (C-25), 29.2 (C-2), 31.3 (C-8), 31.4 (C-7), 33.3 (C-22), 35.4 (C-20), 36.2 (C-10), 36.8 (C-1), 38.3 (C-12), 41.8 (C-13), 45.1 (C-24), 49.6 (C-9), 55.4 (C-17), 56.1 (C-14), 61.1 (C-6'), 70.1 (C-4'), 73.4 (C-2φ), 76.7 (C-5'), 76.8 (C-3), 76.9 (C-3'), 100.8 (C-1'), 121.2 (C-6), 140.4 (C-5).

## RESULTS AND DISCUSSION

Compound **1** was obtained as colorless gum. The  $^1\text{H-NMR}$  spectrum showed two secondary methyl signals at  $\delta$  0.87 (3H, d,  $J = 7.0\text{Hz}$ ) and 0.95 (3H, d,  $J = 7.0\text{Hz}$ ), and a quaternary methyl signal at  $\delta$  0.71 (3H, s), two carbinol proton signals at 3.42 (1H, dd,  $J = 4.5, 12.0\text{Hz}$ ) and 3.71 (1H, t,  $J = 10.0\text{Hz}$ ), and an exomethylene signals at  $\delta$  4.74 (1H, d,  $J = 1.5\text{Hz}$ ) and 5.02 (1H, d,  $J = 1.5\text{Hz}$ ). The  $^{13}\text{C-NMR}$  spectrum demonstrated the presence of 15 carbon signals which contained two olefinic carbon signals at  $\delta$  108.03 and 146.48, and two carbinol carbon signals at  $\delta$  67.25 and 79.28. These observations suggested that compound **1** was a eudesmane sesquiterpene with two secondary alcohol groups, an exomethylene and an isopropyl group. Thus, the structure of compound **1** was determined to be  $1\beta,6\alpha$ -dihydroxy-4(15)-eudesmene. The NMR spectral and physical data of compound **1** were in good agreement with those in the literature (Bohlmann *et al.*, 1983).

Compound **2** was obtained as pale-yellow needle. On the basis of the EI-MS,  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectral data and the comparison of the data in the previous literature, the structure of **2** was established as scopoletin. (Vasconcelos *et al.*, 1998)

Compound **3** was obtained as colorless gum. The molecular formula  $\text{C}_{17}\text{H}_{20}\text{O}_6$  was assigned by HREI-MS. The  $^1\text{H-NMR}$  spectrum showed six olefinic proton signals at  $\delta$  5.08 (1H, d,  $J = 1.0\text{Hz}$ ), 5.25 (1H, d,  $J = 1.0\text{Hz}$ ), 5.71 (1H, d,  $J = 5.5\text{Hz}$ ), 5.87 (1H, dd,  $J = 0.5, 3.5\text{Hz}$ ), 6.01 (1H, d,  $J = 5.5\text{Hz}$ ) and 6.35 (1H, dd,  $J = 0.5, 3.5\text{Hz}$ ) and acetyl signal at  $\delta$  2.17 (3H, s). The  $^{13}\text{C-NMR}$  spectrum demonstrated the presence of 17 carbon signals which was composed of six olefinic carbon signals at  $\delta$  119.02,

125.22, 135.96, 136.92, 140.14, and 144.00, four oxygenated carbon signals at  $\delta$  74.92, 77.86, 82.94, and 87.00, and two carbonyl carbon signals at  $\delta$  169.69 and 170.07. These observations implied that compound **3** was a guaianolide sesquiterpene lactone with two tertiary alcohols and an acetyl group (Singh *et al.*, 1985; Jakupovic *et al.*, 1988). The  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectral data were very similar with 1,4-dihydroxybishopsolicepolide (Singh *et al.*, 1985). The relative stereochemistry of C4-C5-C6-C7-C8 was determined to be similar to that of  $1\alpha,4\alpha$ -dihydroxybishopsolicepolide (Singh *et al.* 1985), which was isolated from *Bishopanthus soliceps*, on the basis of the coupling constants observed in the  $^1\text{H-NMR}$  spectrum. Furthermore, the stereochemistry of **3** was reconfirmed from its 2D NOESY spectrum (Table I). Thus, compound **3** was determined to be  $1\alpha,4\beta$ -dihydroxy- $8\alpha$ -acetoxy-guaia-2,10(14),11(13)-triene-6,12-olide. Although compound **3** is a stereoisomer of  $1\alpha,4\alpha$ -dihydroxybishopsolicepolide, the structure of **3** was not yet reported.

Compound **4** was obtained as colorless gum. The  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectral data were similar to those of compound **3**. The  $^1\text{H-NMR}$  spectrum showed five olefinic proton signals at  $\delta$  5.67 (1H, brs,  $J = 1.5, 5.5\text{Hz}$ ), 5.75 (1H, d,  $J = 3.5\text{Hz}$ ), 5.95 (1H, d,  $J = 5.5\text{Hz}$ ), 5.99 (1H, d,  $J = 5.5\text{Hz}$ ) and 6.32 (1H, d,  $J = 3.5\text{Hz}$ ). The  $^{13}\text{C-NMR}$  spectrum demonstrated the presence of 17 carbon signals which contained six olefinic carbon signals at  $\delta$  122.81, 123.33, 137.93, 138.49, 139.18, and 142.95, four oxygenated carbon signals at  $\delta$  74.24, 75.71, 80.51, and 84.24, and two carbonyl carbon signals at  $\delta$  169.65 and 170.77. Thus, compound **4** was determined to be  $1\alpha,4\beta$ -dihydroxy- $8\alpha$ -acetoxy-guaia-2,9,11(13)-triene-6,12-olide. The NMR spectral and physical data of compound **4** were in good agreement with those in the literature (Jakupovic *et al.*, 1988).

Compound **5** was obtained colorless gum. On the basis of the ESI-MS,  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectral data and the comparison of the data in the previous literature, the structure of **5** was established as  $\beta$ -sitostrol-3-O- $\beta$ -D-glycoside (Kim *et al.*, 1996).

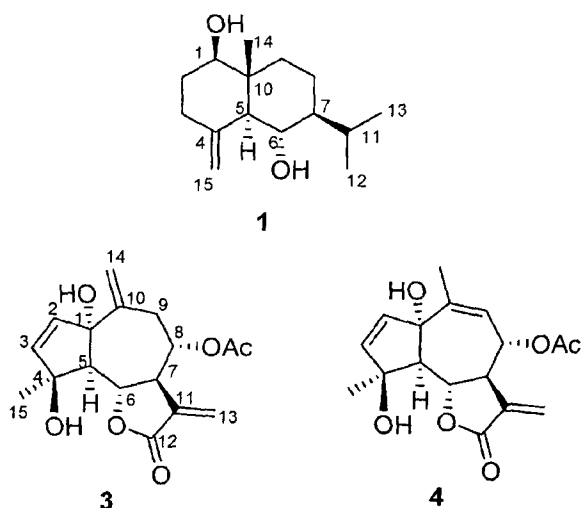


Fig. 1. Structures of compounds **1**, **3**, and **4** isolated from *Artemisia rubripes*

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