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# Vibsane-type diterpenes from leaves and twigs of *Viburnum odoratissimum*

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ABSTRACT

Seven new vibsane-type diterpenes, vibsanols C–H (**1**–**6**) and vibsanin X (**7**), together with seven analogues, were isolated from the leaves and twigs of *Viburnum odoratissimum*. The structures of the new compounds were elucidated by extensive spectroscopic methods. All the new compounds were detected for their cytotoxicity. Compound **1** showed significant cytotoxicity against all the tested cell lines (HL-60, SMMC-7721, A-594, MCF-7, and SW-480), with $IC_{50}$ values of 3.35, 4.41, 5.18, 11.30, and 3.70 μM, respectively. Compounds **4** and **5** also displayed significant cytotoxicity against hepatocellular carcinoma SMMC-7721 cell line, with $IC_{50}$ values of 3.69 and 3.52 μM, respectively.



## 1. Introduction

Vibsane-type diterpenes are quite rare natural products, whose occurrence has been limited to some species of *Viburnum* [1], for example, *Viburnum awabuki* [2–4], *Viburnum odoratissimum* [5–7], *Viburnum suspensum* [8], *Viburnum sieboldii* [9], *Viburnum chingii* [10], and *Viburnum tinus* cv. variegatus [11]. Since Kawazu reported the first isolation of vibsane-type diterpenes in 1980 [12], more than 80 vibsane-type diterpenoids have been isolated [13], which can be further divided into three subtypes, 11-membered ring, 7-membered ring, and rearranged type, represented as vibsanin B [14], vibsanin C [14], and neovibsanin A [15], respectively. The isolation and synthesis of vibsane-type diterpenes have attracted considerable interest, due to their unusual skeletons and a wide range of biological activities [16–19]. Among them, vibsanin B has been proved to target HSP90 to inhibit interstitial leukocyte migration and ameliorates experimental autoimmune encephalomyelitis, that can be used as a promising drug lead for treating inflammation associated diseases [20].

*V. odoratissimum*, a widely planted landscape in many provinces of China, is a rich pool of the vibsane-type diterpenes [5–7]. As a part of our continuous research on biologically active constituents from the *Viburnum* genus [21–23], the leaves and twigs of *V. odoratissimum* were studied and seven new vibsane-type diterpenes, vibsanols C–H (**1**–**6**) and vibsanin X (7), together with seven known analogues were obtained. All the new compounds were detected for their cytotoxicity against five human cancer cell lines. Compound **1** showed significant cytotoxicity against all the tested cell lines (HL-60, SMMC-7721, A-594, MCF-7, and SW-480), with $IC_{50}$ values of 3.35, 4.41, 5.18, 11.30, and 3.70 μM, respectively. Compounds **4** and **5** also displayed significant cytotoxicity against hepatocellular carcinoma SMMC-7721 cell line, with $IC_{50}$ values of 3.69 and 3.52 μM, respectively. Herein, we reported the isolation, structural elucidation, and bioactivity of these isolates.

## 2. Experimental

### 2.1. General

Optical rotations were measured on a JASCO-20C digital polarimeter. IR spectra were obtained on a Tenor 27 spectrometer with KBr pellets. $^{1}H$ and $^{13}C$ NMR spectra were performed on a Bruker AM-400 or DRX-500 spectrometer with TMS as internal standard. Chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. EIMS and HRESMS were taken on a VG Auto Spec-3000 mass spectrometer. Semi-preparative HPLC was performed on an Agilent 1260 apparatus equipped with a diode-array detector and a Zorbax SB-C-18 (Agilent, 9.4 mm × 25 cm) column. Column chromatography (CC) was performed over silica gel (100–200 or 200–300 mesh, Qingdao Marine Chemical Co. Ltd., Qingdao, China) and Sephadex LH-20 (Amersham Pharmacia Biotech, Sweden). Thin-layer chromatography (TLC) was

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carried out on silica gel $GF_{254}$ on glass plates (Qingdao Marine Chemical Inc.) and spots were visualized by heating silica gel plates sprayed with 10% $H_2SO_4$ in EtOH.

### 2.2. Plant material

The leaves and twigs of plants of *V. odoratissimum* were collected in Kunming Botanic Garden of Yunnan Province, People's Republic of China, in July 2006. The sample was identified by Prof. Xiao Cheng, one of the authors. A voucher specimen (KIB-2006715V) was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

### 2.3. Extraction and isolation

The air dried and powdered sample (22 kg) was extracted with 80% acetone/$H_2O$ (24 h × 3), which was then concentrated in vacuo to give deposition portion (700 g) and water-soluble portion. The deposition portion was subjected to a silica gel CC, eluting with petroleum ether–acetone (1:0–0:1) to give fractions I–VI. Faction II (90 g) was chromatographed over silica gel CC (petroleum ether–acetone) to afford three sub-fractions: II-a, II-b, and II-c. Sub-fraction II-a (45 g) was repeatedly purified by silica gel CC (petroleum ether–acetone) and Sephadex LH-20 ($CHCl_3$–MeOH, 1:1) to afford vibsanin A (1 g), vibsanin B (2 mg), vibsanin C (800 mg), and vibsanol A (5 g). Sub-fraction II-b (10 g) was chromatographed over silica gel CC (petroleum ether–acetone) and then purified by semi-preparative HPLC (80% MeOH–$H_2O$) to obtain **6** (9 mg), **7** (65 mg), and vibsanin D (36 mg). Sub-fraction II-c (10 g) was chromatographed over silica gel CC (petroleum ether–acetone), then purified by semi-preparative HPLC (60% MeCN–$H_2O$) to afford **1** (3.5 mg) and vibsanin F (15 mg). Fraction III (76 g) was subjected to silica gel CC (petroleum ether–acetone, 7:3) to give two sub-fractions: III-a and III-b. Fraction III-a was chromatographed over silica gel (petroleum ether–acetone, 8.5:1.5), and then separated by semi-preparative HPLC (45% MeCN–$H_2O$) to get **3** (4.5 mg) and **5** (3.7 mg). Fraction IV was subjected to RP-18 yield (70% MeOH-$H_2O$), Sephadex LH-20 (MeOH), and semi-preparative HPLC (45% MeCN–$H_2O$) to afford **2** (3 mg), **4** (3.5 mg), and (3 mg).

### 2.4. Spectroscopic data

Vibsanol C (**1**): colorless oil; $[\alpha]_D^{20}$ −112.85 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 204 (3.94), 287 (2.69) nm; IR (KBr) $\nu_{max}$: 3434, 2926, 1713, 1646, 1226, 1142 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; ESIMS *m/z* 439 $[M + Na]^+$; HRESIMS *m/z* 439.2464 $[M + Na]^+$ ($C_{25}H_{36}O_5Na$ calcd 439.2460).

Vibsanol D (**2**): colorless oil; $[\alpha]_D^{20}$ −12.79 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 216 (3.88) nm; IR (KBr) $\nu_{max}$: 3430, 2926, 1717, 1645, 1227, 1143 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; ESIMS *m/z* 457 $[M + Na]^+$; HRESIMS *m/z* 457.2566 $[M + Na]^+$ ($C_{25}H_{38}O_6Na$ calcd 457.2566).

Vibsanol E (**3**): colorless oil; $[\alpha]_D^{20}$ + 14.06 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 196 (3.81), 219 (4.04) nm; IR (KBr) $\nu_{max}$: 3425, 2923, 1718, 1699, 1643, 1380, 1226, 987 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; ESIMS *m/z* 487 $[M + Na]^+$; HRESIMS *m/z* 487.2310 $[M + Na]^+$ ($C_{25}H_{36}O_8Na$ calcd 487.2307).

Vibsanol F (**4**): colorless oil; $[\alpha]_D^{20}$ + 16.72 (c 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log ε): 217 (3.93) nm; IR (KBr) $\nu_{max}$: 3431, 2924, 1719, 1639, 1143 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; ESIMS *m/z* 457 $[M + Na]^+$; HRESIMS *m/z* 457.2561 $[M + Na]^+$ ($C_{25}H_{38}O_6Na$ calcd 457.2566).

Vibsanol G (**5**): colorless oil; $[\alpha]_D^{20}$ −6.35 (c 0.1, MeOH); UV (MeOH) λmax (log ε): 192 (3.60), 218 (3.84) nm; IR (KBr) $\nu_{max}$: 3431, 2925, 1720, 1640, 1143 $cm^{-1}$; $^1H$ and $^{13}C$ NMR data, see Tables 1 and 2; ESIMS *m/z* 487 $[M + Na]^+$; HRESIMS *m/z* 487.2303 $[M + Na]^+$ ($C_{25}H_{36}O_8Na$ calcd 487.2307).

Vibsanol H (**6**): colorless oil; $[\alpha]_D^{20}$ −3.16 (c 0.1, MeOH); UV (MeOH) λmax (log ε): 191 (3.06), 203 (3.57) nm; IR (KBr) $\nu_{max}$: 3425, 2967,

**Table 1**
$^1H$ NMR data of **1**–**7**.

| No. | 1[a,b] | 2[a] | 3[a] | 4[a] | 5[b] | 6[c] | 7[a,d] |
|---|---|---|---|---|---|---|---|
| 1a | 5.56 (d, 11.2) | 2.19 (m) | 2.54 (t, 11.5) | 2.22 (m) | 2.42 (t, 11.3) | 2.15 (m) | 2.49 (m) |
| 1b | | 2.04 (m) | 1.98 (dd, 6.6, 12.2) | 1.94 (t, 8.1) | 1.85 (dd, 5.8, 11.3) | 1.89 (dd, 6.6, 12.9) | 2.09 (m) |
| 2 | 4.98 (dd, 11.0, 11.1) | 5.57 (m) | 5.97 (dd, 6.6, 10.8) | 5.52 (m) | 5.82 (dd, 6.3, 10.4) | 5.48 (m) | 5.41 (t, 6.6) |
| 3 | 4.44 (m) | | | | | | |
| 4a | | 2.27 (m) | | 2.24 (m) | | 2.25 (m) | 2.49 (m) |
| 4b | | 2.05 (m) | | 2.02 (m) | | 2.00 (m) | 2.09 (m) |
| 5a | 2.87 (dd, 11.3, 15.7) | 2.28 (m) | 4.32 (d, 8.8) | 2.25 (m) | 4.08 (d, 8.9) | 2.24 (m) | 2.28 (m) |
| 5b | 2.70 (dd, 2.0, 15.8) | 1.16 (m) | | 1.15 (m) | | 1.05 (m) | 0.39 (m) |
| 6 | 3.07 (dd, 2.0, 11.8) | 2.76 (d, 9.8) | 3.17 (d, 8.8) | 2.75 (dd, 2.4, 15.1) | 3.10 (d, 8.8) | 2.69 (d, 11.1) | 3.10 (d, 10.6) |
| 8a | 5.27 (d, 10.0) | 5.14 (d, 8.2) | 5.08 (d, 9.6) | 5.13 (d, 12.3) | 4.97 (d, 9.6) | 2.50 (d, 13.5) | |
| 8b | | | | | | 2.05 (m) | |
| 9 | 5.72 (dd, 10.0, 15.9) | 5.50 (m) | 5.37 (dd, 9.6, 15.9) | 5.51 (m) | 5.26 (dd, 9.6, 15.8) | 5.36 (m) | 5.85 (d, 16.9) |
| 10 | 6.09 (d, 15.9) | 5.86 (d, 13.3) | 5.81 (d, 15.9) | 5.77 (d, 19.9) | 5.60 (d, 15.8) | 5.47 (d, 15.3) | 7.04 (d, 16.9) |
| 12a | 1.41 (2H, m) | 2.19 (m) | 2.14 (m) | 1.43 (2H, m) | 1.46 (m) | 2.11 (m) | 1.37 (2H, m) |
| 12b | | 1.95 (m) | 2.04 (m) | | 1.13 (m) | 1.99 (m) | |
| 13a | 1.88 (2H, m) | 5.56 (m) | 5.53 (m) | 1.48 (m) | 1.27 (2H, m) | 5.53 (m) | 2.08 (2H, m) |
| 13b | | | | 1.25 (m) | | | |
| 14 | 5.05 (m) | 5.63 (d, 13.2) | 5.64 (d, 15.9) | 4.18 (t, 7.8) | 4.06 (t, 6.8) | 5.51 (m) | 5.15 (t, 5.6) |
| 16 | 1.60 (3H, s) | 1.23 (3H, s) | 1.22 (3H, s) | 4.88 (2H, d, 12.5) | 4.78 (2H, m) | 1.29 (3H, s) | 1.65 (3H, s) |
| 17 | 1.53 (3H, s) | 1.23 (3H, s) | 1.22 (3H, s) | 1.67 (3H, s) | 1.59 (3H, s) | 1.29 (3H, s) | 1.60 (3H, s) |
| 18a | 3.76 (t, 9.8) | 4.06 (d, 11.0) | 4.61 (d, 13.8) | 4.05 (d, 16.5) | 4.35 (d, 13.6) | 4.07 (d, 12.9) | 4.50 (d, 12.5) |
| 18b | 3.41 (dd, 5.0, 10,0) | 3.98 (d, 10.7) | 4.35 (d, 13.8) | 3.96 (d, 16.5) | 4.21 (d, 13.6) | 4.00 (d, 12.9) | 4.25 (d, 12.5) |
| 19 | 1.47 (3H, s) | 1.40 (3H, s) | 1.42 (3H, s) | 1.39 (3H, s) | 1.33 (3H, s) | 1.38 (3H, s) | 1.49 (3H, s) |
| 20 | 1.24 (3H, s) | 1.07 (3H, s) | 0.99 (3H, s) | 1.06 (3H, s) | 0.91 (3H, s) | 1.04 (3H, s) | 1.20 (3H, s) |
| 2′ | 5.74 (s) | 5.72 (s) | 5.70 (s) | 5.70 (s) | 5.63 (s) | | |
| 4′ | 2.13 (3H, s) | 2.14 (3H, s) | 2.13 (3H, s) | 2.11 (3H, s) | 2.05 (3H, s) | | |
| 5′ | 1.90 (3H, s) | 1.90 (3H, s) | 1.90 (3H, s) | 1.89 (3H, s) | 1.82 (3H, s) | | |
| OAc | | | | | | | 1.97 (3H, s) |

[a] Compounds were recorded in acetone-$d_6$, δ in ppm and *J* in Hz.
[b] Compounds were recorded in methanol-$d_4$, δ in ppm and *J* in Hz.
[c] Compounds were recorded in $CDCl_3$, δ in ppm and *J* in Hz.
[d] The data were record at the temperature of −20 °C.

**Table 2**
$^{13}$C NMR data of **1**–**7**.[b]

| No. | 1[a] | 2[a] | 3[a] | 4[a] | 5[c] | 6[a] | 7[a,d] |
|---|---|---|---|---|---|---|---|
| 1 | 141.7 CH | 42.6 $CH_2$ | 39.7 $CH_2$ | 42.3 $CH_2$ | 41.1 $CH_2$ | 41.3 $CH_2$ | 39.2 $CH_2$ |
| 2 | 127.0 CH | 119.5 CH | 130.8 CH | 119.3 CH | 131.3 CH | 120.5 CH | 123.7 CH |
| 3 | 52.8 CH | 143.8 qC | 147.3 qC | 143.7 qC | 147.3 qC | 141.8 qC | 139.2 qC |
| 4 | 208.9 qC | 24.1 $CH_2$ | 202.6 qC | 23.9 $CH_2$ | 203.3 qC | 23.7 $CH_2$ | 22.2 $CH_2$ |
| 5 | 44.0 $CH_2$ | 27.3 $CH_2$ | 72.9 CH | 27.2 $CH_2$ | 73.4 CH | 27.0 $CH_2$ | 28.3 $CH_2$ |
| 6 | 59.4 CH | 63.5 CH | 64.6 CH | 63.4 CH | 63.6 CH | 63.4 CH | 62.3 CH |
| 7 | 62.3 qC | 61.5 qC | 62.1 qC | 61.4 qC | 63.3 qC | 60.5 qC | 65.0 qC |
| 8 | 78.3 CH | 78.9 CH | 77.9 CH | 78.8 CH | 78.6 CH | 38.8 $CH_2$ | 197.9 qC |
| 9 | 123.5 CH | 122.3 CH | 123.6 CH | 122.4 CH | 124.3 CH | 121.3 CH | 127.8 CH |
| 10 | 145.1 CH | 147.2 CH | 146.3 CH | 147.5 CH | 147.1 CH | 143.0 CH | 158.9 CH |
| 11 | 42.4 qC | 40.7 qC | 40.9 qC | 40.2 qC | 40.8 qC | 40.0 qC | 42.5 qC |
| 12 | 43.7 $CH_2$ | 41.5 $CH_2$ | 41.7 $CH_2$ | 35.9 $CH_2$ | 35.4 $CH_2$ | 42.3 $CH_2$ | 34.5 $CH_2$ |
| 13 | 23.5 $CH_2$ | 126.9 CH | 126.4 CH | 26.8 $CH_2$ | 26.9 $CH_2$ | 128.0 CH | 23.3 $CH_2$ |
| 14 | 125.2 CH | 138.4 CH | 138.7 CH | 89.9 CH | 90.6 CH | 125.2 CH | 125.3 CH |
| 15 | 131.9 qC | 81.6 qC | 81.5 qC | 145.7 qC | 145.8 qC | 82.1 qC | 131.8 qC |
| 16 | 25.7 $CH_3$ | 25.0 $CH_3$ | 24.9 $CH_3$ | 113.4 $CH_2$ | 114.3 $CH_2$ | 24.5 $CH_3$ | 25.8 $CH_3$ |
| 17 | 17.8 $CH_3$ | 25.0 $CH_3$ | 24.9 $CH_3$ | 17.3 $CH_3$ | 17.0 $CH_3$ | 24.5 $CH_3$ | 17.7 $CH_3$ |
| 18 | 64.8 $CH_2$ | 65.7 $CH_2$ | 63.9 $CH_2$ | 65.7 $CH_2$ | 65.3 $CH_2$ | 66.0 $CH_2$ | 67.7 $CH_2$ |
| 19 | 17.8 $CH_3$ | 17.9 $CH_3$ | 17.7 $CH_3$ | 17.9 $CH_3$ | 17.8 $CH_3$ | 23.3 $CH_3$ | 19.8 $CH_3$ |
| 20 | 22.7 $CH_3$ | 23.8 $CH_3$ | 24.0 $CH_3$ | 23.3 $CH_3$ | 23.6 $CH_3$ | 24.3 $CH_3$ | 24.4 $CH_3$ |
| 1′ | 165.8 qC | 165.7 qC | 165.5 qC | 165.6 qC | 166.7 qC | | |
| 2′ | 116.5 CH | 116.7 CH | 116.4 CH | 116.7 CH | 116.5 CH | | |
| 3′ | 158.4 qC | 157.9 qC | 158.3 qC | 157.8 qC | 159.4 qC | | |
| 4′ | 20.1 $CH_3$ | 20.2 $CH_3$ | 20.2 $CH_3$ | 20.1 $CH_3$ | 20.4 $CH_3$ | | |
| 5′ | 27.2 $CH_3$ | 27.3 $CH_3$ | 27.2 $CH_3$ | 27.2 $CH_3$ | 27.4 $CH_3$ | | |
| OAc | | | | | | | 19.9 $CH_3$170.6 qC |

[a] Compounds were recorded in acetone-$d_6$, $\delta$ in ppm.
[b] Compounds were recorded in methanol-$d_4$, $\delta$ in ppm.
[c] Compounds were recorded in $CDCl_3$, $\delta$ in ppm.
[d] The data were record at the temperature of −20 °C.

2927, 1638, 1463, 1379 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; ESIMS *m/z* 359 $[M + Na]^+$; HRESIMS *m/z* 359.2199 $[M + Na]^+$ ($C_{20}H_{32}O_4Na$ calcd 359.2198).

Vibsanin X (**7**): colorless oil; $[\alpha]_D^{20}$ +40.62 (c 0.1, MeOH); UV (MeOH) λmax (log ε): 202 (3.82), 227 (3.69) nm; IR (KBr) $\nu_{max}$: 2967, 2930, 1739, 1677, 1458, 1376, 1229, 1024 cm$^{-1}$; $^1$H and $^{13}$C NMR data, see Tables 1 and 2; ESIMS *m/z* 383 $[M + Na]^+$; HRESIMS *m/z* 383.2208 $[M + Na]^+$ ($C_{22}H_{32}O_4Na$ calcd 383.2198).

### 2.5. In vitro cytotoxicity assay

The following human tumor cell lines were used: human myeloid leukemia *HL-60*, hepatocellular carcinoma SMMC-7721, lung cancer A-549, breast cancer MCF-7, and colon cancer SW-480 cells. All the cells were cultured in RPMI-1640 or DMEM medium (Hyclone, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) in 5% $CO_2$ at 37 °C. The cytotoxicity assay was performed according to the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] method in 96-well microplates [24]. Briefly, 100 μL adherent cells were seeded into each well of 96-well cell culture plates and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug was added with initial density of $1 \times 10^5$ cells/mL. Each tumor cell line was exposed to the test compound at concentrations of 0.0625, 0.32, 1.6, 8, and 40 μM in triplicates for 48 h, with cisplatin (sigma, USA) as a positive control. After compound treatment, cell viability was detected and cell growth curve was graphed. $IC_{50}$ values were calculated by the Reed and Muench's method [25].

## 3. Results and discussion

### 3.1. Chemistry

The 80% acetone/$H_2O$ extract of *V. odoratissimum* was divided into deposition portion and water dissolved portion. The deposition portion was subjected to normal-phase silica gel, RP-18 silica gel, semipreparative HPLC, and Sephadex LH-20 to afford seven new vibsane-type diterpenes, vibsanols C–H (**1**–**6**) and vibsanin X (7) (Fig. 1), together with seven known analogues. The structures of the known compounds were established by comparing their observed physical data to those reported in the literature, which were identified as vibsanin A [14], vibsanin B [14], vibsanin C [14], vibsanin D [13], vibsanin F [14], vibsanol A [6], and vibsanol B [6].

Vibsanol C (**1**) was obtained as a colorless oil. Its molecular formula, $C_{25}H_{36}O_5$, was deduced from HRESIMS ($[M + Na]^+$ at *m/z* 439.2464), corresponding to eight degrees of unsaturation. The IR spectrum showed the presence of a hydroxy (3434 cm$^{-1}$) and carbonyl (1713 cm$^{-1}$) groups. The $^1$H and $^{13}$C NMR data of **1** (Tables 1 and 2) showed the signals due to six tertiary methyl groups ($\delta_H$ 1.24, 1.47, 1.53, 1.60, 1.90, 2.13), an oxymethylene [$\delta_H$ 3.41 (dd, $J$ = 5.0, 10.0 Hz), 3.76 (t, $J$ = 9.8 Hz)], an oxymethine [$\delta_H$ 5.27 (d, $J$ = 10.0 Hz)], six olefinic protons [$\delta_H$ 4.98 (t, $J$ = 11.1 Hz), 5.05 (m), 5.56 (d, $J$ = 11.2 Hz), 5.72 (dd, $J$ = 10.0, 15.8 Hz), 5.74 (s), 6.09 (d, $J$ = 15.9 Hz)], and a β,β-dimethyl acrylate group (partial unit **e** as shown in Fig. 2). Analysis of the $^1$H–$^1$H COSY and HMBC spectra of **1** provided the additional partial structures **a**–**d** (Fig. 2). The double bonds involved in unit **a** and unit **c** should take *Z* and *E* geometry, respectively, due to their vicinal coupling constants ($J$ = 11.2 and 15.9 Hz). In the HMBC spectrum of **1** (Fig. 2), H-3, H-5, and H-18 correlated with the carbonyl ($\delta_C$ 208.9, s, C-4), which disclosed the connection of the carbonyl to C-3 in unit **a** and C-5 in unit **b**. The signals of both H-6 and H-8 showed cross peaks to a quaternary signal ($\delta_C$ 62.3, s, C-7) and the methyl ($\delta_C$ 17.8, q, C-19) suggested the attachment of C-7 to C-6, C-8, and Me-19. At the same time, the unit **e** was linked to C-8 as evidenced by the HMBC correlation of H-8 with ester carbonyl C-1′ ($\delta_C$ 165.8, s). The signals of H-1, H-10, H-12, and H-20 showed HMBC correlations with a quaternary signal ($\delta_C$ 42.4, s, C-11) that indicated the quaternary signal bonded to C-1 in unit **a**, C-10 in unit **c**, C-12 in unit **d**, and Me-20. Moreover, Me-16 and Me-17 showed HMBC correlations to $\delta_C$ 131.9 (s, C-15) and $\delta_C$ 125.2 (d, C-14), indicating the presence of an isopropyl unit which was attached to C-14 in unit **d**. These HMBC correlations led to the construction of an 11-membered vibsane-type diterpene. Taking into consideration of the degrees of the unsaturation and the $^{13}$C NMR chemical

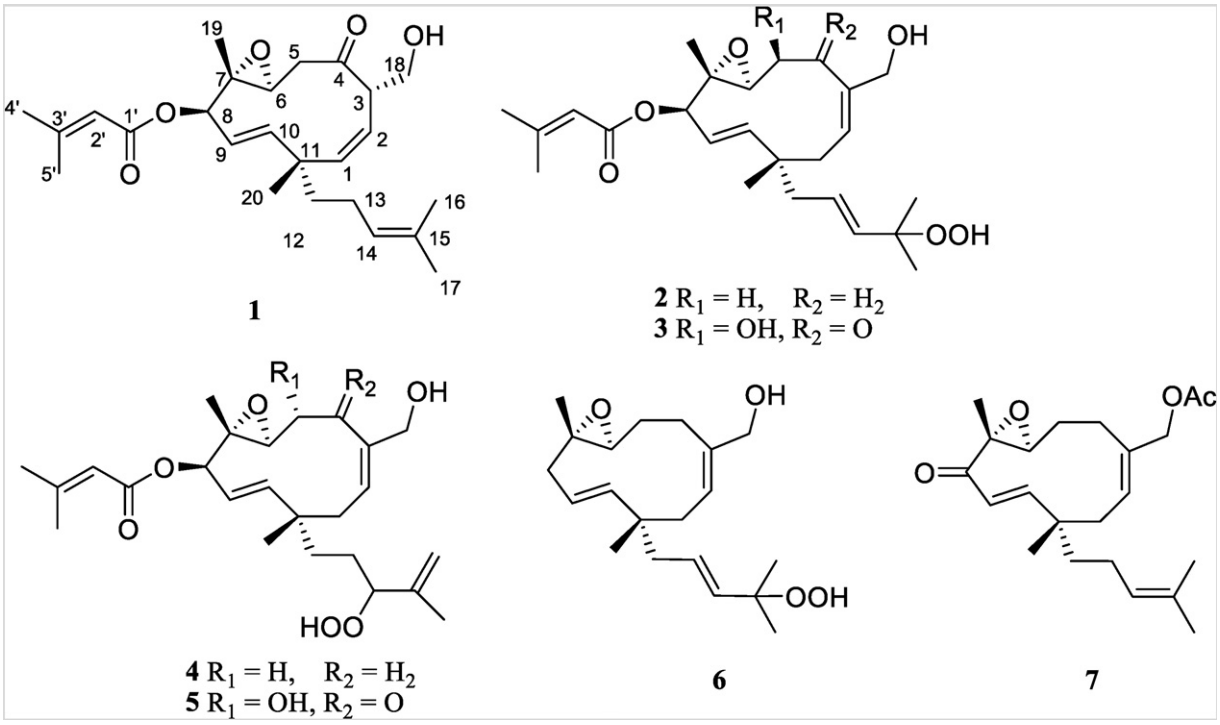


**Fig. 1.** The new compounds isolated from *V. odoratissimum*.

shifts of C-6 ($\delta_C$ 59.4) and C-7 ($\delta_C$ 62.3), an epoxide ring must be formed at C-6 and C-7. Thus, the above analyses established the planar structure of **1** as shown in Fig. 2.

The relative stereochemistry of **1** was elucidated by a ROESY experiment (Fig. 2). ROESY correlations of H-6/H-9, H-6/Me-19, H-3/Me-20, and H-9/Me-20 indicated that H-3, H-6, H-9, Me-19, and Me-20 were on the $\beta$-face of the 11-membered ring, while the epoxy ring at C-6/C-7 was on the $\alpha$-face of the 11-membered ring [5,6]. In addition, ROESY correlations of H-8/H-10 suggested that H-8 and H-10 were on the $\alpha$-face of the 11-membered ring [5,6]. Hence, on the basis of above spectroscopic data, the structure of compound **1** could be represented as vibsanol C.

The spectroscopic data of **2** were found to be very similar to those of vibsanin P, a known compound from *V. awabuki* [3]. Its molecular formula, $C_{25}H_{38}O_6$, as deduced from HRESIMS ($[M + Na]^+$ at $m/z$ 457.2566), suggested the existence of one more oxygen atom than that of vibsanin P [3], implying the presence of a hydroperoxy group in **2**. The hydroperoxy group was suggested to be placed at C-15 as inferred from the low-field chemical shift of C-15 (81.6 for **2** compared with 70.4 for vibsanin P) and the HMBC correlations of $\delta_H$ 1.23 (6H, s, Me-16 and Me-17) and 5.63 (d, $J$ = 13.2 Hz, H-14) with $\delta_C$ 81.6 (s, C-15) [26–27]. The relative stereochemistry of **2** was verified to be the same as that of vibsanin P by the ROESY experiment. Therefore, the structure of **2** was established and named as vibsanol D.

Vibsanol E (**3**) was isolated as a colorless oil. Its molecular formula, $C_{25}H_{36}O_8$, was established by HRESIMS ($[M + Na]^+$ at $m/z$ 487.2307). Preliminary analysis of 1D NMR data (Tables 1 and 2) suggested that **3** had a similar structure to that of **2**. The main differences were that **3** had one more carbonyl and one more hydroxy group than that of **2**, which were placed at C-4 and C-5, as evidenced by the HMBC correlations of $\delta_H$ 4.35 (d, $J$ = 13.8 Hz, H-18b), 4.61 (d, $J$ = 13.8 Hz, H-18a), and 5.98 (dd, $J$ = 6.6, 10.8 Hz, H-2) with $\delta_C$ 202.6 (s, C-4) and of $\delta_H$ 3.17 (d, $J$ = 8.8 Hz, H-6) with $\delta_C$ 72.9 (d, C-5), respectively. In the ROESY spectrum, the correlations of H-6/Me-19, H-9/Me-19, H-9/Me-20, H-8/H-10, and H-10/H-5 were observed, which suggested that H-6, H-9, Me-19, and Me-20 were on the $\beta$-face of the 11-membered ring, while H-5, H-8 and H-10 were on the $\alpha$-face of it. Additionally, the ROESY correlation between H-18 and H-2 indicated the double

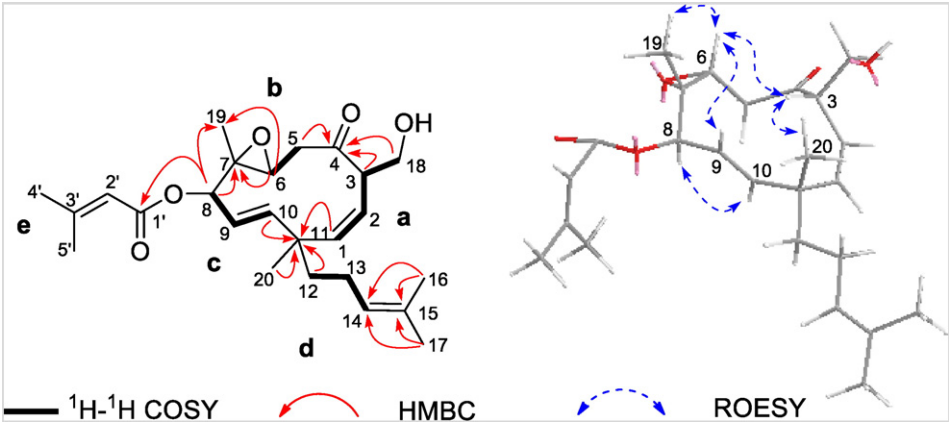


**Fig. 2.** Key 2D NMR correlations of **1**.

bond of C-2/C-3 to be *Z* geometry. On the basis of the above analysis, the structure of compound **3** was established and named as vibsanol E.

Vibsanol F (**4**) had the similar spectroscopic data as those of vibsanin S [21]. Its molecular formula, $C_{25}H_{38}NO_6$, as deduced from HRESIMS ($[M + Na]^+$ at *m/z* 457.2561), suggested the existence of one more oxygen atom than that of vibsanin S [3], implying the presence of a hydroperoxy group at C-14 in **4**, as inferred from the low-field chemical shift of C-14 (89.9 for 4 compared with 75.5 for vibsanin S) and the HMBC correlations of $\delta_H$ 1.67 (3H, s, Me-17) and 4.88 (2H, d, $J$ = 12.5 Hz, H-16) with $\delta_C$ 89.9 (s, C-14) [26–27]. The relative stereochemistry of **4** was verified to be the same as that of vibsanin S by the ROESY experiment. Thus, the structure of **4** was established and named as vibsanol F.

Vibsanol G (**5**), a colorless oil, possessed a molecular formula of $C_{25}H_{36}O_8$, as established by HRESIMS ($[M + Na]^+$ at *m/z* 487.2303), corresponding to eight degrees of unsaturation. Comparison of the NMR data of **5** with those of **4** suggested the similarity of two compounds. The key difference was that **5** had one more carbonyl at C-4 and one more hydroxy group at C-5 than **4**, as deduced from the HMBC correlations of $\delta_H$ 4.21 (d, $J$ = 13.6 Hz, H-18b), 4.35 (d, $J$ = 13.6 Hz, H-18a), and 5.82 (dd, $J$ = 6.3, 10.4 Hz, H-2) with $\delta_C$ 203.3 (s, C-4) and of $\delta_H$ 3.10 (d, $J$ = 8.8 Hz, H-6) with $\delta_C$ 73.4 (d, C-5), respectively. In the ROESY spectrum, the correlations of H-5/H-9, H-6/Me-19, H-9/Me-19, H-9/Me-20, H-8/H-10, and H-10/H-12 were observed, which suggested that H-5, H-6, H-9, Me-19, and Me-20 were on the $\beta$-face of the 11-membered ring, while H-8 and H-10 were on the α-face of it. Moreover, the ROESY correlation between H-18 and H-2 indicated the double bond of C-2/C-3 to be *Z* geometry. So, the structure of compound **5** was established and named as vibsanol G.

Vibsanol H (**6**) was obtained as a colorless oil. Its molecular formula, $C_{20}H_{32}O_4$, was established by HRESIMS ($[M + Na]^+$ at *m/z* 359.2199). Comparison of the 1D and 2D NMR data with those of vibsanin F [14] implied that the double bond of C-14/C-15 in vibsanin F migrated to C13/C14 in **6**, as inferred from the $^1H$–$^1H$ COSY correlations of H-12/H-13/H-14. Meanwhile, C-15 (s, $\delta_C$ 82.1) in **6** was substituted by a hydroperoxy group as deduced from the low-field chemical shift of C-15 and confirmed by the HMBC correlations of $\delta_H$ 1.29 (6H, s, Me-16 and Me-17) with $\delta_C$ 82.1 (s, C-15) [26–27]. The relative stereochemistry of **6** was verified to be the same as that of vibsanin F by the ROESY experiment. Thus, the structure of **6** was established and named as vibsanol H.

Vibsanin X (**7**) had a molecular formula of $C_{22}H_{32}O_4$, as established by HRESIMS ($[M + H]^+$ at *m/z* 383.2208). Comparison of the NMR data of **7** with those of vibsanin F suggested that **7** has one more carbonyl and one more acetyl group, which were placed at C-8 and C-18, respectively, as deduced from the HMBC correlations of $\delta_H$ 3.10 (d, $J$ = 10.6 Hz, H-6) and 7.04 (d, $J$ = 16.9 Hz, H-10) with $\delta_C$ 197.9 (s, C-8) and of $\delta_H$ 4.25 (d, $J$ = 12.5 Hz, H-18b) and 4.50 (d, $J$ = 12.5 Hz, H-18a) with $\delta_C$ 170.6 (s, Ac), respectively. The relative stereochemistry of **7** was verified to be the same as that of vibsanin F [14] by the ROESY experiment. Thus, the structure of **7** was established and named as vibsanin X.

**Table 3**
In vitro evaluation of cytotoxicity of **1–7**.

| Compound | $IC_{50}$ (μM) | | | | |
|---|---|---|---|---|---|
| | HL-60 | SMMC-7721 | A-594 | MCF-7 | SW-480 |
| **1** | 3.4 | 4.4 | 5.2 | 11.3 | 3.7 |
| **2** | 11.4 | 15.0 | 18.2 | >40 | >40 |
| **3** | >40 | >40 | >40 | >40 | >40 |
| **4** | 4.5 | 3.7 | 14.8 | 15.1 | 16.2 |
| **5** | 2.9 | 3.5 | 17.6 | 15.4 | 15.1 |
| **6** | >40 | >40 | >40 | >40 | >40 |
| **7** | >40 | > 40 | >40 | >40 | >40 |
| Cisplatin | 1.0 | 14.8 | 13.6 | 17.1 | 15.6 |

Compounds **1–7** were tested for their cytotoxicity against five human cancer cell lines (Table 3). Vibsanol C (**1**) showed significant cytotoxicity against HL-60, SMMC-7721, A-594, MCF-7, and SW-480, with $IC_{50}$ values of 3.4, 4.4, 5.4, 11.3, and 3.7 μM, respectively. While vibsanol F (**4**) and vibsanol G (**5**) displayed significant cytotoxicity against SMMC-7721, with $IC_{50}$ values of 3.7 and 3.5 μM, respectively. Compounds **3**, **6**, and **7** were noncytotoxic ($IC_{50}$ > 40 μM).

## Conflict of interest

The authors declare that there is no conflict of interest.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2016.01.014.

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