# Six New Dammarane Triterpenoids from *Viburnum cylindricum*

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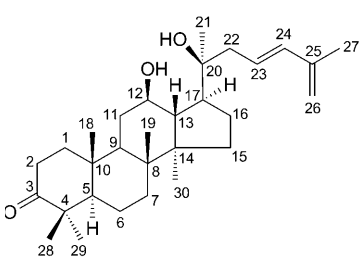
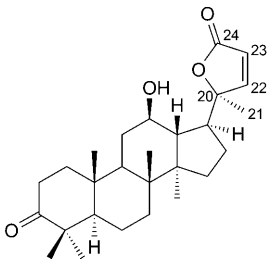
One new dammarane triterpenoid, cylindrictone A (**1**), as well as five new *nor*-dammarane triterpenoids cylindrictone B–F (**2**–**6**) were isolated from the leaves of *Viburnum cylindrium*, together with three known compounds 3$\beta$-hydroxy-hexanordammaran-20-one (**7**), 3$\alpha$-hydroxyoctanordammar-12-en-17-one (**8**), and neoalsogenin B (**9**). Their structures were identified by extensive NMR-spectroscopic (especially 2D-NMR) and mass-spectrometric experiments, and the structure of **3** was further confirmed by a single-crystal X-ray analysis. The compounds exhibited no activity against human promyelocytic leukemia HL60 cell.

**Introduction.** – The plants of the genus *viburnum* are used as traditional Chinese herbal medicine for the treatment of astringent, sedative, and emmengagogue [1], and other health problems [2]. Previous chemical studies established the occurrence of iridoids [3], oleanane-type triterpenoids [4], rearranged dammarane triterpenes [5], phenolic glycosides [6], and vibsane-type diterpenes [7][8]. *Viburnum cylindricum* is distributed in tropical Asia. A literature search revealed that phytochemical and pharmacological studies have rarely been undertaken within this species [9]. As a part of our work, we continued our phytochemical investigations of the genus *viburnum*. The AcOEt-soluble material of a $Me_2CO$ extract of the leaves of *V. cylindricum* furnished six new dammarane-type triterpenoids, **1**–**6**, and three known compounds, namely 3$\beta$-hydroxyhexanordammaran-20-one (**7**) [10], 3$\alpha$-hydroxyoctanordammar-12-en-17-one (**8**) [11], and neoalsogenin B (**9**) [12]. The six new compounds are endowed with five different skeletons, such as normal dammarane triterpenoid, **1**, trinor-dammarane triterpenoid, **2**, **3**, and **4**, hexanordammarane tertripenoid, **5**, and octanordammarane tertripenoid, **6**. In the present article, we report the structural characterization of the compounds **1**–**9** and their anti-cancer activities.

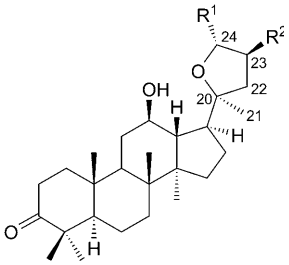
**Results and Discussion.** – Cylindrictone A (**1**), obtained as a white amorphous powder, was shown to have a molecular formula of $C_{30}H_{48}O_3$ by HR-ESI-MS ([$M$ + Na]$^+$, $m/z$ 479.3505; calc. 479.3501). The IR spectrum displayed the presence of OH (3384 cm$^{-1}$) and C=O (1705 cm$^{-1}$) groups, as well as a C=C bond (1608 cm$^{-1}$). The $^1$H-NMR spectrum (*Table 1*) showed signals for seven Me groups, and the $^{13}$C-NMR spectrum (*Table 2*) exhibited signals for 30 C-atoms, including seven quaternary C-



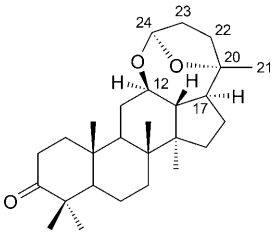
**1**



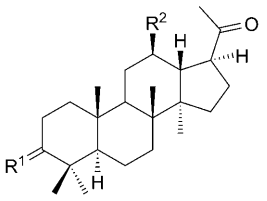
**2**



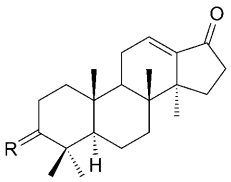
| | $R^1$ | $R^2$ |
|---|---|---|
| **3** | MeO | H |
| **9** | $HOMe_2C$ | $\beta$-OH |



**4**



| | $R^1$ | $R^2$ |
|---|---|---|
| **5** | O | $\beta$-OH |
| **7** | $\beta$-OH, H | H |



| | R |
|---|---|
| **6** | O |
| **8** | $\alpha$-OH, H |

atoms (one ketone C=O ($\delta$(C) 217.9), one oxygenated ($\delta$(C) 74.4), and one olefinic ($\delta$(C) 141.7)), seven CH groups (one oxygenated ($\delta$(C) 70.7)), nine $CH_2$ (one olefinic $\delta$(C) 115.4), and seven Me groups. Comparison of the $^1$H- and $^{13}$C-NMR spectroscopic data of **1** with those of the known compound dammara-23,25-diene-3,12,20-triol [13] showed that the two compounds were very similar. The only difference was the replacement of an oxygenated H–C(3) signal of dammara-23,25-diene-3,12,20-triol by a ketone C-atom in **1**, as indicated by HMBC correlations from Me(28) and Me(29) to C(3), and H–C(1) and H–C(5) to C(3) (*Fig. 1*).

The relative configuration of **1** was determined by analysis of the ROESY spectrum (*Fig. 2*). The $\beta$-orientation of the 12-OH group was deduced from the correlations of Me(30)/H–C(12), H–C(17)/H–C(12), and H–C(9)/H–C(12). The ROESY correlations of Me(21) to H–C(17) confirmed the configuration of 20-OH as being $\beta$-oriented. Accordingly, the structure of **1** was determined as 12$\beta$,20$\beta$-dihydroxy-23,25-diene-3-oxodammarane and named cylindrictone A.

Cylindrictone B (**2**), a white amorphous powder, has the molecular formula $C_{27}H_{40}O_4$ as deduced from the HR-ESI-MS ($[M+Na]^+$, $m/z$ 451.2816; calc. 451.2824). The IR spectrum indicated the presence of OH (3448 cm$^{-1}$) and C=O (1703 cm$^{-1}$) groups, as well as a C=C bond (1639 cm$^{-1}$). The $^{13}$C-NMR spectrum (*Table 2*) determined the 27 skeleton C-atoms in **2** as composed of six Me, seven $CH_2$, seven CH (an oxygenated one and two olefinic ones), five quaternary C-atoms and two C=O groups ($\delta$(C) 217.6 and 172.2). Characteristic $^1$H-NMR signals for Me groups at ($\delta$(H) 1.44 (*s*), 1.01 (*s*), 0.98 (*s*), 0.90 (*s*), 0.89 (*s*), and 0.83 (*s*)) and for the oxygenated CH group ($\delta$(H) 3.61 (*dt* $J$ = 5.2, 10.4)) were observed, which was similar to the known

Table 1. *$^1$H-NMR Spectroscopic Data* (500 MHz) *for Compounds* **1**–**3**

| | **1**[a]) | **2**[a]) | **3**[b]) |
|---|---|---|---|
| $CH_2$(1) | 1.48–1.53 (*m*), 1.97–2.00 (*m*) | 1.43–1.48 (overlap), 1.94–1.97 (*m*) | 1.48–1.54 (*m*), 1.85–1.89 (*m*) |
| $CH_2$(2) | 1.33–1.36 (*m*), 1.56–1.70 (overlap) | 2.52–2.56 (*m*), 2.51–2.53 (*m*) | 2.44–2.50 (*m*), 2.45–2.51 (*m*) |
| H–C(5) | 1.29–1.36 (*m*) | 1.31–1.97 (*m*) | 1.37–1.44 (*m*) |
| $CH_2$(6) | 1.56–1.63 (overlap), 1.47–1.54 (*m*) | 1.52–1.59 (overlap), 1.52–1.56 (overlap) | 1.53–1.61 (overlap), 1.52–1.60 (overlap) |
| $CH_2$(7) | 2.45–2.58 (overlap), 2.54 (*m*) | 1.35–1.50 (*m*), 1.52–1.58 (overlap) | 1.32–1.50 (*m*), 1.53–1.61 (overlap) |
| H–C(9) | 1.52–1.55 (overlap) | 1.52–1.57 (overlap) | 1.50–1.53 (overlap) |
| $CH_2$(11) | 1.83–1.87 (overlap), 1.89–1.92 (*m*) | 1.83–1.88 (*m*), 1.26 (*m*) | 1.51–1.59 (*m*), 1.50–1.55 (overlap) |
| H–C(12) | 3.62 (*dt*, *J* = 5.1, 10.4) | 3.61 (*dt*, *J* = 5.2, 10.4) | 3.47 (*dt*, *J* = 3.6, 8.4) |
| H–C(13) | 1.73–1.82 (*m*) | 1.36–1.45 (overlap) | 2.09–2.18 (*m*) |
| $CH_2$(15) | 1.52–1.58 (overlap), 1.83–1.90 (overlap) | 1.16–1.21 (*m*), 1.55–1.60 (overlap) | 1.77–1.83 (overlap), 1.76–1.85 (overlap) |
| $CH_2$(16) | 1.32–1.40 (*m*), 1.90–1.95 (*m*) | 2.04–2.08 (*m*), 1.71–1.76 (*m*) | 1.80–1.85 (overlap), 1.82–1.90 (*m*) |
| H–C(17) | 2.07–2.16 (*m*) | 2.21–2.29 (*m*) | 1.60–1.72 (*m*) |
| Me(18) | 1.03 (*s*) | 0.90 (*s*) | 0.96 (*s*) |
| Me(19) | 1.19 (*s*) | 0.89 (*s*) | 0.93 (*s*) |
| Me(21) | 1.21 (*s*) | 1.44 (*s*) | 1.35 (*s*) |
| $CH_2$(22) | 2.18–2.32 (*m*), 2.39–2.45 (overlap) | 7.85 (*d*, *J* = 5.7) | 1.78–1.90 (*m*), 1.90–1.98 (*m*) |
| H–C(23) or $CH_2$(23) | 5.74 (*dt*, *J* = 7.5, 7.6, 15.6) | 6.05 (*d*, *J* = 5.7) | 1.95–2.02 (*m*), 2.00–2.09 (*m*) |
| H–C(24) | 6.25 (*d*, *J* = 15.6) | – | 4.93 (*m*) |
| $CH_2$(26) | $H_a$: 4.91 (br. *s*), $H_b$: 4.92 (br. *s*) | – | – |
| Me(27) | 1.86 (*s*) | – | – |
| Me(28) | 1.09 (*s*) | 1.01 (*s*) | 1.01 (*s*) |
| Me(29) | 1.05 (*s*) | 0.98 (*s*) | 0.99 (*s*) |
| Me(30) | 0.90 (*s*) | 0.83 (*s*) | 0.89 (*s*) |
| MeO | – | – | 3.26 (*s*) |

[a]) Spectra were recorded in $CDCl_3$. [b]) Spectra were recorded in $CD_3OD$.

Table 2. *$^{13}$C-NMR Spectroscopic Data* (125 MHz) *for Compounds* **1**–**6**

| | **1**[a]) | **2**[a]) | **3**[b]) | **4**[a]) | **5**[a]) | **6**[a]) |
|---|---|---|---|---|---|---|
| C(1) | 39.7 (*t*) | 39.5 (*t*) | 40.8 (*t*) | 39.7 (*t*) | 39.6 (*t*) | 36.1 (*t*) |
| C(2) | 34.0 (*t*) | 33.8 (*t*) | 34.9 (*t*) | 34.0 (*t*) | 33.8 (*t*) | 33.9 (*t*) |
| C(3) | 217.9 (*s*) | 217.6 (*s*) | 220.6 (*s*) | 217.6 (*s*) | 218.5 (*s*) | 217.0 (*s*) |
| C(4) | 47.4 (*s*) | 47.1 (*s*) | 48.4 (*s*) | 47.3 (*s*) | 47.2 (*s*) | 47.4 (*s*) |
| C(5) | 55.2 (*d*) | 55.0 (*d*) | 56.3 (*d*) | 55.3 (*d*) | 55.0 (*d*) | 55.7 (*d*) |
| C(6) | 19.6 (*t*) | 19.4 (*t*) | 20.7 (*t*) | 19.7 (*t*) | 19.4 (*t*) | 19.4 (*t*) |
| C(7) | 34.0 (*t*) | 33.8 (*t*) | 35.1 (*t*) | 34.0 (*t*) | 34.2 (*t*) | 33.9 (*t*) |
| C(8) | 39.6 (*s*) | 39.3 (*s*) | 40.8 (*s*) | 39.7 (*s*) | 39.5 (*s*) | 36.9 (*s*) |
| C(9) | 49.3 (*d*) | 49.2 (*d*) | 50.6 (*d*) | 49.1 (*d*) | 49.6 (*d*) | 47.4 (*d*) |
| C(10) | 36.8 (*s*) | 36.5 (*s*) | 37.9 (*s*) | 36.9 (*s*) | 36.7 (*s*) | 37.9 (*s*) |
| C(11) | 31.4 (*t*) | 32.4 (*t*) | 32.7 (*t*) | 30.7 (*t*) | 32.3 (*t*) | 24.1 (*t*) |
| C(12) | 70.7 (*d*) | 70.8 (*d*) | 71.9 (*d*) | 73.5 (*d*) | 71.1 (*d*) | 130.6 (*d*) |
| C(13) | 48.3 (*d*) | 49.7 (*d*) | 50.8 (*d*) | 47.3 (*d*) | 50.8 (*d*) | 144.1 (*s*) |
| C(14) | 51.6 (*s*) | 51.8 (*s*) | 53.2 (*s*) | 49.7 (*s*) | 51.0 (*s*) | 47.7 (*s*) |
| C(15) | 30.8 (*t*) | 31.2 (*t*) | 33.1 (*t*) | 31.9 (*t*) | 31.8 (*t*) | 27.0 (*t*) |
| C(16) | 26.4 (*t*) | 26.5 (*t*) | 31.0 (*t*) | 23.7 (*t*) | 26.7 (*t*) | 39.5 (*t*) |
| C(17) | 52.3 (*d*) | 47.0 (*d*) | 50.6 (*d*) | 52.7 (*d*) | 52.8 (*d*) | 216.6 (*s*) |
| C(18) | 15.3 (*q*) | 14.9 (*q*) | 15.5 (*q*) | 15.7 (*q*) | 15.1 (*q*) | 17.0 (*q*) |
| C(19) | 15.9 (*q*) | 15.7 (*q*) | 16.6 (*q*) | 15.8 (*q*) | 15.9 (*q*) | 15.6 (*q*) |
| C(20) | 74.4 (*s*) | 91.8 (*s*) | 80.3 (*s*) | 87.6 (*s*) | 215.3 (*s*) | – |
| C(21) | 27.7 (*q*) | 22.6 (*q*) | 30.5 (*q*) | 26.9 (*q*) | 29.4 (*q*) | – |
| C(22) | 39.0 (*t*) | 161.3 (*d*) | 29.1 (*t*) | 28.8 (*t*) | – | – |
| C(23) | 125.3 (*d*) | 120.6 (*d*) | 33.7 (*t*) | 37.2 (*t*) | – | – |
| C(24) | 136.8 (*d*) | 172.2 (*s*) | 107.4 (*d*) | 102.3 (*d*) | – | – |
| C(25) | 141.7 (*s*) | – | – | – | – | – |
| C(26) | 115.4 (*t*) | – | – | – | – | – |
| C(27) | 18.7 (*q*) | – | – | – | – | – |
| C(28) | 26.7 (*q*) | 26.4 (*q*) | 27.2 (*q*) | 26.8 (*q*) | 26.5 (*q*) | 26.7 (*q*) |
| C(29) | 21.0 (*q*) | 20.7 (*q*) | 21.4 (*q*) | 21.0 (*q*) | 20.8 (*q*) | 21.4 (*q*) |
| C(30) | 16.7 (*q*) | 17.2 (*q*) | 17.9 (*q*) | 16.9 (*q*) | 16.6 (*q*) | 23.6 (*q*) |
| MeO | – | – | 55.4 (*q*) | – | – | – |

[a]) Spectra were recorded in $CDCl_3$. [b]) Spectra were recorded in $CD_3OD$.

compound carbrelactone [14]. Compound **2** differed from that of carbrelactone by having a OH group and a C=C bond. Analysis of the HMBC spectrum of **2** assigned the OH group to C(12) due to the long-range correlations of H–C(9) to C(12), H–C(17) to C(12), and H–C(12) to C(14). The C=C bond was located between C(22) and C(23) because of the presence of HMBC correlations from H–C(22) and H–C(23) to C(24) and C(20).

The relative configuration of **2** was established through an analysis of the ROESY experiment. The $\beta$-orientation of the 12-OH was deduced from the correlations of H–C(9)/H–C(12) and H–C(12)/Me(30). The presence of ROESY correlations of H–C(17)/Me(21) and H–C(12)/Me(21) verified the $\alpha$-orientation of Me(21). Thus, **2** was deduced as 12$\beta$-hydroxy-3-oxo-25,26,27-trinordammar-22-en-24,20-olide and named cylindrictone B.

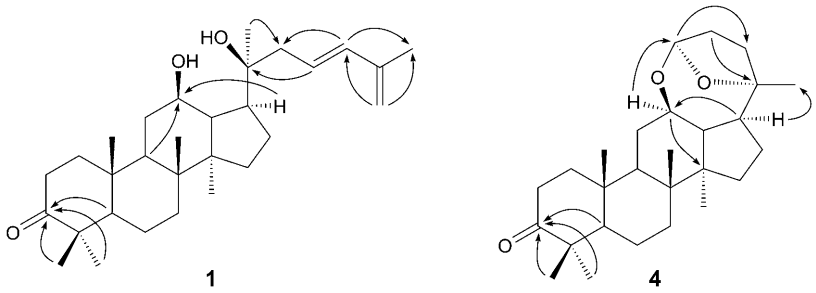



Fig. 1. *HMBC Correlations of compounds* **1** *and* **4**

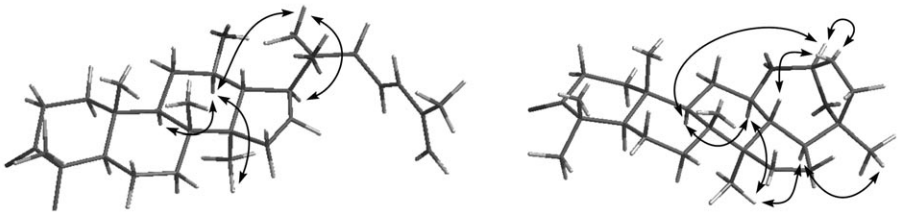



Fig. 2. *ROESY Correlations of compounds* **1** *and* **4**

Cylindrictone C (**3**) was isolated as colorless crystals (in MeOH), and its molecular formula was found to be $C_{28}H_{46}O_4$ by HR-ESI-MS ($[M+H]^+$ *m/z* 447.3479, calc. 447.3474), corresponding to six degrees of unsaturation. The IR spectrum showed an absorption band at 3424 $cm^{-1}$, revealing the presence of a OH group. The $^1$H-NMR spectrum (*Table 1*) and $^{13}$C-NMR data (*Table 2*), along with the DEPT and HSQC experiments, showed that the C-atoms were seven Me (including one MeO group), nine $CH_2$, six CH (including two oxygenated CH groups), and five quaternary C-atoms (including one oxygenated C-atom), and one C=O group at $\delta$(C) 220.6. These NMR data were quite similar to those of **2**, except for the resonances attributed for the side chain. Hence, **3** was expected to be a dammarane with a C=O group at C(3) and OH group at C(12). This assumption was confirmed by the HMBC correlations of Me(28) and Me(29) to C(3) and H–C(17) and H–C(11) to C(12). In the HMBC spectrum, the oxygenated H–C(24) group showed long-range correlations to the oxygenated quaternary C-atom C(20) and MeO ($\delta$(C) 55.4), respectively. These correlations suggested that C(20) and H–C(24) were linked *via* O to form a lactol ring, and the MeO group was placed at C(24).

The relative configuration of **3** was confirmed by a ROESY experiment. The existence of ROESY correlations of H–C(12) to Me(30), and H–C(9) to H–C(12) suggested $\beta$-orientation for the 12-OH group. However, the ROESY spectrum did not provide sufficient information to elucidate the configuration at C(24). A single-crystal X-ray analysis of **3** (*Fig. 3*) was then carried out and clarified the $\alpha$-orientation of the

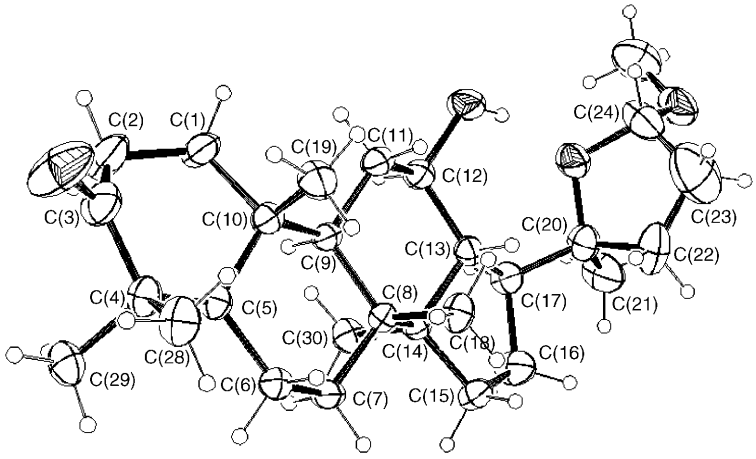



Fig. 3. *X-Ray structure of* **3** *showing the relative configuration*

MeO group on C(24). The structure of **3** was thus elucidated as (5$\alpha$,12$\beta$,17$\beta$)-12-hydroxy-17-[(2*S*,5*S*)-5-methoxy-2-methyltetrahydrofuran-2-yl]-4,4,8,10,14-pentamethylgonan-3-one.

Cylindrictone D (**4**) was obtained as a yellow oil with the molecular formula $C_{27}H_{42}O_3$ based on HR-ESI-MS ($[M+Na]^+$ *m*/*z* 437.3041; calc. 437.3031). The NMR spectra (*Tables 2* and *3*) of **4** disclosed the presence of six Me, nine $CH_2$, six CH groups (two oxygenated, ($\delta$(C) 73.5 C(12)) and 102.3 C(24)), five quaternary C-atoms (one oxygenated $\delta$(C) 87.6 C(20)), and one ketone C=O group ($\delta$(C) 217.6 C(3)), which was similar to those of a known compound 12,24 : 20,24-diepoxydammarane-3,25-diol [15]. The key difference between the two compounds was that the propan-2-ol group of 12,24 : 20,24-diepoxydammarane-3,25-diol was lacking in compound **4**. Further comparison of their NMR spectra exhibited that the oxymethine C-atom signal due to C(3) of 12,24 : 20,24-diepoxydammarane-3,25-diol was replaced by a ketone C-atom ($\delta$(C) 217.6) in **4**, which was confirmed by the HMBC correlation (*Fig. 1*) of Me(28)/C(3), Me(29)/C(3), and H–C(5)/C(3). The relative configurations of H–C(12) and H–C(24) of **4** were deduced from the ROESY experiment. The ROESY correlations (*Fig. 2*) were observed between Me(30)/H–C(12), Me(30)/H–C(17), H–C(9)/H–C(12), H–C(17)/Me(21), and H–C(9)/H–C(24) to establish the $\alpha$-orientation of H–C(12) and H–C(24). On the basis of these evidences, the structure of **4** was characterized as (2a*S*,3*S*,6*R*,7a*R*,8b*R*,14a*R*,14b*R*,14c*R*)-icosahydro-3,8b,12,12,14a,14b-hexamethyl-11*H*-3,6-epoxynaphtha[2′,1′:4,5]indeno[7,1-*bc*]oxocin-11-one, and named as Cylindrictone D.

Cylindrictone E (**5**) showed the molecular formula $C_{24}H_{38}O_3$ as established by HR-ESI-MS ($[M+H]^+$ *m*/*z* 375.2898; calc. 375.2899). The IR absorptions at 3441 and 1704 $cm^{-1}$ indicated the presence of OH and C=O groups, respectively. The resemblance of the NMR spectra of **5** (*Table 2* and *3*) to those of 3$\beta$-acetoxy-hexanordammaran-20-one (**7**) suggested that **5** was a related hexanordammarane triterpenoid. Side-by-side comparison of the NMR data of **5** and **7** indicated that the

Table 3. *$^1$H-NMR Spectroscopic Data* (500 MHz) *for Compounds* **4**–**6**

| | **4**[a]) | **5**[a]) | **6**[a]) |
|---|---|---|---|
| $CH_2$(1) | 1.47–1.55 (*m*), 1.87–1.94 (*m*) | 1.39–1.48 (overlap), 1.88–1.95 (*m*) | 2.39–2.48 (overlap), 2.22–2.31 (*m*) |
| $CH_2$(2) | 2.37–2.46 (*m*), 2.42–2.55 (*m*) | 2.37–2.44 (*m*), 2.46–2.53 (*m*) | 2.42–2.48 (overlap), 2.53–2.59 (*m*) |
| H–C(5) | 1.29–1.38 (*m*) | 1.33–1.38 (overlap) | 1.42–1.47 (overlap) |
| $CH_2$(6) | 1.56–1.63 (*m*), 1.51–1.59 (*m*) | 1.42–1.51 (overlap), 1.47–1.58 (*m*) | 1.39–1.48 (overlap), 1.51–1.60 (*m*) |
| $CH_2$(7) | 1.56–1.64 (*m*), 1.28–1.36 (*m*) | 1.41–1.49 (overlap), 1.31–1.39 (overlap) | 1.47–1.55 (overlap), 1.65–1.74 (*m*) |
| H–C(9) | 1.41–1.48 (*m*) | 1.44–1.52 (overlap) | 1.71–1.78 (*m*) |
| $CH_2$(11) | 1.87 (*d*, *J* = 3, 5.5), 1.85 (*d*, *J* = 3, 5.5) | 1.21–1.28 (*m*), 1.84–1.95 (*m*) | 2.08–2.16 (*m*), 2.19–2.25 (*m*) |
| H–C(12) | 3.79 (*dt*, *J* = 5.6, 10.8) | 3.49 (*dt*, *J* = 5, 10.5) | 6.63 (*dt*, *J* = 3.5, 7.5) |
| H–C(13) | 1.52–1.61 (overlap) | 2.08 (*d*, *J* = 11.3, 22) | – |
| $CH_2$(15) | 1.66–1.74 (*m*), 1.11–1.18 (*m*) | 1.15–1.26 (*m*), 1.59–1.67 (*m*) | 1.92–2.01 (*m*), 1.53–2.02 (*m*) |
| $CH_2$(16) | 1.72–1.79 (*m*), 1.13–1.21 (*m*) | 2.11–2.23 (*m*), 1.67–1.72 (overlap) | 1.91–2.00 (*m*), 1.47–1.59 (overlap) |
| H–C(17) | 2.09–2.15 (*m*) | 2.77–2.85 (*m*) | – |
| Me(18) | 1.03 (*s*) | 1.06 (*s*) | 0.91 (*s*) |
| Me(19) | 0.97 (*s*) | 0.99 (*s*) | 1.10 (*s*) |
| Me(21) | 1.31 (*s*) | 2.24 (*s*) | – |
| $CH_2$(22) | 1.51–1.58 (*m*), 1.88–1.96 (*m*) | – | – |
| $CH_2$(23) | 1.87–1.95 (*m*), 2.19–2.33 (*m*) | – | – |
| H–C(24) | 5.55 (*d*, *J* = 6.2) | – | – |
| Me(28) | 1.09 (*s*) | 1.09 (*s*) | 1.13 (*s*) |
| Me(29) | 1.05 (*s*) | 1.04 (*s*) | 1.09 (*s*) |
| Me(30) | 0.93 (*s*) | 0.91 (*s*) | 1.16 (*s*) |

[a]) Spectra were recorded in $CDCl_3$. [b]) Spectra were recorded in $CD_3OD$.

oxymethine group C(3) in **7** was replaced by a ketone CO group ($\delta$(C) 218.5) in **5**, as indicated by the HMBC correlation of Me(28)/C(3), Me(29)/C(3), and H–C(5)/C(3). Another difference between the two compounds was the replacement of a $CH_2$(12) signal in **7** by an oxygenated CH group ($\delta$(C) 71.1) in **5**, which was confirmed by the HMBC correlations from H–C(9) to C(12), from H–C(11) and H–C(13) to C(12), and from H–C(12) to C(14). H–C(12) was found to be $\alpha$-oriented on the basis of ROESY cross-peaks of Me(30)/H–C(12), H–C(9)/H–C(12), and H–C(12)/H–C(17). Thus, the structure of **5** was established as 12$\beta$-hydroxy-22,23,24,25,26,27-hexanordammarane-3,20-dione, and was given the trival name cylindrictone E.

Cylindrictone F (**6**), a yellow oil, had the molecular formula $C_{22}H_{32}O_2$, as shown by its HR-ESI-MS ($[M+H]^+$ *m*/*z* 329.2480; calc. 329.2480). The IR spectrum showed two C=O bands (1716 and 1702 cm$^{-1}$), and one C=C band (1655 cm$^{-1}$). The presence of two C=O groups and one C=C bond were further supported by the $^{13}$C-NMR signals at $\delta$(C) 217.0, 216.6, 130.6, and 144.1. Five Me, seven $CH_2$, two CH, and four quaternary C-atoms were also observed in the $^{13}$C-NMR spectrum (*Table 2*). The $^1$H- (*Table 3*)

and $^{13}$C-NMR spectra of **6** were similar to those of 3$\alpha$-hydroxyoctanordammar-12-en-17-one (**8**), except that the oxygenated H–C(3) group in **8** was replaced by a C=O group ($\delta$(C) 217.0) in **6**, as revealed by the HMBC correlations between C(3) and the signals for H–C(5), Me(28), and Me(29). Accordingly, the structure of **6** was elucidated to be 20,21,22,23,24,25,26,27-octanordammarane-3,17-dione and named cylindrictone F.

Compounds **1**–**9** were tested for cytotoxicity against human promyelocytic leukemia HL60 cells. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) was used as positive control in this experiment. None of the compounds showed significant cytotoxic activity.

## Experimental Part

*General.* Semiprep. HPLC was performed on an *Agilent 1100* apparatus equipped with a UV detector and a *Zorbax SB-C-18* (*Agilent*, 9.4 mm × 25 cm) column. Column chromatography (CC) was performed either on silica gel ($SiO_2$; 200–300 mesh; *Qingdao Marine Chemical Inc.*, Qingdao, P. R. China), silica gel *H* (10–40 μm; *Qingdao Marine Chemical Inc.*), *Lichroprep RP-18* gel (40–63 μm; *Merck*, Darmstadt, Germany), or *MCI* gel (75–150 μm, *Mitsubinhi Chemical Corporation*, Japan). Fractions were monitored by TLC, and spots were visualized by heating the $SiO_2$ plates sprayed with 10% $H_2SO_4$ in EtOH. M.p.: *XRC-1* micro melting point apparatus, uncorrected. $[\alpha]_D$: *Horiba SEPA-300* polarimeter. UV Spectra: *Shimadzu UV-2401A* spectrometer. IR Spectra: *Bio-Rad FTS-135* spectrometer with KBr pellets. 1D- and 2D-NMR: *Bruker AM-400* and *DRX-500* spectrometers with $Me_4Si$ as internal standard. Unless specified, chemical shifts ($\delta$) were expressed in ppm with reference to the solvent signals. MS: *VG Autospec-3000* spectrometer or *Finnigan MAT 90* instrument.

*Plant Material.* The fronds of *V. cylindricum* were collected in Songming County, Kunming city of Yunman Province, P. R. China, in April 2006. The sample was identified by Prof. *Xiao Cheng*, and a voucher specimen (KIB 06041918) has been deposited with the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

*Extraction and Isolation.* The air-dried and powdered leaves of *V. cylindricum* (3.0 kg) were extracted with $Me_2CO$ (3 × 8 l, each 24 h) at r.t., and filtered. The filtrate was evaporated to give a residue, which was suspended in $H_2O$ (3 l), and then extracted with petroleum ether (PE) (3 × 2 l), AcOEt (4 × 2 l), and BuOH (3 × 2 l), successively. The AcOEt extract (185 g) was decolorized on *MCI* gel (eluted with 95% EtOH) and then subjected to CC ($SiO_2$; PE/$Me_2CO$, gradient system) to obtain fractions 1–9. *Fr. 1*, eluted by CC ($SiO_2$; PE/$Me_2CO$, increasing polarity) was then subjected to *RP-18* (65% MeOH/$H_2O$) to yield **5** (205 mg). *Fr. 2* (3 g) was applied to $SiO_2$, and eluted with PE/i-PrOH (20:1 to 2:1) and $CHCl_3$/MeOH (100:1), after semiprep. HPLC with MeOH/MeCN/$H_2O$ (54:2:46), **6** (4.2 mg) and **7** (10 mg) were obtained. *Fr. 3* (10 g), eluted with CC (PE/AcOEt, 20:1 to 5:1), was then purified by CC (PE/$CHCl_3$, 3:7) to yield compounds **3** (2.6 mg) and **4** (2.8 mg). *Fr. 6* (5 g) was subjected to a *RP-18* gel column with a gradient elution (40% to 80% MeOH/$H_2O$) to provide subfractions *6a*–*6d*. *Subfr. 6a* was eluted by CC($SiO_2$; $CHCl_3$/i-PrOH, 80:1) and further purified with *Sephadex LH-20* (MeOH) to obtain **1** (8 mg) and **9** (3 mg). *Subfr. 6d* (500 mg) provided **2** (9 mg) and **8** (12 mg) after CC over $SiO_2$ (PE/$CHCl_3$/i-PrOH, 60:40:1).

*Cylindrictone A* (*=(12β,23*E*)-12,20-Dihydroxydammara-23,25-dien-3-one*; **1**). White amorphous powder. M.p. 256–257°. $[\alpha]_D^{26} = +17.2$ ($c = 0.31$, $CHCl_3$). UV ($CHCl_3$): 241 (0.59). IR (KBr): 3384, 2950, 2875, 1705, 1630, 1608, 1112, 1460, 1384. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 457 ($[M+H]^+$). HR-ESI-MS (pos.): 479.3505 ($[M+Na]^+$, $C_{30}H_{48}NaO_3^+$; calc. 479.3501).

*Cylindrictone B* (*=(5α,12β,17β)-12-Hydroxy-4,4,8,10,14-pentamethyl-17-[(2*S*)-2-methyl-5-oxo-2,5-dihydrofuran-2-yl]gonan-3-one*; **2**). White amorphous powder ($CHCl_3$). M.p. 273–274°. $[\alpha]_D^{26} = 0$ ($c =$ 0.36, $CHCl_3$). UV ($CHCl_3$): 198 (0.07). IR (KBr): 3448, 2948, 2875, 1757, 1703, 1608, 1461, 1384, 1249, 1109. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 429 ($[M+H]^+$). HR-ESI-MS: 451.2816 ($[M+Na]^+$, $C_{27}H_{40}NaO_4^+$; calc. 451.2824).

*Cylindrictone C* (=*(5α,12β,17β)-12-Hydroxy-17-[(2*S,5S*)-5-methoxy-2-methyltetrahydrofuran-2-yl]-4,4,8,10,14-pentamethylgonan-3-one*; **3**). Colorless crystals (MeOH). M.p. 178–179°. $[\alpha]_D^{26} = +25$ ($c =$ 0.10, $CHCl_3$). UV (MeOH): 204 (0.45). IR (KBr): 3424, 1628, 1384. $^1$H- and $^{13}$C-NMR: *Tables 1* and *2*. FAB-MS (pos.): 447 ($[M+H]^+$). HR-ESI-MS: 447.3479 ($[M+H]^+$, $C_{28}H_{47}O_4^+$; calc. 447.3474).

*Cylindrictone D* (=*(2a*S,*3*S,*6*R,*7a*R,*8b*R,*14a*R,*14b*R,*14c*R*)-Icosahydro-3,8b,12,12,14a,14b-hexamethyl-11*H*-3,6-epoxynaphtho[2′,1′:4,5]indeno[7,1-*bc*]oxocin-11-one*; **4**). Yellow oil. $[\alpha]_D^{26} = +15.0$ ($c =$ 0.10, $CHCl_3$). UV ($CHCl_3$): 199 (0.09). IR (KBr): 3426, 1629, 1426, 1385, 1152, 876. $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. ESI-MS (pos.): 415 ($[M+H]^+$). HR-ESI-MS: 437.3041 ($[M+Na]^+$, $C_{27}H_{42}NaO_3^+$; calc. 437.3031).

*Cylindrictone E* (=*(5α,12β,17β)-17-Acetyl-12-hydroxy-4,4,8,10,14-pentamethylgonan-3-one*; **5**). White amorphous powder ($CHCl_3$). M.p. 236–237°. $[\alpha]_D^{26} = +51.7$ ($c = 0.16$, $CHCl_3$). UV ($CHCl_3$): 199 (0.66) IR (KBr): 3441, 2967, 2947, 2871, 1704, 1461, 1384, 1364, 1176, 1044, 1011. $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. FAB-MS (pos.): 375 ($[M+H]^+$). HR-ESI-MS: 375.2898 ($[M+H]^+$, $C_{24}H_{39}O_3^+$; calc. 375.2899).

*Cylindrictone F* (=*(5α)-4,4,8,10,14-Pentamethylgon-12-ene-3,17-dione*; **6**). Yellow oil. $[\alpha]_D^{26} = +83.3$ ($c = 0.16$, $CHCl_3$). UV ($CHCl_3$): 191 (0.29). IR (KBr): 3423, 2948, 1716, 1702, 1655,1452, 1416, 1378, 1224, 1002. $^1$H- and $^{13}$C-NMR: *Tables 2* and *3*. EI-MS: 329 ($[M+H]^+$). HR-ESI-MS: 329.2480 ($[M+H]^+$, $C_{22}H_{33}O_2^+$; calc. 329.2480).

*Crystallographic Data for* **3**[1]). $C_{28}H_{46}O_4$, $M = 446.65$, orthorhombic, space group $P2_12_12_1$, $a =$ 7.6228(1) Å, $b = 11.1482(1)$ Å, $c = 30.037(1)$ Å, $V = 2552.6(5)$ Å$^3$, $Z = 4$, $d = 1.162$ g/cm$^3$, crystal dimensions $0.22 \times 0.21 \times 0.15$ mm, measured on a *Bruker apex II* diffractometer with a graphite monochromator ($\omega$ scans, $2\theta_{max} = 50.0°$), Mo$K_\alpha$ radiation. The total number of independent reflections measured was 5988, of which 3079 were observed ($|F|^2 \geq 2\sigma|F|^2$). Final indices: $R_F = 0.068$, $R_w = 0.201$ ($W = 1/\sigma|F|^2$). The crystal structure (**3**) was solved by direct methods using SHELXL-97 [16] and expanded using difference *Fourier* techniques, refined by the program and method NOMCSDP [17] and full-matrix least-squares calculations.

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[1]) Crystallographic data for the structure of **3** has been deposited with the *Cambridge Crystallographic Data Centre* (deposition number: CCDC-656472). Copies of those data can be obtained free of charge *via* www.ccdc.cam.ac.uk/conts/retrieving.html (or from the *Cambridge Crystallographic Data Centre*, 12 Union Road, Cambridge CB21EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

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