# Chemical Constituents from *Clerodendrum bungei* and Their Cytotoxic Activities

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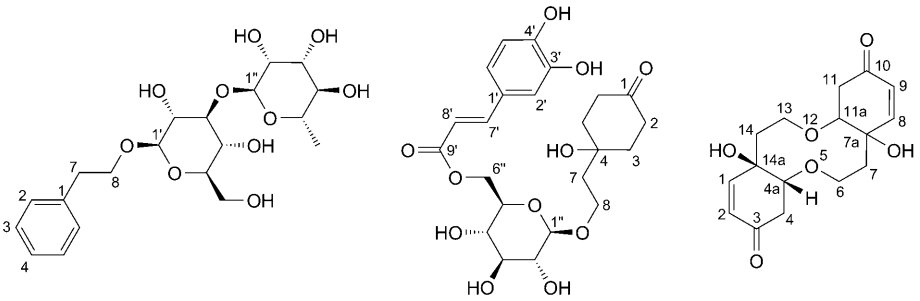
A new phenylethanoid glycoside, two new cyclohexylethanoids, one new phenolic glycoside, and a new farnesane-type sesquiterpenoid, namely 2-phenylethyl 3-*O*-(6-deoxy-α-L-mannopyranosyl)-β-D-glucopyranoside (**1**), 6″-*O*-[(*E*)-caffeoyl] rengyoside B (**2**), clerodenone A (**3**), 2-({6-*O*-[(4-hydroxy-3-methoxyphenyl)carbonyl]-β-D-glucopyranosyl}oxy)-2-methylbutanoic acid (**4**), 2-{(2*S*,5*R*)-5-[(1*E*)-4-hydroxy-4-methylhexa-1,5-dien-1-yl]-5-methyltetrahydrofuran-2-yl}propan-2-yl β-D-glucopyranoside (**5**), together with 16 known compounds, were isolated from the roots of *Clerodendrum bungei*. All structures were elucidated by spectroscopic methods. The new compounds showed modest *in vitro* inhibition of the proliferation of the HeLa human cervical carcinoma cell line (CCL-2), with $IC_{50}$ values in the range of 3.5–8.7 μM, adriamycin being used as positive control, with an $IC_{50}$ value of 0.026 ± 0.001 μM.

**Introduction.** – The genus Clerodendrum (Verbenaceae) contains more than 30 species distributed in China, some of which have been used as Traditional Chinese Medicine (TCM), such as *Clerodendrum indicum* for treating malaria and rheumatism [1], *Clerodendrum inerme* possessing antimicrobial and protecting cardiovascular system activity [2], and *Clerodendrum calamitosum* for treating calculus in bladder, kidney, and gall as a diuretic [3]. The characteristic chemical constituents of this genus are phenylpropanoid and phenylethanoid glycosides, flavonoids, diterpenoids, and iridoids [4].

*Clerodendrum bungei* STEUD. is a small shrub mainly distributed in south of China. Local inhabitants have used its stems and leaves as a folk medicine to be a detoxifying and detumescent drug [5] for a long time. Preparations of the leaves and branches of *C. bungei* have been used in folk medicine to treat boils, hemorrhoids, eczema, and hypertension, and the roots are used to alleviate rheumatism, beriberi, hypertension, and prolapse of the uterus [6]. Several types of constituents including diterpenoids [6][7], phenylethanoid glycosides [8], steroids and triterpenoids [9][10] have been identified from this plant. In our continuing chemical studies and screening of bioactive components from Chinese medicinal plants [11], five new compounds along with sixteen known ones were isolated from the aqueous acetone extract of the roots of *C. bungei*, and their *in vitro* cytotoxic activities against the HeLa human cervical carcinoma cell line (CCL-2) were investigated. This article reports on the structural elucidation and cytotoxic activity of new compounds **1**–**5**.

**Results and Discussion.** – *Structure Elucidation.* Compound **1** was obtained as a yellow amorphous powder, with the molecular formula $C_{20}H_{30}O_{10}$ determined from the HR-ESI-MS (*m*/*z* 453.1721 ([*M* + Na]$^+$, calc. 453.1737)). The H-atom and H-atom-



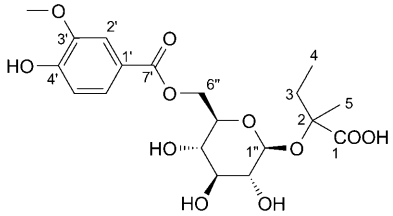
**1**[1])



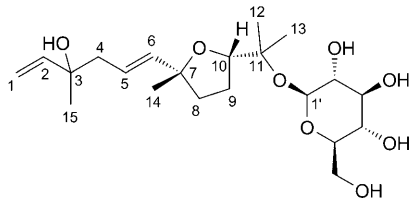
**2**[1])



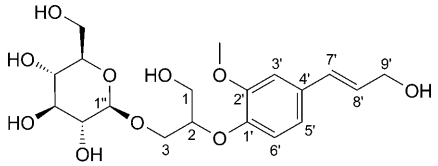
**3**



**4**[1])



**5**[1])



**6**[1])

bearing C-atom NMR signals of **1** were assigned unambiguously by the HSQC experiment. The $^1$H- and $^{13}$C-NMR spectral data (*Table 1*) displayed signals attributed to two sugar units, a $\beta$-D-glucopyranose and an $\alpha$-L-rhamnopyranose, which were identified from the two anomeric H-atoms ($\delta$(H) 4.35 ($d$, $J$ = 7.9) and 5.21 ($d$, $J$ = 1.3)), two anomeric C-atoms ($\delta$(C) 104.5 and 102.9), and some other characteristic NMR resonances. The 1D $^1$H and 2D $^1$H,$^1$H-COSY spectra showed the presence of a Ph group and a $CH_2CH_2O$ group, and the correlations between the H-atom signal at $\delta$(H) 2.96 ($CH_2CH_2O$) and the aromatic C-atom signal at $\delta$(C) 130.3 indicated a $PhCH_2CH_2O$ group. The glycosidic linkages were determined from the following HMBC correlations: H–C($1_{Glc}$) ($\delta$(H) 4.35)/C(8) ($\delta$(C) 72.0), and H–C($1_{Rha}$) ($\delta$(H) 5.21)/C($3_{Glc}$) ($\delta$(C) 84.8). The remaining HMBC correlations are shown in the *Figure*. Therefore, the structure of compound **1** was elucidated as 2-phenylethyl 3-*O*-(6-deoxy-$\alpha$-L-mannopyranosyl)-$\beta$-D-glucopyranoside.

Compound **2**, a brown amorphous powder, was shown to have a molecular formula of $C_{23}H_{30}O_{11}$ by HR-ESI-MS ($m/z$ 505.1675, $[M + Na]^+$). The $^1$H- and $^{13}$C-NMR spectra (*Table 1*) were very similar with those of rengyoside B [12], except for

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[1]) The absolute configuration of the glucose and rhamnose residues is assumed as D and L, resp., from biogenetic considerations.

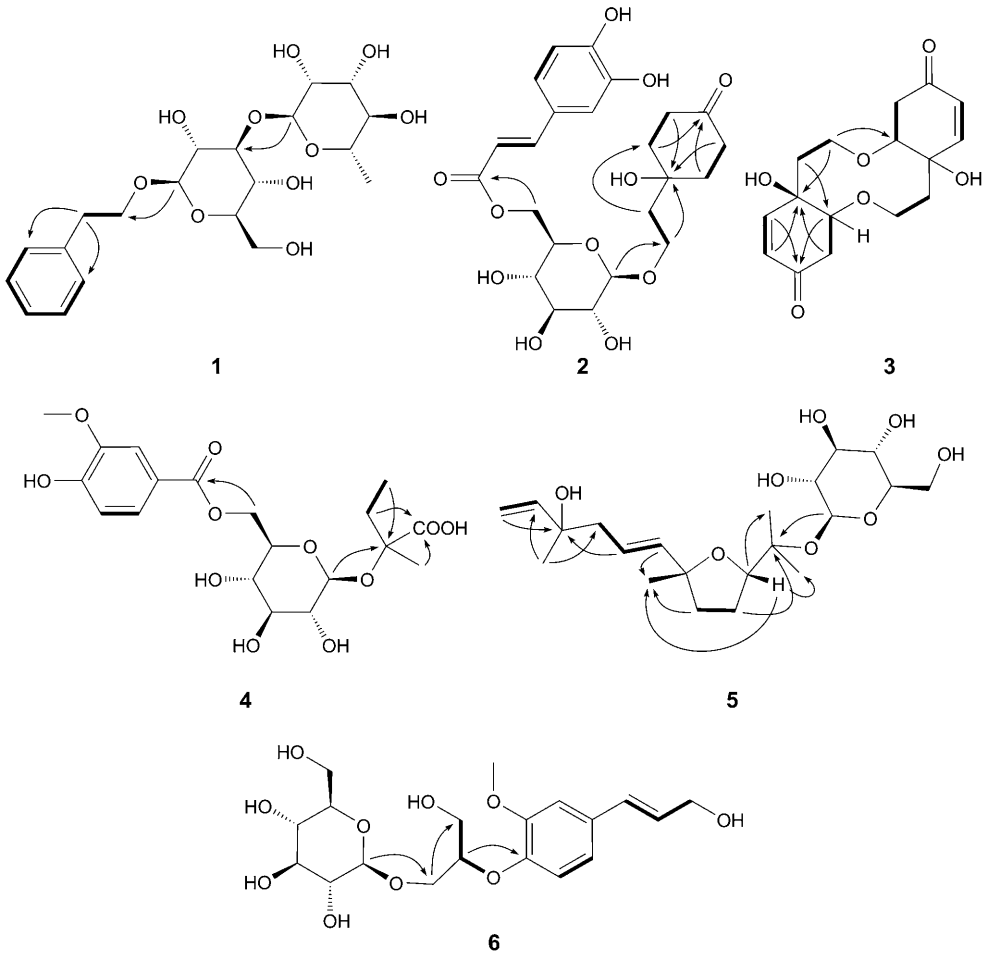


Figure. *Key HMBC* (H → C) *and* $^1H,^1H$*-COSY* (—) *interactions of compounds* **1**–**6**

additional signals arising from some aromatic and olefinic H- and C-atoms. Its $^1$H-NMR spectrum exhibited an *ABX* signal pattern typical of a 1,3,4-substituted Ph group at $\delta$(H) 7.09 (*d*, *J* = 2.0), 6.98 (br. *d*, *J* = 7.5), 6.82 (*d*, *J* = 7.7), and two *doublet*s due to (*E*)-olefinic H-atoms at $\delta$(H) 7.60 (*d*, *J* = 15.5) and 6.32 (*d*, *J* = 16.0). With the HMBC cross-peaks between the phenolic H-atoms and the olefinic C(7′) ($\delta$(C) 147.5), and (*E*)-olefinic H-atoms with the ester CO signal at $\delta$(C) 169.4, a (*E*)-caffeoyl moiety was deduced. The HMBC correlation of the glycosidic H-atoms $CH_2$(6″) ($\delta$(H) 4.54 (*dd*, *J* = 11.5, 2.5) and 4.40 (*dd*, *J* = 11.5, 6.5)) to the carboxylic C-atom ($\delta$(C) 169.4) indicated that the (*E*)-caffeoyl group was linked to the C(6″) of the glucose moiety of rengyoside B. Thus, compound **2** was established as 6″-*O*-[(*E*)-caffeoyl] rengyoside B, which corresponds to 2-(1-hydroxy-4-oxocyclohexyl)ethyl 6-*O*-[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]-$\beta$-D-glucopyranoside.

Table 1. *$^1H$- and $^{13}C$-NMR Spectral Data* ($CD_3OD$) *of Compounds* **1**, **2**, *and* **5**. $\delta$ in ppm, $J$ in Hz.

| | **1** | | | **2** | | | **5** | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | | $\delta$(H) | $\delta$(C) | | $\delta$(H) | $\delta$(C) |
| C(1) | | 140.3 | C(1) | | 215.3 | C(1) | 5.25 (*dd*, | 112.4 |
| C(2) | 7.30 (br. *d*, | 130.3 | C(2) | 2.64–2.66 (*m*), | 38.0 | | $J$ = 17.5, 1.6), | |
| | $J$ = 7.2) | | | 2.16–2.21 (*m*) | | | 5.19 (*dd*, | |
| C(3) | 7.28 (br. *dd*, | 129.6 | C(3) | 2.04–2.06 (*m*), | 38.1 | | $J$ = 10.7, 2.0) | |
| | $J$ = 7.2, 6.8) | | | 1.82–1.85 (*m*) | | C(2) | 5.95 (*dd*, | 146.5 |
| C(4) | 7.20–7.24 (*m*) | 127.5 | C(4) | | 70.6 | | $J$ = 17.8, 10.9) | |
| C(5) | 7.28 (br. *dd*, | 129.6 | C(5) | 2.04–2.06 (*m*), | 38.0 | C(3) | | 74.0 |
| | $J$ = 7.2, 6.8) | | | 1.82–1.85 (*m*) | | C(4) | 2.28 (*d*, $J$ = 7.2) | 46.6 |
| C(6) | 7.30 (br. *d*, | 130.3 | C(6) | 2.64–2.66 (*m*), | 38.1 | C(5) | 5.74 (*dt*, | 124.7 |
| | $J$ = 7.2) | | | 2.16–2.21 (*m*) | | | $J$ = 15.6, 7.2) | |
| C(7) | 2.96 (*t*, $J$ = 7.5) | 37.5 | C(7) | 1.95 (*t*, $J$ = 6.6) | 42.3 | C(6) | 5.70 (*d*, $J$ = 15.6) | 140.1 |
| C(8) | 3.77–3.90 (*m*) | 72.0 | C(8) | 4.06–4.11 (*m*), | 67.4 | C(7) | | 84.7 |
| Glc: | | | | 3.81–3.84 (*m*) | | C(8) | 1.86–1.89 (*m*) | 39.3 |
| C(1′) | 4.35 (*d*, $J$ = 7.9) | 104.5 | C(1′) | | 127.9 | C(9) | 1.93–1.99 (*m*) | 38.6 |
| C(2′) | 3.32–3.35 (*m*) | 75.9 | C(2′) | 7.09 (*d*, $J$ = 2.0) | 116.9 | C(10) | 4.05–4.08 (*m*) | 87.0 |
| C(3′) | 3.54 (*t*, $J$ = 8.3) | 84.8 | C(3′) | | 147.1 | C(11) | | 81.0 |
| C(4′) | 3.38–3.40 (*m*) | 70.3 | C(4′) | | 149.7 | C(12) | 1.28 (*s*) | 21.1 |
| C(5′) | 3.33–3.36 (*m*) | 78.1 | C(5′) | 6.82 (*d*, $J$ = 7.7) | 115.1 | C(13) | 1.25 (*s*) | 24.1 |
| C(6′) | 3.93 (*dd*, | 62.9 | C(6′) | 6.98 (br. *d*, $J$ = 7.5) | 123.3 | C(14) | 1.37 (*s*) | 27.5 |
| | $J$ = 2.3, 12.0), | | C(7′) | 7.60 (*d*, $J$ = 15.5) | 147.5 | C(15) | 1.33 (*s*) | 26.9 |
| | 3.72 (*dd*, | | C(8′) | 6.32 (*d*, $J$ = 16.0) | 115.5 | Glc: | | |
| | $J$ = 5.0, 12.0) | | C(9′) | | 169.4 | C(1′) | 4.53 (*d*, $J$ = 7.7) | 99.0 |
| Rha: | | | Glc: | | | C(2′) | 3.16–3.21 (*m*) | 75.5 |
| C(1″) | 5.21 (*d*, $J$ = 1.3) | 102.9 | C(1″) | 4.36 (*d*, $J$ = 7.2) | 104.8 | C(3′) | 3.37–3.41 (*m*) | 78.3 |
| C(2″) | 3.71–3.75 (*m*) | 72.4 | C(2″) | 3.23–3.25 (*m*) | 75.3 | C(4′) | 3.28–3.32 (*m*) | 72.1 |
| C(3″) | 3.99–4.00 (*m*) | 72.6 | C(3″) | 3.41–3.43 (*m*) | 78.2 | C(5′) | 3.28–3.31 (*m*) | 77.9 |
| C(4″) | 3.43–3.47 (*m*) | 74.2 | C(4″) | 3.38–3.40 (*m*) | 72.1 | C(6′) | 3.83–3.87 (*m*), | 63.1 |
| C(5″) | 3.38–3.39 (*m*) | 70.5 | C(5″) | 3.57–3.59 (*m*) | 75.8 | | 3.68–3.70 (*m*) | |
| C(6″) | 1.30 (*d*, $J$ = 6.3) | 18.2 | C(6″) | 4.54 (*dd*, | 64.9 | | | |
| | | | | $J$ = 11.5, 2.5), | | | | |
| | | | | 4.40 (*dd*, | | | | |
| | | | | $J$ = 11.5, 6.5) | | | | |

Clerodenone A (**3**) was obtained as an orange oil, and its molecular formular was determined by HR-EI-MS as $C_{16}H_{20}O_6$, which, combined with the presence of only eight C-atom signals in the $^{13}C$-NMR spectrum, suggested that **3** was a dimer. Analysis of the $^1H$- and $^{13}C$-NMR (*Table 2*), DEPT, and HSQC spectra revealed that half of the molecule, $C_8H_{10}O_3$, possessed a ketone CO group ($\delta$(C) 197.6), an oxygenated quaternary C-atom ($\delta$(C) 75.0), an O-bearing CH group ($\delta$(C) 81.1, $\delta$(H) 4.16 (*dt*, $J$ = 5.6, 1.4)), two olefinic CH groups ($\delta$(C) 148.8, $\delta$(H) 6.72 (*dd*, $J$ = 10.2, 1.9); $\delta$(C) 128.1, $\delta$(H) 5.92 (*d*, $J$ = 10.4)), and three $CH_2$ groups ($\delta$(C) 66.1, $\delta$(H) 3.94–4.02, 3.80–3.88; $\delta$(C) 39.8, $\delta$(H) 2.72 (*dd*, $J$ = 17.2, 4.5), 2.52 (*dd*, $J$ = 16.8, 5.2); $\delta$(C) 39.3, $\delta$(H) 2.22–2.31, 2.09–2.18)). $^1H,^1H$-COSY Correlations revealed the connections of H–C(1/8) ($\delta$(H) 6.72) with H–C(2/9) ($\delta$(H) 5.92), $CH_2$(4/11) ($\delta$(H) 2.72 and 2.52) with H–C(4a/11a) ($\delta$(H) 4.16), and $CH_2$(7/14) ($\delta$(H) 2.22–2.31, 2.09–2.18) with

Table 2. *$^1H$- and $^{13}C$-NMR Spectral Data of Compounds* **3**, **4**, *and* **6**. $\delta$ in ppm, $J$ in Hz.

| | **3**[a]) | | | **4**[b]) | | | **6**[b]) | |
|---|---|---|---|---|---|---|---|---|
| | $\delta$(H) | $\delta$(C) | | $\delta$(H) | $\delta$(C) | | $\delta$(H) | $\delta$(C) |
| C(1) | 6.72 (*dd*, *J* = 10.2, 1.9) | 148.8 | C(1) | | 182.7 | C(1) | 3.89–3.90 (*m*) | 63.3 |
| C(2) | 5.92 (*d*, *J* = 10.4) | 128.1 | C(2) | | 86.2 | C(2) | 4.63–4.66 (*m*) | 81.3 |
| C(3) | | 197.6 | C(3) | 1.77–1.87 (*m*) | 31.9 | C(3) | 4.14 (*dd*, | 70.6 |
| C(4) | 2.72 (*dd*, *J* = 17.2, 4.5), | 39.8 | C(4) | 0.84 (*t*, *J* = 7.2) | 10.1 | | *J* = 4.8, 11.0), | |
| | 2.52 (*dd*, *J* = 16.8, 5.2) | | C(5) | 1.40 (*s*) | 25.2 | | 4.00 (br. | |
| C(4a) | 4.16 (*dt*, *J* = 5.6, 1.4) | 81.1 | C(1′) | | 124.0 | | *d*, *J* = 11.0) | |
| C(6) | 3.94–4.02 (*m*), | 66.1 | C(2′) | 7.64 (br. *s*) | 115.9 | C(1′) | | 148.6 |
| | 3.80–3.88 (*m*) | | C(3′) | | 149.8 | C(2′) | | 152.2 |
| C(7) | 2.22–2.31 (*m*), | 39.3 | C(4′) | | 153.1 | C(3′) | 7.19 (br. *s*) | 113.0 |
| | 2.09–2.18 (*m*) | | C(5′) | 7.03 (*d*, *J* = 8.1) | 117.8 | C(4′) | | 134.2 |
| C(7a) | | 75.0 | C(6′) | 7.67 (*d*, *J* = 8.9) | 127.1 | C(5′) | 7.08 (br. *d*, *J* = 8.5) | 122.5 |
| C(8) | 6.72 (*dd*, *J* = 10.2, 1.9) | 148.8 | C(7′) | | 170.6 | C(6′) | 7.14 (br. *d*, *J* = 8.5) | 118.8 |
| C(9) | 5.92 (*d*, *J* = 10.4) | 128.1 | MeO | 3.96 (*s*) | 58.7 | C(7′) | 6.63 (*d*, *J* = 16.0) | 133.1 |
| C(10) | | 197.6 | Glc: | | | C(8′) | 6.39 (*dt*, | 129.8 |
| C(11) | 2.72 (*dd*, *J* = 17.2, 4.5), | 39.8 | C(1″) | 4.72 (*d*, *J* = 7.7) | 100.0 | | *J* = 16.0, 6.0) | |
| | 2.52 (*dd*, *J* = 16.8, 5.2) | | C(2″) | 3.44–3.46 (*m*) | 76.0 | C(9′) | 4.30 (*d*, *J* = 5.8) | 65.0 |
| C(11a) | 4.16 (*dt*, *J* = 5.6, 1.4) | 81.1 | C(3″) | 3.58–3.63 (*m*) | 78.6 | MeO | 3.92 (*s*) | 58.4 |
| C(13) | 3.94–4.02 (*m*), | 66.1 | C(4″) | 3.55–3.57 (*m*) | 73.0 | Glc: | | |
| | 3.80–3.88 (*m*) | | C(5″) | 3.81–3.87 (*m*) | 76.1 | C(1″) | 4.51 (*d*, *J* = 7.9) | 105.3 |
| C(14) | 2.22–2.31 (*m*), | 39.3 | C(6″) | 4.65 (*dd* | 66.8 | C(2″) | 3.32–3.37 (*m*) | 75.8 |
| | 2.09–2.18 (*m*) | | | *J* = 12.1, 2.5), | | C(3″) | 3.42–3.44 (*m*) | 78.6 |
| C(14a) | | 75.0 | | 4.51 (*dd*, | | C(4″) | 3.44–3.47 (*m*) | 72.2 |
| | | | | *J* = 11.5, 7.4) | | C(5″) | 3.53–3.56 (*m*) | 78.3 |
| | | | | | | C(6″) | 3.88–3.92 (*m*), | 63.0 |
| | | | | | | | 3.75–3.78 (*m*) | |

[a]) Spectra measured in $CDCl_3$. [b]) Spectra measured in $D_2O$.

$CH_2$(6/13) ($\delta$(H) 3.94–4.02, 3.80–3.88), and the HMBC spectrum showed the cross-peaks from H–C(1/8) and H–C(4a/11a) to C(3/10) ($\delta$(C) 197.6), from H–C(2/9), $CH_2$(4/11), and $CH_2$(6/13) to C(7a/14a) ($\delta$(C) 75.0), and from $CH_2$(7/14) to C(1/8) ($\delta$(C) 148.8) and C(4a/11a) ($\delta$(C) 81.1). These interactions led to the conclusion that **3** was a dimer of 1,6-dihydroxy-1-(2-hydroxyethyl)-2-cyclohexen-4-one [13]. The long-range couplings of H–C(4a/11a) with C(6/13) in the HMBC spectrum supported that a ten-numbered bisether ring *B* connects to rings *A* and *C*. NOESY Correlations of H–C(4a/11a) with H–C(6/13) provided further convincing evidence of the ring connections. The NOESY data of compound **3** (in ($D_6$)DMSO) showed the interactions of HO–C(7a/14a) with H–C(11a/4a), which suggested a relative *cis* spatial arrangement. Therefore, the structure of **3** was identified as 4a,7,7a,11,11a,13,14,14a-octahydro-7a,14a-dihydroxydibenzo[*b*,*g*][1,6]dioxecine-3,10(4*H*,6*H*)-dione, and was given the trivial name clerodenone A. Attempts to grow appropriate crystals of compound **3** for X-ray crystallography were unsuccessful. The relative configuration between the two monomers was still unsolved. Both the optical inactivity and

observation of a single set of NMR signals of compound **3** do not enable to distinguish between a meso compound and a racemate.

The HR-ESI-MS of compound **4** showed a *pseudo*-molecular ion at *m*/*z* 453.1393 $[M + Na]^+$, which, in conjunction with the $^{13}$C-NMR data (*Table 2*), was used to establish a molecular formula of $C_{19}H_{26}O_{11}$. A 3,4-disubstituted benzoyl group was deduced from the signals at $\delta$(H) 7.03 (*d*, *J* = 8.1, H−C(5′)), 7.64 (br. *s*, H−C(2′)), and 7.67 (*d*, *J* = 8.9, H−C(6′)) in the $^1$H-NMR spectrum (*Table 2*). A MeO ($\delta$(H) 3.96) and a OH group were located at C(3′) and C(4′), respectively, from the $^1$H,$^{13}$C-long-range correlations between the MeO group and C(3′) ($\delta$(C) 149.8), and the NOESY between the MeO H-atoms and H−C(2′). In addition, one set of glucopyranose signals, assignable for $\beta$ from $\delta$(H) 4.72 (*d*, *J* = 7.7, H−C(1″)), was found in the $^1$H-NMR spectrum which could be grouped by COSY correlations. The downfield-shifted $CH_2$(6″) ($\delta$(H) 4.65 for $H_a$−C(6″) and 4.51 for $H_b$−C(6″)), and the correlations between $CH_2$(6″) to C(7′) ($\delta$(C) 170.6) in the HMBC spectrum suggested a (3-methoxy-4-hydroxybenzoyl)oxy group attached to C(6″) of the sugar moiety. A 2-hydroxy-2-methylbutanoic acid unit was determined from the following HMBC correlations: Me(4) ($\delta$(H) 0.84)/C(2) ($\delta$(C) 86.2), $CH_2$(3) ($\delta$(H) 1.77–1.87) and Me(5) ($\delta$(H) 1.40)/C(1) ($\delta$(C) 182.7), and Me(5) ($\delta$(H) 1.40)/C(3) ($\delta$(C) 31.9). From the interaction between H−C(1″) ($\delta$(H) 4.72) and C(2) ($\delta$(C) 86.2), the 2-hydroxy-2-methylbutanoic acid unit was deduced to connect with the anomeric C-atom of the glucose unit. The configuration of the 2-hydroxy-2-methylbutanoic acid has not been established. Thus, compound **4** was characterized as 2-({6-*O*-[(4-hydroxy-3-methoxyphenyl)carbonyl]-$\beta$-D-glucopyranosyl}oxy)-2-methylbutanoic acid.

The HR-ESI-MS spectrum of compound **5** showed the *quasi*-molecular ion at *m*/*z* 439.2291 $[M + Na]^+$, according to the molecular formula $C_{21}H_{36}O_8$. The $^1$H-NMR spectrum (*Table 1*) showed, besides the *ABX* system of a vinyl group as three double *doublet*s at $\delta$(H) 5.95 (H−C(2)), 5.25 ($H_a$−C(1)) and 5.19 ($H_b$−C(1)), two H-atoms as a double *triplet* at $\delta$(H) 5.74 (H−C(5)) and a *doublet* at $\delta$(H) 5.70 ($CH_2$(6)). In the aliphatic region of the spectrum, four Me groups were evident at $\delta$(H) 1.37 (Me(14)), 1.33 (Me(15)), 1.28 (Me(12)), and 1.25 (Me(13)). The presence of a *doublet* at $\delta$(H) 4.53 (*d*, *J* = 7.7, H−C(1′)) and two double *doublet*s at $\delta$(H) 3.83–3.87 ($H_a$−C(6′)), 3.68–3.70 ($H_b$−C(6′)), as well as four overlapped H-atoms ranging from 3.41 to 3.16, indicated the presence of a monosaccharide unit as glucopyranose. The $^1$H,$^1$H-COSY experiment showed cross-peaks as (H−C(1)/H−C(2)), $CH_2$(4) ($\delta$(H) 2.28)/H−C(5) ($\delta$(H) 5.74)/H−C(6) ($\delta$(H) 5.70)), and ($CH_2$(8) ($\delta$(H) 1.86–1.89)/$CH_2$(9) ($\delta$(H) 1.93–1.99)/H−C(10) ($\delta$(H) 4.05–4.08)). The $^{13}$C-NMR and DEPT spectra showed signals due to 21 C-atoms, including 15 C-atoms for the aglycone, as four Me, four $CH_2$ (one olefinic), and four CH groups (one O-bearing and three olefinic), as well as three tertiary carbinol C-atoms, and other six C-atoms for the sugar unit. The aglycone structure was established by the HMBC correlations (H−C(2) and $CH_2$(4)/C(15), $CH_2$(1) and H−C(5)/C(3), H−C(6) and $CH_2$(8)/C(14), H−C(5) and $CH_2$(9)/C(7), $CH_2$(8)/C(10), $CH_2$(9)/C(11), H−C(10)/C(12) and C(13)), to be a farnesane-type sesquiterpenoid. The heterocorrelations between H−C(1′) ($\delta$(H) 4.53) and C(11) ($\delta$(C) 81.0) confirmed the linkage of the sugar at C(11). Both the cross-peaks in the HMBC spectrum from H−C(10) ($\delta$(H) 4.05–4.08) to C(7) ($\delta$(C) 84.7), and the correlations in the NOESY experiment from H−C(10) ($\delta$(H) 4.05–4.08) to Me(14)

($\delta$(H) 1.37), indicated a furan ring formed through C(7)–O–C(10). The configuration at C(3) has not been established. From the above evidence, the structure of **5** was established as 2-{(2*S*,5*R*)-5-[(1*E*)-4-hydroxy-4-methylhexa-1,5-dien-1-yl]-5-methyltetrahydrofuran-2-yl}propan-2-yl $\beta$-D-glucopyranoside.

Compound **6** possessed a molecular formula $C_{19}H_{28}O_{10}$ as evidenced by its HR-ESI-MS (*m*/*z* 439.1573 ($[M+Na]^+$, $C_{19}H_{28}NaO_{10}^+$)). The assignments of $^1$H- and $^{13}$C-NMR data (*Table 2*) were based on HSQC, HMBC, and $^1$H,$^1$H-COSY spectra. The $^1$H-NMR spectrum of **6** allowed the assignment of three aromatic H-atoms ($\delta$(H) 7.19 (br. *s*, H–C(3′)), 7.14 (br. *d*, *J* = 8.5, H–C(6′)), 7.08 (br. *d*, *J* = 8.5, H–C(5′))), two olefinic H-atoms ($\delta$(H) 6.63 (*d*, *J* = 16.0, H–C(7′)), 6.39 (*dt*, *J* = 16.0, 6.0, H–C(8′))), three O-bearing $CH_2$ groups ($\delta$(H) 4.30 (*d*, *J* = 5.8, $CH_2$(9′)), 4.14 (*dd*, *J* = 4.8, 11.0, $H_a$–C(3)), 4.00 (br. *d*, *J* = 11.0, $H_b$–C(3)), 3.89–3.90 (*m*, $CH_2$(1))), one O-bearing CH group ($\delta$(H) 4.63–4.66 (*m*, H–C(2))), and one anomeric H-atom ($\delta$(H) 4.51 (*d*, *J* = 7.9, H–C(1″))), indicating a $\beta$-configuration of glucopyranose. The $^1$H,$^1$H-COSY correlations from H–C(7′) through H–C(8′) to $CH_2$(9′), in combination with HMBC correlations from H–C(7′) to C(3′) and C(5′), and from MeO to C(2′), were suggestive of a 4-(3-hydroxypropen-1-yl)-2-methoxyphenyl moiety in **6**. A partial propanol structure $OCH_2CH(O)CH_2OH$ was deduced from the cross-peaks ($CH_2$(1)/H–C(2), H–C(2)/$CH_2$(3)) in the COSY spectrum. This fragment was linked to C(1′) ($\delta$(C) 148.6) and C(1″) ($\delta$(C) 105.3) inferred from the key HMBC cross-peaks (H–C(2)/C(1′), $CH_2$(3)/C(1″)). Consequently, compound **6** was determined to be 3-hydroxy-2-{4-[(1*E*)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenoxy}propyl $\beta$-D-glucopyranoside. The configuration at C(2) has been established. Compound **6** has been previously found in *Urtica dioica*, but only identified as its trimethylsilyl derivative [14].

The additional 15 known compounds were identified as acteoside, campneoside II [8], martynoside [15], stachysoside C [16], verbasoside (descaffeoylverbascoside) [17], dihydrophaseic acid 4′-*O*-$\beta$-D-glucopyranoside [18], 4-acetonyl-3,5-dimethoxy-*p*-quinol [19], cistanoside E [20], $\beta$-D-fructofuranosyl-$\alpha$-D-(6-vanilloyl)glucopyranoside [21], 3-(4-hydroxy-3,5-dimethoxyphenyl)-1,2-propanediol [22], 3,4-dimethoxyphenyl 1-*O*-$\beta$-D-[5-*O*-(4-hydroxybenzoyl)]apiofuranosyl-(1 → 6)-*O*-$\beta$-D-glucopyranoside [23], seguinoside K [24], jionoside D [25], calceolarioside D [26], and *trans*-isoferulic acid [27], by comparison of their spectroscopic data with literature values. Except acteoside, campneoside II, and martynoside, all of them were found for the first time in this plant.

*Biological Studies.* Compounds **1**–**5** were evaluated for their cytotoxic activity against the HeLa human cervical carcinoma cell line (CCL-2) *in vitro* by means of the MTT (3-(4,5-dimethylythiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide) assay [28]. All of the compounds were found to be moderately active to inhibit the proliferation of HeLa cells with the $IC_{50}$ values less than 10 μM (*Table 3*).

## Experimental Part

*General.* Column chromatography (CC): silica gel ($SiO_2$; 200–300 mesh; Qingdao), *Chromatorex C$_{18}$-OPN* (20–45 μm; *Fuji Silysia Chemical Ltd.*), *Chromatorex C$_8$-OPN* (20–45 μm; *Fuji Silysia Chemical Ltd.*), *MCI gel CHP-20P* (75–150 μm, *Mitsubishi Chemical Industries Co., Ltd.*), *TSK gel Toyopearl HW-40F* (30–60 μm; *Toso Co., Ltd.*), and *Diaion HP 20* (*Mitsubishi Chemical Industries Co., Ltd.*). Optical rotations: *Perkin-Elmer 341* polarimeter. UV and IR spectra: *Shimadzu UV-2450* and *Perkin-Elmer 577* spectrophotometer, resp. NMR Spectra: *Varian Mercury* NMR spectrometer, at

Table 3. *Cytotoxicity of Compounds* **1**–**5** *Isolated from* Clerodendrum bungei

| Compound | Cytotoxicity ($IC_{50}$ [μM]) |
|---|---|
| **1** | $4.4 \pm 0.3$ |
| **2** | $7.2 \pm 0.5$ |
| **3** | $3.5 \pm 0.1$ |
| **4** | $8.7 \pm 1.1$ |
| **5** | $4.5 \pm 0.2$ |
| Adriamycin[a]) | $0.026 \pm 0.001$ |

[a]) Positive control.

400 MHz for $^1$H and 100 MHz for $^{13}$C. EI-MS: *Finnigan/MAT-95* spectrometer. LR- and HR-ESI-MS: *Finnigan LCQ-DECA* and *Waters Micromass Q-TOF ultima Globe* spectrometer, resp.

*Plant Material*. Roots of *Clerodendrum bungei* STEUD. were collected from Nanning, Guangxi Province, China, in March 2006, and identified by Prof. *Heming Yang*. A voucher specimen (No. SIMMCB06) is deposited with the Herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, P. R. China.

*Extraction and Isolation*. Air-dried roots (4.5 kg) were pulverized and extracted with 70% aq. acetone at r.t. (10 l, 3 × 2 d). The solvent was removed *in vacuo* to yield 208 g syrup residue. The crude extract was subjected to *Diaion HP 20* CC and eluted with $H_2O$ and 25, 50, 75, and 100% MeOH gradiently to give five fractions (*Fr. A*–*E*). *Fr. B* (30 g) was applied repeatedly to CC over $C_{18}$ (MeOH/$H_2O$, 2 to 30%) and then $SiO_2$ (petroleum ether (PE)/AcOEt, 2 :1) to afford compound **3** (57 mg) and verbasoside (265 mg). *Fr. C* (6.8 g) was subjected to CC (*MCI*; MeOH/$H_2O$ 2 to 40%) to afford four fractions (*Fr. I*–*IV*). *Fr. I* (0.9 g) was further separated by passage over a $C_{18}$ column (MeOH/$H_2O$, 2 to 30%) to give compound **5** (7 mg), acteoside (28 mg), and campneoside II (12 mg). *Fr. II* (1.1 g) was subjected to CC ($SiO_2$; $CHCl_3$/AcOEt 5 :1, 4 :1, 3 :1) to yield dihydrophaseic acid 4′-*O*-*β*-D-glucopyranoside (10 mg) and 4-acetonyl-3,5-dimethoxy-*β*-quinol (8 mg). *Fr. III* (2.0 g) was chromatographed on a $C_{18}$ column eluted with MeOH/$H_2O$ (5 to 60%), and further separated over a $C_8$ column using MeOH/$H_2O$ (5 to 75%) to afford compounds **4** (4 mg) and **6** (18 mg), as well as cistanoside E (50 mg), and *β*-D-fructofuranosyl-*α*-D-(6-vanilloyl)glucopyranoside (10 mg). *Fr. IV* (2.0 g) was passed through a $C_{18}$ column with MeOH/$H_2O$ (5 to 50%) to afford 3-(4-hydroxy-3,5-dimethoxyphenyl)-1,2-propanediol (4 mg), 3,4-dimethoxyphenyl 1-*O*-*β*-D-[5-*O*-(4-hydroxybenzoyl)]apiofuranosyl-(1 → 6)-*O*-*β*-D-glucopyranoside (45 mg), and seguinoside K (44 mg). *Fr. D* (6.5 g) was subjected to $C_{18}$ CC eluted with MeOH/$H_2O$ (5 to 75%), and purified using CC (*HW-40F*; MeOH/$H_2O$ 1 to 10%), resulting in the purification of compounds **1** (16 mg) and **2** (24 mg), jionoside D (48 mg), and calceolarioside D (48 mg). Martynoside (203 mg), stachysoside C (19 mg), and *trans*-isoferulic acid (12 mg) were obtained from *Fr. E* (3.4 g) by CC ($C_{18}$; MeOH/$H_2O$ 5 to 50%).

*2-Phenylethyl 3-*O*-(6-Deoxy-α-*L*-mannopyranosyl)-β-*D*-glucopyranoside* (**1**). Yellow amorphous powder. $[\alpha]_D^{22} = -36.6$ ($c = 0.47$, MeOH). UV (MeOH): 211 (3.95), 256 (2.10). IR (KBr): 3417, 2920, 1630, 1566. $^1$H- and $^{13}$C-NMR ($CD_3OD$): *Table 1*. ESI-MS (pos.): 453.0 ($[M + Na]^+$). ESI-MS (neg.): 429.6 ($[M - H]^-$). HR-ESI-MS: 453.1721 ($[M + Na]^+$, $C_{20}H_{30}NaO_{10}^+$; calc. 453.1737).

*6″-*O*-[(*E*)-Caffeoyl] Rengyoside B* (*=2-(1-Hydroxy-4-oxocyclohexyl)ethyl 6-*O*-[(2*E*)-3-(3,4-Dihydroxyphenyl)prop-2-enoyl]-β-*D*-glucopyranoside*; **2**). Brown amorphous powder. $[\alpha]_D^{22} = -26$ ($c = 0.155$, MeOH). UV (MeOH): 203 (3.69), 294 (3.03), 328 (3.19). IR (KBr): 3415, 2927, 1701, 1601. $^1$H- and $^{13}$C-NMR ($CD_3OD$): *Table 1*. ESI-MS (pos.): 505.1 ($[M + Na]^+$). ESI-MS (neg.): 481.3 ($[M - H]^-$). HR-ESI-MS: 505.1675 ($[M + Na]^+$, $C_{23}H_{30}NaO_{11}^+$; calc. 505.1686).

*Clerodenone A* (*=4a,7,7a,11,11a,13,14,14a-Octahydro-7a,14a-dihydroxydibenzo[*b,g*][1,6]dioxecine-3,10(4*H*,6*H*)-dione*; **3**). Orange oil. $[\alpha]_D^{22} = 0$ ($c = 0.795$, MeOH). UV (MeOH): 230 (3.97). IR (KBr): 3377, 2974, 2887, 1686. $^1$H- and $^{13}$C-NMR ($CDCl_3$): *Table 2*. $^1$H-NMR ((D$_6$)DMSO): 6.77 (*d*, $J = 10.2$, H–C(1/8)); 5.89 (*d*, $J = 10.1$, H–C(2/9)); 5.75 (*s*, HO–C(7a/14a)); 4.03 (*dt*, $J = 5.6$, 1.5, H–C(4a/11a));

3.87–3.71 (*m*, $CH_2$(6/13)); 2.73 (*dd*, *J* = 17.2, 4.5), 2.46 (*dd*, *J* = 17.0, 5.5) ($CH_2$(4/11)); 2.15–2.23 (*m*, $CH_2$(7/14)). EI-MS: 308 (100, $M^+$). HR-EI-MS: 308.1267 ($M^+$, $C_{16}H_{20}O_6^+$; calc. 308.1260).

*2-({6-*O*-[(4-Hydroxy-3-methoxyphenyl)carbonyl]-β-*D*-glucopyranosyl}oxy)-2-methylbutanoic Acid* (**4**). White amorphous powder. $[\alpha]_D^{22} = +8$ (*c* = 0.09, MeOH). UV (MeOH): 221 (3.97), 264 (3.51), 295 (3.14). IR (KBr): 3350, 1662. $^1$H- and $^{13}$C-NMR ($D_2O$): *Table 2*. ESI-MS (pos.): 453.0 ($[M + Na]^+$). ESI-MS (neg.): 429.0 ($[M - H]^-$). HR-ESI-MS: 453.1393 ($[M + Na]^+$, $C_{19}H_{26}NaO_{11}^+$; calc. 453.1373).

*2-{(2*S*,5*R*)-5-[(1*E*)-4-Hydroxy-4-methylhexa-1,5-dien-1-yl]-5-methyltetrahydrofuran-2-yl}propan-2-yl β-*D*-Glucopyranoside* (**5**). White amorphous powder. $[\alpha]_D^{22} = +3.6$ (*c* = 0.055, MeOH). IR (KBr): 3382, 2932. $^1$H- and $^{13}$C-NMR ($CD_3OD$): *Table 1*. ESI-MS (pos.): 439.1 ($[M + Na]^+$). ESI-MS (neg.): 461.3 ($[M + COOH]^-$). HR-ESI-MS: 439.2291 ($[M + Na]^+$, $C_{21}H_{36}NaO_8^+$; calc. 439.2308).

*3-Hydroxy-2-{4-[(1*E*)-3-hydroxyprop-1-en-1-yl]-2-methoxyphenoxy}propyl β-*D*-Glucopyranoside* (**6**). Yellow amorphous powder. $[\alpha]_D^{22} = -19$ (*c* = 0.095, MeOH). UV (MeOH): 221 (3.85), 260 (3.65). $^1$H- and $^{13}$C-NMR ($D_2O$): *Table 2*. ESI-MS (pos.): 439.0 ($[M + Na]^+$). ESI-MS (neg.): 461.1 ($[M + COOH]^-$). HR-ESI-MS: 439.1573 ($[M + Na]^+$, $C_{19}H_{28}NaO_{10}^+$; calc. 439.1580).

*Biological Assay.* The HeLa human cervical carcinoma cell line (CCL-2) was obtained from the American Type Culture Collection (*Manassas*, VA). Cells were cultured in *DMEM* medium supplemented with 10% FBS. Adriamycin, used as pos. control, was purchased from *Sigma*. Cells were seeded in a 96-well plate ($1 \times 10^3$ cells/well) and cultured overnight. Then the tested compound was added at various concentrations, and the wells were incubated for 72 h. Cell proliferation was determined by the MTT assay [28]. The UV/VIS absorbance at 570 nm was measured with a microplate reader. Cytotoxicity was expressed in terms of $IC_{50}$ values as means of three determinations (*n* = 3).

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