Cytotoxic sesquiterpene lactones from *Berlandiera lyatra*

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Abstract: Objective To study the antitumor natural products from *Berlandiera lyatra*. **Methods** Compounds were isolated by liquid/liquid partition and chromatography on Sephadex LH20 and silica gel columns. All process of the fractionation were run with the guidance of cytotoxicity bioassay by MTT method against three human cancer cell lines. The chemical structures of bio-active compounds were identified on the basis of spectroscopic data. **Results** From the $CHCl_3$-soluable fraction of the title plant, two cytotoxic compounds were isolated. They were determined as 3α-epoxypumilin and pumilin. **Conclusion** 3α-epoxypumilin and pumilin were responsible major compounds for the cytotoxicity in the methanolic extract of the plant. Both compounds were isolated from the *B. lyatra* and were reported to be of antitumor activity for the first time.

Key words: *Berlandiera lyatra* Benth.; 3α-epoxypumilin; pumilin; cytotoxicity

Berlandiera lyatra 中具细胞毒活性的倍半萜内酯

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摘 要:目的 研究 *Berlandiera lyatra* 中的抗肿瘤活性化学成分。**方法** 以 MTT 法进行抗肿瘤活性化学成分的追踪,利用硅胶柱色谱和 Sephadex-LH-20 分离化合物,用理化和光谱数据确定化合物结构。**结果** 从 *Berlandiera lyatra* 的 $CHCl_3$ 活性部位中,分离得到了两个细胞毒活性化合物,并确定其为 3α-环氧短小伯兰菊素(3α-epoxypumilin)和短小伯兰菊素(pumilin)。**结论** 3α-环氧短小伯兰菊素和短小伯兰菊素是该植物抗肿瘤的主要活性成分,也是首次从该植物中分离得到的抗肿瘤活性成分。

关键词:*Berlandiera lyatra* Benth.; 3α-环氧短小伯兰菊素;短小伯兰菊素;细胞毒活性

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1 Introduction

Berlandiera lyatra Benth. (Asteraceae), common name chocolate flower or chocolate daisy, is a perennial herbal plant and distributed in southwest USA, Texas State and northwestern Mexico. Its common name revealed its characteristic of yellow daisy-like flowers and a subtle smell of chocolate. In the course of our search for potential novel antitumor agents from desert plants, an extract of *B. lyatra* was found to show reproducible, significant cytotoxicity to three human cell lines tested. Therefore, we had further investigated the cytotoxic constituents by bioassay-guided fractionation, and finally led to the isolation of two known cytotoxic guaianolide-type sesquiterpene lactone compounds, namely, 3α-epoxypumilin (Ⅰ) and pumilin (Ⅱ). Although there were a few of research reports focusing on the guaianolide-type sesquiterpene from the plants of genus *Berlandiera* and its relative genus[1-4], the researches on phy-

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tochemical investigation on anti-tumor activity of the constituents from *B. lyatra* haven't been reported except for the brief mention of its major constituents in a literature[1].

2 Experiment

2.1 General procedures

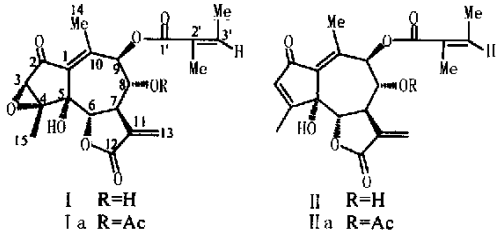
IR Data were recorded on Shimadzu FTIR－8300 spectro-meter with KBr plates; ^{1}H-NMR spectra were recorded on a Varian Unity 300 spectrometer in $CDCl_3$ and pyridine-d_5 with residual solvent as internal standard. Mass spectra were determined with Shimadzu LCMS－AQ 8000 on Supelco (25 cm×2.1 mm, 5 μm) Discovery® C_{18} column, using APCI (+) and APCI (－) or ESI (+) and ESI (－) mode under 3.5 kV or 4.5 kV for ionization. Sephadex LH-20 (Sigma) was employed for gel permeation chromatography. Lower pressure chromatography was performed using Baker silica gel (40 μm) with nitrogen gas to maintain pressure. Merk 25 TLC aluminium sheets (20 cm × 20 cm, silica gel 60 F254) were used for preparative TLC. MeOH, Methyl ethyl ketone (MEK), hexane, dichloromethane (DCM), and other solvents used in the experiment were all GR grade.

2.2 Plant material

The total plant of *B. lyatra* was collected from the Sonoita area, Santa Cruz and Pima County's Anta Rita Mountains, AZ, USA. The identification of the species and collection of plant material were carried out by Dr. Steve McLaughlin and Mrs. Betsy Lewis at Southwestern Center of Natural Products Research and Commercialization, the University of Arizona. A voucher specimen (No. SPM 8144) was kept in the center.

2.3 Extraction and isolation

Aerial plant material was placed in a drying room for three days to remove all moisture. Upon drying, the plant material was ground in a Wiley mill to yield powdered materials (351.5 g). Ground material (100 g) was sequentially extracted twice with 24 h soaks in hexane, MEK, and methanol, respectively. Extracts were evaporated and dried *in vacuum* to yield three initial extracts: Hexane extract (1.656 g), MEK extract (2.154 g), and methanol extract (11.04 g). These extracts were tested in cell line assay. MEK extract was found to be active and further partitioned in hexane/80% aqueous MeOH, then chloroform/60% aqueous MeOH for 80% aqueous MeOH portion. Hexane portion (residue 588.6 mg), chloroform portion (residue 1.289 g) and 60% aqueous MeOH portion (residue 0.278 g) were retested in cell line assay. Chloroform portion indicated cytotoxicity and supplied to further separation. Bioactive chloroform portion (1.0 g) was subject to 15 g Sephadex LH-20 column for permeation, eluting with 300 mL of DCM/hexane (4 : 1), DCM/acetone (3 : 2), DCM/acetone (1 : 4), and DCM/MeOH (1 : 1), collecting fractions in 15 mL/fraction. Based on TLC pattern of fractions in normal silica gel 60 F_{254} plate in DCM/MeOH (100 : 6) and isopropanol/hexane (20 : 100), similar fractions on TLC were combined and supplied for bioassay after evaporating and drying *in vacuum*. Combined fractions 9－14 and 28－30 were found to be active and used to be further purified. The separation and purification of the two combined fractions by silica gel column (eluting with isopropanol/hexane 1 : 10) and preparative TLC (EtOAc/hexane 1 : 2) led to the isolation of 3α-epoxypumilin (Ⅰ) (65 mg) and pumilin (Ⅱ) (8.1 mg). The structures were elucidated by spectroscopic method (HPLC-MS, ^{1}H-NMR) and chemical derivation (acetyl derivation), and comparison of their NMR data with the data previously reported. The chemical structures are seen in Fig. 1.



Ⅰ R=H
Ⅰa R=Ac

Ⅱ R=H
Ⅱa R=Ac

Fig. 1 Chemical structures of 3α-epoxypumilin (Ⅰ) and pumilin (Ⅱ)

3 Identification

Compound Ⅰ: Colorless solid; IR ν_{max}^{KBr} cm^{-1}:

3 421.5, 2 903.1, 1 772.5, 1 714.9, 1 678.0, 1 620.1, 1 407.9, 1 263.3, 1 230.5, 1 110.9, 977.8; HPLC-MS (80% MeOH/H_2O, 0.2 mL/min): t_R = 5.34 min, m/z 391 ([M+H]$^+$, [ESI (+)]; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.21 (4H, m, H-9, 13a, 13b, 3′), 3.90 (3H, m, H-6, 7, 8), 3.80 (1H, s, H-3), 2.20 (3H, s, Me-10), 2.01 (3H, s, Me-2′), 1.97 (3H, d, J=5.6 Hz, Me-3′), 1.79 (3H, s, Me-4); ^{1}H-NMR (300 MHz, pyridin-d_5) δ 7.07 (1H, d, J=9.9 Hz, H-9), 6.51 (1H, dd, J=2.7, 1.0 Hz, H-13b), 6.41 (1H, dd, J=2.7, 1.0 Hz, H-13a), 5.90 (1H, dq, J=7.5, 1.5 Hz, H-3′), 4.56 (1H, dd, J=11.0, 3.7 Hz, H-7), 4.20 (1H, d, J=11.0 Hz, H-6), 4.12 (1H, ddt, J=9.9, 3.7, 1.0 Hz, H-8), 3.84 (1H, s, H-3), 2.41 (3H, brs, H-14), 1.97 (3H, d, J=7.5 Hz, Me-3′), 1.89 (3H, s, H-15), 1.88 (3H, s, Me-2′).

The preparation of 8-acetyl-3α-epoxypumilin (Ⅰa): a mixture of Ⅰ (5 mg), acetic anhydride (0.3 mL), and pyridine (0.3 mL) was kept staying overnight at room temperature (25 ℃) and then evaporated under reduced pressure to give a residue. The residue was chromatographed on 20 cm × 12.3 cm silica gel 60 F_{254} TLC aluminum sheet for preparation and purification of its acetate with DCM/MeOH (10 ∶ 1) as developing solvent to give Ⅰa (1.2 mg). HPLC-MS (80% MeOH/H_2O, 0.3 mL/min): t_R = 3.24 min, m/z 433 {[M+H]$^+$, [APCI (+)]}, 431 {[M−H]$^-$, [APCI (−)]}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.37 (1H, d, J=9.9 Hz, H-9), 6.23 (1H, d, J=7.5 Hz, H-3′), 6.18 (1H, d, J=3.0 Hz, H-13a), 5.45 (1H, d, J=3.0 Hz, H-13b), 5.26 (1H, dd, J=9.9, 6.8 Hz, H-8), 4.10 (1H, dd, J=10.9, 6.8 Hz, H-7), 3.96 (1H, d, J=10.9 Hz, H-6), 3.56 (1H, s, H-3), 2.21 (3H, s, H-14), 2.07 (3H, s, OAc-8), 2.02 (3H, s, Me-3′), 1.88 (3H, s, Me-2′), 1.80 (3H, s, H-15). The data of Ⅰ and Ⅰa were consistent with the those of 3α-epoxypumilin[1] and 8-acetyl-3α-epoxypumilin[2], respectively.

Compound Ⅱ: Colorless crystal (acetone), IR ν_{max}^{KBr} cm^{-1}: 3 448.5, 2 903.1, 1 772.5, 1 710.7, 1 678.0, 1 635.5, 1 616.2, 1 377.1, 1 271.0, 1 230.5, 1 157.2, 1 045.3, 977.8; HPLC-MS (70% MeOH/H_2O, 0.3 mL/min): t_R=4.29 min, m/z 375 {[M+H]$^+$, [APCI (+)]}, 373 {[M+H]$^-$, [APCI (−)]}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.22 (4H, m, H-3, 13a, 13b, 3′), 6.06 (1H, d, J=7.1 Hz, H-9), 3.90 (3H, m, H-6, 7, 8), 2.31 (3H, s, Me-4), 2.27 (3H, s, Me-10), 2.03 (3H, s, Me-3′), 2.00 (3H, s, Me-2′); ^{1}H-NMR (300 MHz, pyridine-d_5) δ: 6.96 (1H, d, J=9.6 Hz, H-9), 6.59 (1H, dd, J=3.3, 1.5 Hz, H-13b), 6.44 (1H, dd, J=3.3, 1.5 Hz, H-13a), 6.31 (1H, d, J=1.2 Hz, H-3), 5.94 (1H, qq, J=7.2, 1.2 Hz, H-3′), 4.50 (1H, dd, J=10.0, 3.0 Hz, H-7), 4.22 (1H, m, H-8), 4.10 (1H, d, J=10.0 Hz, H-6), 2.60 (3H, s, H-14), 2.39 (3H, s, H-15), 2.00 (3H, q, J=7.2 Hz, Me-3′), 1.97 (3H, brs, Me-2′).

The procedure of preparation of 8-acetyl-pumilin (Ⅱa) was similar to that of Ⅰa: pumilin (1.6 mg) was used to yield Ⅱa (0.7 mg). HPLC-MS (70% MeOH/H_2O, 0.3 mL/min): t_R = 6.47 min, m/z 417 {[M+H]$^+$, [APCI (+)]}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 6.28 (1H, d, J=1.5 Hz, H-3), 6.25 (1H, d, J=3.0 Hz, H-13a), 6.22 (1H, d, J=5.6 Hz, H-3′), 6.03 (1H, d, J=13.5 Hz, H-9), 5.51 (1H, d, J=3.0 Hz, H-13b), 5.27 (1H, dd, J=13.5, 3.6 Hz, H-8), 4.03 (1H, dd, J=10.8, 3.6 Hz, H-7), 4.09 (1H, d, J=10.8 Hz, H-6), 2.31 (3H, s, H-14), 2.26 (3H, s, H-15), 2.07 (3H, s, OAc-8), 2.02 (3H, d, J=5.6 Hz, Me-3′), 1.89 (3H, s, Me-2′). The data of Ⅱ and Ⅱa were consistent with those of pumilin[1] and 8-acetyl-pumilin[2], respectively.

4 Bioassay

Assay for cytotoxicity against NCI-H 460 (human lung cancer), MCF-7 (human breast cancer), and SF-268 (human central nerve system cancer) was performed following the general process. Fractions and pure compounds Ⅰ and Ⅱ were assayed with compounds in DMSO and run against taxol as positive control. Cancer cell lines were in-

cubated in 96-well plates for 72 h before addition of MTT. Well absorbances (λ 490 nm) were corrected for background and expressed as percentage of the negative control (DMSO only).

5 Results

Bioassay of the MEK extract from *B. lyatra* exhibited significant activity against three human cancer cell lines: MCI-H460, MCF-7, and SF-268. Meanwhile, the hexane extract before MEK extraction and the MeOH extract after MEK extraction from the same plant material showed no activity against the above three cell lines in 100 μg/mL concentration. Bioactivity-directed fractionation of the MEK extract from *B. lyatra* led to the isolation of the known guaianolide-type sesquiterpene lactones 3α-epoxypumilin (Ⅰ) and pumilin (Ⅱ) as major bioactive constituents responsible for the cytotoxicity. The results of bioassay on the initial extracts, partitioned portions, and fractions from chromatography during our research progress were presented in Table 1. Both compounds showed activity against NCI-H460, MCF-7 and SF-268. The IC_{50} value of compounds Ⅰ and Ⅱ against the three cell lines was listed in Table 2. They did not indicate any selectivity on the cytotoxicity among three cell lines. But compound Ⅰ with lower polarity showed more activity than compound Ⅱ with higher polarity.

Compounds Ⅰ and Ⅱ were earlier isolated from *B. pumilin*, *B. lexana* in family Asteraceae then from *Montanoa tomentosa* subsp. *xanthifolia* and *M. tomentosa* subsp. *rosei* in relative family[1]. Guaianolide-type sesquiterpenes appeared to be common in genus *Berlandiera*, and there were more investigations on their chemistry[1-4]. However, this is the first report of the isolation of Ⅰ and Ⅱ in a bioassay-guided fractionation of a crude plant extract, and no antitumor results of Ⅰ and Ⅱ have been reported yet before.

Table 1 Bioactive data of *B. lyatra* extracts, partitioned portions, and fractions from chromatography against three human cancer cell lines

Sample	Concentration/ (μg·mL^{-1})	Inhibitory rate/%		
		NCI-H460	MCF-7	SF-268
Hexane Extract[a]	100	2.2	10.1	17.8
MEK Extract[a]	100	74.0	92.7	88.3
MeOH Extract[a]	100	−44.9	−0.9	0.1
Hexane portion[b]	10	21.4	35.8	5.1
$CHCl_3$ portion[b]	10	49.4	81.8	59.8
60% MeOH portion[b]	10	22.1	37.1	30.5
Combined fractions 9-14[c]	10	89.0	90.4	77.4
Combined fractions 28-30[c]	10	88.0	91.7	73.6
Taxol	0.1 (μmol·L^{-1})	87.0	88.5	92.3

a, b, and c: samples were assayed in different time

Table 2 IC_{50} Values of compounds Ⅰ and Ⅱ against three human cell lines

Compound	IC_{50}/(μmol·L^{-1})		
	NCI-H460	MCF-7	SF-268
Ⅰ	3.3	2.7	3.1
Ⅱ	16.1	15.4	20.8

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