Structures of Eremophilenolides from the Rhizomes of *Petasites japonicus* MAXIM.

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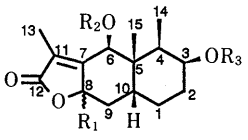
Three new eremophilenolides, 6β-angeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (1), 3β-hydroxy-6β-tigloyloxyeremophil-7(11)-en-12,8β-olide (2) and 3β,6β-diangeloyloxyeremophil-7(11)-en-12,8β-olide (3), were isolated from the dried rhizomes of *Petasites japonicus* MAXIM. (Compositae) with four known sesquiterpenoids. The structures of these compounds were elucidated on the basis of spectroscopic evidence.

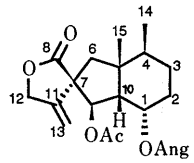
Keywords *Petasites japonicus*; Compositae; sesquiterpenoid; eremophilenolide

The rhizomes of *Petasites japonicus* MAXIM. (Compositae) have been utilized as an agent for the purgation of embryonic poison (taidoku kudashi) since ancient times.[1] The constituents of the rhizomes of this plant have been previously investigated and shown to contain furanoeremophilanes and eremophilenolides.[2] It has been reported that some of the eremophilenolides exhibit anti-allergic and anti-histamic activities.[3] We now wish to report the isolation and structure elucidation of three new eremophilenolides, 6β-angeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (**1**), 3β-hydroxy-6β-tigloyloxyeremophil-7(11)-en-12,8β-olide (**2**) and 3β,6β-diangeloyloxyeremophil-7(11)-en-12,8β-olide (**3**), as well as four known sesquiterpenoid, C-8-epimers of 6β-angeloyloxy-3β,8-dihydroxyeremophilenolides (**4** and **5**), bakkenolide B (fukinolide) (**6**) and isopetasin (**7**). Extraction and separation were carried out as described in the experimental section.

Compound **1**, $C_{20}H_{28}O_5$, $[\alpha]_D^{22}$ −100.3°, was isolated as a colorless gum. The infrared (IR) spectrum suggested the presence of a hydroxyl group (3487 cm^{-1}), an α,β-unsaturated-γ-lactone (1749 cm^{-1}), an α,β-unsaturated ester (1718 cm^{-1}) and a double bond (1649 cm^{-1}). The ultraviolet (UV) spectrum also suggested the presence of an α,β-unsaturated-γ-lactone (λ_{max}^{MeOH}: 218 nm). The proton nuclear magnetic resonance (^{1}H-NMR, Table I) and carbon-13 nuclear magnetic resonance (^{13}C-NMR, Table II) spectra were similar to those of the eremophilenolide derivatives previously isolated from *Farfugium japonicum* (L.) KITAM.[4] and showed signals for a tertiary methyl group, a secondary methyl group and an olefinic methyl group, establishing that the lactone belongs to a sesquiterpene of the eremophilane-type. Naya *et al.*[5] reported that for 8α-methoxyeremophilenolide derivatives, the chemical shifts due to the secondary C-4 methyl group is downfield from those due to the tertiary C-5 methyl group, whereas this relationship is reversed in the 8β-series. They also reported that the homoallylic coupling (J=1.0–1.5 Hz) between the olefinic C-11 methyl group and H-6α found in the 8α-series, is absent in the 8β-series. The value of the optical rotation of the 8β-series, which had a steroidal conformation, was positive, and that of the 8α-series, which had a non-steroidal conformation, was negative. In the ^{1}H-NMR spectrum, **1** showed a singlet of the tertiary C-5 methyl group at δ 0.97 and a doublet of the secondary C-4 methyl group at δ 0.97 (J=7.0 Hz), as well as homoallylic coupling (J=1.5 Hz) of the olefinic C-11 methyl group with H-6 and H-8. Moreover, in the two dimensional (2D) nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum, both the H-6 and H-8 signals showed



	R_1	R_2	R_3
1	···H	Ang	H
2	···H	Tig	H
3	···H	Ang	Ang
4	···OH	Ang	H
4a	···OAc	Ang	Ac
5	—OH	Ang	H
5a	—OAc	Ang	Ac



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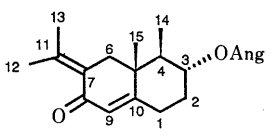

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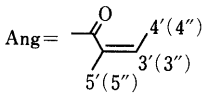

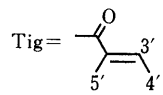

Fig. 1

TABLE I. ^{1}H-NMR Chemical Shifts ($CDCl_3$, 400 MHz)

Proton	**1**	**2**	**3**
3	4.10 dt (J=4.4, 11.4)	4.10 dt (J=4.4, 11.4)	5.11 dt (J=4.4, 11.4)
6	6.15 dd (J=1.5, 1.5)	6.13 dd (J=1.5, 1.5)	6.19 dd (J=1.5, 1.5)
8	4.90 m	4.90 m	4.92 m
9	2.22 ddd (J=2.2, 6.6, 12.0)	2.20 ddd (J=2.2, 6.6, 12.0)	2.23 ddd (J=2.2, 6.6, 12.0)
13	1.80 dd (J=1.5, 1.5)	1.78 dd (J=1.5, 1.5)	1.80 dd (J=1.5, 1.5)
14	0.97 d (J=7.0)	0.96 d (J=7.0)	0.99 d (J=7.0)
15	0.97 s	0.98 s	0.98 s
OCOR			
3′	6.29 qq (J=1.5, 7.3)	6.99 qq (J=1.8, 7.3)	6.24 qq (J=1.5, 7.3)
4′	2.07 qd (J=1.5, 7.3)	1.87 qd (J=1.1, 7.3)	2.07 qd (J=1.5, 7.3)
5′	1.99 qd (J=1.5, 1.5)	1.91 qd (J=1.1, 1.8)	2.00 qd (J=1.5, 1.5)
3″			6.01 qq (J=1.5, 7.3)
4″			1.95 qd (J=1.5, 7.3)
5″			1.86 qd (J=1.5, 1.5)

Coupling constants (J in Hz) are given in parentheses. ′, C-6-angeloyloxy or C-6-tigloyloxy; ″, C-3-angeloyloxy.

TABLE II. ^{13}C-NMR Chemical Shifts ($CDCl_3$, 100 MHz)

Carbon	1	2	3
1	27.1	27.1	26.9
2	28.8	28.7	25.3
3	68.0	68.5	70.8
4	38.8	38.7	35.8
5	45.5	45.6	45.5
6	71.1	71.5	71.0
7	158.3	158.5	158.4
8	77.2	77.6	77.7
9	34.3	34.3	34.4
10	35.3	35.2	35.2
11	122.3	122.4	122.3
12	174.1	174.0	172.2
13	8.3	8.5	8.6
14	20.6	20.4	20.4
15	7.4	7.4	8.2
1′,1″	166.7	166.8	166.8, 165.5
2′,2″	126.5	127.8	126.7, 128.1
3′,3″	141.9	139.8	141.1, 137.6
4′,4″	20.6	12.2	20.6, 20.4
5′,5″	16.1	14.7	15.9, 15.7

′, C-6-angeloyloxy or C-6-tigloyloxy; ″, C-3-angeloyloxy.

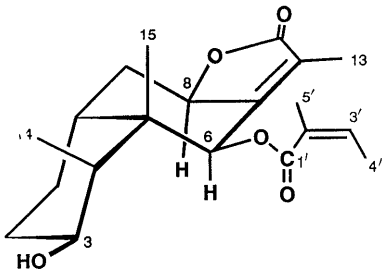

Fig. 2. Stereochemical Structure of Compound **1**

a correlation. Thus, H-6 and H-8 are α oriented. The value of the optical rotation was negative. These data indicated that **1** exists in a non-steroidal conformation (Fig. 2). In the ^{1}H-NMR spectrum, signals at δ 6.29 (1H, qq, J=1.5, 7.3 Hz), 2.07 (3H, qd, J=1.5, 7.3 Hz) and 1.99 (3H, qd, J=1.5, 1.5 Hz) indicated the presence of an angeloyloxyl group.[6] The position of an angeloyloxyl group was confirmed by the ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum. In its spectrum, the cross peak due to long-range coupling was observed between the H-6α signal and the C-1′ signal at δ 166.7, so that an angeloyloxyl group is attached at the C-6β position. The position of a hydroxyl group was confirmed by NOESY spectrum. In its spectrum, each signal of H-6α and H-3α showed a correlation, so that a hydroxyl group is attached at the C-3β position. On the basis of the above-mentioned evidence, the structure of **1** was determined to be 6β-angeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide.

Compound **2**, $C_{20}H_{28}O_5$, $[\alpha]_D^{22}$ −105.5°, was isolated as a colorless gum. The IR spectrum suggested the presence of a hydroxyl group, an α,β-unsaturated-γ-lactone and an α,β-unsaturated ester. The UV spectrum also suggested the presence of an α,β-unsaturated-γ-lactone (λ_{max}^{MeOH}: 216 nm). In comparing the ^{1}H- and ^{13}C-NMR spectral data of **2** with those of **1**, the main skeleton of **2** was in accord with **1** except for the presence of a tigloyloxyl group [δ: 6.99 (1H, qq, J=1.8, 7.3 Hz), 1.87 (3H, qd, J=1.1, 7.3 Hz), 1.91 (3H, qd, J=1.1, 1.8 Hz)][6] in place of an angeloyloxyl group. The position of a tigloyloxyl group was confirmed by the HMBC spectrum. In its spectrum, the cross peak due to long-range coupling was observed between the H-6α signal and the C-1′ signal at δ 166.8, so that a tigloyloxyl group is attached at the C-6β position. The position of a hydroxyl group was in accord with **1** in the NOESY spectrum. On the basis of the above-mentioned evidence, the structure of **2** was determined to be 3β-hydroxy-6β-tigloyloxyeremophil-7(11)-en-12,8β-olide.

Compound **3**, $C_{25}H_{34}O_6$, $[\alpha]_D^{22}$ −120.3°, was isolated as a colorless gum. The IR spectrum suggested the presence of an α,β-unsaturated-γ-lactone and an α,β-unsaturated ester. The UV spectrum also suggested the presence of an α,β-unsaturated-γ-lactone (λ_{max}^{MeOH}: 216 nm). In comparing the ^{1}H- and ^{13}C-NMR spectral data of **3** with those of **1**, the main skeleton of **3** was in accord with **1** except for the presence of one more angeloyloxyl group.[6] The position of this angeloyloxyl group was confirmed by the HMBC spectrum. In its spectrum, the cross peak due to long-range coupling was observed between the H-3α signal and the C-1″ signal at δ 165.5, so that this angeloyloxyl group is attached at the C-3β positions. On the basis of the above-mentioned evidence, the structure of **3** was determined to be 3β,6β-diangeloyloxyeremophil-7(11)-en-12,8β-olide.

Compounds **4** and **5** could not be separated by column chromatography on silica gel. The ^{1}H- and ^{13}C-NMR spectra of the mixture showed the presence of two compounds. On acetylation with acetic anhydride and pyridine, the mixture afforded diacetate which was separated by column chromatography on silica gel to give two compounds (**4a** and **5a**). Compounds **4a** and **5a** were determined to be 3β,8α-diacetoxy-6β-angeloyloxyeremophil-7(11)-en-12,8β-olide and 3β,8β-diacetoxy-6β-angeloyloxyeremophil-7(11)-en-12,8α-olide, respectively, in comparison with published spectral data.[7]

Compounds **6** and **7** were identified as bakkenolide B (fukinolide) and isopetasin, respectively, in comparison with published spectral data.[8,9]

Experimental

General Procedures Optical rotations were determined with a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin Elmer FT-IR 1725X infrared spectrophotometer and UV spectra with a Beckman DU-64 spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded with a JEOL JMN-GSX-400 (400 and 100 MHz, respectively) spectrometer. Chemical shifts were given on a δ (ppm) scale with tetramethylsilane as an internal standard (s, singlet; d, doublet; t, triplet; q, quartet; dd, double doublet; ddd, double double doublet; dt, double triplet; dq, double quartet; qd, quartet doublet; qq, quartet quartet; m, multiplet). The electron impact mass spectrum (EI-MS) and high resolution mass spectrum (HR-MS) were recorded on a JEOL JMS-DX-300 mass spectrometer. Column chromatography was carried out on Kieselgel 60 (Merck; 230—400 mesh). Preparative high performance liquid chromatography (HPLC) was carried out on a Tosoh HPLC system (pump, CCPD; detector, UV-8011) using a TSK gel ODS-120T column (Tosoh) and TSK gel silica-60 column (Tosoh).

Plant Material The dried rhizomes of *Petasites japonicus* were purchased from Tochimototenkaido Co., Ltd. in 1990.

Extraction and Isolation The dried rhizomes of *Petasites japonicus* (3.0 kg) were extracted with MeOH at room temperature for 2 weeks. The MeOH extract was concentrated under reduced pressure and the residue was suspended in a small excess of water. This suspension was extracted, successively, with $CHCl_3$, Et_2O, AcOEt and *n*-BuOH. The $CHCl_3$-soluble fraction was concentrated under reduced pressure to afford a residue

(60.0 g). This residue was chromatographed on a silica gel column using benzene–AcOEt (9 : 1), and the eluate was separated into 16 fractions (frs. 1—16). Fraction 6 was rechromatographed on a silica gel column using *n*-hexane–AcOEt (4 : 1), and the eluate was separated into 11 fractions (frs. 1′—11′). Fraction 3′ was purified by preparative HPLC (Column, TSK gel silica-60, 7.8 mm i.d. × 30 cm; mobile phase, *n*-hexane–acetone (7 : 1); flow rate, 1.0 ml/min; UV detector, 237 nm) to give **7** (25 mg). Fraction 6′ was purified by preparative HPLC (Column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; flow rate, 2.0 ml/min; UV detector, 220 nm) to give **3** (32 mg) and **6** (25 mg), respectively. Fraction 8 was purified by preparative HPLC (Column, TSK gel ODS-120T, 7.8 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (2 : 1); flow rate, 1.5 ml/min; UV detector, 217 nm) to give **1** (50 mg) and **2** (22 mg), respectively. Fraction 9 was rechromatographed on a silica gel column using benzene–AcOEt (9 : 1) to give a mixture (150 mg) of **4** and **5**.

6β-Angeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (1) Colorless gum. $[\alpha]_D^{22}$ −100.3° (c=3.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3487, 1749, 1718, 1649. UV λ_{max}^{MeOH} nm (log ε): 218 (4.2). EI-MS m/z: 348 (M^+). *Anal.* Calcd for $C_{20}H_{28}O_5$: C, 68.94; H, 8.10. Found: C, 68.91; H, 8.12. ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

3β-Hydroxy-6β-tigloyloxyeremophil-7(11)-en-12,8β-olide (2) Colorless gum. $[\alpha]_D^{22}$ −105.5° (c=0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3487, 1751, 1717, 1649. UV ν_{max}^{MeOH} nm (log ε): 216 (4.1). HR-MS m/z: 348.1937 (M^+, Calcd for $C_{20}H_{28}O_5$; 348.1945). ^{1}H-NMR; see Table I. ^{13}C-NMR; see Table II.

3β,6β-Diangeloyloxyeremophil-7(11)-en-12,8β-olide (3) Colorless gum. $[\alpha]_D^{22}$ −120.3°C (c=0.3, $CHCl_3$). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1749, 1720, 1648. UV λ_{max}^{MeOH} nm: 216. HR-MS m/z: 430.2355 (M^+, Calcd for $C_{25}H_{34}O_6$; 430.2355). ^{1}H-NMR: see Table I. ^{13}C-NMR: see Table II.

The Mixture of 6β-Angeloyloxy-3β,8α-dihydroxyeremophil-7(11)-en-12,8β-olide (4) and 6β-Angeloyloxy-3β,8β-dihydroxyeremophil-7(11)-en-12,8α-olide (5) HR-MS m/z: 364.1856 (M^+, Calcd for $C_{20}H_{28}O_6$; 364.1851). ^{1}H-NMR ($CDCl_3$) **4** δ: 0.96 (3H, s, H-15), 0.97 (3H, d, J=7.0 Hz, H-14), 1.77 (3H, d, J=1.5 Hz, H-13), 4.02 (1H, ddd, J=11.7, 4.4, 4.4 Hz, H-3α), 6.10 (1H, d, J=1.5 Hz, H-6α), 6.29 (1H, qq, J=7.3, 1.5 Hz, H-3′), **5** δ: 1.06 (3H, d, J=7.3 Hz, H-14), 1.33 (3H, s, H-15), 3.84 (1H, m, H-3α), 5.67 (1H, s, H-6α), 6.16 (1H, qq, J=7.3, 1.5 Hz, H-3′).

Acetylation of the Mixture of 4 and 5 Acetylation of 150 mg of the mixture with acetic anhydride–pyridine for 1 d at room temperature followed by the usual work up and purification by silica gel column chromatography (benzene–AcOEt (19 : 1)) gave two diacetates (**4a** (120 mg) and **5a** (40 mg)).

3β,8α-Diacetoxy-6β-angeloyloxyeremophil-7(11)-en-12,8β-olide (4a) Colorless gum. $[\alpha]_D^{22}$ −121.6° (c=2.0, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1777, 1729, 1646. UV λ_{max}^{MeOH} nm (log ε): 219 (4.3). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, d, J=6.8 Hz, H-14), 0.97 (3H, s, H-15), 1.85 (3H, d, J=1.5 Hz, H-13), 2.00 (3H, qd, J=1.5, 1.5 Hz, H-5′), 2.02 (3H, s, CH_3COO), 2.08 (3H, dq, J=7.3, 1.5 Hz, H-4′), 2.11 (3H, s, CH_3COO), 4.98 (1H, ddd, J=11.0, 4.4, 4.4 Hz, H-3α), 6.21 (1H, d, J=1.5 Hz, H-6α), 6.27 (1H, qq, J=7.3, 1.5 Hz, H-3′).

3β,8β-Diacetoxy-6β-angeloyloxyeremophil-7(11)-en-12,8α-olide (5a) Colorless gum. $[\alpha]_D^{22}$ +56.9° (c=3.7, $CHCl_3$). IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1775, 1730, 1649. UV λ_{max}^{MeOH} nm (log ε): 221 (4.1). ^{1}H-NMR ($CDCl_3$) δ: 0.96 (3H, d, J=7.0 Hz, H-14), 1.30 (3H, s, H-15), 1.91 (3H, qd, J=1.2, 1.2 Hz, H-5′), 2.00 (3H, dq, J=7.0, 1.2 Hz, H-4′), 2.04 (3H, s, CH_3COO), 2.08 (3H, s, CH_3COO), 4.99 (1H, ddd, J=3.0, 3.0, 2.9 Hz, H-3α), 5.77 (1H, s, H-6α), 6.12 (1H, qq, J=7.0, 1.2 Hz, H-3′).

Bakkenolide B (Fukinolide, 6) IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 3089, 1773, 1736, 1654, 905. UV λ_{max}^{MeOH}: 215. EI-MS m/z: 390 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 0.90 (3H, d, J=7.0 Hz, H-14), 1.13 (3H, s, H-15), 1.78 (3H, d, J=1.5 Hz, H-5′), 1.80 (3H, d, J=7.0 Hz, H-4′), 1.93 (3H, s, CH_3COO), 2.82 (1H, dd, J=5.0, 10.5 Hz, H-10), 4.67 (2H, t, J=1.5 Hz, H-12), 5.15 (1H, m, H-1), 5.17 (1H, d, J=1.1 Hz, H_a-13), 5.21 (1H, d, J=1.1 Hz, H_b-13), 5.79 (1H, d, J=11.0 Hz, H-9α), 5.96 (1H, qq, J=7.0, 1.5 Hz, H-3′).

Isopetasin (7) IR $\lambda_{max}^{CHCl_3}$ cm^{-1}: 1711, 1663, 1628. EI-MS m/z: 316 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, d, J=7.0 Hz, H-14), 1.05 (3H, s, H-15), 1.86 (3H, d, J=1.0 Hz, H-13), 1.90 (3H, d, J=1.5 Hz, H-5′), 1.99 (3H, dq, J=7.3, 1.5 Hz, H-4′), 2.10 (3H, d, J=2.3 Hz, H-12), 2.93 (1H, d, J=13.9 Hz, H-6), 4.92 (1H, dt, J=11.2, 4.3 Hz, H-3), 5.78 (1H, d, J=1.7 Hz, H-9), 6.07 (1H, qq, J=7.3, 1.5 Hz, H-3′).

Acknowledgements The authors are grateful to Dr. S. Suzuki and Dr. K. Hisamichi (Tohoku College of Pharmacy) for the measurements of mass spectra and NMR spectra, respectively.

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