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Notes

Isolation of a New Labdane-type Diterpene from *Vitex rotundifolia*

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Vitex rotundifolia L. fil (Verbenaceae) is widely distributed along the sandy beaches of Korea, China and Japan. Traditionally in Korea, seeds and fruits of *V. rotundifolia* are used for treatment of various allergic diseases as well and for alleviation of the symptoms of various other ailments including rhinitis, sinusitis, migraine, or even the common cold.[1] Several flavonoids and terpenoids such as vitexicarpin, artemetin, rotundifuran and ferruginol – some of which show antiproliferative and antioxidant effects *in vitro* – have previously been isolated from this plant.[1-15] We have previously reported three known flavonoids from the *V. rotundifolia*.[16] In our continuing, further study, we isolated a new labdane-type diterpene (**1**) and five known diterpenes (**2**-**6**) from *V. rotundifolia*; our procedures and findings are reported in this paper (Figure 1).

The chemical structures of known compounds including two labdane-type diterpenes, (5*S**,6*R**,8*R**,9*R**,10*S**)-6-acetoxy-9-hydroxy-15-methoxy-13(14)-labden-16,15-olide (**2**)[2] and vitexilactone (**3**),[3,4] and three halimane-type diterpenes,[5] vitetrifolin F (**4**), vitetrifolin E (**5**) and vitetrifolin D (**6**) were determined by comparison of the obtained spectroscopic data with those reported in the literature (Figure 1).

Compound **1** was obtained as a pale yellow solid. The molecular formula of **1** was determined to be $C_{22}H_{34}O_6$

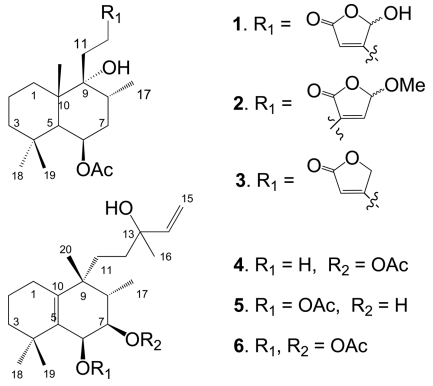

Figure 1. Chemical structure of compounds **1**-**6** isolated from *Vitex rotundifolia*.

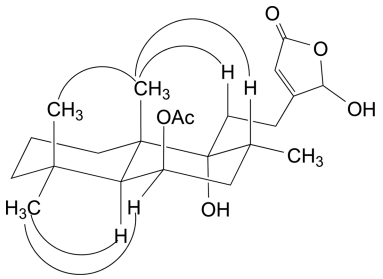

Figure 2. Key NOESY correlations of compound **1**.

by a combination of high resolution ESIMS ($[M-H]^-$ *m/z* 393.2286) and ^{13}C NMR spectrometry. The IR spectrum of **1** exhibited the presence of hydroxy (3509 cm^{-1}), acetoxyl (1734 cm^{-1}), and α,β-unsaturated γ-lactone (1779 cm^{-1}) groups. The ^1H and ^{13}C NMR spectrum of **1** contained signals for three tertiary methyl groups [δ_H 1.26, 1.01, 0.96 (each 3H, s); δ_C 19.0, 23.7, 33.7], one secondary methyl group [δ_H 0.91 (3H, d, J = 6.6 Hz); δ_C 16.2], one acetyl group [δ_H 2.05 (3H, s); δ_C 22.0], one oxygenated methine proton [δ_H 5.37 (1H, q, J = 2.5 Hz); δ_C 69.7 (d)] and γ-hydroxy-α,β-unsaturated γ-lactone [δ_H 5.98, 5.86 (1H, s), δ_C 98.9 (d), 117.3 (d), 170.5 (s)]. The ^1H NMR spectrum of **1** was similar to that of vitexilactone (**3**), except for appearance of an oxymethine signal at δ 5.98 (1H, s) instead of an oxygenated methylene (δ 4.73, d, J = 1.7 Hz) assigned to H-16 of **3**.

This suggestion was supported by the ^{13}C NMR spectral data. Comparing the ^{13}C NMR spectra of **1** with those of **3**, the signals of C-14 and C-16 in **3** were shifted downfield from δ 114.8 and 73.2 of **3** to δ 117.3 and 98.9 of **1**, respectively, whereas those of C-13 and C-15 were shifted upfield from δ 170.9 and 170.9 of **3** to δ 170.3 and 170.5 of **1**, respectively, and other carbon signals were quite similar to those of **3** (Table 1). These ^1H and ^{13}C NMR spectroscopic data were assigned by the ^1H-^1H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) experiments (Table 1). The relative stereochemistry in **1** was

Table 1. ^{1}H and ^{13}C NMR data for compounds **1** and **3** isolated from *Vitex rotundifolia*

No	1		3	
	δ_H	δ_C	δ_H	δ_C
1	1.45 (2H, m)	33.7 t	1.43 (2H, m)	33.7 t
2	1.48 (2H, m)	18.6 t	1.47 (2H, m)	18.7 t
3	1.35 (2H, m)	43.6 t	1.25 (2H, m)	43.6 t
4	-	34.1 s	-	34.1 s
5	1.51 (1H, m)	47.8 d	1.55 (1H, d, J = 2.5 Hz)	47.7 d
6	5.37 (1H, q, J = 2.5, 2.5 Hz)	69.7 d	5.35 (1H, q, J = 2.5 Hz)	69.8 d
7	1.58 (2H, m)	36.1 t	1.61 (2H, m)	36.1 t
8	2.13 (1H, m)	32.2 d	2.11 (1H, m)	32.1 d
9	-	77.6 s	-	76.4 s
10	-	43.7 s	-	43.8 s
11	2.13 (1H, m) 1.79 (1H, m)	31.5 t	1.95 (1H, ddd, J = 14.3, 9.4, 7.2 Hz) 1.72 (1H, ddd, J = 14.3, 9.4, 7.2 Hz)	31.7 t
12	2.53 (2H, m)	24.3 t	2.48 (2H, dd, J = 9.4, 7.2 Hz)	25.5 t
13	-	170.3 s	-	170.9 s
14	5.86 (1H, s)	117.3 d	5.82 (1H, s)	114.8 d
15	-	170.5 s	-	170.9 s
16	5.98 (1H, s)	98.9 d	4.73 (2H, d, J = 1.7 Hz)	73.2 t
17	0.91 (3H, d, J = 6.6 Hz)	16.2 q	0.88 (3H, d, J = 6.6 Hz)	16.2 q
18	0.96 (3H, s)	33.7 q	0.95 (3H, s)	33.7 q
19	1.01 (3H, s)	23.7 q	0.99 (3H, s)	23.8 q
20	1.26 (3H, s)	19.0 q	1.23 (3H, s)	19.1 q
6-CO$\underline{CH_3}$	2.05(3H, s)	22.0 q	2.04(3H, s)	22.0 q
6-$\underline{CO}CH_3$	-	169.6 s	-	170.3 s

Measured in $CDCl_3$ at 300 and 75 MHz, respectively. Assignments were aided by ^{1}H gDQCOSY, TOCSY, DEPT, gHMQC, and gHMBC experiments.

examined from its NOESY spectral data. The NOE correlation between H-20/H-19, H-20/H-11a, and H-20/H-8 was observed in **1**. In addition, NOE correlations between H-18/H-19, H-18/H-6, and H-18/H-5 were observed (Figure 2). On the basis of these data, the configurations at C-5, C-6, C-8, C-9 and C-10 were determined to be S^*, R^*, R^*, R^* and S^*, respectively. Thus, **1** was determined as (5S^*,6R^*,8R^*,9R^*,10S^*)-6-acetoxy-9,16-dihydroxy-13(14)-labden-16,15-olide. However, the configuration of the hydroxy group at C-16 was not determined.

Experimental

General Experimental Procedures. Optical rotations were determined on a Perkin-Elmer polarimeter 341. NMR spectra were recorded in CD_3OD and $CDCl_3$ on a Varian Mercury 300 instrument at 300 MHz for ^{1}H and 75 MHz for ^{13}C using standard pulse sequence programs. All chemical shifts were recorded with respect to TMS as an internal standard. Mass spectra were obtained at the Korean Basic Science Institute, Seoul, Korea. Column chromatography was carried out on RP 18 (YMC-Pack ODS-A, 12 nm, S-5 μm, 250 × 10 mmI.D., YMC, USA) and silica gel (YMC-Pack SIL, 12 nm, S-5 μm, 250 × 10 mmI.D., YMC, USA). High performance liquid chromatography (HPLC) was performed on a Dionex P580 HPLC system equipped with a Varian 350 RI detector. All solvents used were spectral grade or were distilled from glass prior to use.

Plant Material. The halophyte *V. rotundifolia* used for this study was collected from the Muan-gun, Jeollanamdo, Korea in July of 2008. The species was identified by Dr. Sung-Gi Moon by its morphological character. A voucher specimen (08H-6) is deposited at the Herbarium of the Division of Marine Environment and Bioscience, Korea Maritime University, Busan, Korea.

Extraction and Isolation. The air-dried sample of *V. rotundifolia* was chopped into small pieces and extracted successively for 48 h with CH_2Cl_2 (3 L × 2) and MeOH (3 L × 2), in turn. The combined crude extract (145.1 g) was evaporated under reduced pressure and then the residue was partitioned between CH_2Cl_2 and water. The organic layer was further partitioned between 85% aqueous MeOH and *n*-hexane, and the aqueous layer was fractionated with *n*-BuOH and H_2O. The resulting four fractions were evaporated to dryness *in vacuo*, to yield *n*-hexane (33.6 g), 85% aqueous MeOH (21.0 g), *n*-BuOH (39.0 g), and water (47.8 g) fractions, respectively. The portion of the 85% aqueous MeOH (21.0 g) fraction was subjected to C_{18} reversed-phase vacuum flash chromatography and eluted with gradient system of MeOH-water of decreasing polarity (50, 60, 70, 80, 90, 100%; 800mL each) to provide 6 fractions. Fraction 4 was separated by reversed-phase HPLC (ODS-A, 78% aq. MeOH) to give 6 subfractions (4-1~6), in order of elution. Subfraction 4-2 and 4-3 were separately purified by reversed-

phase HPLC with same solvent (ODS-A, 50% aq. CH_3CN) to give **1** (2.5 mg) and **4** (15.0 mg), respectively. Subfraction 4-4 was separated by reversed-phase HPLC with 63% aq. CH_3CN to afford **3** (18.0 mg). Similarly, subfraction 4-5 was applied on reversed-phase HPLC with 63% aq. CH_3CN to give **5** (55.7 mg) and one mixture (4-5-1). Subfraction 4-5-1 was applied on normal-phase HPLC eluting with 25% EtOAc in hexane to give **2** (2.9 mg). Subfraction 4-6 was subjected reversed-phase HPLC with 60% aq. CH_3CN to afford **6** (69.9 mg).

(5*S,6*R**,8*R**,9*R**,10*S**)-6-Acetoxy-9,16-dihydroxy-13(14)-labden-16,15-olide (1):** A pale yellow solid, $[\alpha]_{20}^{D}$ +17.0 (*c* = 0.42, MeOH). HR-ESI-MS (negative-ion mode) *m/z*: 393.2286 $[M-H]^-$ (calcd for $C_{22}H_{33}O_6$: 393.2277); ^{1}H-NMR and ^{13}C-NMR, see Table 1.

(5*S,6*R**,8*R**,9*R**,10*S**)-6-Acetoxy-9-hydroxy-15-methoxy-13(14)-labden-16,15-olide (2):** An amorphous white solid, $[\alpha]_{20}^{D}$ −7.2 (c, 0.21, MeOH). ESI-MS (negative-ion mode) *m/z* 407 $(M-H)^-$, EI-MS *m/z*: 348 $(M-CH_3COOH)^+$.

Vitexilactone (3): A pale yellow solid, $[\alpha]_{20}^{D}$ −3.0 (*c* = 0.37, MeOH). EI-MS *m/z*: 318 $(M-CH_3COOH)^+$. ^{1}H-NMR and ^{13}C-NMR, see Table 1.

Vitetrifolin D (4): A colorless syrup, $[\alpha]_{20}^{D}$ +104 (*c* = 1.28, MeOH). EI-MS *m/z*: 346 $(M-CH_3COOH)^+$, 286 $(M-CH_3COOH\times2)^+$.

Vitetrifolin E (5): A colorless solid, $[\alpha]_{20}^{D}$ +60 (*c* = 0.38, MeOH). EI-MS *m/z*: 304 $(M-CH_3COOH)^+$.

Vitetrifolin F (6): A colorless syrup, $[\alpha]_{20}^{D}$ +83 (*c* = 0.22, MeOH). EI-MS *m/z*: 304 $(M-CH_3COOH)^+$.

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