Antiproliferative Sesquiterpene Lactones from the Roots of *Inula helenium*

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The MeOH extract of the roots of *Inula helenium* showed a high inhibitory activity for cell growth against MK-1, HeLa and B16F10 cell lines. Significant activity was found in the hexane-soluble fraction. From the hexane-soluble fraction, seven sesquiterpenes, namely, one germacrane (4β,5α-epoxy-1(10),11(13)-germacradiene-8,12-olide), one elemane (igalane), and five eudesmanes (alantolactone, isoalantolactone, 11α,13-dihydroalantolactone, 11α,13-dihydro-isoalantolactone, 5-epoxyalantolactone) were isolated. *In vitro* antiproliferative activities of the isolates against MK-1, HeLa and B16F10 cells are reported.

Key words *Inula helenium*; Compositae; antiproliferative activity; sesquiterpene lactone; igalan; alantolactone

Inula helenium (Compositae) is a widely occurring perennial herb in Europe and East Asia. Its roots have been traditionally used as a diaphoresis and a diuretic expectorant agent in Europe, as a fragrance agent for home medicines in Japan,[1] and as agents of tuberculotic enterorrhea, chronic enterogastritis and bronchitis and a preservative in China.[2] Native Americans used infusion and decoctions of this roots to treat lung disorders and against tuberculosis.[3] The investigation of the genus *Inula* has shown the sesquiterpene lactone and essential oil groups,[4—6] and some phenolic acids and flavonoids were evaluated as other constituents of this genus.[7,8] Many sesquiterpenes were isolated from *Inula helenium*.[9—11] The principal ingredient, alantolactone has strong anthelmintic and antibacterial activities.[9]

As part of our screening for antiproliferative constituents in natural resources, we examined the roots of *I. helenium* grown in Tibet. The MeOH extract of the roots of this plant showed antiproliferative activities against three tumor cell lines: human gastric adenocarcinoma cells (MK-1), human uterus carcinoma (HeLa) and mouse melanoma (B16F10) cells.

The MeOH extract was partitioned with *n*-hexane, $CHCl_3$, EtOAc, and then *n*-BuOH. The *n*-hexane fraction showed strong antiproliferative activity against MK-1, HeLa and B16F10 cells, while the EtOAc and *n*-BuOH fractions had very low activity as shown in Fig. 1. The *n*-hexane fraction was fractionated in the manner described in the Materials and Methods section, and seven sesquiterpenes were isolated. The antiproliferative activities of the fractions and seven isolated compounds against MK-1, HeLa, and B16F10 cell lines will be discussed.

MATERIALS AND METHODS

Material Dried roots were obtained from Mr. Hiroyuki Kaito of Soma Co., 7–9–1 Tanimati, Tyuo-ku, Osaka 542, Japan on December 7, 1999. A voucher specimen (KPU-001952) is deposited in the herbarium of the Department of Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University, Japan.

Cells MK-1 cells were provided by Prof. M. Katano of Faculty of Medicine, Kyushu University, and HeLa and B16F10 cells were supplied by the Cell Resource Center of the Biomedical Research Institute of Development, Aging and Cancer, Tohoku University.

Determination of Antiproliferative Activity Inhibition of the cellular growth was estimated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay described by Mosmann.[12] The detailed procedure is shown in the previous paper.[13]

Extraction and Isolation The dried roots (1.5 kg) of *Inula helenium* grown in Tibet were extracted with MeOH at room temperature for 2 weeks. The MeOH extract was then filtered and evaporated under reduced pressure to obtain a viscous mass (555.1 g). This material was suspended with H_2O and partitioned with *n*-hexane, $CHCl_3$, EtOAc and *n*-BuOH to give each organic fraction, respectively. The antiproliferative activities (GI_{50} μg/ml) of these organic fractions are shown in Fig. 1. The H_2O fraction exhibited no activity.

The *n*-hexane fraction (142 g) was subjected to column chromatography on silica gel (800 g) to give 7 fractions using an increasing polarity solvent system (*n*-hexane–EtOAc gradient and MeOH) as follows: fraction 1 (fr. 1, 44.2 g), fr. 2 (34.5 g), fr. 3 (25.4 g), fr. 4 (7.9 g), fr. 5 (2.4 g), fr. 6 (2.7 g), fr. 7 (15.9 g). Fraction 3 was chromatographed over silica gel [*n*-hexane–EtOAc (20 : 1→10 : 1→5 : 1→1 : 1)→EtOAc→MeOH] to give eight fractions (frs. 3-1—3-8). Fraction 3-2 (1.3 g) was separated by silica gel [*n*-hexane–EtOAc (4 : 1)] and reversed-phase silica gel column chromatography [Cosmosil 75C_{18}-open (Nacalai Tesque Co., Ltd.), MeOH–H_2O (3 : 2)] to give compound **2** (22.8 mg). Fraction 3-3 (4.5 g) was separated by reversed-phase silica gel column chromatography [MeOH–H_2O (3.5 : 1)→MeOH] and recycled HPLC [Jaigel 310 (250×20 mm i.d.×2, Nihonbunseki Kogyo Co., Ltd.), MeOH] to give compounds **4** (830 mg) and **5** (486.5 mg). Fraction 3-5 (1.05 g) was separated by silica gel column chromatography [*n*-hexane–EtOAc (4.5 : 1)] to give compound **6** (25.4 mg). Fraction 3-7 (690 mg) was separated by silica gel column chromatography [$CHCl_3$–*n*-hexane (3 : 1)] to afford compound **1** (10.4 mg). Fraction 4 was subjected to silica gel column chromatography [*n*-hexane–EtOAc (8 : 1→3 : 1→1 : 1)→EtOAc→MeOH] to give seven fractions (frs. 4-1—4-7). Fraction 4-2 (1.05 g) was separated

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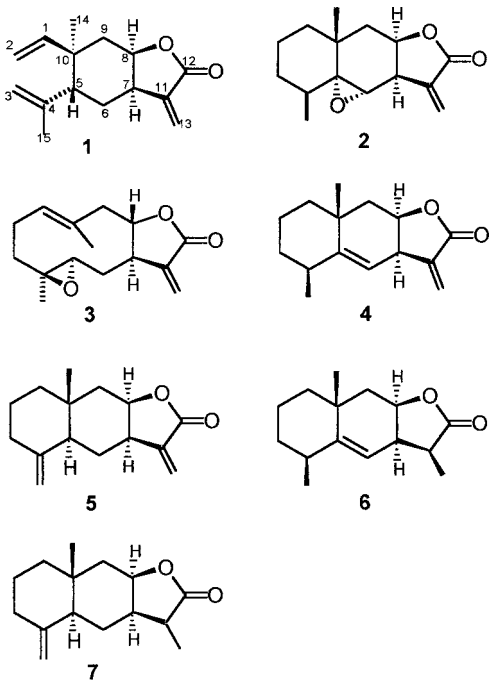
by reversed-phase silica gel column chromatography [MeOH–H_2O (3 : 2→6 : 1)] and silica gel column chromatography [*n*-hexane–EtOAc (3 : 1)] to give compound **3** (36.7 mg). Purification of fraction 4-3 (800 mg) on silica gel column chromatography using *n*-hexane–EtOAc (3 : 1) gave **7** (56.4 mg).

RESULTS AND DISCUSSION

^{1}H- and ^{13}C-NMR spectra of **1** exhibited signals from three olefinyl carbon groups [δ_C 113.0, 145.7, δ_C 111.0, 147.3, and δ_C 137.3, 120.4], two methyl protons [δ_H 1.04 (d, J=0.5 Hz) and 1.71 (dd, J=0.7, 1.6 Hz)] and four methylene protons [δ_H 1.28 (dd, J=10.6, 13.5 Hz), 1.93 (ddd, J=0.7, 6.0, 13.5 Hz), 1.95 (m), 2.16 (ddd, J=6.5, 12.4, 13.5 Hz), 4.68 (dd, J=0.7, 0.7 Hz), 4.90 (dd, J=1.6, 1.6 Hz), 4.89 (dd, J=0.9, 17.5 Hz), and 4.93 (dd, J=0.9, 10.8 Hz)]. From distortionless enhancement by polarization transfer (DEPT), heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond connectivity (HMBC) experiments, the structure of **1** was deduced as 1,3,11(13)-elematrien-8,12-olide. The relative stereochemistry was elucidated by nuclear Overhauser effect (NOE). Thus, **1** was identified as 1,3,11(13)-elematrien-8β,12-olide, igalan.[14,15] ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **4** except for the chemical shifts of C-5 (δ_C 67.4) and C-6 [δ_C 61.1, δ_H 2.92 (d, J=2.5 Hz)] in **2**. The NOE experiment suggested that the H-8 and H-7 were cis conformation and C-15 methyl group was oriented at β. This confirmed that the structure of **2** was 5α-epoxyalantolactone.[9] The ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY) experiment enabled spectral assignment and identification of the rough structure of **3**. Compound **3** was assigned as 4β,5α-epoxy-1(10),11(13)-germacradiene-8,12-olide[16] by HMBC and NOE experiments. This is the first time **1**—**3** has been isolated from *I. helenium*. Compounds **4**—**7** were identified as alantolactone,[17—21] isoalantolactone,[20,21] 11α,13-dihydroalantolactone,[22,23] and 11α,13-dihydroisoalantolactone[9,17—19] by comparison of their spectral data with those reported in the literature, respectively.



The MeOH extract of the root of *Inula helenium* was partitioned with *n*-hexane, $CHCl_3$, EtOAc and then *n*-BuOH. Activity-guided fractionation of MeOH extract led to the isolation of seven sesquiterpenes, **1**—**7** from the *n*-hexane fraction. The antiproliferative activity of each fraction and isolate against three tumor cell lines, MK-1, HeLa and B16F10 was determined by MTT assay,[12] and their 50% growth inhibition

Table 1. The Antiproliferative Activity (GI_{50}, μM) against MK-1, HeLa, and B16F10 Cell Lines *in Vitro* (n=4)

Compound	MK-1	HeLa	B16F10
1	6.9	13	4.3
2	6.9	6.5	3.6
3	12	33	14
4	6.9	6.9	4.7
5	44	41	29
6	>427	>427	>427
7	>427	>427	44
5-Fu	19.2	12.3	1.1

5-Fu (5-Fluorouracil) was used as a positive control.

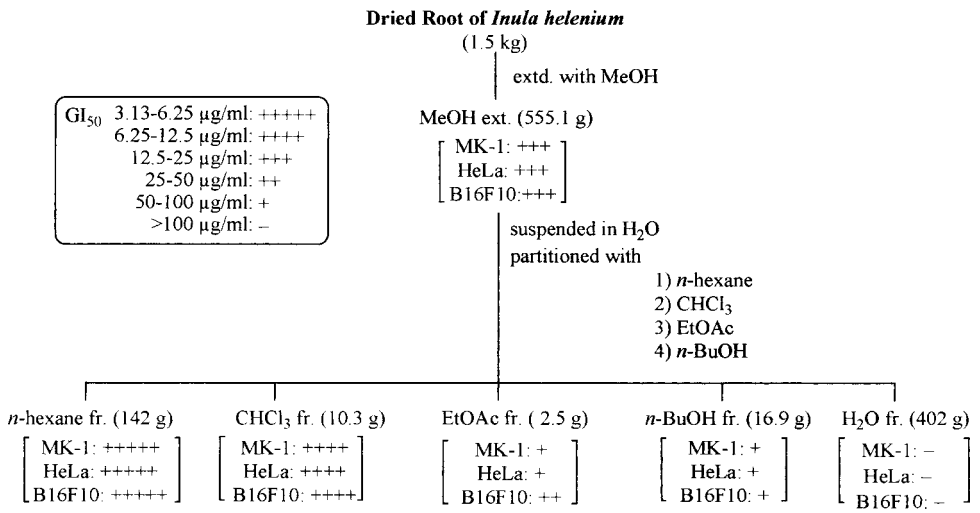


Fig. 1. Fractionation Scheme of MeOH Extract of *Inula helenium* and Antiproliferative Activities

(GI_{50}, μg/ml) values are listed in Fig. 1 and Table 1.

The *n*-hexane fraction showed strong antiproliferative activity against MK-1, HeLa and B16F10 cells followed by $CHCl_3$, while the EtOAc and *n*-BuOH fraction had a very low activity (Fig. 1). The water fraction did not show the antiproliferative activity for any cell lines. This suggests that the aliphatic compounds exhibited the antiproliferative activity.

The antiproliferative activities of compounds **2** and **4** were stronger than those of other sesquiterpenes (**1**, **3**, **5**—**7**). The activity against MK-1 and B16F10 of **1** exhibited almost the same potency as **2** and **4**, but that of **1** against HeLa cells showed about half those of **2** and **4**. The GI_{50} values of compounds **3** and **5** against the three cell lines are larger than those of **1**, **2** and **4**. The 11,13-dihydro compounds, **6** and **7** were inactive ($>427\,\mu$M) except for the activity (GI_{50} values: $44\,\mu$M) of **7** against B16F10. Compounds **1**—**5** which possessed the lactone of 11,13-dehydro type in the molecules exhibited the strong activities against the three cell lines. These results suggest that the 11,13-dehydro lactone moiety of these sequiterpenes contributed to the antiproliferative activity.

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