Sesquiterpenoids Isolated from *Eupatorium glehnii*. Isolation of Guaiaglehnin A, Structure Revision of Hiyodorilactone B, and Genetic Comparison

Motoo Tori,*[a] Norie Morishita,[a] Naoko Hirota,[a] Yoshinori Saito,[a] Katsuyuki Nakashima,[a] Masakazu Sono,[a] Masami Tanaka,[a] Akiko Utagawa,[b] and Hiroshi Hirota[c]

[a] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University; Yamashiro-cho, Tokushima 770–8514, Japan:* [b] *Tama University Hijirigaoka High School; Hijirigaoka, Tama 206–0022, Japan: and* [c] *RIKEN Genomic Sciences Center; Suehiro-cho, Tsurumi-ku, Yokohama 230–0045, Japan.*

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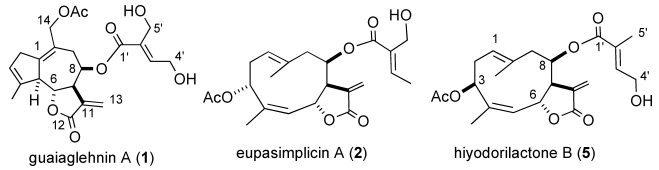
A new sesquiterpenoid substituted with unsaturated ester, guaiaglehnin A (1), along with 15 previously known compounds, were isolated from the methanol extract of the terrestrial part of *Eupatorium glehnii* (Compositae) collected in Nagano Prefecture, Japan, the results of which supported the previous study by Takahashi *et al.* The chemical constituents of *E. glehnii* collected in Nagano Prefecture and those collected in Tokushima or Hokkaido are quite different, depending on collection site, although the species are identical. The base sequences of three different samples were identical. Diversity in the chemical components was detected, though no diversity existed in the DNA sequence. In this study, eupasimplicin A (2) was also isolated, whose presence in the extract of *E. chinense simplicifolium* was recorded but not in an article. The side chain geometry of hiyodorilactone B (5) was revised to be *E*.

Key words *Eupatorium glehnii*; compositae; guaianolide; germacranolide; *atpB-rbcL*; internal transcribed spacer

Eupatorium glehnii is found throughout Hokkaido, Honshu, and Shikoku Island in Japan at high altitude (normally between 1000 m and 1800 m).[1,2] Takahashi and colleagues isolated hiyodorilactones A—F from *E. sachalinense*,[3—6] the name of which was subsequently changed to *E. glehnii*.[7—11] Hiyodorilactones show cytotoxicity,[3,4] so we were interested in reinvestigating *E. glehnii*,[3—11] collected in Tokushima and Hokkaido, Japan, and studying its chemical constituents. From the ethyl acetate-soluble fraction of the methanol extract, we found four new germacranolides having unsaturated esters at the 8-position (which we named eupaglehnins A, B, C, and D[8]), two new chlorine-containing germacranolides (which we named eupaglehnins E and F[7,8]) and 2α-acetoxyepitulipinolide (the first time from a natural source). Hiyodorilactones from the extracts collected in the Tokushima and Hokkaido areas were not isolated. We collected the same plant in Nagano Prefecture where Takahashi *et al.* had collected their samples in the early 1970s.[3,4,12] We found hiyodorilactones and one new compound from the Nagano sample. This study describes the elucidation of the structure of this new terpenoid, structure revision of hiyodorilactone B,[3] and characterization of eupasimplicin A,[13,14] based mainly on two-dimensional (2D) NMR techniques, and biological activity, as well as comparison of these species in terms of their chemical constituents and base sequences.

Results and Discussion

The ethyl acetate soluble fraction of the methanol (MeOH) extract of *E. glehnii* collected in Nagano Prefecture was subjected to silica-gel column chromatography followed by Sephadex LH-20 and high-pressure liquid chromatography (HPLC) to yield guaiaglehnin A (**1**) along with 15 previously known compounds.



Guaiaglehnin A (**1**) showed a quasi-molecular ion peak at *m*/*z* 419 and its molecular formula was determined to be $C_{22}H_{26}O_8$ (by HR-CI-MS). The infrared (IR) spectrum indicated a hydroxy group (3500 cm^{-1}), and some carbonyl (1740, 1735, 1720 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** displayed the signals of only one methyl group (δ 1.99) attached to the sp^2 carbon, an acetyl group (δ 2.00), an exomethylene (δ 5.46, 6.20), two protons attached to the sp^2 carbons, and eight protons attached to the carbon-bearing oxygen functions. The ^{13}C-NMR spectrum indicated eight olefinic carbons and three carbonyl carbons. Therefore, the degree of unsaturation was ten and this compound must be tricyclic. The HMBC spectrum shown in Fig. 1 clearly demonstrates ^3J and ^2J correlations between H-15 and C-3, C-4, and C-5, between H-5 and C-1 and C-10, between H-13 and C-7 and C-12, between H-8 and C-6, C-10, and C-1′, and between H-14 and C-1″ as well as other correlations shown in Fig. 1a. The ^{1}H–^{1}H COSY correlations were observed for H-2/H-3 and H-5/H-6/H-7/H-8/H-9 spin systems. These observations indicated that **1** was a guaiane-type with the acetoxyl group at the C-14 position. The ^3J correlation between H-6 and C-12 was not observed, but a γ-lactone was assumed to be at C-6 and C-7 positions. The chemical shift of C-6 of known compound **3** was δ 80.0 and that of H-6 was δ 4.64, and the signal of C-6 of compound **1** appeared at δ 79.0 and that of H-6 at δ 4.17, both at similar positions. The ester attached to the C-8 position was 4-hydroxy-2-hydroxymethyl-2-butenoate because two protons at δ 4.43 (H-4′) coupled with a proton at δ 6.86 (H-3′) and two protons resonating at δ 4.32 was assigned to H-5′. The HMBC correlations were observed between H-5′ and C-1′, C-2′, and C-3′, between H-4′ and C-2′ and C-3′, and between H-3′ and

* To whom correspondence should be addressed. e-mail: tori@ph.bunri-u.ac.jp

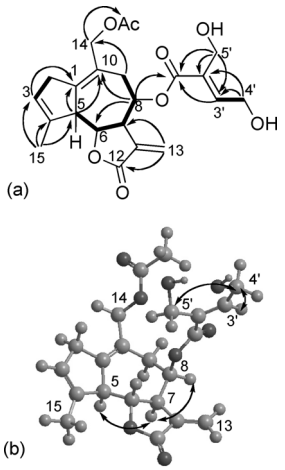


Fig. 1. (a) Selected HMBC (Arrows) and ^{1}H–^{1}H COSY (Bold Lines) Correlations and (b) NOE (Arrows) Correlations for Guaiaglehnin A (**1**)

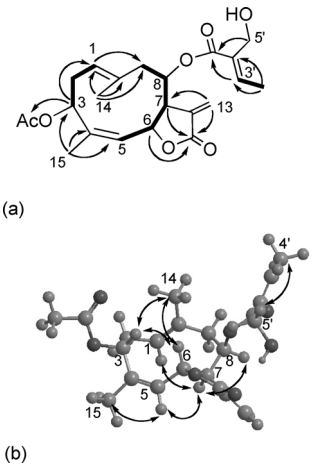


Fig. 2. (a) Selected HMBC (Arrows) and ^{1}H–^{1}H COSY (Bold Lines) Correlations and (b) NOE (Arrows) Correlations for Eupasimplicin A (**2**)

C-1′. The stereochemistry was deduced from the NOESY spectrum. The correlations were observed between H-5 and H-7, and between H-7 and H-8, between H-3′ and H-4′, and between H-4′ and H-5′, establishing the stereochemistry depicted in Fig. 1b. The CD maximum was observed at 256.2 nm ($\Delta\varepsilon$ −1.31), which was compared with known guaianolides[15] to show the absolute configuration of **1** (Fig. 1). Because this compound was hitherto unknown, **1** was named guaiaglehnin A.

Compound **2** has the molecular formula $C_{22}H_{28}O_7$ (by HR-MS), the degree of unsaturation being nine. The ^{13}C-NMR spectrum displayed the signals of eight olefinic carbons and three carbonyl carbons, as well as four carbons bearing an oxygen function. The IR spectrum indicated a lactone (1760 cm^{-1}) and esters. The HMBC spectrum showed the correlations between H-14 (δ 1.89) and C-1 (δ 124.4), C-9 (δ 43.3), and C-10 (δ 135.9), between H-15 (δ 1.80) and C-3 (δ 70.6), C-4 (δ 135.9), and C-5 (δ 125.4), between H-13 (δ 6.37) and C-7 (δ 48.7) and C-12 (δ 169.2), between H-4′ (δ 1.93) and C-2′ (δ 131.4) and C-3′ (δ 142.4), and between H-5′ (δ 4.33) and C-1′ (δ 166.4). The lactone moiety must be at the C-6 and C-7 positions, because H-6 (δ 5.27) and H-7 (δ 2.98) had ^{3}J correlations between C-12 (δ 169.2), and H-7 coupled with H-6 and H-8 (Fig. 2a). Therefore, this must have a ten-membered ring containing a γ-lactone substituted with 2-hydroxymethyl-2-butenoyl group. The acetoxyl group must be at C-3 because H-3 has ^{3}J correlation with the acetyl carbonyl group. The position of the unsaturated ester was not indicated by the HMBC spectrum, but it was assumed to be at the C-8 position because the chemical shift of H-8 resonated at δ 5.27 in the lower field. The stereochemistry was deduced by the NOESY spectrum, as shown in Fig. 2b. The most stable conformation was calculated by CONFLEX[16—18] and was compared with the NOESY correlations. Because the NOEs between H-1 and H-7, between H-7 and H-8, and between H-7 and H-5 were observed, these protons must be below the ten-membered carbocycle. The NOEs between H-3 and H-6, between H-3 and H-14, and between H-14 and H-6 implied that these protons are above the ten-membered carbocycle. The geometry of the Δ^4 double bond was *Z* due to the NOE between H-5 and H-15. These results supported the calculated conformation. The unsaturated ester was determined to be 5′-hydroxytiglate because NOEs between H-4′ and H-3′ and between H-4′ and H-5′ were observed. Therefore, the structure of this compound was established as depicted in the formula. Literature survey revealed that this compound was once isolated from *E. chinense simplicifolium* and named eupasimplicin A (**2**).[13,14] The data in the experiments is worth recording because a report was not published.

Hiyodorilactone B (**5**)[3] was also isolated and characterized with high-resolution NMR spectroscopy. We used 800 MHz NMR along with benzene-shift to confirm the relative structure except for the side chain because the signals of H-1, 3, 5, and 8 are overlapped, and methyl groups are also close to each other. The geometry of the side chain must be changed to *E* because NOE between H-4′ and H-5′ was detected. The similar revision of the *E*,*Z*-geometry was reported in the case of eupachifolin-C (**3**).[19,20]

The other 13 compounds were identified as eupachifolin-C (**3**),[19,20] hiyodorilactones A (**4**), C (**6**), and D (**7**),[3,4] eupaformosanin (**8**),[21] 3β-acetoxy-8β-tigloyloxyheliangolide (**9**),[22] 4*E*-deacetyl chromolaenide-4′-*O*-acetate (**10**),[23] 5′-deoxyeupaformosanin (**11**),[24] deacetyl hiyodorilactone D (**12**),[25] *E*,*E*,*E*-3-hydroxymethyl-7,11,15-trimethylhexadeca-2,6,10-triene-1,14,15-triol (**13**),[26] borneol glucoside (**14**),[27] thymol glucoside (**15**),[28—30] and eugenol glucoside (**16**).[31,32]

Hiyodorilactones[3,4] from *E. glehnii* collected in Nagano Prefecture were isolated, but these substances were not found from the sample collected in Tokushima and Hokkaido.[8] The name of *E. glehnii* was chosen by Kawahara,[9] who studied the classification of *Eupatorium* by applying gene technology and reclassified the related species.[10,11] Thus, the old name *E. sachalinense* is no longer, although the plant is the same as that studied by Takahashi *et al.* in 1978.[3,4,12] All of the collected plants in Tokushima, Hokkaido, and Nagano were identified as *E. glehnii*.[9] We isolated less oxygenated germacranolides from the Tokushima and Hokkaido samples; hiyodorilactones, which have more oxygen functions, were isolated from the Nagano sample. We have previously isolated two chlorine-containing compounds from the Tokushima sample.[7,8] The diversity observed is illustrated in Fig. 3. We then studied the DNA sequence of the *atpB–rbcL* region in the plastid genome and the internal transcribed spacers (ITSs) of the ribosomal RNA gene on the nuclear genome.[33]

Table 1. ^{1}H- and ^{13}C-NMR Spectral Data of Guaiaglehnin A (**1**), Eupasimplicin A (**2**), and Hiyodorilactone B (**5**)

Position	Guaiaglehnin A (**1**)		Eupasimplicin A (**2**)		Hiyodorilactone B (**5**)	
	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C	δ_H (mult., *J* in Hz)	δ_C
1	—	144.0	5.08 (t, 7.6)	124.4	5.22 (d, 11.0))	125.2
2	3.15 (br d, 18)	37.2	2.09 (m)	30.6	2.74 (br d, 13.5)	43.3
	3.26 (br d, 18)	—	2.75 (m)	—	2.47 (br t, 13.5)	—
3	5.59 (m)	126.1	5.61 (dd, 11.6, 5.0)	70.6	5.26 (t, 3.6)	76.8
4	—	139.9	—	135.9	—	136.3
5	3.51 (d, 10)	57.1	5.21 (d, 10.7)	125.4	5.22 (d, 11.0)	126.3
6	4.17 (t, 10)	79.0	5.27 (d, 10.7)	74.2	5.94 (d. 11.0)	75.7
7	3.12 (m)	55.4	2.98 (t, 2.2)	48.7	2.99 (br s)	48.5
8	5.70 (br d, 6.0)	66.1	5.26 (br s)	79.4	5.25 (br s)	78.8
9	2.47 (br d, 15.6)	33.1	2.40 (dd, 14.2, 3.0)	43.3	2.74 (br d, 13.5)	29.3
	2.87 (dd, 15.6, 6.0)	—	2.73 (dd, 14.2, 3.2)	—	2.30 (m)	—
10	—	124.3	—	135.9	—	135.5
11	—	135.1	—	137.2	—	137.4
12	—	168.8	—	169.2	—	169.8
13	5.46 (d, 3.0)	120.0	6.37 (d, 2.2)	124.7	6.35 (d, 2.2)	124.6
	6.20 (d, 3.0)	—	5.77 (d, 2.2)	—	5.78 (d, 1.6)	—
14	4.45 (d, 12)	66.8	1.89 (s)	18.6	1.80 (s)	19.3
	4.50 (d, 12)	—	—	—		
15	1.99 (s)	17.7	1.80 (s)	18.1	1.83 (s)	23.0
1′	—	165.9	—	166.4	—	166.1
2′	—	131.6	—	131.4	—	127.2
3′	6.86 (t, 6.0)	144.4	6.94 (q, 7.3)	142.4	6.82 (t, 5.0)	142.4
4′	4.43 (d, 6.0)	59.2	1.93 (d, 7.3)	14.6	4.33 (br s)	59.6
5′	4.32 (d, 5.5)	57.2	4.33 (d, 6.0)	56.9	1.80 (s)	12.5
Ac	2.00 (s)	20.9	2.11 (s)	21.2	2.17 (s)	21.1
	—	171.3	—	170.2	—	169.6
OH	—	—	2.34 (t, 6.0)	—		

Measured at 60 MHz (^{1}H-NMR) and 150 MHz (^{13}C-NMR) in $CDCl_3$.

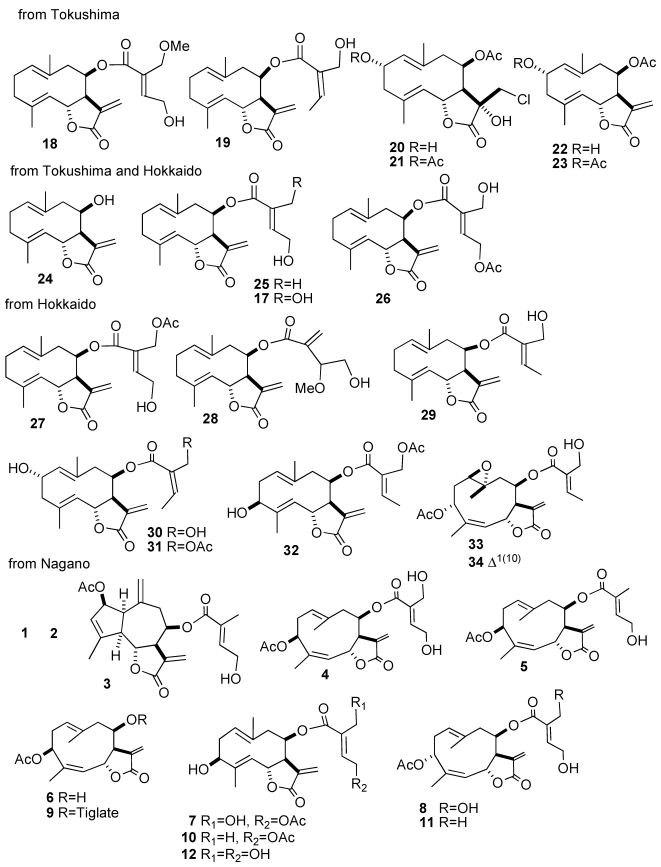


Fig. 3. Distribution of Germacranolides, Heliangolides and Guaianolides in Three Different Collections of *E. glehnii*

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   1  tatcataata taataataaa gtaaaaaaga tctaattttt ttgcgaaaat tatcgcattc
  61  aaaagaaatg tccgatgaca agttgatcgg ttaattcaaa aa----tggg agttagcaca
 121  cgattttgtt agtaccatcc aaccgaatcc aatttaattg tttacttatt caatttcaat
 181  gaatgaattt gaaagttcaa ccaacccatt ttcaaaatat caagtagatg aataagaatc
 241  ttgataaaat ctttcatttg tctatcatta tagacaatcc catctatatt atctatggaa
 301  ttcgaacctg aactctattt acgattcagt atttctatat catcggctct tcttatttat
 361  agtgatttac gtctagtctg ttgttgttgt ····t--ctt ttcataaaaa tattccacat
 421  attttcaaat ctaggattta catatacaac ata******* ***aatttct tagtatttgg
 481  gtgattttta ggtatttcga t--aaaaaaa -----t---- -tggggttgc gccatatata
 541  tgaaagagta tacaataatg atgtatttgc cgaatcaaat accatggtct -aataatcaa
 601  gcattctgat tagttgataa ttttactatt agttgggaat tttgtgaaag gttcctgtaa
 661  aaagtttcat taacgcctaa ttcatgtcga gtagaccttg ttgttgtgag aattcttaat
 721  tcatgagttg tagggaggga ttt

********** = tatcatttacaatatatatcactgtcaaggggg
···· = ttttttttttt
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Fig. 4. Base Sequence in *atpB–rbcL* Intergenic Region of Three *E. glehnii* Samples

The results for the *atpB–rbcL* region are shown in Fig. 4. Among three samples, the 420th base was C in the Nagano sample, T in the Tokushima sample, and T in the Hokkaido sample. All three samples were identical in the case of ITS1 and ITS2. The results were different from those of Ito *et al.*[34] in that one C was inserted in the ribosomal 18S subunit region and four bases (T192C, C197T, C203T, C207T) were different in the ITS2 region. These data indicate that there is no diversity in the base sequences of the three samples of *E. glehnii*, but that there is diversity in the chemical constituents. We do not consider this diversity to depend on the harvest season because we collected the plants in the flowering season, but it presumably depends on the region in which it grows.

In an attempt to find the compounds that are cytotoxic against HL-60 cells,[35] we found that hiyodorilactone A (**5**) had moderate activity (IC_{50} of 3.75 μM). Hiyodorilactone B

(**6**) showed slightly higher activity (IC_{50} of 1.95 μM). Eupatoriopicrin (**18**)[5,6,36—39] isolated from the Hokkaido sample was the most effective compound (IC_{50} of 1.3 μM) among those tested. We have previously investigated the chemical constituents of *E. fortunei*,[40] but only derivatives of aromatic substance were isolated. We are currently studying the related *Eupatorium* species, collecting plant samples from Japan and China.

Experimental

General Experimental Procedures The IR spectra were measured using a JASCO FT/IR-5300 spectrophotometer. The ^{1}H-, ^{13}C-, and 2D NMR spectra were produced using a Varian Unity 600 (600 MHz), or a Bruker AVANCE-800 (800 MHz) spectrometer. The mass spectra (including high-resolution mass spectra) were recorded on a JEOL JMS-700 MStation. Specific rotations were measured using a JASCO DIP-140. A Chemcopak Nucleosil 50-5 was used for HPLC (JASCO pump system). Silica gel 60 (70—230 mesh, Merck) was used for column chromatography, and silica gel 60 F_{254} plates (Merck) were used for thin-layer chromatography.

CONFLEX program (BARISTA) was purchased from CONFLEX Corporation and run on Windows XP. The initial parameters were created with BARISTA, and a conformation search within 5 kcal/mol was carried out to find the global minimum conformation with MMFF94S parameter.[16—18]

DNeasy Plant Mini Kit (Qiagen) was used to purify DNA from plant materials; glass milk (Gentra Systems) was used to purify the DNA further. HotStarTaq polymerase (Qiagen) was used for the polymerase chain reaction (PCR). A High Pure PCR Product Purification Kit (Roche Diagnostics) was used to purify the PCR product after separation by agarose gel electrophoresis. DNA sequencing reactions were carried out with BigDye Terminator v1.1 Cycle Sequencing Kit (ABI) and analyzed on an ABI PRISM 310 Genetic Analyzer. The primers used for PCR and nucleotide sequencing were: ITS2B, 5′ CTCGATGGTTCACGGGAT 3′; ITS3, 5′ GCATCGATGAAGAACGCAGC 3′; ITS4, 5′ TCCTCCGCTTATTGATATGC 3′; ITS5m, 5′ GGAAGGAGAAGTCGTAACAAGG 3′; *ast-atpB*, 5′ GCTGTACCTCACAAGTCACATTAATTGGTTGACCA 3′; *ast-rbcL*, 5′ GGTTGAGGAGTTACTCGAAATGCTGCCAAGATATC 3′; Eg1, 5′ CCTGAACTCTATTTACGATTCAG 3′; Eg2, 5′ CTGAATCGTAAATAGAGTTCAGG 3′; Eg3, 5′ ATCTAGGATTTACATATACAACATA 3′; Eg4, 5′ TTCCCCCTTGACAGTGATATA 3′; and Eg5, 5′ AGACCATGGTATTTGATTCGGC 3′.

Extraction and Isolation *E. glehnii* was collected in Fujimi-cho, Nagano Prefecture (1997).[9] The half-dried (overnight) plant (4.4 kg) was extracted with MeOH at room temperature for three weeks. The solvent was evaporated to afford a residue (318 g), which was partitioned between EtOAc and H_2O–MeOH. The EtOAc-soluble fraction (77 g) was subjected to silica-gel column chromatography (hexane : EtOAc, in a gradient to EtOAc : MeOH, in a gradient) to give frs. Frs. were further separated by silica-gel column chromatography (hexane : EtOAc and $CHCl_3$: MeOH, in a gradient) and Sephadex LH-20 ($CHCl_3$: MeOH = 1 : 1) followed by HPLC ($CHCl_3$: EtOAc) to afford a new compound **1** (1.1 mg) and 15 previously known compounds: **2** (3.2 mg), **3** (1.7 mg), **4** (590.4 mg), **5** (37.3 mg), **6** (43.9 mg), and **7** (126.6 mg), **8** (9.3 mg), **9** (71.5 mg), **10** (29.6 mg), **11** (27.5 mg), **12** (38.6 mg), **13** (10.3 mg), **14** (4.5 mg), **15** (10.6 mg), and **16** (32.6 mg).

Determination of DNA Sequence DNA was purified from dried leaves with a DNeasy Plant Mini Kit, and then with glass milk. PCR amplification of the *atpB–rbcL* intergenic region was carried out with primers *ast-atpB* and *ast–rbcL*. The primers were newly designed based on *atpB* and *rbcL* sequences of Asteraceae species in the database. In comparison with previously published primers, the present ones were further inside the coding regions, which facilitated complete sequencing of the intergenic region. Forty cycles of amplification were carried out, with each cycle consisting of a 30 s denaturation at 95 °C, a 30 s annealing at 40 °C, and a 1 min extension at 72 °C. PCR products, *ca.* 1100 bp, were separated by agarose gel electrophoresis and purified with the High Pure PCR Product Purification Kit. Nucleotide sequencing was carried out with ITS2B, ITS3, ITS4, ITS5m, *ast-atpB*, *ast-rbcL*, Eg1, Eg2, Eg3, Eg4, and Eg5.

Guaiaglehnin A (**1**): Colorless oil; $[\alpha]_D^{23}$ −75° (c=0.1, $CHCl_3$). CD (2.6×10^{-6} M) $\Delta\varepsilon$ −1.31 (256.2 nm, $CHCl_3$). FT-IR (ATR) cm^{-1}: 3500, 1740, 1735, and 1720. ^{1}H- and ^{13}C-NMR (Table 1). CI-HR-MS (CH_4) m/z: 419.1679 $[M+H]^+$ (Calcd for $C_{22}H_{27}O_8$: 419.1706). CI-MS (CH_4) m/z: 419 $[M+H]^+$, 391, 369, 341, 257, 211, 136 (base).

Eupasimplicin A (**2**): Colorless oil; $[\alpha_D^{22}$ −44.3° (c=0.32, $CHCl_3$). CD (4.0×10^{-6} M) $\Delta\varepsilon$ +3.94 (251.6 nm, $CHCl_3$). FT-IR (ATR) cm^{-1}: 3500, 1760, 1710, and 1660. ^{1}H- and ^{13}C-NMR (Table 1). EI-HR-MS m/z: 404.1807 (M^+) (Calcd for $C_{22}H_{28}O_7$: 404.1835). EI-MS m/z: 404 (M^+), 404, 345, 288, 246, 229 (base), 211, 183, 157, and 119.

Hiyodorilactone B (**5**): Colorless oil; $[\alpha]_D^{21}$ −123.9° (c=1.15, $CHCl_3$). CD (2.8×10^{-6} M) $\Delta\varepsilon$ +8.01° (260.3 nm, $CHCl_3$). FT-IR (ATR) cm^{-1}: 3450, 1760, 1740, 1710, and 1660. ^{1}H- and ^{13}C-NMR (Table 1).

Evaluation for *in vitro* Cytotoxicity HL-60 cells (1×10^4 cells/well in 96-well plate) were incubated with the chemicals at the indicated concentration for 24 h. Cell viability was determined by WST-8 assay. Data were mean±S.D. (n=4).

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References and Notes

1) Schmidt G. J., Schilling E. E., *Am. J. Bot.*, **87**, 716—726 (2000).
2) Ito M., Watanabe K., Kita Y., Kawahara T., Crawford D. J., Yahara T., *J. Plant Res.*, **113**, 79—89 (2000).
3) Takahashi T., Eto H., Ichimura T., Murae T., *Chem. Lett.*, **1978**, 1345—1348 (1978).
4) Takahashi T., Ichimura T., Murae T., *Chem. Pharm. Bull.*, **27**, 2539—2543 (1979).
5) Woerdenbag H. J., Meijer C., Mulder N. H., de Vries E. G. E., Hendriks H., Malingre Th. M., *Planta Med.*, **52**, 112—114 (1986).
6) Woerdenbag J., Moskal T. A., Pras N., Malingré Th. M., *J. Nat. Prod.*, **56**, 849—856 (1993).
7) Tori M., Takeichi Y., Kuga H., Nakashima K., Sono M., *Heterocycles*, **52**, 1075—1078 (2000).
8) Tori M., Takeichi Y., Kuga H., Nakashima K., Sono M., *Chem. Pharm. Bull.*, **50**, 1250—1254 (2002).
9) The plant was identified by Dr. Takayuki Kawahara, Hokkaido Branch of Forestry and Forest Products Research Institute, Forestry Agency, Ministry of Agriculture, Forestry and Fisheries, to whom many thanks are due. According to his research, the plant name should be changed to *E. glehnii* based on gene identification methodology.[10,11]
10) Kawahara T., Yahara T., Watanabe K., *Bot. Mag. Tokyo*, **102**, 165—179 (1989).
11) Kawahara T., Yahara T., Watanabe K., *Plant Species Biol.*, **4**, 37—46 (1989).
12) Private communication with Professor Emeritus Takeyoshi Takahashi.
13) Takahashi T., Utagawa A., Murae T., Abstracts, 23rd Symposium on the Chemistry of Terpenes, Essential Oils, and Aromatics, Tottori, Japan, Chemical Society of Japan,1979, pp. 276—278.
14) Utagawa A., "Structural and synthetic studies of germacranolide derivatives of Eupatorium chinense simplicifolium," Thesis, The University of Tokyo, 1986.
15) Lee K. H., Simpson R. F., Geissman T. A., *Phytochemistry*, **8**, 1515—1521 (1969).
16) Goto H., Osawa E., *J. Am. Chem. Soc.*, **111**, 8950—8951 (1989).
17) Goto H., Osawa E., *J. Chem. Soc., Perkin Trans. 2*, **1993**, 187—198 (1993).
18) Goto H., Ohta K., Kamakura T., Obata S., Nakayama N., Matsumoto T., Osawa E., Conflex Corp., Tokyo, 2004.
19) Yang S.-P., Huo J., Wang Y., Lou L.-G., Yue J.-M., *J. Nat. Prod.*, **67**, 638—643 (2004).
20) Ito K., Sakakibara Y., Haruna M., *Chem. Lett.*, **1979**, 1473—1476 (1979).
21) Lee K.-H., Kimura T., Haruna M., McPhail A. T., Onan K. D., Huang H.-C., *Phytochemistry*, **16**, 1068—1070 (1977).
22) Jakupovic J., Pathak V. P., Bohlmann F., Gage D., Dillon M. O., *Phytochemistry*, **25**, 2563—2565 (1986).
23) Boeker R., Jakupovic J., Bohlmann F., King R. M., Robinson H., *Phytochemistry*, **25**, 1669—1672 (1986).
24) Bohlmann F., Zdero C., King R. M., Robinson H., *Phytochemistry*, **22**, 2860—2862 (1983).
25) Jakupovic J., Sun H., Bohlmann F., King R. M., *Planta Med.*, **53**, 97—

98 (1987).
26) Perez A. L., Ortega A., Romo de Vivar A., *Phytochemistry*, **27**, 3897—3901 (1998).
27) Kaneda N., Nakanishi H., Kuraishi T., Katori T., *Yakugaku Zasshi*, **103**, 1133—1139 (1983).
28) Skopp von K., Horster H., *Planta Med.*, **29**, 208—215 (1976).
29) Ahmed A. A., Jakupovic J., *Phytochemistry*, **29**, 3658—3661 (1990).
30) Shimoda K., Kondo Y., Nishida T., Hamada Ha., Nakajima N., Hamada Hi., *Phytochemistry*, **67**, 2256—2261 (2006).
31) Mulkens A., Kapetanidis I., *J. Nat. Prod.*, **51**, 496—498 (1988).
32) Fujita T., Nakayama M., *Phytochemistry*, **31**, 3265—3267 (1992).
33) Hanai R., Gong X., Tori M., Kondo S., Otose K., Okamoto Y., Nishihama T., Murota A., Shen Y., Wu S., Kuroda C., *Bull. Chem. Soc. Jpn.*, **78**, 1302—1308 (2005).
34) Ito M., Watanabe K., Kita Y., Kawahara T., Yahara T., DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/), DDBJRELEASE, AB032033, **2000**.
35) Green L. M., Reade J. L., Ware C. F., *J. Immunol. Methods*, **70**, 257—268 (1984).
36) Dolejs L., Herout V., *Collect. Czech. Chem. Commun.*, **27**, 2654—2661 (1962).
37) Doskotch R. W., El-Feraly R. W., *J. Org. Chem.*, **35**, 1928—1936 (1970).
38) Drozdz B., Grabarczyk H., Samek Z., Holub M., Herout V., Sorm F., *Collect. Czech. Chem. Commun.*, **37**, 1546—1554 (1972).
39) Woerdenbag H. J., Lemstra W., Hendriks H., Malingré Th. M., Konings A. W. T., *Planta Med.*, **53**, 318—322 (1987).
40) Tori M., Ohara Y., Nakashima K., Sono M., *J. Nat. Prod.*, **64**, 1048—1051 (2001).