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Labdane-type Diterpenoids from the Fruits of *Vitex rotundifolia*

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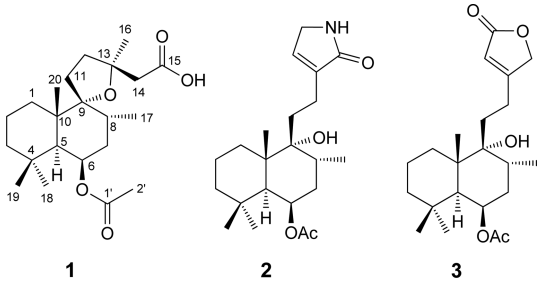
Vitex rotundifolia (Verbenaceae), synonymous *Vitex trifolia* Linn. var. *simplicifolia*, is native to seashores of Japan, Korea peninsula, and east China. Its mature fruits are used as traditional Chinese medicine (TCM) for the treatment of colds, headaches, migraine, cancer, inflammations and eye pain.[1] Several kinds of compounds, including diterpenoids,[2,3] flavonoids,[4-7] lignans,[8] iridoid and phenolic glucoside,[9] have been obtained from the fruits of this plant. Polymethoxy flavonoids in this plant can inhibit proliferation of several cancer cells by inducing apoptosis and G2/M arrest.[4,5] In the course of screening for bioactive constituents from this TCM, we have isolated norlabdane diterpene vitrifolin A, and evaluated its inhibitory activity on NO production in RAW264.7 cells.[10] Continued investigation of the diterpenoid components resulted in the isolation of a new labdane, vitrifolin B (**1**), and two known ones, vitexlactam A (**2**)[11] and vitexilactone (**3**),[12,13] from the fruits of *V. rotundifolia* (Fig. 1). This paper reports the isolation and structure elucidation of these compounds.

Vitrifolin B (**1**) was obtained as colorless crystal. Its molecular formula was determined to be $C_{22}H_{36}O_5$ by the quasi molecular ion peak at *m/z* 403.2451 ($[M + Na]^+$, Calcd for $C_{22}H_{36}O_5Na^+$: 403.2455) in HR-ESI-MS spectrometry, suggesting five unsaturated degrees. Its IR spectrum showed the absorption bands of hydroxyl (3414 cm^{-1}) and ester carbonyl (1739 cm^{-1}) moieties. The highfield in ^{1}H-NMR spectrum (Table 1) displayed the signals of six methyls respectively at δ_H 2.05 (s), 1.40 (s), 1.26 (s), δ_H 0.99 (s), 0.95 (s), 0.91 (d, J = 6.8 Hz). The chemical shifts and coupling patterns of these signals were very similar to that of labdane-type diterpenpids previously isolated from the same genus.[3,14,15] With the aids of HMBC and HMQC correlations, the signals δ_H 2.05, and δ_C 20.9 and 169.2 can be assigned to an acetyl group. Compared with the data in literature,[16] the ^{1}H-, ^{13}C-NMR and DEPT spectra of **1** (Table 1) were very similar to that of dihydrogrindelic acid, except for the presence of acetyl group, and an oxygenated methane signal at δ_H 5.41 (1H, m) and δ_C 68.9 (CH). Therefore, vitrifolin B (**1**) was supposed to be the derivative of dihydrogrindelic acid with an acetyloxy group.

Table 1. ^{1}H-, ^{13}C-NMR and DEPT data of vitrifolin B (**1**) ($CDCl_3$, δ ppm, TMS)[a,b]

No.	δ_H mult (*J* in Hz)	δ_C
1	1.42 m, 1.49 m	33.8 t
2	1.51 m , 1.53 m	17.7 t
3	1.17 ddd (13.4, 13.4, 3.6), 1.33 m	43.0 t
4	-	33.2 s
5	1.34 d (2.3)	48.7 d
6	5.41 (1H, m)	68.9 d
7	1.52 m (overlapped), 1.59 m (overlapped)	35.8 t
8	2.18 m	30.3 d
9	-	94.4 s
10	-	41.9 s
11	1.78 ddd (13.1, 8.7, 2.1), 2.23 m	28.1 t
12	1.87 m, 1.92 m	37.7 t
13	-	81.2 s
14	2.62 d (15.4), 2.78 d (15.4)	46.4 t
15	-	170.0 s
16	1.40 s	26.3 q
17	0.91 d (6.8)	16.9 q
18	0.95 s	32.2 q
19	0.99 s	22.7 q
20	1.26 s	19.1 q
OAc		
1'	-	169.2 s
2'	2.05 s	20.9 q

[a]Measured at 500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR. [b]Assigned by ^{1}H-^{1}H COSY, HMQC and HMBC spectra.



1 2 3

Figure 1. The structures of **1**-**3**.

The above conclusion and the structure of vitrifolin B (**1**) were finally confirmed by the 2D NMR spectra. The ^{1}H-^{1}H COSY spectrum displayed the correlations through δ_H 1.42 and 1.49 (H-1) to δ_H 1.17 and 1.33 (H-3), δ_H 1.34 (H-5) to δ_H 2.18 (H-8) and then to δ_H 0.91 (CH_3-17), which suggested

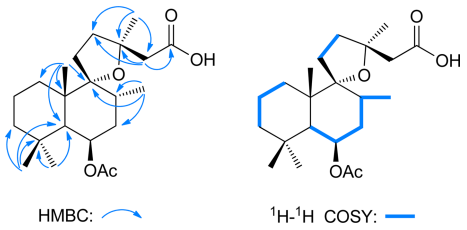


Figure 2. The key HMBC correlations and partial structures assigned by ^{1}H-^{1}H COSY of vitrifolin B (**1**).

the presence of two substructures, CH_2(1)-CH_2(2)-CH_2(3), and CH(5)-CH(6)-CH_2(7)-CH(8)-CH_3(17). (Fig. 2). The HMBC correlations of CH_3-18 and CH_3-19 with C-3, C-4, C-5, CH_3-20 with C-1, C-5, C-9, C-10, CH_3-17 with C-7, C-8 and C-9 (Fig. 2), along with the above substructures obtained by ^{1}H-^{1}H COSY spectrum, established the A and B rings of a labdane skeleton (Fig. 2). The downfield shift of the signal of sole oxygenated methine (δ_H 5.41) and ^{1}H-^{1}H COSY correlation between δ_H 1.34 (H-5) and δ_H 5.41 (H-6) also suggested the location of acetyloxy group at C-6.[3,10] Along with the five unsaturated degrees, the HMBC correlations of CH_3-20 and CH_3-17 with C-9 (94.4 ppm), and CH_3-16 with C-12 and C-13 (81.2 ppm) indicated that an ether linkage must be restricted between C-9 and C-13. The HMBC correlations of AB system signals at δ_H 2.62 and 2.78 (respectively, d, J = 15.4 Hz, H_2-14) with C-12, C-13 and C-15 suggested the placement of the methylene between the carboxylic group and a substituted carbon at C-13. The configuration of methyl group at C-10 (CH_3-17) was assigned as β orientation under the biogenetical consideration of normal labdane skeleton isolated from Verbenaceae species.[2,3,14,15] Other relative configurations of **1** ware assigned by its NOESY correlations and analysis of coupling constant (Fig. 3). The NOESY correlation between CH_3-18 and H-5, and H-5 and CH_3-17 suggested the *trans*-configuration of two rings and α orientation of CH_3-17. The NOESY correlation between CH_3-17 and H-11 suggested the β orientation of H-11. The CH_3-16 was assigned as α orientation by the NOESY correlation between H-14 and CH_3-17. The acetyloxy group at C-6 was assigned as β orientation by the small coupling constant between H-5 and H-6 ($J_{5,6}$ = 2.3 Hz).[15] Thus, the structure of **1** was identified (Fig. 1), and named as vitrifolin B.

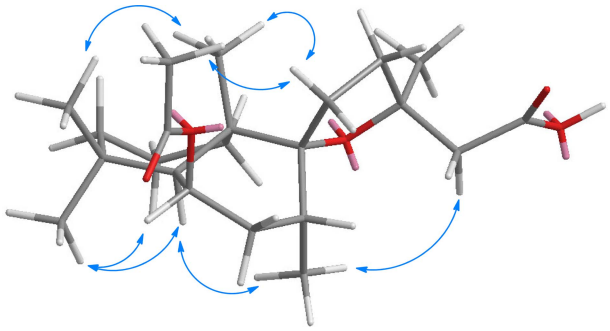


Figure 3. The key NOESY correlations for vitrifolin B (**1**).

Experimental Section

General Procedures. Melting points were determined on Kofler melting point apparatus and uncorrected. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were obtained from Nicolet NEXUS 670 FT-IR spectrometer. ^{1}H-, ^{13}C-NMR (DEPT) and 2D NMR were recorded on Bruker AVANCE 500 spectrometer. HR-ESI-MS spectrum was obtained on Bruker APEX spectrometer. Silica gel used for column chromatography (CC) and silica GF_{254} for thin layer chromatography (TLC) were purchased from *Qingdao Marine Chemical Factory* in China. Silica gel C-18 used for low pressure CC was purchased from *Merck*. Spots were detected on TLC under UV light at 254 nm or by heating after spraying with 5% H_2SO_4 in EtOH.

Plant Material. The fruits of *V. rotundifolia* were collected from Wendeng, Weihai, Shandong Province, P. R. China, in September 2010, and identified by Associate Prof. Hong Zhao (Shandong University at Weihai). A voucher specimen (No. KY201001) is deposited at the herbarium in the Laboratory of Botany, Marine College, Shandong University at Weihai.

Extraction and Isolation. The powered fruits (35 kg) of *V. rotundifolia* were extracted with MeOH at room temperature (7 days × 3). The extract was partitioned with petroleum ether (60-90 ºC), $CHCl_3$ and H_2O. The $CHCl_3$ soluble fraction (1204 g) was separated by silica gel CC eluting with hexane-acetone gradient (20:1 to 2:1) to give four fractions (Fr1-Fr4). Fr2 (hexane-acetone, 10:1, 155 g) was isolated by silica gel CC with hexane-EtOAc gradient (20:1 to 2:1) to afford subfractions f_1-f_4. Compound **3** (72 mg) was purified from f_1 (hexane-EtOAc, 20:1). Fr3 (hexane-acetone, 5:1, 232 g) was isolated by silica gel CC with hexane-EtOAc gradient (20:1 to 2:1) to afford subfractions f_5-f_8. Subfraction f_5 (hexane-EtOAc, 15:1) was subject to a silica gel CC purification with hexane-acetone(10:1) elution, and further purified by C-18 CC (H_2O-MeOH 1:1) to give **2** (68 mg). Compounds **1** (45 mg) was obtained by repeated silica gel CC (hexane-acetone, 10:1) and C-18 CC (H_2O-MeOH, 1:1) from f_6 (hexane-EtOAc, 5:1). The R_f values of compounds **1** and **2** were 0.16 and 0.27 respectively when they were checked by TLC in silica gel developed with hexane-acetone (10:1).

Vitrifolin B (1). Colorless crystal; mp 171-173 ºC; $[\alpha]_D^{20}$ = +32 (*c* 0.625, CH_3OH); IR (KBr): ν_{max} 3414, 2964, 2928, 1739, 1686, 1463, 1384, 1261, 1098, 1051, 1020, 801, 637, 615 cm^{-1}; HR-ESI-MS *m/z*: 403.2451 ($[M + Na]^+$, Calcd for $C_{22}H_{36}O_5Na^+$: 403.2455). ^{1}H-NMR (500 MHz, $CDCl_3$) and ^{13}C-NMR (DEPT) (125 MHz, $CDCl_3$): see Table 1.

Vitexlactam A (2). Colorless needle crystal; mp 205-207 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 6.70 (1H, brs, H-14), 5.32 (1H, brs, H-6), 3.90 (2H, brs, H_2-15), 1.97 (3H, s, H_3-2'), 1.14 (3H, s, H_3-20), 0.92 (3H, s, H_3-19), 0.89 (3H, s, H_3-18), 0.85 (3H, d, J = 6.7 Hz, H_3-17); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 32.7 (C-1), 17.8 (C-2), 42.7 (C-3), 32.9 (C-4), 46.5 (C-5), 69.6 (C-6), 35.2 (C-7), 32.6 (C-8), 76.2 (C-9), 42.9 (C-10), 31.3 (C-11), 20.8 (C-12), 139.6 (C-13), 136.2 (C-

14), 45.5 (C-15), 173.8 (C-16), 15.5 (C-17), 31.2 (C-18), 22.7 (C-19), 17.9 (C-20), 169.5 (C-1'), 20.9 (C-2').

Vitexilactone (3). Colorless needle crystal; mp 143 - 145 ºC; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 5.77 (1H, m, H-14), 5.32 (1H, m, H-6), 4.68 (2H, brs, H_2-16), 1.98 (3H, s, H_3-2'), 1.18 (3H, s, H_3-20), 0.94 (3H, s, H_3-19), 0.89 (3H, s, H_3-18), 0.83 (3H, d, J = 6.8 Hz, H_3-17); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 31.0 (C-1), 17.5 (C-2), 42.5 (C-3), 32.9 (C-4), 46.6 (C-5), 68.7 (C-6), 34.9 (C-7), 32.5 (C-8), 75.4 (C-9), 42.7 (C-10), 30.5 (C-11), 24.3 (C-12), 170.1 (C-13), 113.9 (C-14), 173.0 (C-15), 72.1 (C-16), 15.0 (C-17), 31.0 (C-18), 22.6 (C-19), 17.9 (C-20), 169.4 (C-1'), 20.8 (C-2').

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