SHORT PAPER

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Two new sesquiterpenes from the Chinese herb *Halenia elliptica* and their antibacterial and antitumour activity†

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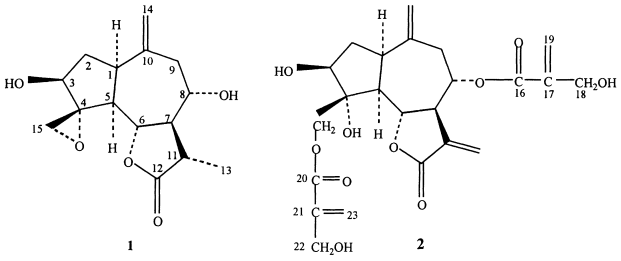
Two new sesquiterpenes halenin A (**1**) and halenin B (**2**) have been isolated from Chinese herb *Halenia elliptica* and their structures established by spectrosiopic methods. Compounds **1** and **2** showed significant antibacterial and antitumour activity.

Keywords: sesquiterpenes, *Halenia elliptica*, antibacterial, antitumour

A number of dihydroxypolymethoxyxanthones which have been isolated from the leaves of *Halenia elliptica* of Chinese origin,[1] have been shown to possess marked hepatoprotective activity.[2] Under a programme of screening Chinese medicinal plants for wide range of biological activity at our laboratory, two new sesquiterpenes were isolated from the acetone extract of this herb. This appears to be the first reported occurrence of sesquiterpenes in the family *Halenia*. We present herein the isolation and structural elucidation of two new sesquiterpenes, halenin A (**1**) and halenin B (**2**), and their antibacterial and antitumour activity.

The chopped dry whole plant of *Halenia elliptica* was extracted with acetone. This was followed by carefully column chromatographic separation giving compounds **1** and **2**. Compound **1** was obtained as colourless gum, $[\alpha]^2{}_D{}^4$ +43.5°(c 1.1, $CHCl_3$). HREIMS gave a molecular ion peak at *m*/*z* 280.1305 corresponding to the molecular formula $C_{15}H_{20}O_5$ (calcd. 280.1301). Its IR spectra showed absorption bands for hydroxyl groups (3470 cm^{-1}), double bonds (1645 cm^{-1}) and carbonyl groups (1785 cm^{-1}). The ^1H NMR spectrum exhibited a doublet methyl signal at δ 1.18 (3H, *J*=7.5 Hz), exomethylene proton signals at δ 5.18 (1H, s); δ 4.87(1H, s), and five protons on oxygenated carbons at δ 4.52 (t, *J* = 10.0 Hz), 3.98 (br), 3.79 (m), 3.31 (*J* = 12.0 Hz) and 3.07 (d, *J* = 12.0 Hz), respectively. The ^{13}C NMR spectrum of **1** showed signals for 15 carbons, and the DEPT spectra indicated the presence of one methyl, four methylenes, seven methines, and three quaternary carbons. Taking into account of the degree of unsaturation of 6, compound **1** was considered to be a guaiane-type sesquiterpene derivative[3–5] with an epoxy ring, a lactone carbonyl group and an exomethylene (Scheme 1). A comparison of the chemical shifts and multiplicities of the ^1H and ^{13}C NMR signals of compound **1** and 3 β, 8 α-dihydroxy-11 β H-11,13-dihydrocostuslactone,[6] a known guaiane-type sesquiterpene, showed that the signals were closely related except for the absence of the signals for an exomethylene double bond in compound **1**. These appeared instead as two doublets for two hydrogens at δ 3.31 and δ 3.07. This was further supported by the ^{13}C NMR spectral data which showed the presence of two olefinic carbons in compound **1** while there are four such carbons in 3 β, 8 α-dihydroxy-11 β H-11,13-dihydrocostuslactone. A survey of the literature revealed that the two doublets at δ 3.31 and δ 3.07 may be attributed to the presence of a 15-methylene when the 4,15 double bond is epoxidised with an δ-oriented oxirane ring at C-4[7]. Hence the structure of this compound could be depicted as **1**. Confirmation of this structure was established by 2D NMR experiments. The ^1H-^1H COSY and HMQC spectra confirmed correlations of-$CH(3)$–$CH_2(2)$–$CH(1)$–$CH(5)$ –$CH(6)$–$CH(7)$–($CH(11)$–$CH_3(13)$)–$CH(8)$–$CH_2(9)$–, and showed allylic couplings between H-1, H-9 and H-14. HMBC spectra showed long range correlations for C-4/H-3, H-5, H-15; C-10/H-1, H-9, H-14 and C-12/H-11, H-13. The NOESY spectrum exhibited clear correlations between H-1 and H-3, H-3 and H-5, H-5 and H-7, H-6 and H-8, H-6 and H-11, H-8 and H-13. Therefore, the structure of **1** was confirmed as 4α, 15-epoxy-8α-hydroxy-11α,13-dihydrozaluzanin C and named as halenin A.

Compound **2** was colourless needles, $[\alpha]^2{}_D{}^4$ +33.4 (c 0.70, $CHCl_3$). HREIMS gave a molecular ion peak at *m*/*z* 464.1686 corresponding to the molecular formula $C_{23}H_{28}O_{10}$ (calcd. 464.1682). Its IR spectrum showed the presence of an α, β-unsaturated-γ-lactone, ν_{max} 1763 cm^{-1}, and this was further confirmed from the IR bands at 1660, 1405 and 810 cm^{-1}, which are typical of this conjugated moiety.[8] The ^1H NMR spectrum displayed two characteristic low-field doublets (δ 5.52 and 6.16, *J* = 3.4Hz), which are due to the protons of an exomethylenic double bond in conjugation with a lactone carbonyl. The 1D (^1H, ^{13}C / DEPT) and 2D (COSY, HMQC, HMBC) NMR spectra of **2** were similar to those of **1** except the absence of the methyl group and the epoxide, and the appearance of a new exomethylenic double bond and two hydroxymethylenelacrylate. The location of two hydroxymethylenelacrylate were assigned to C-8 and C-15 based on



Scheme 1

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† This is a Short Paper, there is therefore no corresponding material in *J Chem. Research (M)*.

Table 1 Antitumour activity of halenin A and halenin B[a]

	SMMC-7721	B16	Hela
Halenin A	85.1 ± 2.1	87.2 ± 3.3	84.9 ± 4.1
Halenin B	65.6 ± 1.5	70.1 ± 2.4	68.4 ± 3.1
Vincristine	63.2 ± 1.8	70.7 ± 2.8	67.2 ± 2.2

[a]Activities are expressed as IC_{50} (50 % inhibitory concentration) in μg/ml.

Table 2 Antibacterial activity of halenin A and halenin B[a]

	B. subtilis	*E. coli*	*S. aureus*
Halenin A	12.5	14.3	13.5
Halenin B	11.8	13.4	14.8
Chloramphenicol	14.5	14.9	15.1

[a]Activities are expressed as the diameter of the inhibitory zone in mm.

the HMBC spectrum which showed correlation of C-16/ H-8, H-18, H-19; C-20/ H-15, H-22, H-23. Therefore, the structure of **2** was assigned to be 8a**,** 15-dihydroxymethylenelacrylate-dihydrozaluzanin C and named as halenin B.

Halenin A and B exhibited significant *in vitro* cytotoxic activity against human hepatoma cells (SMMC-7721), human uterine cervix carcinoma cells (Hela) and mouse melanotic carcinoma cells (B16) (Table 1), as well as antibacterial activity against *B. subtilis, E. coli* and *S. aureus* (Table 2). It is seen from Tables 1 and 2 that the antitumour activity of halenin B is comparable to that of antitumour drug vincristine, and the antibacterial activity of both halenin A and B is comparable to chloramphenicol.

Experimental

Optical rotation was measured on a Perkin-Elmer 241 polarimeter. The IR spectra were taken on a Nicolet 170SX IR spectrometer. ^1H, ^{13}C and 2D NMR spectra were recorded on a Bruker AM 400 NMR spectrometer with TMS as internal standard. HREIMS spectra were obtained on a VG ZAB-HS mass spectrometer.

Extraction and isolation procedure: The whole plant of *Halenia elliptica* was collected in the suburb of Lanzhou city, Gansu, China. The chopped whole plant material (2.5 kg) was extracted repeatedly (3 times, 7 days each time) with acetone at room temperature to give a residue (55g) after evaporation. This residue was separated by silica gel (200–300 mesh) column chromatography with a gradient elution of petroleum ether-acetone (20:1, 15:1, 10:1, 5:1, 3:1, 1:1, 0:1). The gummy crude extract containing **1** and **2** was obtained from the fraction of petroleum ether-acetone (5:1) and subjected to gel filtration (Sephadex, LH-20) followed by silica gel (200–300 mesh) column chromatography eluted with petroleum ether–AcOEt (4:1) to give **1** (20 mg) and **2** (25 mg).

Halenin A (**1**): colourless gum, $[\alpha]^2{}_D{}^4$ +43.5°(c 1.1, $CHCl_3$); HREIMS: M^+ Found: 280.1305, Calcd for $C_{15}H_{20}O_5$: 280.1301; ν_{max} / cm^{-1}: 3470, 1785, 1645; δ_H (400 MHz, $CDCl_3$, TMS): 5.18 s (H-14a), 4.87 s (H-14b), 4.52 t, *J*=10.0 Hz (H-6), 3.98 br (H-3), 3.79 m (H-8), 3.36 m (H-1), 3.31 d, *J*=12.0 Hz (H-15a), 3.07 d, *J*=12.0 Hz (H-15b), 2.84 dd, *J*=12.0, 5.0 Hz (H-9α), 2.71 dq, *J*=7.0, 7.5 Hz (H-11), 2.37 ddd, *J*=11.1, 10.0, 7.0 Hz (H-7), 2.30 m (H-2β), 2.19 dd, *J*=12.5, 8.5 Hz (H-9β), 2.08 dd, *J*=10.0, 9.0 Hz (H-5), 1.69 m (H-2α), 1.18 d, *J*=7.5 Hz (3H, H-13); δ_C (100MHz, $CDCl_3$, TMS) (C-1 to C-15): δ46.27, 37.99, 75.50, 68.58, 53.18, 76.71, 55.20, 75.65, 40.20, 144.53, 42.63, 178.45, 15.69, 114.65, 48.58.

Halenin B (**2**): colourless needles, m.p.199–200°C; $[\alpha]^2{}_D{}^4$ +33.4° (c 0.70, $CHCl_3$); HREIMS: M^+ Found: 464.1686, Calcd for $C_{23}H_{28}O_{10}$: 464.1682; ν_{max} / cm^{-1}: 3450, 1763, 1660, 1405, 810 cm^{-1}; δ_H (400 MHz, $CDCl_3$, TMS): 6.16 d, *J*=3.4Hz (H-13a), 5.52 d, *J*=3.4Hz (H-13b), 6.33 br (H-19a), 6.25 br (H-23a), 5.96 br (H-19b), 5.90 br (H-23b), 5.17 s (H-14a), 5.03 m (H-8), 4.96 s (H-14b), 4.56 dd, *J*=11.0, 10.5 Hz (H-6), 4.38 br (H-18), 4.35 br (H-22), 4.18 m (H-3), 4.03 d, *J*=10.0Hz (H-15a), 3.96 d, *J*=10.0Hz (H-15b), 3.38 m (H-1), 3.20 m (H-7), 2.79 dd, *J*=14.5, 5.0 Hz (H-9β), 2.45 m (H-2β), 2.33 dd, *J*=14.5, 2.3 Hz (H-9α), 2.26 dd, *J*=11.0, 8.0 Hz (H-5), 1.78 m (H-2α); δ_C (100MHz, $CDCl_3$, TMS) (C-1 to C-23): 45.50, 36.78, 76.38, 84.72, 56.86, 76.69, 46.69, 74.28, 36.08, 142.25, 136.84, 168.69, 122.81, 117.64, 66.07, 165.08, 139.80, 62.36, 126.68, 166.31, 140.12, 63.05, 127.18.

Cytotoxicity assay: The cytotoxicity of halenin A and B was tested in three cell lines: SMMC-7721 (human hepatoma), B16 (mouse melanoma) and Hela (human carcinoma of uterine cervix). Cells were cultured at 37 °C under a humidified atmosphere of 5% CO_2 in RPMI 1640 medium supplemented with 10% fetal calf serum and dispersed in replicate 96-well plates with 1×10^4 cells/well for 24 hours. halenin A or halenin B (10–400 μmol/l) were then added. After 48-h exposure to the toxins, cell viability was determined by the methylthiazolyltetrazolium bromide (MTT) colorimetric assay[9] by measuring the absorbance at 595 nm with an ELISA reader.

Antibacterial assay: The paper-disk method[10] was used for antimicrobial tests. A 10 μg portion of halenin A, halenin B or chloramphenicol (used as positive control) was applied onto a paper disk, and the paper disk was air-dried. Then the disks were placed on agar plates that had been seeded with *B.subtilis*, *E.coli* and *S.aureus*, respectively, and incubated at 37°C for 24h. The antibacterial activity was determined by measuring the diameter of the inhibitory circles. Each test was performed in duplicate.

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