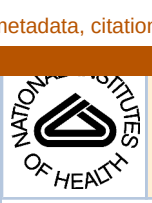

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Altaicalarins A–D, Cytotoxic Bisabolane Sesquiterpenes from *Ligularia altaica*

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Abstract

Four new bisabolane sesquiterpenes, altaicalarins A–D (**1–4**) and three known analogues (**5–7**) were isolated from the roots and rhizomes of *Ligularia altaica*. The structures were elucidated by spectroscopic methods including 2D NMR techniques, and the structure of **1** was confirmed by single-crystal X-ray diffraction. The isolated compounds were also evaluated for cytotoxic activity against human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB), and vincristine-resistant nasopharyngeal (KBVIN) cell lines, and **1** was found to show significant cytotoxicity with EC_{50} values of 3.4, 0.8, 1.0, and 0.9 μg/mL, respectively.

Ligularia altaica DC. (Asteraceae) is widely distributed in the Altaica mountains of China, and its roots and rhizomes are used as a folk medicine in the Xinjiang region, China for easing breathing, stimulating blood flow, reducing inflammation, stopping cough, and eliminating phlegm.[1] In prior investigations, flavonoids, triterpenes, sterols, benzofurans, and eremophilane sesquiterpenes were reported.2[−]4 As part of our program to discover anticancer agents from Chinese herbs, a phytochemical investigation of the roots and rhizomes of this plant led to the isolation and characterization of four new (**1–4**) and three known [1β,8-diangloyloxy-3β,4β,10,11- diepoxybisabol-7(14)-ene (**5**),[5] 1β-acetoxy-1β,8-diangloyloxy-10,11-dihydroxy-3,4- epoxybisabol-7(14)-ene (**6**),[6] and bisabolone (**7**)7] bisabolane sesquiterpenes. Herein we report the isolation and structural elucidation of the new compounds, as well as their in vitro cytotoxicity evaluation of all isolates against human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB), and vincristine-resistant nasopharyngeal (KBVIN) cell lines.

Results and Discussion

An EtOH extract of the roots and rhizomes of *L. altaica* was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. Repeated column chromatography

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Supporting Information Available: NMR spectra of the new compounds **1–4**, as well as crystallographic data of **1**. This material is available free of charge *via* the Internet at http://pubs.acs.org.

of the combined EtOAc and petroleum ether portions on silica gel and RP_{18} gel, followed by preparative TLC, yielded four new (**1–4**) and three known (**5–7**) bisabolane sesquiterpenes.

Compound **1** was obtained as a colorless solid and its molecular formula was determined as $C_{22}H_{28}O_6$ with nine degrees of unsaturation by HREIMS (*m/z* 388.1897). The IR spectrum showed absorption bands for OH (3423 cm^{-1}), ester carbonyl (1740 cm^{-1}), and double bond (1694 cm^{-1}) moieties. The ^1H and ^{13}C NMR spectra (Table 1 and Table 2) displayed the characteristic signals of an angeloyloxy group [δ_H 6.08 (1H, qq, *J* = 7.0, 1.5 Hz), 1.97 (3H, dq, *J* = 7.0, 1.1 Hz), 1.90 (3H, dq, *J* = 1.5, 1.1 Hz); δ_C 167.3 (C), 127.9 (C), 138.1 (CH), 15.7 (CH_3), 20.6 (CH_3)],[8] and an acetoxy group [δ_H 2.30 (3H, s); δ_C 170.3 (C), 20.7 (CH_3)], in accordance with the significant MS fragment peaks at *m/z* 288 [*M*-AngOH]$^+$ and *m/z* 246 [*M*-AngOH-HOAc]$^+$. Apart from these two ester groups, the ^{13}C NMR and DEPT spectra of **1** revealed 15 skeletal carbon signals, including three methyl, two methylene, three methine, seven quaternary carbons, and the typical carbon signals for a terminal double bond [δ_C 144.4 (C) and 115.0 (CH_2)]. The ^1H NMR data of **1** displayed signals accounting for 18 protons: two hydroxy groups [δ_H 5.47, 5.58 (broad s)], one aromatic [δ_H 2.11, 3H, s], two allylic [δ_H 1.52, 1.65 (each 3H s)] methyls, two methylenes, including one exocyclic methylene [δ_H 5.09, 5.30 (each 1H, br s)], and three methines, including an oxymethine proton [δ_H 5.61, dd, *J* = 7.8, 7.8 Hz], a trisubstituted olefinic proton [δ_H 5.07, dd, *J* = 7.4, 1.5 Hz], and an aromatic proton [δ_H 6.36 (1H, s)]. HMBC correlations were found between the proton at δ_H 5.07 (1H, dd) with carbons at δ_C 25.7 (C-12) and 17.8 (C-13), the protons at δ_H 1.65 (3H, s) and 1.52 (3H, s) with the carbon at δ_C 119.2 (C-10), and the proton at δ_H 2.24 (1H, dd) with carbons at δ_C 119.2 (C-10) and 134.1 (C-11), which proved the presence of a prenyl group (Figure 1). The above spectroscopic data were in agreement with those reported for an aromatic bisabolane sesquiterpene with an angeloyloxy group, an acetoxy group, and two hydroxy groups.[9]

Furthermore, in the HMBC spectrum of **1** (Figure 1), the correlations of the proton at δ_H 5.61 (1H, d) with carbons at δ_C 167.3 (OAng, C-1'), 144.4 (C-7), 119.2 (C-10), 115.0 (C-14), and 32.2 (C-9) indicated attachment of the angeloyloxy group to C-8, whereas the correlations of the proton at δ_H 6.36 (1H, s) with carbons at δ_C 129.7 (C-1), 112.3 (C-3), and 146.5 (C-4) together with the correlations of the methyl protons at δ_H 2.11 (3H, s) with carbons at δ_C 152.1 (C-2), 112.3 (C-3), and 146.5 (C-4) indicated that the two hydroxy groups and an acetoxy group might be located at C-2, C-4, and C-1, respectively. However, the absolute configuration at C-8 could not be determined. The structure of **1** was further confirmed by X-ray crystallography (Figure 2). Therefore, **1** was established as 1-acetoxy-2,4-dihydroxy-8-angeloyloxybisabola-1,3,5,7(14),10(11)- pentaene, named as altaicalarin A.

The HREIMS of **2** gave *m/z* 330.1825 in accordance with the molecular formula $C_{20}H_{26}O_4$ and eight unsaturation degrees. The IR spectrum showed absorptions for hydroxy (3260 cm^{-1}), ester carbonyl (1703 cm^{-1}), and double bond (1674 cm^{-1}) moieties. Most of the NMR data of **2** were similar to those of **1** (Table 1 and Table 2), except that signals for an acetoxy group were absent. ^1H NMR signals for a pair of proton doublets [δ_H 6.63, 6.48 (each 1H, d, *J* = 7.8 Hz)] and the HMBC correlations of the proton at δ_H 5.83 (H-OH) with carbons at δ_C 124.0 (C-3), 142.9 (C-4), and 140.5 (C-5), and of the proton at δ_H 9.00 (−OH) with carbons at C-4 and C-5, indicated that the two hydroxy groups should be located at C-4 and C-5, respectively (Figure 1). The configuration of **2** was the same as that of **1**, because they both had a positive optical rotation. Thus, **2** was determined as 4,5-dihydroxy-8-angeloyloxybisabola-1,3,5,7(14),10(11)-pentaene, named as altaicalarin B.

Compound **3**, colorless gum, has a molecular formula of $C_{22}H_{30}O_6$ on the basis of HRESIMS data (*m/z* 413.1931 [*M*+Na]$^+$). The ^1H and ^{13}C NMR spectra (Table 1 and Table 2) displayed signals for angeloyloxy and acetoxy groups. The ^{13}C NMR and DEPT spectra revealed 15 additional skeletal carbon signals: three methyl, three methylene, five methine, and four

quaternary carbons. To accommodate 8 degrees of unsaturation, compound **3** was proposed to have a monocyclic sesquiterpene skeleton with a terminal double bond [δ_H 5.12, 5.26 (each 1H, br s); δ_C 113.0 (CH_2) and 147.5 (C)], an epoxy group [δ_H 3.45 (1H, dd, J = 4.3, 4.3 Hz); δ_C 64.5 (CH), 61.4 (C)], a carbonyl group (δ_C 201.3), an angeloyloxy group, and an acetoxy group. ^{1}H-^{1}H COSY 2D-NMR data indicated the two main structural sequences, –CH–CH_2–CH–CH– and –CH–CH_2–CH–, which can be further connected with other fragments based on the following HMBC correlations: H-15/C-4; H-2/C-3; H-5/C-4, C-7; H-8/C-7; H-12, H-13/C-10 (Figure 3). Thus, **3** was determined as a bisabolane sesquiterpene.[10]

In the HMBC spectrum of **3**, correlations of the proton at δ_H 5.70 (1H, d) with carbons at δ_C 169.7 (OAc), 201.3 (C-4), and 43.8 (C-6), and of the proton at δ_H 5.10 (1H, dd) with carbons at δ_C 166.8 (OAng, C-1'), 147.5 (C-7), 118.9 (C-10), 113.0 (C-14), and 32.0 (C-9) indicated that the acetoxy and angeloyloxy groups are attached to C-5 and C-8, respectively. The HMBC correlations of the proton at δ_H 3.45 (1H, d) with carbons at δ_C 31.8 (C-1), 61.4 (C-3), and 43.8 (C-6) indicated that the epoxy group should be assigned at C-2 and C-3. The location of the carbonyl group at C-4 was established by HMBC correlations of the protons at δ_H 1.45 (3H, s) and 5.70 (1H, d) with carbon at δ_C 201.3 (C-4).

The stereo-structure of **3** was deduced from the ^{1}H NMR coupling constants and the ROESY spectrum (Figure 3). When H-6 is assumed to be in an α–orientation,[11] H-5 should be β-axial due to a large $^3J_{5,6}$ value (12.9 Hz), and the proton at δ_H 2.25 should be H-1 and α-oriented due to a smaller $^3J_{1,6}$ value (8.2 Hz). In the ROESY spectrum, the obvious correlation of H-2 with H-1α and H-15 revealed that the epoxy group should be 2β,3β-oriented. The relative configuration at C-8 could not be determined from the spectroscopic data. Thus, the structure of **3** was determined as 5α-acetoxy-8-angeloyloxy-2β,3β-epoxybisabola-7(14),10(11)-dien-4-one, named as altaicalarin C.

The molecular formula of **4** was determined as $C_{27}H_{36}O_9$ based on the HRESIMS (*m/z* 527.2250 [*M*+Na]$^+$). The IR spectrum indicated the presence of ketone (1755 cm^{-1}) and ester carbonyl groups (1720 cm^{-1}), and a double bond (1647 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (Table 1 and Table 2) displayed signals for one acetoxy and two angeloyloxy groups. Apart from the three ester groups, the ^{1}H, ^{13}C NMR, and ^{1}H-^{1}H COSY spectra (Figure 3) of **4** indicated that the basic skeleton of compound **4** was similar to that of **3**, except for an epoxy group [δ_H 2.76 (1H, t, J = 5.8 Hz); δ_C 60.8 (CH), 58.3 (C)] at C-10 and C-11 in **4**, rather than a corresponding trisubstituted double bond in **3**.

The locations of the two epoxy groups at C-2, C-3 and C-10, C-11 were confirmed by HMBC correlations of the proton at δ_H 3.45 (1H, s) with carbons at δ_C 71.6 (C-1), 61.4 (C-3), and 48.5 (C-6), and of the proton at δ_H 2.76 (1H, dd) with carbons at δ_C 72.6 (C-8), 33.5 (C-9), and 24.6 (C-12). The HMBC correlations of the proton at δ_H 5.70 (1H, d) with carbons at δ_C 169.4 (OAc), 199.5 (C-4), and 48.5 (C-6), the proton at δ_H 5.50 (1H, d) with carbons at δ_C 166.2 (OAng), 65.6 (C-2), 61.4 (C-3), and 72.8 (C-5), and the proton at δ_H 5.43 (1H, dd) with carbons at δ_C 166.0 (OAng), 145.5 (C-7), and 60.8 (C-10), indicated that the acetoxy group is at C-5 and the two angeloyloxy groups are at C-1 and C-8. (Figure 3)

The relative configuration of the stereogenic centers of the cyclohexane ring in **4** was the same as those in **3**, based on the ^{1}H NMR coupling constants ($J_{5,6}$ = 11.7, $J_{6,1}$ = 7.8 Hz) and the ROESY correlations between H-1/H-2 and H-2/H-15. The relative configurations at C-8 and C-10 could not, however, be determined from the spectroscopic data Thus, the structure of **4** was determined as 1β,8-diangeloyloxy-5α-acetoxy-2β,3β;10,11-diepoxybisabola-7(14)-en-4-one, named as altaicalarin D.

All isolates were evaluated for their in vitro cytotoxicity against human lung carcinoma (A-549), human breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx

(KB), and vincristine-resistant nasopharyngeal (KBVIN) cell lines according to a described procedure.[12] The results are listed in Table 3. The most potent compound, **1**, exhibited significant cytotoxicity against all four cell lines with EC_{50} values of 3.4 (A549), 0.8 (MCF-7), 1.0 (KB), and 0.9 (KBVIN) μg/mL. Compound **2**, which differs from **1** in the number and position of the aromatic hydroxy and ester groups, showed only weak cytotoxicity against the latter three cancer cell lines. Among the compounds without an aromatic ring, **6–7** showed no activity (EC_{50} >20 μg/mL) against the cell line panel, **3** showed weak activity against all four cell lines while **4** and **5** showed weak activity only against KBVIN. Generally, the compounds with a C-10-C-11 double bond (**1–3**) showed broader cytotoxicity compared with compounds with a C-10-C-11 epoxy group (**4–5**).

Experimental Section

General Experimental Procedures

Melting points were measured on an XT-4 micro-melting point apparatus and are uncorrected. Optical rotations were recorded on a JASCO P-1020 polarimeter at room temperature. UV spectra were measured on a Shimadzu UV-260 spectrophotometer in absolute MeOH. IR spectra were recorded on an Avatar 360 FT-IR ESP spectrometer in CH_2Cl_2. Mass spectra were determined on an HP5989A mass spectrometer for EIMS, a Waters Micromass GCT mass spectrometer for HREIMS, and a Bruker Daltonics APEXIII 7.0 TESLA FTMS mass spectrometer for HRESIMS. ^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer in $CDCl_3$. Analytical and preparative TLC were run on silica gel plates (GF_{254}, Yantai Institute of Chemical Technology, Yantai, China). Spots were observed under UV light and visualized by spraying with 10% H_2SO_4, followed by heating. Column chromatography was performed on silica gel (200–300 mesh and 300–400 mesh; Qingdao Marine Chemical Factory, Qingdao, China) and Lichroprep RP_{18} gel (40–60 μm, Merck, Darmstadt, Germany). X-ray crystallographic analysis was carried out on a Bruker Smart Apex CCD diffractometer with graphite-monochromated Mo Kα radiation (λ= 0.71073 Å). The structure was solved by direct methods using the program SHELXS. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were located by geometry and riding on the related atoms during refinements with a temperature factor of 1.2 or 1.5 times of the latter's.

Plant Material

The roots and rhizomes of *L. altaica* were collected in August 2005 on the Altaica mountains (altitude 2000 m) in Xinjiang, China. The identity of the plant material was verified by Professor Ping Yan at Shihezi University and a voucher specimen (WQ-LA-05-2) has been deposited in the Herbarium of Materia Medica, Department of Pharmacognosy, School of Pharmacy, Fudan University, Shanghai, People's Republic of China.

Extraction and Isolation

The dried and powdered materials (5.0 kg) were extracted three times with 95% EtOH at reflux temperature and filtered. The filtrate was evaporated in vacuo to give a residue (360 g), a portion of which (350 g) was suspended in H_2O (2 L) and partitioned successively with petroleum ether (3×1.5 L) and EtOAc (3×1.5 L). The combined EtOAc (160 g) and petroleum ether (50 g) extracts were chromatographed on Si gel (200–300 mesh, 2 kg, 10×120 cm) column, eluted successively with petroleum ether-acetone (50:1, 30:1, 15:1, 9:1, 7:1, 5:1, 3:1, 2:1, 1:1) to yield fractions 1–6. Subsequently, fractions 1, 2, 3, 5 and 6 were subjected to silica gel CC with petroleum ether-EtOAc (80:1, 25:1, 15:1, 5:1, 3:1), respectively. Fraction 1 (26 g) yielded **7** (740 mg). Fraction 2 (28 g) gave two fractions 2a and 2b. Fraction 2a (16 g) was applied to silica gel CC with petroleum ether-EtOAc (30:1) to afford **5** (287 mg), and fraction 2b (6 g) was chromatographed similarly, followed by CC on RP_{18} gel with MeOH-H_2O (80:20) to give **2** (25 mg). Fraction 3 (27 g) gave two fractions 3a and 3b. Fraction 3b (7 g) was applied to

silica gel CC with petroleum ether-EtOAc (9:1) to afford **4** (68 mg). Fraction 5 (25 g) gave two fractions 5a and 5b. Fraction 5a (5 g) was applied to silica gel CC with petroleum ether-EtOAc (6:1) to give **6** (262 mg), and fraction 5b (6 g) was applied to silica gel CC with petroleum ether-EtOAc (5:1), followed by prep TLC with petroleum ether-acetone (12:1) to give **3** (138 mg). Fraction 6 (39 g) afforded **1** (66 mg), after further purification over prep TLC with $CHCl_3$-acetone (20:1).

1-Acetoxy-2,4-dihydroxy-8-angeloyloxybisabola-1,3,5,7(14),10(11)-pentaene (1)—colorless blocks (acetone); mp. 122–123°C; $[\alpha]_D^{22}$ +129 (*c* 0.3, MeOH); IR υ_{max} (KBr): 3423, 1740, 1694, 1644, 1434, 1239, 1098 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 284 (sh), 211 (1.36); For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1 and Table 2; EIMS *m/z* 388 $[M]^+$ (2.6), 288 $[M\text{-AngOH}]^+$ (26.7), 246 $[M\text{-AngOH-AcOH}]^+$ (97.0), 231 (60.6), 177 (100), 83 (39.1), 55 (30.3); HREIMS *m/z* 388.1897 (calcd for $C_{22}H_{28}O_6$, 388.1886); Crystal data:[13] $C_{22}H_{28}O_6$, M_r = 388.44, monoclinic, space group *C* 2, a = 17.066 (4) Å, b = 6.9212 (15) Å, β = 100.536 (4) °, c = 19.004 (4) Å, *V* = 2206.9 (8) $Å^3$, *Z* = 4, D_{calc} = 1.169 Mg/m^3. The final *R* value were R1 = 0.0564 and wR2 = 0.1220 for 6925 observed reflections [$I>2\sigma(I)$].

4,5-Dihydroxy-8-angeloyloxybisabola-1,3,5,7(14),10(11)-pentaene (2)—colorless gum, $[\alpha]_D^{22}$ +23.3 (*c* 0.6, MeOH); IR υ_{max} (CH_2Cl_2): 3260, 2921, 1703, 1674, 1463, 1257, 739 cm^{-1}; UV λ_{max} (MeOH) nm (log ε): 280 (sh), 210 (1.26); For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1 and Table 2; EIMS *m/z* 330 $[M]^+$ (2.3), 230 $[M\text{-AngOH}]^+$ (33.7), 215 $[M\text{-AngOH-Me}]^+$ (42.3), 193 $[M\text{-AngOH-H}-2\times H_2O]+$ (9.4), 161 (100), 83 (68.8), 55 (87.1); HREIMS *m/z* 330.1825 (calcd for $C_{20}H_{26}O_4$, 330.1831).

5α-Acetoxy-8-angeloyloxy-2β,3β-epoxybisabola-7(14),10(11)-dien-4-one (3)—colorless gum, $[\alpha]_D^{22}$ −26.3 (*c* 0.5, MeOH); IR υ_{max} (CH_2Cl_2): 1735, 1646, 1459, 1378, 1142, 736 cm^{-1}; For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1 and Table 2 ; EIMS *m/z* 331 (2.7), 228 (2.9), 173 (10.1) , 97 (100); HRESIMS: *m/z* 413.1931 $[M+Na]^+$ (calcd for $C_{22}H_{30}O_6Na$, 413.1934).

1β,8-Diangeloyloxy-5α-acetoxy-2β,3β;10,11-diepoxybisabola-7(14)-en-4-one (4)—colorless gum, $[\alpha]_D^{22}$ +44.4 (*c* 0.2, MeOH); IR ν_{max} (CH_2Cl_2): 1757, 1720, 1647, 1457, 1378, 1230, 1148, 1040, 754 cm^{-1}; For ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Table 1 and Table 2 ; EIMS *m/z* 405 $[M+H\text{-AngOH}]^+$ (2.7), 263 $[M+H\text{-2AngOH-AcOH}]^+$ (1.2), 219 (4.6), 83 (100); HRESIMS: *m/z* 527.2250 $[M+Na]^+$ (calcd for $C_{27}H_{36}O_9Na$, 527.2251).

Growth Inhibition Assay

Drug stock solutions were prepared in DMSO and stored at −70 °C. Upon dilution into culture medium, the final DMSO concentration was ≤1% DMSO (v/v), a concentration without effect on cell replication. The human tumor cell line panel consisted of lung carcinoma (A-549), breast adenocarcinoma (MCF-7), epidermoid carcinoma of the nasopharynx (KB), and vincristine-resistant nasopharyngeal (KBVIN). Etoposide was used as control. Cell culture and other procedures were the same as those reported previously.[12] The EC_{50} value is the concentration that inhibited growth by 50% following two days of continuous exposure.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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13. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Center. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

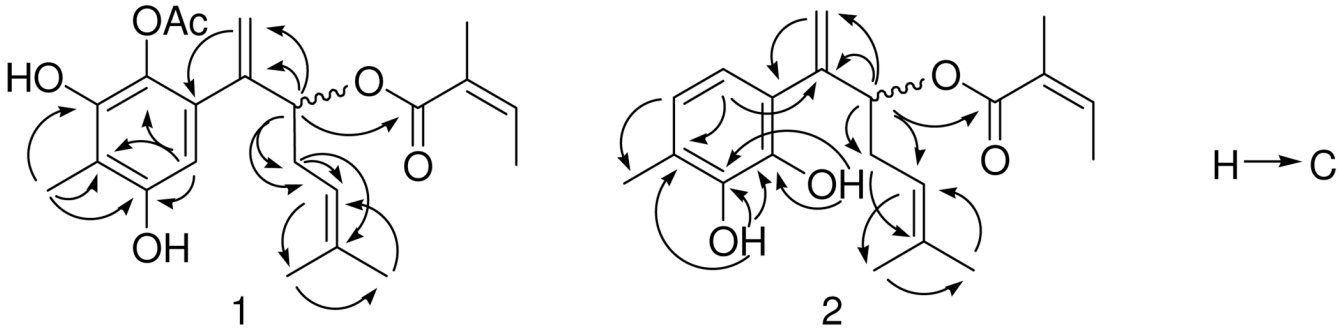


Figure 1.
Key HMBC correlations of **1** and **2**

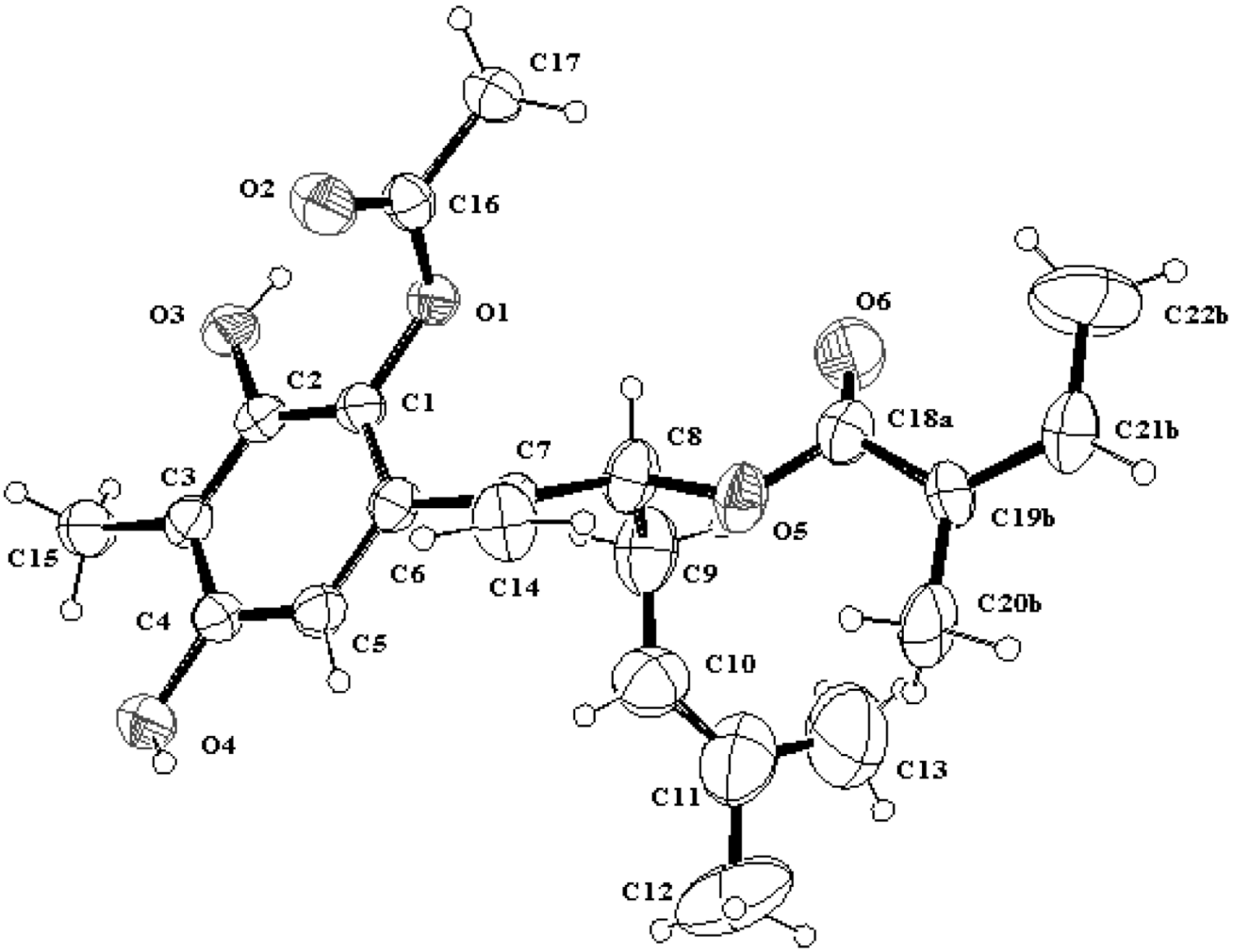


Figure 2.
X-ray crystal structure of 1

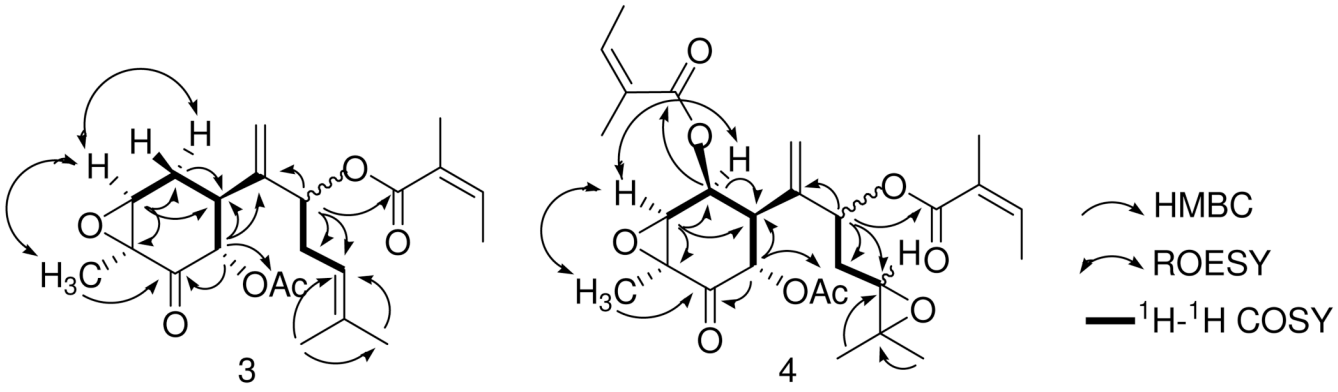
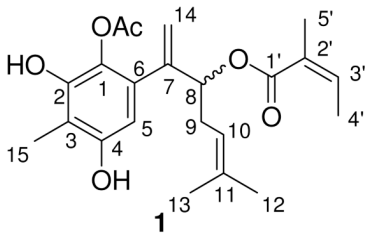


Figure 3.
Key HMBC, ^{1}H-^{1}H COSY, and ROESY correlations of **3** and **4**



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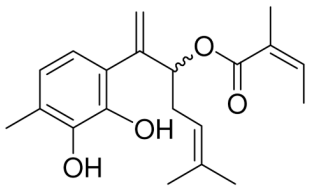
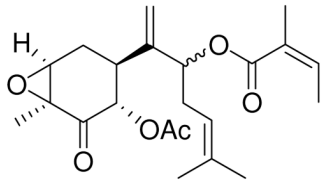
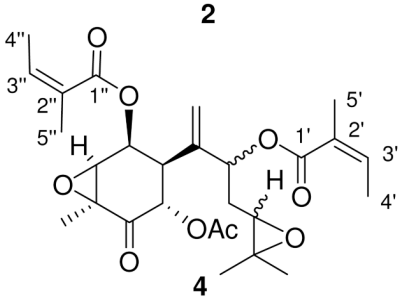


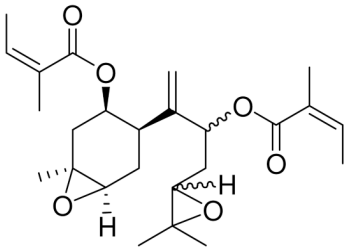
2



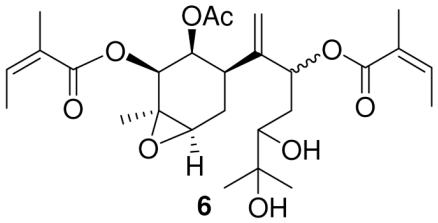
3



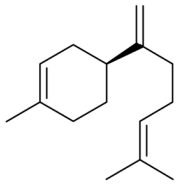
4



5



6



7

Table 1

^{1}H NMR (400MHz) Data of Compounds **1**–**4** ($CDCl_3$, δ ppm, *J* in Hz)

	1	**2**	**3**	**4**
1	-	6.48, d (7.8)	2.55, ddd (8.2, 7.4, 4.3)	5.50, d (7.8)
			2.20, ddd (15.6, 8.2, 7.4)	
2	-	6.63, d (7.8)	3.45, d (4.3)	3.45, s
3	-	-	-	-
4	-	-	-	-
5	6.36, s	-	5.70, d (12.9)	5.70, d (11.7)
6	-	-	2.75, ddd (15.6, 12.9, 8.2)	2.93, dd (11.7, 7.8)
7	-	-	-	-
8	5.61, dd (7.8, 7.8)	5.09, dd (7.0, 7.0)	5.10, dd (7.8, 6.2)	5.43, dd (5.1, 5.1)
9a	2.29, overlapped	2.42, overlapped	2.34, dd (6.6, 6.6)	1.87, dd (5.9, 5.1)
9b	2.24, dd (7.4, 7.4)	2.40, overlapped	2.34, dd (6.6, 6.6)	1.83, dd (5.9, 4.7)
10	5.07, dd (7.4, 1.5)	5.10, dd (7.4, 2.7)	5.05, dd (7.1, 1.5)	2.76, t (5.8)
11	-	-	-	-
12	1.65, s	1.66, s	1.62, s	1.23, s
13	1.52, s	1.57, s	1.68, s	1.26, s
14a	5.30, br s	5.30, br s	5.26, br s	5.40, br s
14b	5.09, br s	5.15, br s	5.12, br s	5.31, br s
15	2.11, s	2.26, s	1.45, s	1.46, s
H-OH	5.47, 5.58, bl s	5.83, 9.00, br s		
OAc	2.30, s		2.03, s	2.05, s
OAng				
3'	6.08, qq (7.0, 1.5)	6.25, qq (7.0, 1.5)	6.09, qq (7.0, 1.5)	6.11, qq (7.0, 1.5)
3"				6.17, qq (7.4, 1.5)
4'	1.97, dq (7.0, 1.1)	2.05, dq (7.0, 1.5)	1.98, dq (7.0, 1.1)	1.98, dq (7.0, 1.1)
4"				1.98, dq (7.4, 1.5)
5'	1.90, dq (1.5, 1.1)	1.96, dq (1.5, 1.5)	1.89, dq (1.5, 1.1)	1.90, dq (1.5, 1.1)
5"				1.90, dq (1.5, 1.5)

Table 2

^{13}C NMR (100MHz) Data of Compounds **1–4** ($CDCl_3$, δ ppm)

	1	2	3	4
1	129.7 (s)	120.2 (d)	31.8 (t)	71.6 (d)
2	152.1 (s)	121.6 (d)	64.5 (d)	65.6 (d)
3	112.3 (s)	124.0 (s)	61.4 (s)	61.4 (s)
4	146.5 (s)	142.9 (s)	201.5 (s)	199.5 (s)
5	107.9 (d)	140.5 (s)	74.8 (d)	72.8 (d)
6	130.7 (s)	123.2 (s)	43.8 (d)	48.5 (d)
7	144.4 (s)	147.3 (s)	147.5 (s)	145.5 (s)
8	74.8 (d)	76.5 (d)	75.6 (d)	72.6 (d)
9	32.2 (t)	33.2 (t)	32.0 (t)	33.5 (t)
10	119.2 (d)	119.1 (d)	118.9 (d)	60.8 (d)
11	134.1 (s)	134.7 (s)	134.5 (s)	58.3 (s)
12	25.7 (q)	25.7 (q)	25.7 (q)	24.6 (q)
13	17.8 (q)	17.9 (q)	18.0 (q)	18.8 (q)
14	115.0 (t)	114.8 (t)	113.0 (t)	114.8 (t)
15	8.5 (q)	15.4 (q)	14.8 (q)	14.3 (q)
OAc				
1'	170.3 (s)		169.7 (s)	169.4 (s)
2'	20.7 (q)		20.2 (q)	20.2 (q)
OAng				
1'	167.3 (s)	169.8 (s)	166.8 (s)	166.0 (s)
1"				166.2 (s)
2'	127.9 (s)	127.1 (s)	127.8 (s)	126.6 (s)
2"				127.3 (s)
3'	138.1 (d)	140.9 (d)	138.6 (d)	139.6 (d)
3"				140.9 (d)
4'	15.6 (q)	15.9 (q)	15.7 (q)	15.8 (q)
4"				15.9 (q)
5'	20.6 (q)	20.3 (q)	20.5 (q)	20.5 (q)
5"				20.4 (q)

Table 3

Cytotoxicity Data of Compounds **1**–**7** (EC_{50} μg/mL)

Compound	Cell Line			
	A549	MCF-7	KB	KBVIN
1	3.4	0.8	1.0	0.9
2	>20	10.8	11.8	15.9
3	11.4	7.7	10.4	7.6
4	>20	>20	>20	18.9
5	>20	>20	>20	11.4
6	>20	>20	>20	>20
7	>20	>20	>20	>20
Etoposide	0.4	16.4	3.9	8.8