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Songaricalarins A–E, Cytotoxic Oplopane Sesquiterpenes from *Ligularia songarica*#

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Abstract

Five new highly oxygenated oplopane sesquiterpenes, songaricalarins A–E (**1–5**), and two known analogues (**6** and **7**) were isolated from the roots and rhizomes of *Ligularia songarica*. Their structures and configurations were elucidated by spectroscopic methods, including 2D-NMR techniques, and the structure of **1** was confirmed by single-crystal X-ray diffraction. All compounds were evaluated for in vitro cytotoxic activity against cultured A-549, MCF-7, KB, and KBVIN cells, and **4** exhibited cytotoxicity with EC_{50} values of 4.9, 0.8, 3.4, and 3.2 μg/mL, respectively.

Ligularia songarica (Fisher) Y. Ling (Asteraceae) is distributed widely in the Altaica mountains of mainland China, and its roots and rhizomes are used as a folk medicine in the Xinjiang region, for easing breathing, stimulating blood flow, reducing inflammation, stopping coughs, and eliminating phlegm.[1] In prior investigations, triterpenes, sterols, and bisabolane and eremophilane sesquiterpenes have been reported.[2–4] As part of our program to discover anticancer agents from Chinese herbs, a phytochemical investigation on this plant led to the isolation and characterization of five new oplopane sesquiterpenes (**1–5**) and two known analogues 7β-[(3'-ethylcrotonoyl)oxy]-1α-[(2'-methylbutanoyl)oxy]-3,14-dehydro-*E*-notonipetranone (**6**)[5] and (3*S*,4*R*,5*S*,6*R*,7*S*,9*R*,11*S*,14*S*)-14α-acetoxy-7-[(4-acetoxy-4-methylsenecioyl)oxy]-6-[(2- methylbutanoyl)oxy]-11,12-epoxyoplop-8(10)-en-2-

[#]Dedicated to Dr. Lester A. Mitscher, of the University of Kansas, for his pioneering work on the discovery of bioactive natural products and their derivatives

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ASSOCIATED CONTENT

Supporting Information

NMR spectra of the new compounds **1–5**, as well as crystallographic data of **1** are available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

one (**7**).[6] Herein, we report the isolation and structure elucidation of the new compounds, as well as the in vitro cytotoxicity evaluation of all isolates obtained 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. songarica* was suspended in H_2O and partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. Repeated column chromatography of the combined EtOAc and petroleum ether portions on silica gel and C_{18} RP columns, followed by preparative TLC, yielded five new (**1–5**) and two known (**6** and **7**) oplopane sesquiterpenes.

Compound **1** was obtained as colorless plates. The HRESIMS gave *m/z* 545.2725 $[M+Na]^+$, in accordance with the molecular formula, $C_{28}H_{42}O_9Na$. The IR spectrum showed absorption bands for OH (3485 cm^{-1}), ester carbonyl (1712 cm^{-1}), and double bond (1646 cm^{-1}) moieties. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) displayed characteristic signals for an acetoxy group [δ_H 1.91 (3H, s); δ_C 170.5 (C), 21.2 (CH_3)], a (2-methylbutanoyl)oxy (OMebu) group [δ_H 2.45 (1H, m), 1.52, 1.84 (2H, m), 0.93 (3H, t, J = 7.3 Hz), 1.18 (3H, d, J = 7.3 Hz); δ_C 175.7 (CO), 41.9 (CH), 26.9 (CH_2), 11.9 (CH_3), 16.2 (CH_3)],[7] and a (4-methylsenecioyl)oxy (OMesen) group [δ_H 5.66 (1H, brs), 2.15 (2H, q, J = 7.3 Hz), 1.02 (3H, t, J = 7.3 Hz), 2.11 (3H, s); δ_C 166.1 (CO), 115.6 (CH), 161.9 (C), 34.1 (CH_2), 12.2 (CH_3), 18.6 (CH_3)],[8] in accordance with the significant EIMS fragment peaks at *m/z* 408 $[M–MesenOH]^+$, 348 $[408–AcOH]^+$, and 306 $[408–MebuOH]^+$. Apart from these three ester groups, the NMR spectra of **1** showed resonances for an olefinic methylene [δ_H 5.19, 5.30 (2 × brs, 1H each); δ_C 114.2 (CH_2), 141.8 (C)] and an epoxide [δ_H 2.65, 2.80 (d, J = 3.9 Hz, 1H each); δ_C 53.6 (CH_2), 56.9 (C)]. Moreover, the NMR signals indicated nine CH (including five oxygenated methines), and two Me groups, in which one [δ_H 1.32 (s); δ_C 16.8 (CH_3)] was attached to a tertiary C-atom and the other [δ_H 1.48 (d, J = 6.8 Hz); δ_C 16.8 (CH_3)] to a secondary C-atom. To accommodate an index of hydrogen insufficiency of eight, compound **1** was proposed as having a bicyclic sesquiterpene skeleton, with an epoxy group and an exocyclic C=C bond, in agreement with an oplopanoid sesquiterpene skeleton.[9]

The ^1H and^1H-COSY NMR analyses showed clear signals for the partial structure shown in Figure 1, which was supported further by the following HMBC correlations: H-3 with C-2, C-4, C-5, and C-14; H-4 with C-3, C-5, and C-9; H-9 with C-2, C-4, and C-8; H-10 with C-7, C-8, and C-9 (Figure 1). These data confirmed that **1** is an oplopanol derivative. The positions of the two hydroxy groups at C-1 and C-6 were indicated by the following HMBC correlations: H-2, H-4, and OH (δ_H 3.37) with C-1, as well as H-5, H-7, and OH (δ_H 4.11) with C-6. The positions of the three ester groups at C-14, C-2, and C-7 were inferred from the HMBC correlations between H-14, H-2, and H-7 (δ_H 5.17, 5.25, 5.48, respectively) with the ester C=O resonances at δ_C 170.5, 175.7, and 166.1 of the OAc, OMebu, and OMesen groups, respectively (Figure 1).

The relative configuration of **1** was deduced by analysis of the ^1H-^1H coupling constants and ROESY correlations. When H-4 is assumed to be in a β–orientation, H-3, H-9, and H-5 should be α-axial due to the large $^3J_{4,3}$, $J_{4,5}$, and $J_{4,9}$ (12.6 Hz) coupling constants. Furthermore, $J_{6,5}$ and $J_{6,7}$ (10.2, 2.9 Hz, respectively) indicated that H-6 and H-7 are on the same side of the molecule as H-4, while $J_{2,3}$ and $J_{2,1}$ (3.9, 3.4 Hz, respectively) indicated that H-2 and H-1 are on the opposite side from H-4. Finally, a ROESY correlation of H-4 with H-13 revealed that H-13 is located on the same side as H-4 (Figure 1). Based on the X-ray crystallographic data (Figure 2), the orientation of the OAc group at C-14 is α. The

absolute configuration of **1**, i.e., (1*S*,2*R*,3*S*,4*S*,5*S*,6*R*,7*S*,9*R*,11*S*,14*S*), was deduced by circular dichroism (CD) spectroscopy, in which a negative Cotton effect was observed at 358 nm (Δε−22).[10] Therefore, **1** was established as (1*S*,2*R*,3*S*,4*S*,5*S*,6*R*,7*S*,9*R*,11*S*,14*S*)-14α-acetoxy-1,6-dihydroxy-2-[(2-methylbutanoyl)oxy]-7-[(4-methylsenecioyl)oxy]-11,12-epoxyoplop-8(10)-ene,[11] and given the name songaricalarin A.

The HRESIMS of compound **2** displayed a $[M+Na]^+$ signal at *m/z* 487.2457, in accordance with the molecular formula, $C_{25}H_{36}O_8Na$. Based on comparison of the EIMS and NMR spectra of **2** (Tables 1 and 2) with those of **1**, one OH and the OMebu group in **1** were replaced by H and AcO groups, respectively, in **2**. The only hydroxy group in **2** was positioned at C-6 due to the HMBC correlations of H-5 and H-7 with C-6 (δ_C 74.1). The positions of the three ester groups at C-14, C-2, and C-7 were inferred from the HMBC correlations between H-14, H-2, and H-7 (δ_H 5.11, 5.50, 5.57, respectively) with the ester C=O resonances at δ_C 170.3, 170.6, and 165.9 of the two OAc and OMesen groups, respectively. The absolute configuration of **2** (songaricalarin B), i.e., (2*R*,3*S*,4*S*,5*S*,6*R*,7*S*,9*R*,11*S*,14*S*), was deduced from a negative Cotton effect at 357 nm (Δε−12) in the CD spectrum.[10]

Compound **3** was obtained as colorless gum. The HRESIMS showed a $[M+Na]^+$ signal at *m/z* 619.2651, in accordance with a molecular formula of $C_{31}H_{45}O_9ClNa$. In addition, a series of characteristic isotopic fragment peaks in the EI mass spectrum of **3** with a ratio of 3:1 at *m/z* 229/231 and 201/203, supported the presence of a chlorine atom.[12] Comparison of the NMR spectra of **3** (Tables 1 and 2) with those of the known compound **6**[5] showed initially that the signals for an exocyclic C=C bond were absent, and were replaced by those for a CH_2-Cl group [δ_H 3.55, 3.92 (each 1H, brd, J = 11.9 Hz); δ_C 73.0 (C), 48.5 (CH_2)], and an OH group [δ_H 2.60], which was connected to C-8 as indicated by the HMBC correlations between H-10 and OH (δ_H 2.60) with C-7, C-8, and C-9 (Figure 1). Second, a methyl group in **6** was replaced by an epoxide [δ_H 2.59, 2.81 (d, J = 4.4 Hz, 1H each); δ_C 51.8 (CH_2), 56.2 (C)] in **3**, with the location inferred from the HMBC correlations of H-12 with C-5, C-11, and C-13 and of H-13 with C-5, C-11, and C-12 (Figure 1). The positions of the three ester groups, located at C-1, C-6, and C-7, were determined from the HMBC correlations of H-1 and H-6 (δ_H 5.28, 5.45, respectively) with the ester C=O resonances at δ_C 175.3 and 175.7, respectively, of the two OMebu groups and of H-7 (δ_H 5.39) with the ester C=O resonance at δ_C 164.3 of the OMesen group (Figure 1).

The relative configuration of **3** was deduced by analysis of the ^1H-^1H coupling constants and ROESY correlations. The coupling constants $J_{4,9}$, $J_{4,5}$, $J_{10a,10b}$, and $J_{5,6}$ (all ca. 11.9 Hz), as well as $J_{1,9}$ and $J_{6,7}$ values of 4.7 Hz and 3.1 Hz, respectively, in the ^1H NMR spectrum, together with the cross-peaks between H-9 and OH, H-4 and H-13, H-4 and H-6, and H-6 and H-7 in the ROESY spectrum, indicated that H-4, H-6, H-7, and H-10 are *trans*-oriented with respect to H-9, H-1, H-5, and OH (Figure 1). The absolute configuration of **3**, i.e., (1*S*,4*S*,5*S*,6*R*,7*S*,8*R*,9*R*,11*S*), was deduced from the CD spectrum, in which a negative Cotton effect was observed at 310 nm (Δε−12).[10] Compound **3** was named songaricalarin C.

The HRESIMS of **4** showed a $[M+Na]^+$ signal at *m/z* 451.2456, in accordance with the molecular formula, $C_{26}H_{36}O_5Na$. Comparing the NMR spectra of **4** (Tables 1 and 2) with those of the known compound **6**[5] showed that an isopropyl group in **6** was replaced by an isopropylene group [δ_H 4.86, 4.92 (each 1H, brs), 1.76 (3H, s); δ_C 146.7 (C),δ_C 112.8 (CH_2), 19.1 (CH_3)] in **4**. The relative configuration of **4** was assigned by analysis of the ^1H-^1H coupling constants and ROESY correlations. When H-4 is assumed to be in a β–orientation, H-9 and H-1 should be α-oriented due to the large $^3J_{4,9}$ (10.5 Hz) and small $^3J_{1,9}$ (4.3 Hz) coupling constants respectively. Clear ROESY correlations of H-4 with H-13 and of H-4 with H-7 indicated that the orientations of H-5 and H-7 should be α and β,

respectively. Since the absolute configurations of **1–3** had already been established, it was assumed that this configuration is also retained in their close congener **4** (songaricalarin D).

The molecular formula of **5** was determined as $C_{28}H_{40}O_8$ by HRESIMS (*m/z* 527.2624 [M +Na]$^+$). The NMR spectra of **5** (Tables 1 and 2) were compared with those of the known compound **7**,[6] and it was evident that a 4-acetoxy-4-methylsenecioyloxy group in **7** was replaced by a 4-methylsenecioyloxy group [δ_H 5.63 (1H, brs), 2.15 (2H, q, *J* = 7.0 Hz), 1.06 (3H, t, *J* = 7.0 Hz), 2.11 (3H, s); δ_C 165.3 (CO), 113.8 (CH), 163.1 (C), 33.7 (CH_2), 11.7 (CH_3), 18.8 (CH_3)] in **5.** This postulate was confirmed by a significant ESIMS base peak at *m/z* 390 [M–MesenOH]$^+$. The positions of the three ester groups, located at C-14, C-6, and C-7, were inferred from the HMBC correlations between H-14, H-6, and H-7 (δ_H 5.12, 5.15, 5.78, respectively) with the ester C=O resonances at δ_C 170.7, 176.0, and 165.3 of the OAc, OMebu, and OMesen groups, respectively. Compound **5** with a specific optical rotation of $[\alpha]_D^{22}$+15.6 (*c* 2.2, MeOH) is similar to compound **7** with a value of $[\alpha]_D^{22}$+16.7 (*c* 3.0, $CHCl_3$),[6] and thus, they have the same relative and absolute configurations. Compound **5** was named songaricalarin E.

All isolates were evaluated for 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 previously described procedure.[13] The results are given in Table 3. Compound **4** exhibited the highest potency against all four cancer cell lines with EC_{50} values of 4.9, 0.8, 3.4, and 3.2 μg/mL, respectively. Compounds **3–7** with a keto group at C-2 showed higher cytotoxicity against the four cancer cell lines used, compared with compounds **1** and **2** without a keto group at C-2, which were inactive or weakly active. This finding suggests that the keto group at C-2 might be important in mediating the cytotoxicity of oplopane sesquiterpenes. In addition, compound **3**, in which chlorine and hydroxy groups were added across the C8, C10 double bond, was less potent against the A-549 and MCF-7 than against the two KB cell lines. This result suggests that a chlorine atom might result in differential sensitivity between cell lines, or that the double bond might be involved.

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. CD Spectra were recorded on a JASCO J-715 spectropolarimeter [$\gamma([\theta])$ in nm]. NMR spectra were recorded on a Bruker DRX-400 spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). Mass spectra were determined on an HP5989A mass spectrometer for EIMS and a Bruker Daltonics APEXIII 7.0 TESLA FTMS mass spectrometer for HRESIMS. Analytical and preparative TLC were run on silica gel plates (GF_{254}, Yantai Institute of Chemical Technology, Yantai, People's Republic of 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, People's Republic of 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 (γ0.71073 Å).

Plant Material

The roots and rhizomes of *L. songarica* were collected in August 2008 in the Tianshan Mountains (altitude 550 m) in Xinjiang, People's Republic of China. The identity of the plant material was verified by Professor Ping Yan at Shihezi University and a voucher specimen (DFC-WQ-LS-08-2) 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 material (7.3 kg) was extracted three times with 95% EtOH at reflux temperature and filtered. The filtrate was evaporated in vacuo to give a residue (560 g), a portion of which (500 g) was suspended in H_2O (2 L) and partitioned successively with petroleum ether (60–90 °C, 3×1.5 L) and EtOAc (3×1.5 L). The combined EtOAc (40 g) and petroleum ether (340 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 1–8. Fraction 4 (20 g) was applied to silica gel CC with petroleum ether-EtOAc (7:1) to give three subfractions 4a, 4b, and 4c. Subfraction 4a (3 g) was applied to silica gel CC with petroleum ether-EtOAc (7:1) to afford **5** (46 mg), subfraction 4b (2 g) was applied to silica gel CC with petroleum ether-acetone (7:1) to afford **1** (36 mg), and subfraction 4c (6 g) was chromatographed similarly, followed by CC on C_{18} RP gel with MeOH-H_2O (4:1) to give **7** (76 mg). Fraction 5 (18 g) was applied to silica gel CC with petroleum ether-EtOAc (6:1) to give two subfractions, 5a and 5b. Subfraction 5a (5 g) was applied to silica gel CC with petroleum ether-EtOAc (6:1) to give **6** (77 mg), and subfraction 5b (6 g) was applied to silica gel CC with petroleum ether-EtOAc (6:1), followed by prep TLC with petroleum ether-acetone (20:1) to give **4** (11 mg). Fraction 7 (26 g) was applied to silica gel CC with petroleum ether-acetone (5:1) to give two subfractions, 7a and 7b. Subfraction 7a (5 g) was applied to silica gel CC with petroleum ether-EtOAc (4:1) to give **3** (12 mg), and subfraction 7b (6 g) was applied to silica gel CC with petroleum ether-EtOAc (4:1), followed by preparative TLC with $CHCl_3$-acetone (20:1) to afford **2** (3.5 mg).

(1*S*,2*R*,3*S*,3a*R*,5*S*,6*R*,7*S*,7a*S*)-2-[(2-Methylbutanoyl)oxy]-1-(1α-acetoxyethyl)octahydro-3, 6-bishydroxy-4-methylidene-7-[(2*S*)-2-methyloxiran-2-yl]-1*H*-inden-5-yl(2*E*)-3-methylpent-2-enoate (Songaricalarin A, 1)—colorless plates (acetone); mp 102–103 °C; $[\alpha]_D^{22}$ +188 (*c* 0.1, $CHCl_3$); IR (KBr) υ_{max} 3485, 2972, 1712, 1646, 1375, 1246, 1148, 873, 751 cm^{-1}; CD (*c* 0.05, MeOH) $\Delta\varepsilon_{250}$ +27, $\Delta\varepsilon_{358}$ −22; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; EIMS *m/z* (%) 408 (2.7), 390 (1.1), 348 (4.1), 330 (1.2), 306 (1.7), 246 (5.4), 97 (100); HRESIMS *m/z* 545.2725 $[M+Na]^+$ (calcd $C_{28}H_{42}O_9Na$, 545.2721);

Crystal data:[14] $C_{28}H_{42}O_9$, M_r = 522.62, monoclinic, space group $P2_1$, *a* = 6.3820 (13) Å, *b* = 14.364 (3) Å, β = 101.352 (3) °, *c* = 15.810 (3) Å, *V* = 1421.0 (5) $Å^3$, *Z* = 2, D_{calc} = 1.221 Mg/m^3. The final *R* values were *R1* = 0.0616 and w*R2* = 0.1544 for 5435 observed reflections [$I>2\sigma(I)$].

(1*S*,2*R*,3a*R*,5*S*,6*R*,7*S*,7a*S*)-2-Acetoxy-1-(1α-acetoxyethyl)octahydro-6-hydroxy-4-methylidene-7-[(2*S*)-2-methyloxiran-2-yl]-1*H*-inden-5-yl(2*E*)-3-methylpent-2-enoate (Songaricalarin B, 2)—colorless gum; $[\alpha]_D^{22}$ +214 (*c* 0.3, $CHCl_3$); CD (*c* 0.05, MeOH) $\Delta\varepsilon_{225}$ +13, $\Delta\varepsilon_{357}$ −12; IR (CH_2Cl_2) υ_{max} 3467, 2969, 1736, 1648, 1368, 1251, 1144, 1081, 735 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data,

see Tables 1 and 2, respectively; EIMS *m/z* (%) 350 (2.3), 290 (4.4), 230 (9.4), 212 (4.9), 173 (12), 97 (100); HRESIMS *m/z* 487.2457 $[M+Na]^+$ (calcd $C_{25}H_{36}O_8Na$, 487.2454).

(3*S*,3a*R*,4*R*,5*S*,6*R*,7*S*,7a*S*)-1-(1-Ethylidene)octahydro-3,6-bis[(2-methylbutanoyl)oxy]-4-chloromethyl-4-hydroxy-7-[(2*S*)-2-methyloxiran-2-yl]-2-oxo-1*H*-inden-5-yl(2*E*)-3-methylpent-2-enoate (Songaricalarin C, 3)—colorless gum; $[\alpha]_D^{22}$ − 13.4 (*c* 0.12, $CHCl_3$); CD (*c* 0.05, MeOH) $\Delta\varepsilon_{224}$ −14, $\Delta\varepsilon_{250}$ +14, $\Delta\varepsilon_{310}$ −12; IR (CH_2Cl_2) υ_{max} 3443, 2970, 1731, 1644, 1461, 1133, 737 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; EIMS *m/z* (%) 596 (0.7), 278 (1.5), 231 (1.0) , 229 (2.4), 203 (0.8), 201 (2.2), 173 (1.6), 157 (1.5), 97 (100); HRESIMS *m/z* 619.2651 $[M+Na]^+$ (calcd $C_{31}H_{45}O_9ClNa$, 619.2644).

(3*S*,3a*R*,5*S*,7*S*,7a*S*)-1-(1-Ethylidene)octahydro-3-[(2-methylbutanoyl)oxy]-4-methylidene-7-propylene-2-oxo-1*H*-inden-5-yl(2*E*)-3-methylpent-2-enoate (Songaricalarin D, 4)—colorless gum; $[\alpha]_D^{22}$ − 139.2 2 (*c* 1.1, MeOH); IR (CH_2Cl_2) υ_{max} 2968, 1743, 1644, 1459, 1377, 1218, 1140, 997 cm^{-1}; for ^1H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 451.2456 $[M+Na]^+$ (calcd $C_{26}H_{36}O_5Na$, 451.2455).

(1*S*,3a*R*,5*S*,6*R*,7*S*,7a*R*)-1-(1α-Acetoxyethyl)octahydro-6-[(2-methylbutanoyl)oxy]-4-methylidene-7-[(2*S*)-2-methyloxiran-2-yl]-2-oxo-1*H*-inden-5-yl(2*E*)-3-methylpent-2-enoate (Songaricalarin E, 5)—colorless gum; $[\alpha]_D^{22}$+15.6 (*c* 2.2, MeOH); IR (CH_2Cl_2) υ_{max} 3466, 2970, 1724, 1647, 1460, 1374,1244, 1141, 1037 cm^{-1}; for ^1H NMR and ^{13}C NMR spectroscopic data, see Tables 1 and 2, respectively; HRESIMS *m/z* 527.2624 $[M+Na]^+$ (calcd $C_{28}H_{40}O_8Na$, 527.2615).

Growth Inhibition Assays

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 a positive control. Cell culture and other procedures were the same as those reported previously.[13] The EC_{50} value is the concentration that inhibited growth by 50% following two days of continuous exposure.

X-ray Crystallography

The structure was solved by direct methods using the program SHELXS, then refined by SHELXS, with refinement of F^2 against all reflections. All esds (except the esd in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. 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 the latter.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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11. The atom labels referred to throughout the manuscript correspond to the "molecular" structure and not the "crystallographic" structure. As the *IUPAC Recommendations on Nomenclature of Organic Compounds* provides changes in the numbering of corresponding positions due to changes in the substituents apart from the backbone, the depicted oplopane numbering is used throughout to ensure easier reading and data comparison. For systematic names, see Experimental Section.
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14. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (857033). 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 deposit@ccdc.cam.ac.uk).

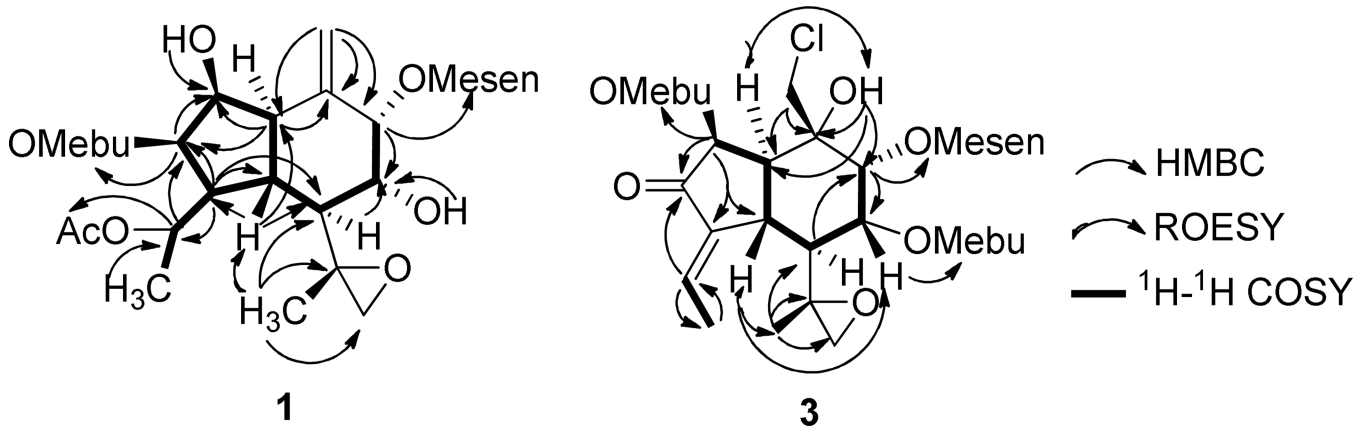


Figure 1.
Key HMBC, ^{1}H-^{1}H COSY, and ROESY correlations of **1** and **3**

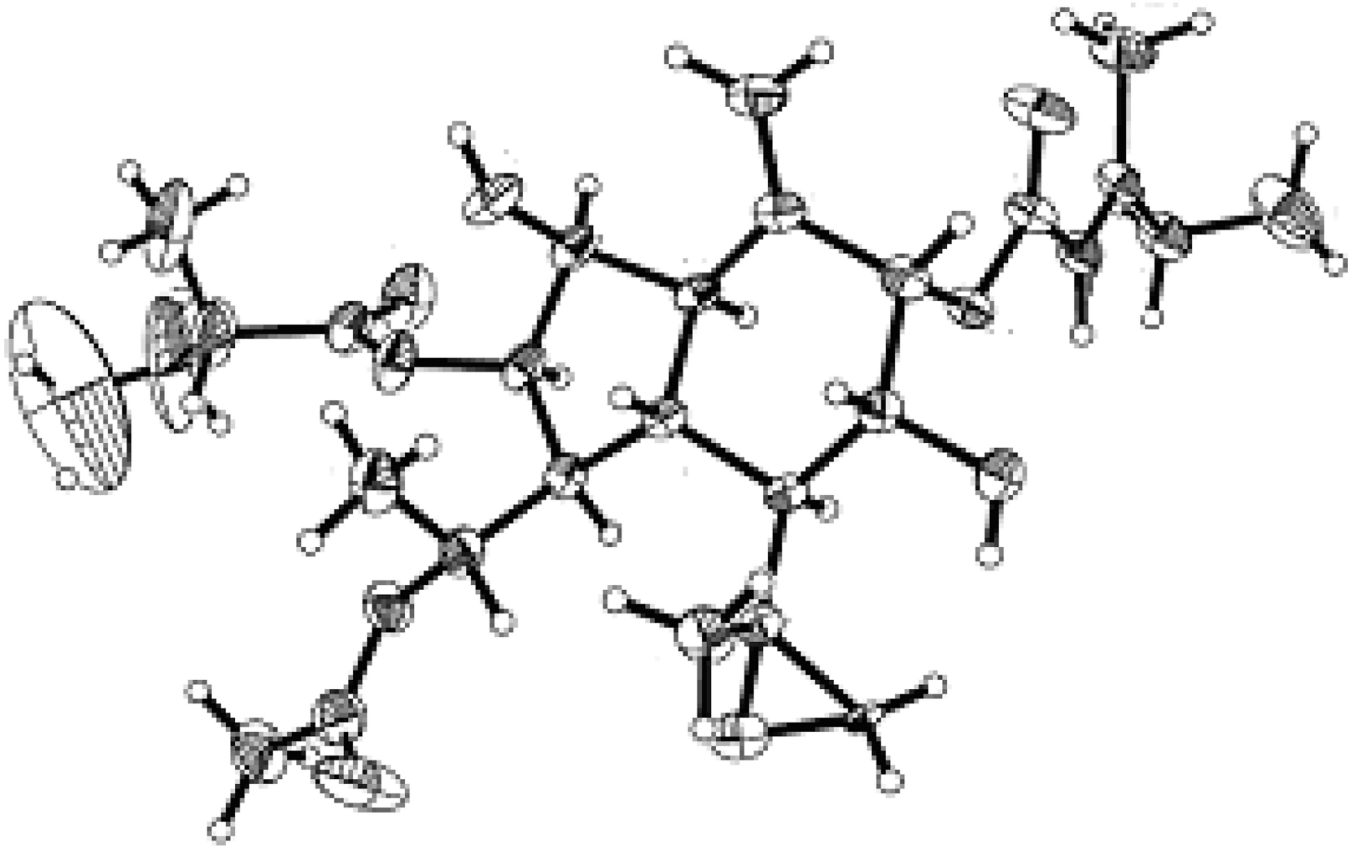
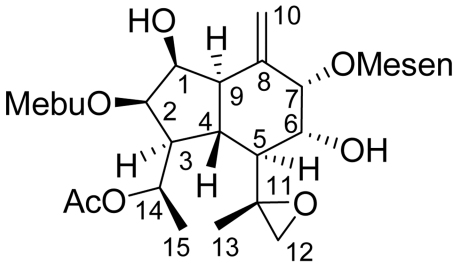


Figure 2.
X-ray crystal structure of **1**



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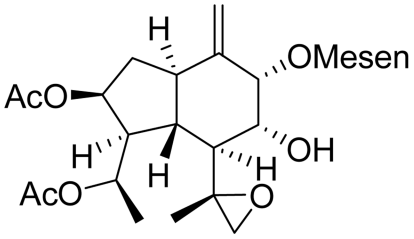

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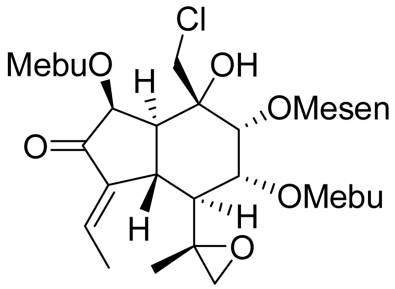

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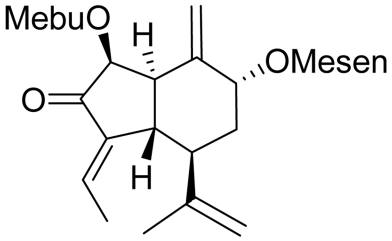

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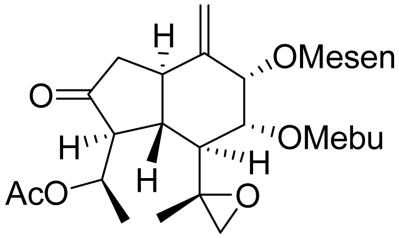

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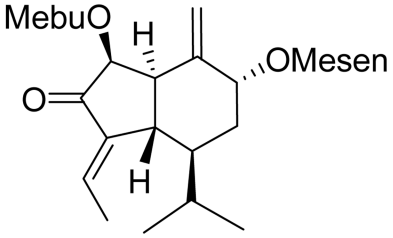

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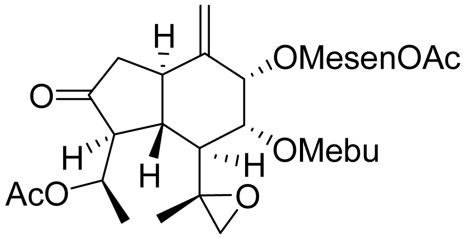

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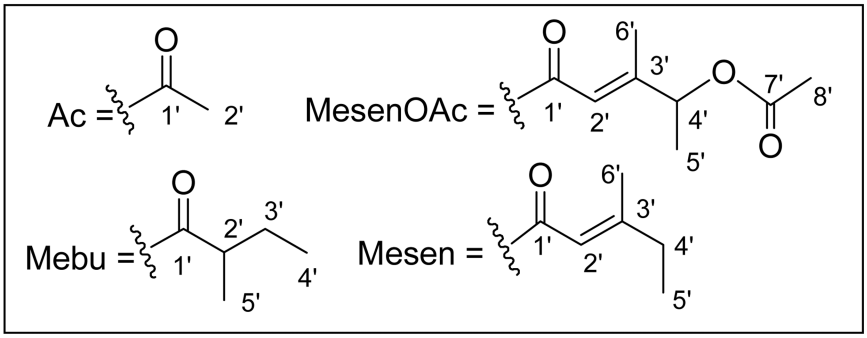

Table 1

^{1}H NMR (400 MHz) Data of Compounds 1–5

	1 [a]	2 [b]	3 [b]	4 [b]	5 [b]
1	4.33 dd, (3.4, 3.9)	1.66 m	5.28 d, (4.7)	5.53 d, (4.3)	2.15 dd, (15.2, 11.4)
	-	2.11 m	-	-	2.44 dd, (15.2, 6.6)
2	5.25 dd, (3.4, 3.9)	5.50 m	-	-	-
3	2.69 ddd, (10.2, 4.3, 3.9)	2.59 ddd, (10.0, 3.9, 3.9)	-	-	2.63 dd, (11.4, 3.6)
4	2.03 ddd, (12.6, 12.6, 10.2)	1.38 ddd, (11.0, 11.0, 10.0)	3.01 dd, (11.9, 11.9)	2.78 dd, (10.5, 10.2)	1.53 ddd, (11.4, 11.4, 11.4)
5	1.54 dd, (12.6, 10.2)	1.47 dd, (11.0, 10.5)	2.14 dd, (11.9, 11.9)	2.65 (1H, m)	2.00 dd, (11.4, 10.2)
6	3.76 dd, (10.2, 2.9)	3.83 dd, (10.5, 3.5)	5.45 dd, (11.9, 3.1)	1.78, 1.98 (m)	5.15 dd, (10.2, 3.6)
7	5.48 d, (2.9)	5.57 d, (3.5)	5.39 d, (3.1)	5.50 dd, (2.7, 3.1)	5.78 d, (3.6)
8	-	-	-	-	-
9	2.38 brd, (12.6)	2.24 m	2.05 dd, (11.9, 4.7)	2.62 dd, (10.5, 4.3)	2.63 ddd, (11.4, 10.2, 6.6)
10	5.19 brs	4.92 brs	3.55 d, (11.9)	4.83 brs	4.90, brs
	5.30 brs	5.22 brs	3.92 d, (11.9)	5.30 brs	5.28, brs
11	-	-	-	-	-
12	2.65 d, (3.9)	2.79 d, (3.5)	2.59 d, (4.4)	4.86, brs	2.68 d, (3.9)
	2.80 d, (3.9)	2.81 d, (3.5)	2.81 d, (4.4)	4.92, brs	2.80 d, (3.9)
13	1.32 s	1.33 s	1.48 s	1.76 s	1.22 s
14	5.17 dq, (6.8, 4.3)	5.11 dq, (6.6, 3.9)	6.50 dq, (7.4, 2.7)	6.43 dq, (7.4, 2.3)	5.12 dq, (6.6, 3.6)
15	1.48 d, (6.8)	1.45 d, (6.6)	2.22 d, (7.4)	2.12 d, (7.4)	1.23 d, (6.6)
OH	3.37 at C(1); 4.11 at C(6)		2.60 at C(8)		
	AcO : 1.91 s	AcO: 1.99 s	MebuO:		AcO: 2.10 s
	MebuO:	AcO: 2.09 s	(two groups)	MebuO:	MebuO:
	2' 2.45 m		2' 2.36 m; 2.33 m	2' 2.40 m	2' 2.40 m
	3' 1.52 m		3' 1.65 m; 1.46 m	3' 1.45 m	3' 1.47 m
	1.84 m		1.70 m; 1.69 m	1.66 m	1.75 m
	4' 0.93 t, (7.3)		4' 0.89; 0.88 t, (7.4)	4' 0.88 t, (7.4)	4' 0.89 t, (7.8)
	5' 1.18 d, (7.3)		5' 1.12; 1.15 d, (7.0)	5' 1.13 d, (7.0)	5' 1.15 d, (6.6)
	MesenO:	MesenO:	MesenO:	MesenO:	MesenO:
	2' 5.66 brs	2' 5.63 brs	2' 5.62 brs	2' 5.64 brs	2' 5.63 brs
	4' 2.15 q, (7.3)	4' 2.15 q, (7.4)	4' 2.20 q, (7.0)	4' 2.17 q, (7.4)	4' 2.15 q, (7.0)
	5' 1.02 t, (7.3)	5' 1.07 t, (7.4)	5' 1.08 t, (7.0)	5' 1.07 t, (7.4)	5' 1.06 t, (7.0)
	6' 2.11 brs	6' 2.14 brs	6' 2.15 brs	6' 2.15 brs	6' 2.11 brs

[a] Solvent: acetone-d_6.

[b] Solvent: $CDCl_3$.

Table 2

^{13}C NMR (100MHz) Data of Compounds 1–5

	1 [a]	2 [b]	3 [b]	4 [b]	5 [b]
1	70.8(d)	33.5(t)	71.6(d)	72.2 (d)	41.7 (t)
2	76.0(d)	72.9(d)	199.1(s)	199.8 (s)	212.6 (s)
3	46.2(d)	45.3(d)	136.9(s)	137.7 (s)	56.4 (d)
4	43.4(d)	47.3(d)	38.7(d)	44.6 (d)	45.7 (d)
5	52.2(d)	51.5(d)	44.8(d)	44.2 (d)	48.5 (d)
6	73.9(d)	74.1(d)	71.9(d)	38.4 (t)	72.7 (d)
7	77.2(d)	75.4(d)	69.1(d)	75.5 (d)	72.1 (d)
8	141.8(s)	143.5(s)	73.0(s)	140.2 (s)	142.2 (s)
9	46.2(d)	41.6(d)	45.4(d)	45.2 (d)	40.8 (d)
10	114.2(t)	111.7(t)	48.5(t)	113.5 (t)	113.0 (t)
11	56.9(s)	56.3(s)	56.2(s)	146.7 (s)	54.9 (s)
12	53.6(t)	53.5(t)	51.8(t)	112.8 (t)	52.8 (t)
13	16.8(q)	16.3(q)	23.0(q)	19.1 (q)	15.2 (q)
14	71.0(d)	70.0(d)	139.7(d)	138.5 (d)	68.5 (d)
15	16.8(q)	16.1(q)	15.3(q)	14.9 (q)	15.1 (q)
	AcO:	AcO:			AcO:
	170.5(s)	170.3(s)			170.7(*s*)
	21.2(q)	21.1(q)	MebuO:		21.1(*q*)
	MebuO:	AcO:	(two groups)	MebuO:	MebuO:
	1' 175.7(s)	170.6(s)	1' 175.3;175.7 (s)	1' 175.4 (s)	1' 176.0 (*s*)
	2' 41.9(d)	21.4(q)	2' 41.0; 40.8 (d)	2' 40.9 (d)	2' 41.1 (*d*)
	3' 26.9(t)		3' 26.3; 26.5 (t)	3' 26.7 (t)	3' 26.2 (*t*)
	4' 11.9(q)		4' 11.6; 11.4 (q)	4' 11.4 (q)	4' 11.5 (*q*)
	5' 16.2(q)		5' 16.1; 16.2 (q)	5' 16.4 (q)	5' 16.1 (*q*)
	MesenO:	MesenO:	MesenO:	MesenO:	MesenO:
	1' 166.1(s)	1' 165.9(s)	1' 164.3(s)	1' 165.6 (s)	1' 165.3 (*s*)
	2' 115.6(d)	2' 113.9(d)	2' 113.0(d)	2' 114.4 (d)	2' 113.8 (*d*)
	3' 161.9(s)	3' 163.1(s)	3' 164.7(s)	3' 162.3 (s)	3' 163.1 (*s*)

	1 [a]		2 [b]		3 [b]		4 [b]		5 [b]
4'	34.1(t)	4'	33.8(t)	4'	33.0(t)	4'	33.7 (t)	4'	33.7 (*t*)
5'	12.2(q)	5'	11.8(q)	5'	11.8(q)	5'	11.8 (q)	5'	11.7 (*q*)
6'	18.6(q)	6'	19.0(q)	6'	19.0(q)	6'	18.8 (q)	6'	18.8 (*q*)

[a] Solvent: acetone-d_6.

[b] Solvent: $CDCl_3$.

Table 3

Cytotoxicity Data of Compounds 1–7

compound	EC_{50} (μg/mL)			
	A549	**MCF-7**	**KB**	**KBVIN**
1	>20	10.4	17.4	10.4
2	>20	>20	>20	>20
3	10.6	12.1	4.7	3.7
4	5.0	0.8	3.4	3.2
5	6.2	5.3	4.9	5.7
6	5.2	4.0	4.3	3.3
7	3.6	4.6	4.7	4.9
etoposide	0.4	16.4	3.9	8.8