Triterpenoids and Diterpenoids from *Viburnum chingii*

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Two new oleanane triterpenoids (1—2) and one new vibsane-type diterpenoid (3), together with 7 known compounds (4—10), were isolated from the leaves of *Viburnum chingii*. The structures of compounds 1—3 were elucidated by means of spectroscopic methods including extensive 1D- and 2D-NMR technique. Cytotoxicity of compounds 1—10 were tested against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 cell lines. Compound 3 showed significant cytotoxicity against HL-60, SK-BR-3, and PANC-1 cell lines.

Key words *Viburnum chingii*; oleanane triterpenoid; vibsane diterpenoid

Phytochemical studies have revealed that *Viburnum* species mainly contained triterpenoids,[1,2] iridoids,[3] vibsane-type diterpenes,[4,5] lignans,[6] and phenolic glycosides.[7] *Viburnum chingii* P. S. Hsu is mainly distributed in southwest of Yunnan province, China. A literature search revealed that no phytochemical study has been undertaken within this species. As a part of our work, we chemically examined the acetone extract of *V. chingii* leaves. Two new oleanane triterpenoids (**1**—**2**) and one new vibsane-type diterpenoid (**3**) were isolated from this plant, along with seven known compounds, vibsaol B (**4**),[8] 2α,3β-dihydroxy-20(29)-lupene (**5**),[9] 6α-hydroxy-3-on-20(29)-lupen-28-oic acid (**6**),[10] 3,6-dion-20(29)-lupen-28-oic acid (**7**),[10] hederagenin acid (**8**),[11] castanopsone (**9**),[12] and 3β,6α-dihydroxy-20(29)-lupene (**10**).[13] Compounds **1**—**10** were tested cytotoxicity against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 cell lines. Herein, we describe the isolation, structure elucidation of **1**—**3**, and cytotoxicity of **1**—**10**.

The acetone extract of *V. chingii* leaves was successively subjected to silica gel chromatography, Sephadex LH-20 as well as RP-18 to afford three new compounds (**1**—**3**) and 7 known ones (**4**—**10**).

Compound **1**, white amorphous powder, had a molecular formula of $C_{31}H_{52}O_3$ according to its positive high resolution-electrospray ionization-mass spectra (HR-ESI-MS) (495.3802, $[M+Na]^+$) and ^{13}C-NMR analysis, demanding 6 degrees of unsaturation. The IR showed the presence of hydroxyl groups (3440 cm^{-1}) and double bond (1631 cm^{-1}). The ^{13}C- and distortionless enhancement by polarization transfer (DEPT) spectral data showed seven quaternary carbons (an olefinic one), seven methines (an olefinic one and three oxygen bearing ones), eight methylenes, eight methyls, and one methoxy group (Table 1). The ^{1}H-NMR and heteronuclear single quantum coherence (HSQC) spectra also confirmed the observations in the ^{13}C-NMR spectrum. The ^{13}C- and ^{1}H-NMR spectroscopic data accounted for all the hydrogens except for two ones, indicating the presence of two free hydroxyls. Apart from one degree of unsaturation from the double bond, the remaining five ones in **1** were assumed to be the presence of five rings. Considering the characteristic of ^{13}C- and ^{1}H-NMR data, **1** was ascribed to be derivate of oleanene triterpenoids. The double bond was located between C-12 and C-13 because H-15, H-18, and H-19 showed the heteronuclear multiple bond connectivity (HMBC) correlations with the olefinic quaternary carbon (δ_C 151.5, C-13). The HMBC correlations from H-9 and H-18 to the olefinic methine (δ_C 119.2, C-12) further confirmed the location of the double bond. The methoxy group exhibited HMBC correlation with C-11 identified by the hydrogen spin system of H-11/H-12 from ^{1}H–^{1}H correlation spectroscopy

Table 1. ^{1}H- and ^{13}C-NMR Data for Compounds **1**—**2** in $CDCl_3$ (δ in ppm, J in Hz)

No.	**1**		**2**	
	δ_H	δ_C	δ_H	δ_C
1	4.36 (1H, br s)	73.0	4.15 (1H, br s)	72.8
2	1.93, 2.04 (each 1H, m)	34.6	1.90, 2.01 (each 1H, m)	32.2
3	3.72 (1H, dd, 6.0, 14.5)	72.9	3.78 (1H, dd, 4.5, 12.0)	72.9
4		41.9		39.0
5	1.51 (1H, m)	47.6	1.43 (1H, m)	44.3
6	1.58, 1.66 (each 1H, m)	18.2	1.53, 1.68 (each 1H, m)	17.4
7	1.28, 1.69 (each 1H, m)	33.2	1.37, 1.71 (each 1H, m)	31.6
8		42.2		42.5
9	1.75 (1H, m)	46.1		150.2
10		43.1		45.0
11	3.75 (1H, dd, 4.0, 8.2)	76.6	5.73 (1H, d, 7.0)	117.0
12	5.46 (1H, d, 4.0)	119.2	5.56 (1H, d, 7.0)	119.9
13		151.5		149.0
14		41.0		40.7
15	1.11, 1.79 (each 1H, m)	26.4	1.03, 1.85 (each 1H, m)	25.6
16	1.02, 1.85 (each 1H, m)	27.1	0.88, 1.98 (each 1H, m)	26.9
17		32.0		32.1
18	2.07 (1H, m)	46.9	2.14 (1H, m)	45.7
19	0.98, 1.64 (each 1H, m)	46.7	1.07, 1.63 (each 1H, m)	46.7
20		31.1		31.1
21	1.33, 1.59 (each 1H, m)	39.1	1.18, 1.47 (each 1H, m)	39.0
22	1.13, 1.32 (each 1H, m)	36.7	1.11, 1.33 (each 1H, m)	36.9
23	1.02 (3H, s)	28.5	1.07 (3H, s)	28.2
24	0.80 (3H, s)	15.0	0.82 (3H, s)	15.5
25	1.22 (3H, s)	25.5	1.22 (3H, s)	25.5
26	0.88 (3H, s)	17.8	1.02 (3H, s)	20.3
27	1.00 (3H, s)	18.1	1.14 (3H, s)	20.9
28	0.89 (3H, s)	23.7	0.91 (3H, s)	23.6
29	0.83 (3H, s)	33.1	0.88 (3H, s)	33.1
30	0.90 (3H, s)	24.7	0.87 (3H, s)	21.7
OCH_3	3.25 (3H, s)	56.0		

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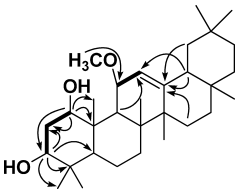

Fig. 1. Selected ^{1}H–^{1}H COSY (——) and HMBC (⟶) of Compound **1**

(COSY) spectrum, suggesting that methoxy group was attached at C-11 doubtlessly. In turn, one hydroxyl group was deduced to be linked to C-3 as a hydrogen on oxygen bearing carbon at δ_H 3.72 (dd, J=6.0, 14.5 Hz) showed the HMBC correlations with C-2, C-4, C-5, and C-23. The other hydroxyl, however, was assigned to C-1 due to the hydrogen spin system of H-3/H-2/H-1 obtained from the ^{1}H–^{1}H COSY spectrum as well as the HMBC correlations from H-1 (δ_H 4.36) to C-2, C-3, C-10, and C-25 (Fig. 1). Therefore, the planar structure of **1** was unambiguously established. The relative configuration of **1** was determined by the analysis of rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum and coupling constants. H-3 was assigned as the α-orientation inferred from the large H-3 coupling constants (6.0, 14.5 Hz). H-1 appeared as a broad singlet. This suggested that the H-1 was in the β-orientation, which can be confirmed by the ROESY correlation of H-1/Me-25. In addition, the ROESY correlations of H-9/OMe, H-9/Me-27, and H-11/Me-26 suggested that OCH_3 was in the α-configuration. Accordingly, **1** was elucidated as 1α,3β-dihydroxy-11α-methoxy-olean-12-ene.

Compound **2** was obtained as a white amorphous powder. It had a molecular formula of $C_{30}H_{48}O_2$ on the basis of the positive HR-ESI-MS ($[M+Na]^+$, m/z 463.3553). The ^{13}C-NMR suggested the presence of 30 carbon atoms due to eight methyl groups, eight methylenes, six methines (including two olefinic ones and two oxygen bearing ones) as well as eight quaternary carbons (including two olefinic ones) according to DEPT experiments. The ^{1}H-NMR spectrum showed the presence of two vinyl hydrogens as an AB pair of doublets at δ_H 5.73 (d, J=7.0 Hz) and 5.56 (d, J=7.0 Hz) and two hydrogens on oxygen-bearing carbons at δ_H 4.15 (br s) and 3.78 (dd, J=4.5, 12.0 Hz). Furthermore, the ^{1}H-NMR also confirmed the presence of eight tertiary methyl groups. The NMR data of **2** showed many similarities to those of **1**. The remarkable differences were the absent from one methine (C-9), methoxy group, and one oxygenated methine (C-11) in **1** and the presence of two olefinic carbons consisting of a double bond in **2**. On the basis of those observations, it was reasonable to conclude that a molecule of MeOH was eliminated from **1**, while a pair of double bond was formed between C-9 and C-11 instead, leading to the presence of two vinyl hydrogen signals as an AB pair of doublets in the ^{1}H-NMR. 2D-NMR experiments further verified the speculation. The orientations of H-1 and H-3 in **2** were the same as those of **1** since they presented similar chemical shifts (C-1, C-3) and coupling constants (H-1, H-3). **2** was established as 1α,3β-dihydroxy-olean-9(11),12-diene.

Compound **3**, colorless oil, was assigned a molecular formula of $C_{26}H_{38}O_6$ as deduced from the negative HR-ESI-MS ($[M+Cl]^-$, m/z 481.2350)[14,15] and ^{13}C-NMR data. Its IR showed the absorption typical of hydroxyl (3426 cm^{-1}) and

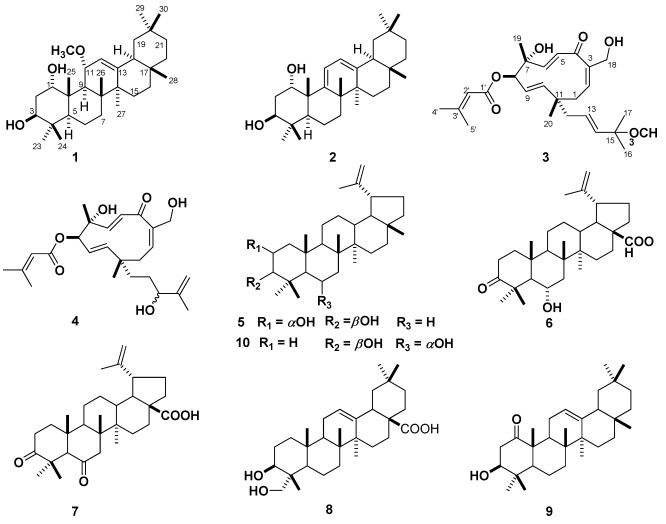

Fig. 2. Structures of Compounds **1**—**10**

Table 2. Cytotoxicity of Compounds **1**—**10**

Compounds	IC_{50} (μM)				
	HL-60	SMMC-7721	A-549	SK-BR-3	PANC-1
1	>40	>40	>40	>40	>40
2	18.7	19.8	18.7	34.5	23.4
3	1.1	5.8	8.4	1.0	2.0
4	5.5	>40	23.5	9.3	17.4
5	11.2	24.6	18.9	33.9	27.4
6	>40	>40	>40	>40	>40
7	>40	>40	>40	>40	>40
8	>40	>40	>40	>40	>40
9	17.9	19.6	17.5	18.9	25.2
10	13.9	19.9	16.7	26.9	27.5
cis-Platinum	2.0	16.8	18.8	15.1	14.0

carbonyls (1723, 1705 cm^{-1}). Its ^{13}C-NMR and DEPT spectral data demonstrated a total of 26 signals consisting of two carbonyls, five quaternary carbons, nine methines, three methylenes, six methyls, and one methoxy group. In the ^{1}H-NMR, signals of six methyls and one methoxy group were observed. The ^{13}C- and ^{1}H-NMR data of **3** almost imitated those of vibsanol A.[8] The only difference was the presence of the methoxy group in **3**, suggesting that one hydroxyl group in vibsanol A was replaced by the methoxy group in **3**. The HMBC correlation of methoxy group with C-15 (δ_C 74.8) suggested that methoxy group was affirmatively attached at C-15. The relative stereochemistry of **3** was inferred to be consistent with that of vibsanol A based on the side by side comparisons of chemical shifts and coupling constants.[8] Therefore, compound **3** was elucidated as 15-*O*-methyl-vibsanol A.

Cytotoxicity of compounds **1**—**10** were evaluated against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 knubbly cells. Among the ten compounds, **3** showed the most potent cytotoxicity against the tested cell lines with IC_{50} ranging 1.0 to 8.4 μM. It is worthy noting that **3** exerted potent cytotoxicity against HL-60, SK-BR-3, and PANC-1 cell lines, presenting IC_{50} 1.1, 1.0, and 2.0 μM, respectively. In addition, compounds **2**, **4**, **5**, **9**, and **10** demonstrated weak cytotoxicity against all the tested cell lines except for **4** showing non-cy-

totoxicity to SMMC-7721 cell. Compounds **1**, **6**, **7**, and **8** were inactive.

Experimental

General Procedure Optical rotation was measured on a Horiba SEPA-300 polarimeter. IR spectra were obtained with a Tensor 27 FT-IR spectrometer with KBr pellets. UV data were measured on a UV 210A spectrometer. The ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded on Bruker AV-400 or DRX-500 spectrometers at room temperature (δ in ppm, J in Hz). Mass spectra were carried out on a VG Auto spec-3000 spectrometer or on a Finnigan MAT 90 instrument. Silica gel (200—300 mesh, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), Silica gel H (60 μm, Qingdao Marine Chemical Ltd., Qingdao, People's Republic of China), Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden), and Lichroprep RP-18 gel (43—63 μm, Merck, Darmstadt, Germany) were used for column chromatography. Fractions were monitored using TLC, and spots were visualized by heating silica gel plates immersed with 15% H_2SO_4 in ethanol. Solvents were distilled before use.

Plant Material The leaves of *V. chingii* were collected in Caojian town, Yunnan province, People's Republic of China, in November 2008. The sample was identified by Prof. Xiao Cheng, Kunming Institute of Botany, Yunnan, People's Republic of China. A voucher specimen (20081109) was deposited at Kunming Institute of Botany, Chinese Academy of Sciences, People's Republic of China.

Extraction and Isolation The dried and powdered leaves of *V. chingii* (4.5 kg) were extracted with Me_2CO (3×15 l, 3 d, each) at room temperature. The extract was evaporated to dryness under reduced pressure to obtain a residue (600 g). The residue was suspended in water (1200 ml) and then extracted with ethyl acetate (3×2.4 l). The ethyl acetate extract (232 g) was subjected to silica gel column chromatography eluted with gradient petroleum ether–Me_2CO (10 : 0, 9 : 1, 8 : 2, 7 : 3, 6 : 4, 0 : 10) to yield fractions 1—6. Fraction 3 (19.2 g) was further purified by silica gel column chromatography using petroleum ether–Me_2CO (8 : 2) as eluents to provide subfractions 3.1—3.5. Subfraction 3.2 (4.1 g) was subjected to Sephadex LH-20 ($CHCl_3$–MeOH 1 : 1) and then further purified by silica gel column chromatography eluted with $CHCl_3$–Me_2CO (100 : 1) to yield compounds **1** (7 mg), **2** (25 mg), **5** (10 mg), and **6** (8 mg). Subfraction 3.3 (3.6 g) was separated by silica gel column chromatography eluted with petroleum ether–Me_2CO (7 : 3) followed by RP18 (MeOH–H_2O, 70 : 30, 75 : 25, 80 : 20, 85 : 15, 100 : 0) to get **7** (15 mg), **8** (22 mg), **9** (6 mg), and **10** (55 mg). Continuing purification of Fraction 4 by RP-18, Sephadex LH-20, and silica gel obtained **3** (5 mg) and **4** (7 mg).

Compound (**1**): White amorphous powder. $[\alpha]_D^{23.3}$ +261.8 (c=0.19, $CHCl_3$). IR (KBr) ν_{max} cm^{-1}: 3440, 2946, 2869, 1631, 1462, 1377, 1365, 1050. ^{13}C- and ^{1}H-NMR see Table 1. EI-MS *m/z*: 472 $[M]^+$. Positive HR-ESI-MS *m/z*: 495.3802 $[M+Na]^+$ (Calcd for $C_{31}H_{52}O_3Na^+$, 495.3809).

Compound (**2**): White amorphous powder. $[\alpha]_D^{28.2}$ +117.4 (c=0.16, $CHCl_3$). UV λ_{max}($CHCl_3$) nm (log ε): 286 (3.26). IR (KBr) ν_{max} cm^{-1}: 3425, 2966, 2869, 1630, 1461, 1375, 1365, 1078, 1050. ^{13}C- and ^{1}H-NMR see Table 1. EI-MS *m/z*: 440 $[M]^+$. Positive HR-ESI-MS *m/z*: 463.3553 $[M+Na]^+$ (Calcd for $C_{30}H_{48}O_2Na^+$, 463.3552).

Compound (**3**): Colorless oil. $[\alpha]_D^{28.2}$ +56.0 (c=0.30, $CHCl_3$). λ_{max} ($CHCl_3$) nm (log ε): 240 (3.78). IR (KBr) ν_{max} cm^{-1}: 3426, 2968, 2934, 1723, 1705, 1649, 1450, 1380, 1226, 1144. ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 43.5 (C-1), 128.7 (C-2), 145.1 (C-3), 202.3 (C-4), 128.5 (C-5), 154.8 (C-6), 74.1 (C-7), 81.6 (C-8), 123.5 (C-9), 143.0 (C-10), 41.3 (C-11), 40.6 (C-12), 125.1 (C-13), 139.1 (C-14), 74.8 (C-15), 25.9 (C-16), 26.1 (C-17), 64.9 (C-18), 18.4 (C-19), 23.2 (C-20), 167.2 (C-1′), 115.2 (C-2′), 159.5 (C-3′), 20.5 (C-4′), 27.6 (C-5′), 50.3 (OCH_3). ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.86 (1H, t, J=12.6, 16.0 Hz, H-1α), 2.06 (1H, t, J=12.6, 16.0 Hz, H-1b), 5.97 (1H, d, J=12.6 Hz, H-2), 6.07 (1H, d, J=15.8 Hz, H-5), 6.58 (1H, d, J=15.8 Hz, H-6), 5.35 (1H, d, J=9.0 Hz, H-8), 5.20 (1H, t, J=9.0, 15.6 Hz, H-9), 5.73 (1H, d, J=15.6 Hz, H-10), 2.20 (1H, m, H-12a), 2.36 (1H, m, H-12b), 5.46 (1H, m, H-13), 5.48 (1H, d, J=16.8 Hz, H-14), 1.24 (3H, s, Me-15), 1.25 (3H, s, Me-16), 1.28 (3H, s, Me-17) 4.19 (d, J=15.8 Hz, H-18a), 4.41 (d, J=15.8 Hz, H-18b), 1.25 (3H, s, Me-19), 1.00 (3H, s, Me-20), 5.77 (1H, s, H-2′), 2.19 (3H, s, Me-4′), 1.93 (3H, s, Me-5′), 3.10 (3H, s, OCH_3). Negative FAB-MS *m/z*: 445 $[M-H]^-$. Negative HR-ESI-MS: *m/z* 481.2350 $[M+Cl]^-$ (Calcd for $C_{26}H_{38}O_6Cl^-$, 481.2356).

Cytotoxicity Assay Cytotoxicity of compounds **1**—**10** against HL-60, SMMC-7721, A-549, SK-BR-3, and PANC-1 cell lines was determined using the methyl thiazol tetrazalium (MTT) method with minor modifications.[16—18] Cells were seeded in 96-well plates 24 h before treatment and continuously exposed to different concentrations of compounds (0.064, 0.32, 1.6, 8, 40 μM). After 48 h, 20 μl of MTT, 5 mg/ml solution were added to each well, which were incubated for another 4 h at 37 °C. Then 100 μl resolving buffer (20% sodium dodecyl sulfate (SDS), 50% *N*,*N*-dimethylformamide (DMF)) was added to each well. After 12 h incubation at 37 °C, the optical density (OD) value of each well was read at 595 nm with a plate reader. All assays were carried out in triplicate and *cis*-platinum was used as positive control. Control wells, treated with dimethyl sulfoxide (DMSO) alone, were included in all the experiments: growth inhibition (%)= $(OD_{control}-OD_{treated})/OD_{control}\times100$. The cytotoxicity of compounds on tumor cells was expressed as IC_{50} values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) calculated by Reed and Muench's method.[19]

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References

1) Kagawa M., Minami H., Nakahara M., Takahashi H., Takaoka S., Fukuyama Y., *Phytochemistry*, **47**, 1101—1105 (1998).
2) Machida K., Kikuchi M., *Tetrahedron Lett.*, **37**, 4157—4159 (1996).
3) Hase T., Iwagawa T., Dave M. N., *Phytochemistry*, **24**, 1323—1327 (1985).
4) Fukuyama Y., Minami H., Ichikawa R., Takeuchi K., Kodama M., *Phytochemistry*, **42**, 741—744 (1996).
5) Fukuyama Y., Fujii H., Minami H., Takahashi H., Kubo M., *J. Nat. Prod.*, **69**, 1098—1100 (2006).
6) Fukuyama Y., Nakahara M., Minami H., Kodama M., *Chem. Pharm. Bull.*, **44**, 1418—1420 (1996).
7) Zhu X. D., Wang Q., Wang Y. F., Ju P., Luo S. D., *Acta Bot. Yunnan*, **28**, 91—94 (2006).
8) Shen Y. C., Prakash C. V. S., Wang L. T., Chien C. T., Hung M. C., *J. Nat. Prod.*, **65**, 1052—1055 (2002).
9) Ageta H., Ageta T., *Chem. Pharm. Bull.*, **32**, 369—372 (1984).
10) Kuroyanagi M., Shiotsu M., Ebihara T., Kawal H., Ueno A., Fukushima S., *Chem. Pharm. Bull.*, **34**, 4012—4017 (1986).
11) Kizu H., Tomimori T., *Chem. Pharm. Bull.*, **30**, 3340—3346 (1982).
12) Kagawa M., Minami H., Nakahara M., Takahashi H., Takaoka S., Fukuyama Y., *Phytochemistry*, **47**, 1337—1341 (1998).
13) Hisham A., Kumar G. J., Fujimoto Y., Hara N., *Phytochemistry*, **52**, 789—794 (1996).
14) Asbury G. R., Klasmeier J., Hill H. H. Jr., *Talanta*, **50**, 1291—1298 (2000).
15) Zhu J. H., Cole R. B., *J. Am. Soc. Mass Spectrom.*, **11**, 932—941 (2000).
16) Mosmman T., *J. Immunol. Methods*, **65**, 55—63 (1983).
17) Alley M. C., Scudiero D. A., Monks A., Hursey M. L., Czerwinski M. J., Fine D. L., Abbott B. J., Mayo J. G., Shoemaker R. H., Boyd M. R., *Cancer Res.*, **48**, 589—601 (1988).
18) Zhou J. J., Yue X. F., Han J. X., Yang W. Y., *Chin. J. Pharm.*, **24**, 455—457 (1993).
19) Reed L. J., Muench H., *Am. J. Hyg.*, **27**, 493—497 (1938).