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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 1β,6α-dihydroxy-4(15)-eudesmene (**1**), scopoletin (**2**), 1α,4β-dihydroxy-8α-acetoxy-guaia-2,10(14), 11(13)-triene-6,12-olide (**3**), 1α,4β-dihydroxy-8α-acetoxy-guaia-2,9,11(13)-triene-6,12-olide (**4**), and β-sitosterol-3-*O*-β-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 $CDCl_3$, Bruker AMX 500 and Varian UNITY INOVA 500. IR: in CCl_4, 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_{254} plates and RP-18 F_{254s} plates. LPLC: Merck Lichroprep Lobar®-A Si 60 (240×10 mm)

Plants meterial

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 systemof *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 ($CHCl_3$: Acetone=10:1) and HPLC ($CHCl_3$: MeOH=30:1) to yield compound **1** (8 mg). Fraction W5 (12 g) was chromatographed with Sephadex LH-20 (CH_2Cl_2:MeOH= 1:1) to give three subfractions (W51-W53). Subfraction W52 (6 g) was rechromatographed with Sephadex LH-20 (CH_2Cl_2:MeOH=1:1) to give five subfractions (W521-W525). Subfraction W522 (3.3 g) was chromatographed with silica gel column ($CHCl_3$: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 ($CHCl_3$: EtOAc=5:1) to afford compounds **3** (4 mg) and **4** (5 mg). Fraction W6 (6.2 g) was chromatographed with silica gel column ($CHCl_3$:MeOH=20:1) to give four subfractions (W61-W64). Subfraction W64 (1.5 g) was chromatographed with Sephadex LH-20 (CH_2Cl_2: 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° (c 0.95, $CHCl_3$); IR ν_{Max}^{NaCl} (cm^{-1}): 3019, 2400, 1215, 928, 757, 669; EI-MS *m/z* (rel. int.): 238 (M^+, 15), 220 (75), 202 (27), 177 (44), 159 (47), 133 (32), 121 (60), 107 (75), 83 (100); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.71 (3H, s, H-14), 0.87 (3H, d, *J* = 7.0Hz, H-12), 0.95 (3H, d, *J* = 7.0Hz, H-13), 1.21 (1H, dd, *J* = 3.0, 10.0Hz, H-9a), 1.28 (1H, td, *J* = 4.0, 10.0Hz, H-9b), 1.55 (2H, m, H-3a, 8a), 1.75 (1H, d, *J* = 10.0Hz, H-5), 1.86 (2H, m, H-2), 1.92 (1H, ddd, *J* = 2.5, 3.0, 12.5Hz, H-8b), 2.07 (1H, td, *J* = 2.5, 5.5Hz, H-3b), 2.24 (1H, td, *J* = 2.5, 7.0Hz, H-11), 2.33 (1H, ddd, *J* = 2.5, 5.0, 13.0Hz, H-7), 3.42 (1H, dd, *J* = 4.5, 11.5Hz, H-1), 3.71 (1H, t, *J* = 10.0Hz, H-6), 4.74 (1H, d, *J* = 1.5Hz, H-15b), 5.02 (1H, d, *J* = 1.5Hz, H-15a); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 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^+)^{1}H-NMR (500 MHz, $CDCl_3$): δ 3.97 (3H, s, -OCH_3), 6.13 (1H, s, -OH), 6.29 (1H, d, *J* = 9.5Hz, H-3), 6.86 (1H, s, H-5), 6.94 (1H, s, H-8), 7.61 (1H, d, *J* = 9.5Hz, H-4) ^{13}C-NMR (125 MHz, $CDCl_3$): δ 56.65 (-OCH_3), 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, $[\alpha]^{24}{}_D$ +6.6° (c 0.95, $CHCl_3$); UV λ_{max}^{MeOH} nm (log) 214; IR ν_{Max}^{NaCl} (cm^{-1}): 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^+, 11), 305 (57), 263 (61), 242 (36), 230 (57), 199 (33), 171 (35), 135 (30), 121 (31), 98 (100); ^{1}H-NMR (500 MHz, $CDCl_3$): Table I ^{13}C-NMR (125 MHz, $CDCl_3$): Table I.

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

Colorless gum, $[\alpha]_D^{24}$ +12.4° (c 0.95, $CHCl_3$); UV λ_{max}^{MeOH} nm (log) 216; ν_{Max}^{NaCl} (cm^{-1}): 2933, 2362, 1750, 1235, 1020, 751; EI-MS *m/z* (rel. int.): 320 (M^+, 8), 305 (10), 260 (66), 217 (57), 200 (100), 171 (75), 165 (73), 121 (64) ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.56 (3H, brs, H-15), 1.88 (3H, s, H-14), 2.16 (3H, s, OAc), 2.43 (1H, d, *J* = 10.0Hz, H-5), 4.14 (1H, td, *J* = 2.0, 6.5Hz, H-7), 4.46 (1H, t, *J* = 10.0Hz, H-6), 5.21 (1H, dd, *J* = 4.5, 9.5Hz, H-8), 5.67 (1H, brq, *J* = 1.5, 5.5Hz, H-9), 5.75 (1H, d, *J* = 3.5Hz, H-13b), 5.95 (1H, d, *J* = 5.5Hz, H-2), 5.99 (1H, d, *J* = 5.5Hz, H-3), 6.32 (1H, d, *J* = 3.5Hz, H-13a) ^{13}C-NMR (125 MHz, $CDCl_3$): δ 21.52 (-C=O-CH_3), 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).

Table I. ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$) spectral data of Compound **3**

Position	δ_C	δ_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-14b	9
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	

β-Sitosterol-3-*O*-β-D-glycoside (5)

Colorless oil, ESI-S *m/z*: 576 (M^+); ^{1}H-NMR (300 MHz, DMSO-d_6): δ 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* = 5.0Hz, H-1'), and 5.41 (1H, m, H-6); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 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-2¢), 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}H-NMR spectrum showed two secondary methyl signals at δ 0.87 (3H, d, *J* = 7.0Hz) and 0.95 (3H, d, *J* = 7.0Hz), and a quaternary methyl signal at δ 0.71 (3H, s), two carbinol proton signals at 3.42 (1H, dd, *J* = 4.5, 12.0Hz) and 3.71 (1H, t, *J* = 10.0Hz), and an exomethylene signals at δ 4.74 (1H, d, *J* = 1.5Hz) and 5.02 (1H, d, *J* = 1.5Hz). The ^{13}C-NMR spectrum demonstrated the presence of 15 carbon signals which contained two olefinic carbon signals at δ 108.03 and 146.48, and two carbinol carbon signals at δ 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 1β,6α-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}H- and ^{13}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 $C_{17}H_{20}O_6$ was assigned by HREI-MS. The ^{1}H-NMR spectrum showed six olefinic proton signals at δ 5.08 (1H, d, *J* = 1.0Hz), 5.25 (1H, d, *J* = 1.0Hz), 5.71 (1H, d, *J* = 5.5Hz), 5.87 (1H, dd, *J* = 0.5, 3.5Hz), 6.01 (1H, d, *J* = 5.5Hz) and 6.35 (1H, dd, *J* = 0.5, 3.5Hz) and acetyl signal at δ 2.17 (3H, s). The ^{13}C-NMR spectrum demonstrated the presence of 17 carbon signals which was composed of six olefinic carbon signals at δ 119.02, 125.22, 135.96, 136.92, 140.14, and 144.00, four oxygenated carbon signals at δ 74.92, 77.86, 82.94, and 87.00, and two carbonyl carbon signals at δ 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}H- and ^{13}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 1α,4α-dihydroxybishopsolicepolide (Singh *et al.* 1985), which was isolated from *Bishopanthus soliceps*, on the basis of the coupling constants observed in the ^{1}H-NMR spectrum. Furthermore, the stereochemistry of **3** was reconfirmed from its 2D NOESY spectrum (Table I). Thus, compound **3** was determined to be 1α,4β-dihydroxy-8α-acetoxy-guaia-2,10(14),11(13)-triene-6,12-olide. Although compound **3** is a stereoisomer of 1α,4α-dihydroxybishopsolicepolide, the structure of **3** was not yet reported.

Compound **4** was obtained as colorless gum. The ^{1}H-NMR and ^{13}C-NMR spectral data were similar to those of compound **3**. The ^{1}H-NMR spectrum showed five olefinic proton signals at δ 5.67 (1H, brs, *J* = 1.5, 5.5Hz), 5.75 (1H, d, *J* = 3.5Hz), 5.95 (1H, d, *J* = 5.5Hz), 5.99 (1H, d, *J* = 5.5Hz) and 6.32 (1H, d, *J* = 3.5Hz). The ^{13}C-NMR spectrum demonstrated the presence of 17 carbon signals which contained six olefinic carbon signals at δ 122.81, 123.33, 137.93, 138.49, 139.18, and 142.95, four oxygenated carbon signals at δ 74.24, 75.71, 80.51, and 84.24, and two carbonyl carbon signals at δ 169.65 and 170.77. Thus, compound **4** was determined to be 1α,4β-dihydroxy-8α-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}H- and ^{13}C-NMR spectral data and the comparison of the data in the previous literature, the structure of **5** was established as β-sitostrol-3-*O*-β-D-glycoside (Kim *et al.*, 1996).

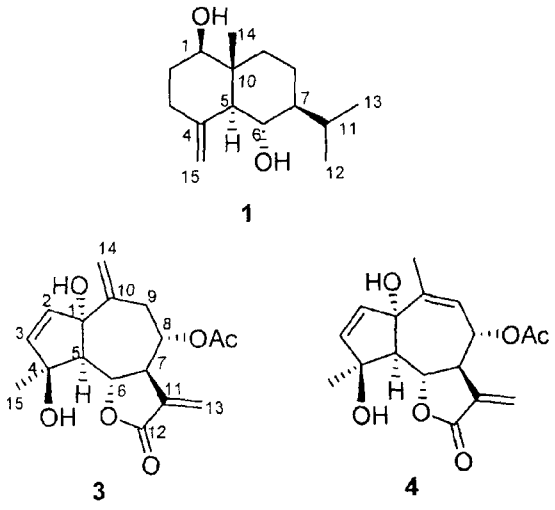

Fig. 1. Structures of compounds **1**, **3**, and **4** isolated from *Artemisia rubripes*

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