Three New Sesquiterpenes with Cytotoxic Activity from *Dobinea delavayi*

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Abstract

Three new sesquiterpenes dobinins A–C (**1**–**3**), together with five known compounds, were isolated from the root of *Dobinea delavayi*. The structures of the three new compounds were determined on the basis of spectroscopic analysis including 1D-, 2D-NMR and MS techniques as well as by comparison of the spectral data with those of analogous compounds reported in the literatures. Compounds **1**–**3** were screened for antitumor activity *in vitro* and exhibited definite cytotoxic activity against the human tumor cell line HL-60 with IC_{50} levels of 8.0×10^{-5}, 4.7×10^{-5}, and 5.1×10^{-5} M, respectively.

Key words

Dobinea delavayi · Podoaceae · sesquiterpenes · anti-cancer activity

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Dobinea Buch.-Ham. ex D. Don is a special genus in the Podoaceae family. There are only two *Dobinea* species endemic to eastern Asia, among which *Dobinea delavayi* has been placed in three different families, the Podoaceae, the Sapindaceae and the Anacardiaceae [1]. The roots of *D. delavayi* have been used against tumors in traditional Chinese medicine. A previous chemical study on this plant showed the presence of two eremophilane sesquiterpenes [2]. In the search for new bioactive constituents against tumors from *D. delavayi,* the chemical investigation of its roots, collected from Kunming, Yunnan Province, People's Republic of China, led to the isolation of eight sesquiterpenes including three new ones. This paper describes the isolation and structural elucidation of three new sesquiterpenes as well as their screening for antitumor activity.

Dobinin A (**1**) possessed a molecular formula of $C_{20}H_{28}O_5$, with seven degrees of unsaturation, as evidenced by HRESIMS (*m/z* 349.2012 $[M + H]^+$) and ^{13}C NMR spectra. The IR spectrum displayed the presence of hydroxyls (3457 cm^{-1}), ester carbonyls (1750, 1712 cm^{-1}), and double bonds (1686 cm^{-1}). The ^1H NMR spectrum of compound **1** clearly showed five methyls at δ 1.16 (3H, s), 1.27 (3H, s), 1.80 (3H, s), 1.92 (3H, s), and 2.00 (3H, d, J = 2.0 Hz). The ^{13}C NMR and DEPT spectroscopic data (**Table 1**) revealed the presence of five methyls, four methylenes, four methines (two oxygenated), and seven quaternary carbons, including four double bond carbons (δ 120.6, 165.8; 129.5, 138.9) and two ester carbonyls (δ 177.2, 169.0). Comparison of its similar NMR data with those of compound **4** indicated that the planar structure of **1** was determined to be the type of compound **4** [3]. The only difference was that the chemical shift of one methine (C-8) in **1,** δ 79.9, replaced the quaternary carbon δ 103.3 in compound **4**, which suggested the loss of one hydroxyl at C-8 in **1**.

In the HMBC spectrum, the correlations of H-5′ (δ 1.92, s) with C-1′ (δ 169.0), C-2′ (δ 129.5), and C-3′ (δ 138.9), and H-3 (δ 4.73, dd, J = 6.0, 12.0 Hz) with C-1′ were observed, indicating the presence of angeloyloxy attached to C-3. The presence of α,β-unsaturated lactone was proved by the correlations of H-8 (δ 4.96, d, J = 7.5 Hz) and H-13 (δ 1.80, s) with C-7 (δ 165.8), C-11 (δ 120.6), and C-12 (δ 177.2). The β-orientations of CH_3-14, CH_3-15, and H-8 were established by key ROESY correlations of H-8/H-14 and H-14/H-15 and combined with their comparison of chemical shifts with those reported in the literature [3]. The α-orientations of H-3 and H-5 were confirmed by the key NOE of H-3 (δ 4.73, dd, J = 6.0, 12.0 Hz)/H-5 (δ 1.40, m). Based on the above evidences, compound **1** was identified as 3β-angeloyloxy-4α-hydroxy-eudesm-7(11)-en-8α,12-olide (3) and named dobinin A.

Dobinin B (**2**) was obtained as white powder and had a molecular formula of $C_{22}H_{30}O_6$, with eight degrees of unsaturation, as established from a pseudomolecular ion peak at *m/z* 391.2150 $[M + H]^+$ in the HRESIMS spectrum. The IR absorption bonds at 1759 and 1649 cm^{-1} and UV absorption at 218 nm suggested the presence of an α,β-unsaturated γ-lactone ring in **2**. The ^1H NMR and ^{13}C NMR (DEPT) spectroscopic data (**Table 1**) revealed the presence of six methyls, four methylenes, four methines, and eight quaternary carbons, among which included four double bond carbons (δ 120.7, 161.1; 127.7, 138.1) and three ester carbonyls (δ 174.4, 170.2, 168.8). The gross structure of **2** was very similar to that of **1** except for one more acetyl (δ 170.2, 22.5) determined by analysis of their ^{13}C NMR data. In the HMBC spectrum, a weak correlation of H-15 (δ 1.35) with -CO (δ 170.2) was observed, which confirmed the linkage of the additional acetyl to C-4. The same orientations of H-3, H-5, H-8, and CH_3-14 as those in **1** were established by key ROESY correlations of H-8/H-14 and H-3/H-5. The α-orientation of CH_3-15 was established by analysis of their chemical shifts at C-4 and C-15 with those reported in the literature [4]. By all evidences, the structure of compound **2** was established as 3β-angeloyloxy-4α-acetoxy-eudesm-7(11)-en-8α,12-olide and named dobinin B.

Dobinin C (**3**) was isolated as white powder. Its molecular formula $C_{20}H_{26}O_5$, with eight degrees of unsaturation, was deduced from a pseudomolecular ion peak at *m/z* 347.1850 $[M + H]^+$ in the HRESIMS spectrum. The IR absorption bonds at 1769 and 1652 cm^{-1} and UV absorption at 219 nm also implied the presence of an α,β-unsaturated γ-lactone ring. The ^{13}C NMR (DEPT) spectroscopic data (**Table 1**) revealed the presence of five methyls, three methylenes, four methines, and eight quaternary carbons, which included six double bond carbons (δ 150.7, 121.6; 149.5, 120.7; 129.5, 139.0) and two ester carbonyls (δ 173.1, 168.9). Comparison of spectral data with those of **1** showed that the structure of **3** was similar to **1**, but one more double bond in **3** replaced the one methylene and one oxygenated methine in **1**. In the HMBC spectrum, the correlations of H-9 (1H, δ 5.62, s) with C-1 (δ 37.4), C-5 (δ 52.9), C-7 (δ 150.7), C-8 (δ 149.5), and C-14 (δ 19.8) were observed, suggestive of a double bond between C-8 and C-9. The orientations of H-3, H-5, CH_3-14, and CH_3-15 were also established to be the same as those in **1** by key ROESY cross-peaks of H-15/H-14 and H-3/H-5. Based on the

Table 1 NMR spectroscopic data of compounds **1**–**3** (**1**–**2** in CD_3OD, **3** in $CDCl_3$, δ in ppm, J in Hz).

	1		2		3	
	δ C	δ H (J/Hz)	δ C	δ H (J/Hz)	δ C	δ H (J/Hz)
1	38.8	1.42, m; 1.61, m	37.2	1.43, dd, 13.5, 3.5; 1.55, d, 13.5	37.4	1.65, d, 4.0; 1.74, m
2	26.0	1.73, m; 1.83, m	25.1	1.69, m; 1.90, overlapped	26.3	1.71,m; 1.92, m
3	81.6	4.73, dd, 6.0, 12.0	73.3	5.78, dd, 5.0,12.0	81.5	4.78, dd, .0,14.0
4	74.8	–	86.8	–	74.3	–
5	55.0	1.40, m	47.2	2.75, dd, 13.5, 3.3	52.9	1.88, m
6	23.2	α-H 3.10, dd, 4.5, 17.0; β-H 2.38, t, 17.0	22.6	α-H 2.65, dd, 14.0, 3.0 β-H 2.28, m	20.2	α-H 3.08, m β-H 2.56, m
7	165.8	–	161.1	–	150.7	–
8	79.9	4.96, dd, 7.5	77.5	4.76, dd, 12.0, 6.0	149.5	–
9	51.3	α-H 1.04, t, 14.5; β-H 2.23, dd, 7.5, 14.5	50.1	α-H 1.09, overlapped; β-H 2.18, dd, 12.0, 6.0	120.7	5.62, s
10	36.5	–	35.7	–	37.8	–
11	120.6	–	120.7	–	121.6	–
12	177.2	–	174.4	–	173.1	–
13	8.1	1.80, s	8.1	1.74, s	8.3	1.88, s
14	18.9	1.16, s	19.1	1.11, s	19.8	1.12, s
15	17.4	1.27, s	16.8	1.35, s	17.6	1.31, s
1′	169.0	–	168.8	–	168.9	–
2′	129.5	–	127.7	–	129.5	–
3′	138.9	6.14, q, 2.0	138.1	6.05, m	139.0	6.13, m
4′	16.1	2.00, d, 2.0	15.7	1.93, s	16.1	1.92, d, 1.5
5′	20.8	1.92, s	20.6	1.86, s	20.8	1.88, d, 2.0
4-$CH_3\underline{C}O$			170.2	–		
4-$\underline{C}H_3CO$			22.5	1.95, s		

above evidences, the structure of compound **3** was identified as 3β-angeloyloxy-4α-hydroxyl-eudesm-7(11),8(9)-dien-8,12-olide and named dobinin C.

Five known compounds had similar spectral characteristics as compounds **1**–**3** and were identified to be 3β-angeloyloxy-4α,8β-dihydroxy-eudesm-7(11)-en-8α,12-olide (**4**) [3], 3β-angeloyloxy-4β-acetoxy-8β-hydroxy-eudesm-7(11)-en-8α,12-olide (**5**) [5], 1β-angeloyloxy-6β,8β-dihydroxy-10β-methoxyeremophil-7(11)-en-8α,12-olide (**6**) [6], 1β-angeloyloxy-6β,8α-dihydroxy-10α-methoxyeremophil-7(11)-en-8β,12-olide (**7**) [6], and 6β,8β-dimethoxy-10β-hydroxyeremophil-7(11)-en-8β,12-olide (**8**) [6] (**Fig. 1**), respectively, by detailed comparison of their spectral data with those reported in the literatures.

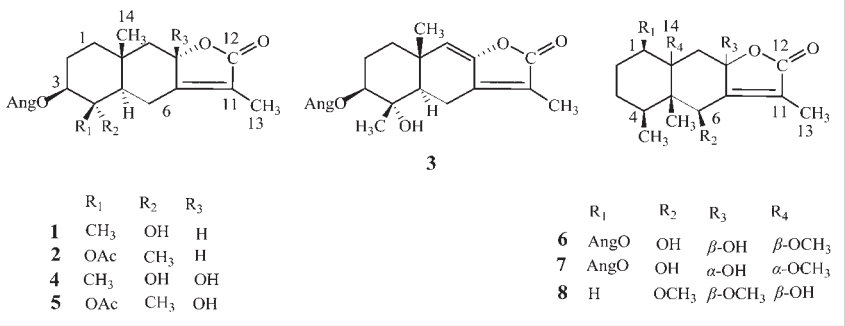
The antitumor test of the three new compounds **1**–**3** against two tumor cell lines, HL-60 and A-549, were evaluated and showed that compounds **1**-**3** exhibited cytotoxicities against the tumor cell line HL-60 at IC_{50} levels of 8.0×10^{-5}, 4.7×10^{-5} and 5.1×10^{-5} M (with the positive control cisplatin, 5.5×10^{-6} M), respectively, but all three new compounds revealed no cytotoxic activity against the tumor cell line A-549.

Materials and Methods

The root of *D. delavayi* was collected in Kunming, Yunnan Province, People's Republic of China, in September 2009, and authenticated by Professor Hua Peng. A voucher specimen (KUN 0865826) has been deposited in the Herbarium of Kunming Institute of Botany, Chinese Academy of Sciences. The air-dried and powdered root of *D. delavayi* (10 kg) was extracted with 95% EtOH (3 × 20 L, total amount 60 L) under reflux for 3 h. The combined extract was evaporated *in vacuo* to yield the EtOH extract (910 g), which was dissolved in H_2O (10 L) and then extracted successively with EtOAc (3 × 5 L) and *n*-BuOH (3 × 5 L) to yield an EtOAc extract (500 g) and an *n*-BuOH extract (210 g), respectively. The EtOAc extract was applied to silica gel column chromatography (12 × 150 cm, 200–300 mesh, 4.0 kg) eluted with petroleum ether-acetone (60 : 1–7 : 1, 25 L) to give three mixture fractions. Fr. 3 (15 g) was purified by silica gel column (6 × 120 cm, 200–300 mesh, 350 g) with CH_3Cl/CH_3OH (5 : 1–1 : 1, 3.2 L) and RP-18 (3 × 60 cm, 10–100% aqueous CH_3OH) to give four subfractions. Sub-Fr. 1 (500 mg) was purified on Sephadex LH-20 (1.5 × 120 cm, $CH_3OH : CH_3Cl$ = 1 : 1, 1.2 L), and then subjected to semipreparative HPLC ($CH_3CN : H_2O$ = 50 : 50, 2 mL/min, 25 °C, detector wavelength 210 nm), which gave compounds **1** (7 mg) and **4** (5 mg). Sub-Fr. 2 (2.0 g) was purified by repeated silica gel column chromatography (3.0 × 60 cm, 60 g) and RP-18 (2 × 50 cm, 20–100% aqueous CH_3OH) to give compounds **2** (30 mg), **3** (9 mg), and **5** (5 mg). Sub-Fr. 3 (12.0 g) was purified by repeated silica gel column chromatography (5 × 100 cm, 180 g) and RP-18 (3 × 60 cm, 40–100% aqueous CH_3OH, 1 L) and then on Sephadex LH-20 (2.5 × 150 cm, $CH_3OH : CH_3Cl$ = 1 : 1, 1.5 L) to obtain compounds **6** (6 mg), **7** (5 mg), and **8** (5 mg).

Dobinin A (**1**): white powder, mp 138–140 °C; $[\alpha]_D^{21}$ +88.4 (*c* 0.350, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 197 (4.0772), 219 (4.3774); IR (KBr) ν 3457, 1750, 1712, 1686, 1236, 1035 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): see **Table 1**; HR ESIMS: 349.2012 $[M + H]^+$ (calcd. for $C_{20}H_{29}O_5$, 349.2015); ESIMS: 349 $[M + H]^+$ (3), 330 (6), 305 (25), 248 (15), 166 (55), 83 (100).

Dobinin B (**2**): white powder, mp 158–159 °C; $[\alpha]_D^{21}$ −70.9 (*c* 0.328, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 240 (3.468), 226 (2.816), 218 (2.909), 194 (3.081); IR (KBr) ν 1759, 1716, 1649, 1237, 1045 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$): see **Table 1**; HR ESIMS: 391.2150 $[M + H]^-$ (calcd. for $C_{22}H_{31}O_6$, 391.2121); ESIMS: 391 $[M + H]^+$ (25), 330 (50), 265 (40), 230 (70), 83 (100).



	R_1	R_2	R_3
1	CH_3	OH	H
2	OAc	CH_3	H
4	CH_3	OH	OH
5	OAc	CH_3	OH

3

	R_1	R_2	R_3	R_4
6	AngO	OH	β-OH	β-OCH_3
7	AngO	OH	α-OH	α-OCH_3
8	H	OCH_3	β-OCH_3	β-OH

Fig. 1 Chemical structures of compounds **1–8**.

Dobinin C (**3**): white powder, mp 140–142 °C; $[\alpha]_D^{21}$ −63.8 (*c* 0.900, CH_3OH); UV (CH_3OH) λ_{max} (log ε) 218 (4.100), 274 (4.277); IR (KBr) *v* 3484, 1769, 1712, 1652, 1236, 1021 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (100 MHz, CD_3OD): see ◉ **Table 1**; HR ESIMS: 347.1850 $[M + H]^+$ (calcd. for $C_{20}H_{27}O_5$, 347.1859); ESIMS: 347 $[M + H]^+$ (8), 328 (5), 318 (3), 246 (7), 83 (100).

Anticancer activity: Three tested compounds [with purities of 95% (**1**), 96% (**2**), and 98% (**3**)] were dissolved with DMSO to a stock concentration of 10 mM and then diluted to the required concentrations with the medium. Cytotoxicity of the compounds against two human tumor cell lines, HL-60 (leukemia) and A-549 (lung carcinoma), were measured. Briefly, cells were placed in 96-well plates 12 h before treatment with an initial density of 5000 cells/well and continuously exposed to different concentrations (40, 8, 1.6, 0.32, and 0.064 mM) of the compounds for 48 h, with cisplatin (Sigma, 99%) as the positive control. Inhibition rates of cell proliferation after compound treatment were determined by the MTT (methyl-thiazol-tetrozolium) assay, as described previously [7,8], and the IC_{50}s were calculated using the Reed and Muench method [9].

Supporting information

The original spectrums of NMR, UV, and IR for three new compounds (**1**–**3**) and general experimental procedures are available as Supporting Information.

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Conflict of Interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service, and/or company that could be construed as influencing the position presented in, or the review of, the entitled manuscript.

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