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Structures of New Eremophilane Derivatives from the Rhizomes of *Petasites japonicus* MAXIM.[1)]

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Eight new eremophilenolides, 6β-angeloyloxy-3β,9α-dihydroxyeremophil-7(11)-en-12,8β-olide (1), 6β-angeloyloxy-3β,9β-dihydroxyeremophil-7(11)-en-12,8β-olide (2), 6β-angeloyloxy-3β,8β,9β-trihydroxyeremophil-7(11)-en-12,8α-olide (3), 6β-(3′-chloro-2′-hydroxy-2′-methylbutyloyloxy)-3β-hydroxyeremophil-7(11)-en-12,8β-olide (4), 6β-epoxyangeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (5), 3β-hydroxy-6α-methoxyeremophil-7(11)-en-12,8β-olide (6), 3β-hydroxy-6β-methoxyeremophil-7(11)-en-12,8α-olide (7) and 8β-hydroxy-3-oxoeremophil-7(11)-en-12,8α-olide (8) were isolated from the dried rhizomes of *Petasites japonicus* MAXIM. (Compositae) together with a new eremophilane-type sesquiterpenoid, 3β-hydroxy-8-oxoeremophil-6-en-12-oic acid methyl ester (9). Compound 4 is the first chlorine-containing eremophilenolide derivative isolated from natural sources. The structures of these compounds were elucidated by spectroscopy.

Key words *Petasites japonicus*; Compositae; eremophilenolide; eremophilane-type sesquiterpenoid

The rhizomes of *Petasites japonicus* MAXIM. (Fuki in Japanese, Compositae) have been used for the treatment of tonsillitis, contusions and poisonous-snake bite in China.[2)] In previous papers, we reported the structural elucidation of nor-sesquiterpenoid,[1)] eremophilenolides,[3)] triterpenoids,[4)] anthraquinones[4)] and phenolic compounds.[5)] We now wish to report the isolation and structural elucidation of eight new eremophilenolides, 6β-angeloyloxy-3β,9α-dihydroxyeremophil-7(11)-en-12,8β-olide (**1**), 6β-angeloyloxy-3β,9β-dihydroxyeremophil-7(11)-en-12,8β-olide (**2**), 6β-angeloyloxy-3β,8β,9β-trihydroxyeremophil-7(11)-en-12,8α-olide (**3**), 6β-(3′-chloro-2′-hydroxy-2′-methylbutyloyloxy)-3β-hydroxyeremophil-7(11)-en-12,8β-olide (**4**), 6β-epoxyangeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (**5**), 3β-hydroxy-6α-methoxyeremophil-7(11)-en-12,8β-olide (**6**), 3β-hydroxy-6β-methoxyeremophil-7(11)-en-12,8α-olide (**7**) and 8β-hydroxy-3-oxoeremophil-7(11)-en-12,8α-olide (**8**), as well as a new eremophilane-type sesquiterpenoid, 3β-hydroxy-8-oxoeremophil-6-en-12-oic acid methyl ester (**9**). Extraction and isolation were carried out as described in the Experimental section.

Compound **1** was isolated as a colorless oil, $[\alpha]_D$ −32.4°. The molecular formula was determined to be $C_{20}H_{28}O_6$ by high-resolution (HR)-MS. The IR spectrum of **1** suggested the presence of a hydroxyl group (3423 cm^{-1}), an α,β-unsaturated-γ-lactone (1752, 1681 cm^{-1}) and an α,β-unsaturated ester (1719, 1646 cm^{-1}). The ^1H- (Table 1) and ^{13}C-NMR (Table 2) spectra indicated the presence of a tertiary methyl group [δ_H 0.97 (s, H-15), δ_C 20.8 (C-15)], a secondary methyl group [δ_H 0.98 (d, J=7.0 Hz, H-14), δ_C 7.3 (C-14)] and an olefinic methyl group [δ_H

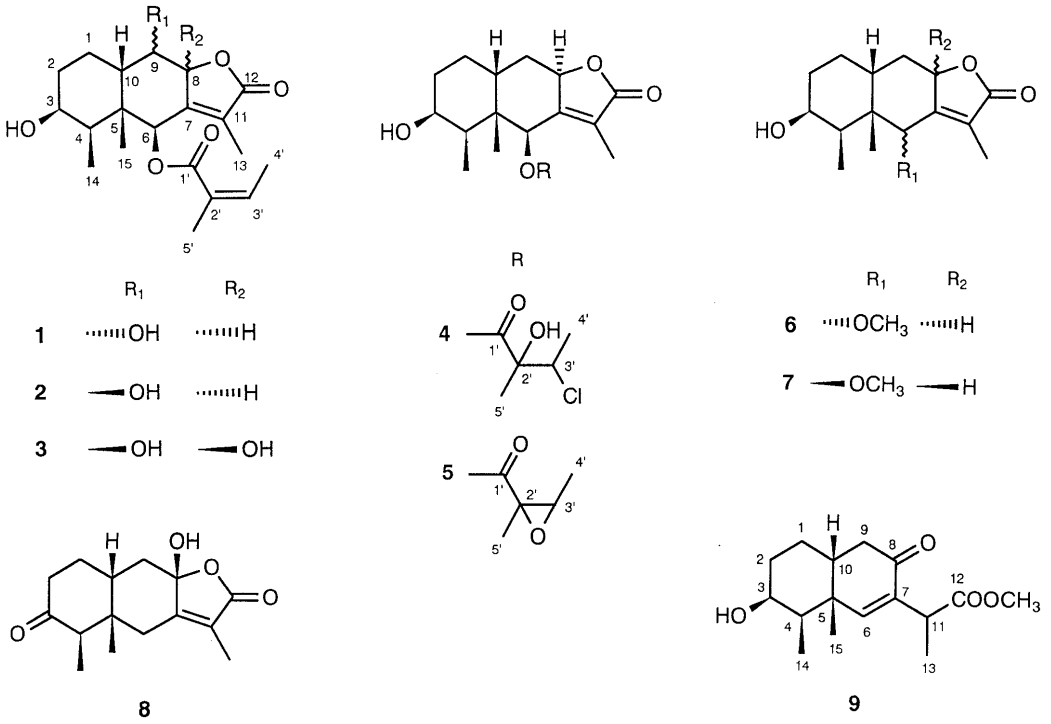

Chart 1

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

Proton	1	2	3[a]	4	5	6	7	8	9
1	α 1.39 dddd (13.6, 13.2, 13.2, 4.0) β 2.17 dddd (13.6, 4.8, 4.0, 3.7)								
2	β 1.54 dddd (13.2, 12.8, 11.4, 4.0)								
3	4.12 ddd (11.7, 4.4, 4.4)	4.08 ddd (11.7, 4.4, 4.4)	3.85 ddd (2.6, 2.6, 2.6)	4.03 ddd (10.7, 4.4, 4.4)	4.15 ddd (11.4, 4.4, 4.4)	4.74 ddd (11.7, 4.8, 4.8)	3.79 ddd (2.9, 2.9, 2.9)		3.74 ddd (9.9, 4.4, 4.4)
4	1.93 m					1.94 m		2.37 q (6.6)	1.88 qd (7.0, 4.4)
6	6.19 br s	6.12 br s	5.56 s	5.96 br s	6.26 br s	3.88 s	4.01 s	α 2.74 d (13.9) β 2.28 dd (13.9, 1.7)	6.47 br s
8	4.83 br d (9.2)	4.95 br s		4.89 m	4.87 m	5.01 m	4.93 ddq (11.0, 7.0, 1.8)		
9	3.73 dd (9.2, 5.1)	4.24 dd (4.4, 2.2)	3.81 d (10.6)	2.24 ddd (12.7, 6.4, 2.0)	2.22 ddd (12.8, 6.6, 2.2)	2.22 ddd (12.8, 6.6, 2.2)			α 2.30 dd (17.2, 4.0) β 2.66 dd (17.2, 4.8)
10									2.03 m
11									3.62 qd (7.3, 1.1)
13	1.80 dd (1.5, 1.5)	1.84 dd (1.8, 1.8)	2.02 s	1.85 dd (1.5, 1.5)	1.91 dd (1.8, 1.8)	1.89 d (1.8)	1.89 d (1.8)	1.87 d (1.7)	1.28 d (7.3)
14	0.98 d (7.0)	0.98 d (7.3)	1.06 d (7.0)	1.00 d (7.3)	0.98 d (7.3)	0.99 d (7.0)	1.01 d (7.0)	0.97 d (6.6)	0.99 d (7.0)
15	0.97 s	1.12 s	1.36 s	0.98 s	0.97 s	0.80 s	1.34 s	1.00 s	1.24 s
3′	6.29 qq (7.3, 1.5)	6.28 qq (7.3, 1.5)	6.13 qq (7.3, 1.5)	4.35 q (6.3)	3.17 q (5.1)				
4′	2.07 dq (7.3, 1.5)	2.07 dq (7.3, 1.5)	2.02 dq (7.3, 1.5)	1.64 d (6.3)	1.39 d (5.1)				
5′	1.98 dq (1.5, 1.5)	1.99 dq (1.5, 1.5)	1.90 dq (1.5, 1.5)	1.54 s	1.66 s				
OCH_3						3.20 s	3.21 s		3.66 s

Coupling constants (J in Hz) are given in parentheses. *a*) In $CDCl_3$ with small amounts of CD_3OD.

Table 2. ^{13}C-NMR Chemical Shifts ($CDCl_3$, 100 MHz)

Carbon	1	2	3[a]	4	5	6	7	8	9
1	20.6[b]	25.8	15.6	27.1	27.2	28.4	20.8	26.5	26.5
2	28.2	28.1	27.6	27.8	28.6	29.5	27.8	36.7	28.0
3	68.1	67.9	71.5	67.7	67.8	69.5	71.8	211.3	70.6
4	39.1	39.7	34.6	39.2	39.1	43.6	33.3	45.1	44.8
5	45.2	45.7	41.8	45.8	45.9	43.8	42.8	45.2	40.1
6	70.8	71.3	70.2	75.2	73.0	84.3	79.0	34.6	154.3
7	154.9	155.2	150.4	157.4	157.3	160.1	160.3	157.1	136.6
8	82.3	79.0	104.9	77.1[c]	77.1	76.3	78.2	103.0	197.6
9	73.6	72.8	72.3	34.6	34.3	35.3	34.7	38.9[f]	40.1
10	41.0	40.8	42.5	35.1	35.3	35.7	34.3	38.9[f]	35.7
11	123.8	123.6	130.5	122.3	122.9	124.9	124.4	123.9	37.8
12	173.9	174.1	171.2	174.1[d]	173.9	174.3	174.3	171.5	175.1
13	8.3	8.4	9.1	9.0	8.8	8.3	8.6	8.3	16.0
14	7.3	7.5	12.9	7.5	7.4	7.9	12.8	7.7	7.4
15	20.8	22.2	19.0	20.0	20.2	24.7	18.8	12.3	25.2
1′	166.6	166.7	167.1	174.1[d]	169.3				
2′	126.3	126.5	127.3	77.1[c]	60.4[e]				
3′	142.2	142.0	139.8	63.2	60.4[e]				
4′	16.1	16.1	15.9	18.0	13.6				
5′	20.6[b]	20.6	20.6	22.5	19.5				
OCH_3						57.3	57.0		52.0

a) In $CDCl_3$ with small amounts of CD_3OD. *b*–*f*) Signals were overlapped.

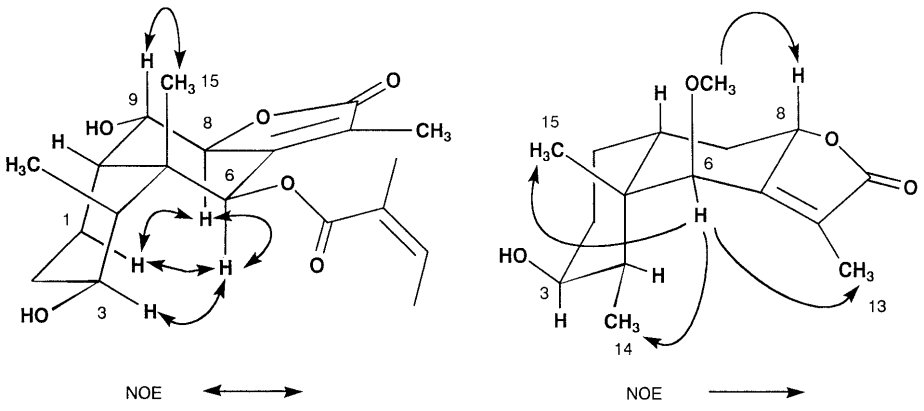
1.80 (dd, J=1.5, 1.5 Hz, H-13), δ_C 8.3 (C-13)], which are characteristic of an eremophilenolide derivative.[3] Other signals in the ^{1}H- and ^{13}C-NMR spectra, obtained with the aid of ^{1}H–^{1}H shift correlation spectroscopy (^{1}H–^{1}H COSY), ^{13}C–^{1}H shift correlation spectroscopy (^{13}C–^{1}H COSY) and ^{1}H-detected heteronuclear multiple bond correlation (HMBC) spectrum, indicated to two hydroxy-bearing methines [δ_H 3.73 (dd, J=9.2, 5.1 Hz, H-9β), 4.12 (ddd, J=11.7, 4.4, 4.4 Hz, H-3α), δ_C 73.6 (C-9), 68.1 (C-3)], an oxygenated methine [δ_H 4.83 (br d, J=9.2 Hz,

H-8α), δ_C 82.3 (C-8)], an angeloyloxyl group [δ_H 1.98 (dq, J=1.5, 1.5 Hz, H-5′), 2.07 (dq, J=7.3, 1.5 Hz, H-4′), 6.29 (qq, J=7.3, 1.5 Hz, H-3′), δ_C 16.1 (C-4′), 20.6 (C-5′), 126.3 (C-2′), 142.2 (C-3′), 166.6 (C-1′)], an angeloyloxy-bearing methine [δ_H 6.19 (br s, H-6α), δ_C 70.8 (C-6)] and an α,β-unsaturated-γ-lactone [δ_C 123.8 (C-11), 154.9 (C-7), 173.9 (C-12)]. These spectral data and molecular formula suggested that the most likely structure of this compound is **1**, as illustrated. The stereostructure was determined from the nuclear Overhauser effect correlation spectroscopy (NOESY) spectrum. The NOESY cross-peaks observed between H-6α and H-1α; H-6α and H-3α; H-6α and H-8α, and between H-8α and H-1α implied a *cis*-junction of the A/B rings which had a non-steroidal conformation (Fig. 1). The coupling patterns and constants for H-3 [δ_H 4.12 (ddd, J=11.7, 4.4, 4.4 Hz)] and H-9 [δ_H 3.73 (dd, J=9.2, 5.1 Hz)] suggested that the hydroxyl groups at C-3 and C-9 are β and α configurations, respectively, and thus was supported by the NOESY cross-peaks between H-3α and H-6α, and between H-9β and H-15 (Fig. 1). The configuration of the angeloyloxyl group at C-6 was determined to be β from the NOESY spectrum, in which cross-peaks were observed between H-6α and H-3α, and between H-6α and H-8α (Fig. 1). On the basis of the above evidence, the structure of **1** was determined to be 6β-angeloyloxy-3β,9α-dihydroxyeremophil-7(11)-en-12,8β-olide.

Compound **2** was isolated as a colorless oil, $[\alpha]_D$ −39.9°. The molecular formula was determined to be $C_{20}H_{28}O_6$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3442 cm^{-1}), an α,β-unsaturated-γ-lactone (1756 cm^{-1}) and an α,β-unsaturated ester (1718, 1646 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **2** were closely related to those of **1**. Naya *et al.*[6] reported homoallylic coupling (J=1.0—1.8 Hz) between the olefinic methyl group (H-13) and H-6α found in the 8α-methoxyeremophil-7(11)-en-12,8β-olide derivatives, which had a non-steroidal conformation, while this long-range coupling was absent in 8β-methoxyeremophil-7(11)-en-12,8α-olide derivatives, which had a steroidal conformation. The ^{1}H-NMR spectrum of **2** showed the homoallylic coupling (J=1.8 Hz) of the olefinic methyl group (H-13) with H-6α. Thus, **2** exists in a non-steroidal conformation. In the ^{1}H-NMR spectrum of **2**, the coupling pattern and constants for H-9 [δ_H 4.24 (dd, J=4.4, 2.2 Hz)] suggested that the hydroxyl group at C-9 has a β configuration. In the ^{13}C-NMR spectrum of **2**, the C-15 signal appeared at δ 22.2, 1.4 ppm lower field than that of **1**. This is due to the δ_1-hydroxy substituent effect[7] of the 9β-hydroxyl group, which adopts a 1,3-*syn* periplanar arrangement with respect to the C-15. Thus, **2** was the 9-epimer of **1**. Based on this evidences, the structure of **2** was determined to be 6β-angeloyloxy-3β,9β-dihydroxyeremophil-7(11)-en-12,8β-olide.

Compound **3** was isolated as an amorphous powder, $[\alpha]_D$ +41.2°. The molecular formula was determined to be $C_{20}H_{28}O_7$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3445 cm^{-1}), an α,β-unsaturated-γ-lactone (1757 cm^{-1}) and an α,β-unsaturated ester (1718, 1644 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **3** were virtually identical to those of 6β-angeloyloxy-3β,8β-dihydroxyeremophil-7(11)-en-12,8α-olide[3a,8] except for the presence of one more hydroxyl group. In the ^{1}H-NMR spectrum of **3**, the homoallylic coupling between the olefinic methyl group (H-13) and H-6α is lacking. In the NOESY spectrum, nuclear Overhauser effects (NOEs) were seen between H-6α and H-13; H-6α and H-14; between H-6α and H-15. These data indicate that **3** exists in a steroidal conformation. The ^{13}C-NMR spectrum of **3** showed a signal due to a hemi-ketal carbon [δ_C 104.9 (C-8)], suggesting that the lactone was γ-hydroxylated. The hydroxyl group at C-8 has a β configuration, which was supported by the NOESY cross-peaks between H-6α and H-13; H-6α and H-14; between H-6α and H-15. In the HMBC spectrum, the hydroxy-bearing methine at δ 3.81 showed connectivity to C-1 at δ 15.6 and C-10 at δ 42.5, indicating that this hydroxyl group is attached at the C-9. The coupling pattern and constant for H-9 [δ_H 3.81 (d, J=10.6 Hz)] suggested that the hydroxyl group at C-9 has a β configuration. From the above data, the structure of **3** was determined to be 6β-angeloyloxy-3β,8β,9β-trihydroxyeremophil-7(11)-en-12,8α-olide. Compounds **1**—**3** are the first eremophilenolide derivatives isolated from the genus *Petasites* plants having the 9-hydroxyl group.

Compound **4** was isolated as a colorless oil, $[\alpha]_D$ −55.6°.



1 **7**

Fig. 1

The electron ionization (EI)-MS of **4** showed a pair of molecular ion peaks at *m/z* 400 and 402 in a 3 : 1 ratio, indicating the presence of a chlorine atom in the molecule. The molecular formula was determined to be $C_{20}H_{29}ClO_6$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3447 cm^{-1}), a saturated ester (1750 cm^{-1}) and an α,β-unsaturated-γ lactone (1750 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **4** were virtually identical to those of 6β-angeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide (**10**).[3a] The ^{1}H-NMR spectrum of **4** showed the homoallylic coupling (J=1.5 Hz) of the olefinic methyl group (H-13) with H-6α. In the NOESY spectrum, NOEs were seen between H-3α and H-6α; and between H-6α and H-8α. These data indicate that **4** exists in a non-steroidal conformation. The ^{1}H- and ^{13}C-NMR spectra of **4** showed the presence of a 3′-chloro-2′-hydroxy-2′-methylbutyloyloxyl group [δ_H 1.54 (s, H-5′), 1.64 (d, J=6.3 Hz, H-4′), 4.35 (q, J=6.3 Hz, H-3′), δ_C 18.0 (C-4′), 22.5 (C-5′), 63.2 (C-3′), 77.1 (C-2′), 174.1 (C-1′)][9] in place of the angeloyloxyl group of **10**. The EI-MS gave a fragment ion peak at *m/z* 248, indicating loss of 3′-chloro-2′-hydroxy-2′-methylbutylic acid from the molecular ion peak at *m/z* 400. The structure of 3′-chloro-2′-hydroxy-2′-methylbutyloyloxyl group was confirmed by the HMBC spectrum, in which the tertiary methyl group (H-5′) showed connectivity to C-1′, C-2′ and C-3′, and the secondary methyl group (H-4′) showed connectivity to C-2′ and C-3′. The absolute configuration of C-2′ and C-3′ could not be determined. Based on this evidence, the structure of **4** was determined to be 6β-(3′-chloro-2′-hydroxy-2′-methylbutyloyloxy)-3β-hydroxyeremophil-7(11)-en-12,8β-olide. Compound **4** is the first chlorine-containing eremophilenolide derivative isolated from natural sources.

Compound **5** was isolated as a colorless oil, $[\alpha]_D$ −96.2°. The molecular formula was determined to be $C_{20}H_{28}O_6$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3423 cm^{-1}), a saturated ester (1750 cm^{-1}) and an α,β-unsaturated-γ-lactone (1750 cm^{-1}). In comparing the ^{1}H- and ^{13}C-NMR spectra of **5** with those of **4**, the main skeleton of **5** was in accord with **4**. The ^{1}H-NMR spectrum of **5** showed homoallylic coupling (J= 1.8 Hz) of the olefinic methyl group (H-13) with H-6α. Thus, **5** exists in a non-steroidal conformation. The ^{1}H- and ^{13}C-NMR spectra of **5** showed the presence of an epoxyangeloyloxyl group [δ_H 1.39 (d, J=5.1 Hz, H-4′), 1.66 (s, H-5′), 3.17 (q, J=5.1 Hz, H-3′), δ_C 13.6 (C-4′), 19.5 (C-5′), 60.4 (C-2′ and C-3′), 169.3 (C-1′)][10] in place of a 3′-chloro-2′-hydroxy-2′-methylbutyloyloxyl group of **4**. The EI-MS of **5** showed a fragment ion peak at *m/z* 248, indicating loss of epoxyangelic acid from the molecular ion peak at *m/z* 364. Comparison of ^{1}H- and ^{13}C-NMR data for epoxyangeloyloxyl group with publishid data[10] confirmed the structure of the epoxyangeloyloxyl moiety of **5**. The structure of this acyl group was further confirmed by the HMBC spectrum, in which the tertiary methyl group (H-5′) showed connectivity to C-1′, C-2′ and C-3′, and the secondary methyl group (H-4′) showed connectivity to C-2′ and C-3′. The absolute configuration of C-2′ and C-3′ could not be determined. From the above data, the structure of **5** was determined to be 6β-epoxyangeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-olide. Compound **5** is the first eremophilenolide derivative with an epoxyangeloyloxyl group isolated from natural sources.

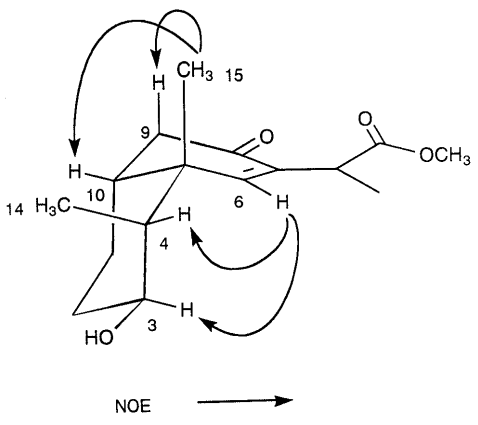
Compound **6** was isolated as a colorless oil, $[\alpha]_D$ −54.4°. The molecular formula was determined to be $C_{16}H_{24}O_4$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3475 cm^{-1}) and an α,β-unsaturated-γ-lactone (1751 cm^{-1}). Detailed analysis of the ^{1}H- and ^{13}C-NMR spectra of **6** revealed a gross structure, including conformation, identical with that of 3β-hydroxy-6β-methoxyeremophil-7(11)-en-12,8β-olide (**11**), which had a non-steroidal conformation.[3b] However, the chemical shift and coupling pattern for the olefinic methyl group (H-13) differ significantly between the two compounds. The ^{1}H-NMR spectrum of **11**[3b] showed the homoallylic coupling of the olefinic methyl group [δ 1.97 (dd, J=1.5, 1.5 Hz, H-13)] with H-6α. In addition, in the ^{1}H-NMR spectrum of **6**, the homoallylic coupling between the olefinic methyl group [δ 1.89 (d, J=1.8 Hz, H-13)] and H-6α is lacking. In the NOE difference spectra, the NOE between H-6α and H-3α was not observed, while NOEs were seen between H-6β and H-4α; H-6β and H-13; and between H-6β and H-15. These data indicate that the methoxyl group at C-6 has an α configuration. Thus, the structure of **6** was determined to be 3β-hydroxy-6α-methoxyeremophil-7(11)-en-12,8β-olide. Compound **6** is the first 6α-substituted eremophil-7(11)-en-12,8β-olide derivative from natural sources.

Compound **7** was isolated as a colorless oil, $[\alpha]_D$ +95.7°. The molecular formula was determined to be $C_{16}H_{24}O_4$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3455 cm^{-1}) and an α,β-unsaturated-γ-lactone (1747 cm^{-1}). The ^{1}H-NMR spectrum of **7** was closely related to that observed for **6** except for a signal due to the tertiary methyl group [δ 1.34 (s, H-15)]. Naya *et al.*[6] reported that, for 8β-methoxyeremophil-7(11)-en-12,8α-olide derivatives, which have a steroidal conformation, the chemical shifts due to the tertiary methyl group (H-15) are downfield from those due to the secondary methyl group (H-14), whereas this relationship is reversed in the 8α-series, which has a non-steroidal conformation. These variations in the chemical shifts may be explained similarly in terms of the effect due to the alteration in geometry of the skeleton observed in the steroid field.[6] The ^{1}H-NMR spectrum of **7** showed a singlet of the tertiary methyl group (H-15) at δ 1.34 and a doublet of the secondary methyl group (H-14) at δ 1.01 (J=7.0 Hz). Furthermore, in the NOE difference spectra, NOEs were observed between H-6α and H-13; H-6α and H-14; H-6α and H-15; and between the 6β-methoxyl group and H-8β. These data indicate that **7** exists in a steroidal conformation (Fig. 1). The position of the hydroxyl group was determined to be at the C-3β by comparing the chemical shift, coupling pattern and constants of the hydroxy-bearing methine proton of **7** with those of 3β-hydroxy-6β-acyleremophil-7(11)-en-12,8α-olides.[8] The position of the methoxyl group was determined to be at the C-6β from the HMBC spectrum. Based on this evidence, the structure of **7** was determined to be 3β-hydroxy-6β-methoxyeremophil-7(11)-en-12,8α-olide.

Compound **8** was isolated as a colorless needles, mp 204—206 °C, $[\alpha]_D$ +48.4°. The molecular formula was

determined to be $C_{15}H_{20}O_4$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3346 cm^{-1}), an α,β-unsaturated-γ-lactone (1753 cm^{-1}) and a six-membered ring ketone (1709 cm^{-1}). The ^1H- and ^{13}C-NMR spectra showed signals due to a tertiary methyl group [δ_H 1.00 (s, H-15), δ_C 12.3 (C-15)], a secondary methyl group [δ_H 0.97 (d, J=6.6 Hz, H-14), δ_C 7.7 (C-14)], an olefinic methyl group [δ_H 1.87 (d, J=1.7 Hz, H-13), δ_C 8.3 (C-13)], an AB-type methylene [δ_H 2.28 (dd, J=13.9, 1.7 Hz, H-6β), 2.74 (d, J=13.9 Hz, H-6α), δ_C 34.6 (C-6)], an α,β-unsaturated-γ-lactone [δ_C 123.9 (C-11), 157.1 (C-7), 171.5 (C-12)], a hemi-ketal carbon [δ_C 103.0 (C-8)] and a carbonyl carbon [δ_C 211.3 (C-3)]. Thus, the structure of **8** was 8 (α or β)-hydroxy-3-oxoeremophilenolide. The HMBC spectrum supported this structure. In the NOESY spectrum, NOEs were seen between H-6α and H-13; H-6α and H-14; H-6α and H-15; and between H-13 and H-14. Based on this evidence, the structure of **8** was determined to be 8β-hydroxy-3-oxoeremophil-7(11)-en-12,8α-olide, which had a steroidal conformation.

Compound **9** was isolated as a colorless oil, $[\alpha]_D$ −9.3°. The molecular formula was determined to be $C_{16}H_{24}O_4$ by HR-MS. The IR spectrum suggested the presence of a hydroxyl group (3452 cm^{-1}), a saturated ester (1733 cm^{-1}) and an α,β-unsaturated ketone (1671 cm^{-1}). The ^1H- and ^{13}C-NMR spectra showed signals due to two secondary methyl groups [δ_H 0.99 (d, J=7.0 Hz, H-14), 1.28 (d, J=7.3 Hz, H-13), δ_C 7.4 (C-14), 16.0 (C-13)], a tertiary methyl group [δ_H 1.24 (s, H-15), δ_C 25.2 (C-15)], two secondary methyl-bearing methines [δ_H 1.88 (qd, J=7.0, 4.4 Hz, H-4α), 3.62 (qd, J=7.3, 1.1 Hz, H-11), δ_C 44.8 (C-4), 37.8 (C-11)], a methine [δ_H 2.03 (m, H-10), δ_C 35.7 (C-10)], a methylene [δ_H 2.30 (dd, J=17.2, 4.0Hz, H-9α), 2.66 (dd, J=17.2, 4.8 Hz, H-9β), δ_C 40.1 (C-9)], a carbomethoxyl group [δ_H 3.66 (s, $COCH_3$), δ_C 52.0 (OCH_3), 175.1 (C-12)], a hydroxy-bearing methine [δ_H 3.74 (ddd, J=9.9, 4.4, 4.4 Hz, H-3α, δ_C 70.6 (C-3)], an olefin [δ_H 6.74 (br s, H-6), δ_C 154.3 (C-6)] and a carbonyl group [δ_C 197.6 (C-8)]. These spectral data and molecular formula suggested that the most likely structure of this compound is **9**, as illustrated. The stereostructure was determined by NOE difference spectra. An NOE was observed between H-6 and H-3α, suggesting that the hydroxyl group at C-3 has a β configuration. Furthermore, a *cis*-junction of the A/B rings which had a non-steroidal conformation, was suggested by the NOEs between H-15 and H-9β; H-15 and H-10; H-6 and H-3α; and between H-6 and H-4α (Fig. 2). The absolute configuration of the secondary C-11 methyl group could not be determined. From the above data, the structure of **9** was determined to be 3β-hydroxy-8-oxoeremophil-6-en-12-oic acid methyl ester.



9

Fig. 2

Experimental

General Procedures Melting points were determined with a Yanagimoto micromelting apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-360 digital polarimeter. IR spectra were recorded with a Perkin-Elmer FT-IR 1725X IR spectrophotometer and UV spectra with a Beckman DU-64 spectrophotometer. ^1H- and ^{13}C-NMR spectra were recorded with a JEOL JNM-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; br s, broad singlet; d, doublet; br d, broad doublet; dd, double doublet; ddd, double double doublet; dddd, double double double doublet; dq, double quartet; ddq, double double quartet; q, quartet; qd, quartet doublet; qq, quartet quartet; m, multiplet). The EI-MS and HR-MS were recorded on a JEOL JMS-DX 303 mass spectrometer. Column chromatography was carried out on Kieselgel 60 column (Merck; 230—400 mesh). Preparative HPLC was carried out on a Tosoh HPLC system (pump, CCPD; detector, UV-8011) using a TSK gel ODS-120T column (Tosoh).

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

Extraction and Isolation The dried rhizomes of *Petasites japonicus* (3.0 kg) were extracted three times with MeOH at room tempertaure 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 (112.5 g). This residue (60.0 g) was chromatographed on a silica-gel column using benzene–AcOEt (9:1, 8:2, 7:3) and $CHCl_3$–MeOH (8:2), and the eluate was separated into 4 fractions (frs. 1—4). Fraction 4 was rechromatographed on a silica-gel column using benzene–AcOEt (6:4, 5:5, 4:6, 3:7) and $CHCl_3$–MeOH (9:1, 8:2), and the eluate was separated into 4 fractions (frs. 1′—4′). Fraction 2′ was rechromatographed on a silica-gel column using *n*-hexane–acetone (5:4, 5:5, 4:5, 3:6) and acetone, and the eluate was separated into 5 fractions (frs. 1″—5″). Fraction 3″ was rechromatographed on a silica gel column using benzene–AcOEt (3:2, 2:2), and the eluate was separated into 7 fractions (frs. 1‴—7‴). Fraction 6‴ was purified by preparative HPLC (Column, TSK gel ODS-120T, 21.5 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (8:9); flow rate, 5.0 ml/min; UV detector, 220 nm) to give **5** (0.5 mg), a mixture of **6** and **7** (5.0 mg), **8** (1.2 mg) and **9** (2.9 mg). The mixture of **6** and **7** was purified by preparative HPLC (Column, TSK gel ODS-120T, 21.5 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (2:3); flow rate, 4.0 ml/min; UV detector, 220 nm) to give **6** (2.4 mg) and **7** (1.9 mg). Fraction 4″ was purified by preparative HPLC (Column, TSK gel ODS-120T, 21.5 mm i.d. × 30 cm; mobile phase, MeOH–H_2O (1:1); flow rate, 4.0 ml/min; UV detector, 220 nm) to give **1** (12.3 mg), **2** (6.0 mg), **3** (3.6 mg) and **4** (1.4 mg).

6β-Angeloyloxy-3β,9α-dihydroxyeremophil-7(11)-en-12,8β-olide (1) Colorless oil. $[\alpha]_D^{22}$ −32.4° (c=1.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3423, 1752, 1719, 1681, 1646. UV λ_{max}^{MeOH} nm (log ε): 221 (4.0). HR-MS m/z: 364.1915 (M^+, Calcd for $C_{20}H_{28}O_6$; 364.1886). ^1H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

6β-Angeloyloxy-3β,9β-dihydroxyeremophil-7(11)-en-12,8β-olide (2) Colorless oil. $[\alpha]_D^{22}$ −39.9° (c=0.6, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3442, 1756, 1718, 1646. UV λ_{max}^{MeOH} nm (log ε): 218 (4.1). HR-MS m/z: 364.1839 (M^+, Calcd for $C_{20}H_{28}O_6$; 364.1886). ^1H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

6β-Angeloyloxy-3β,8β,9β-trihydroxyeremophil-7(11)-en-12,8α-olide (3) Amorphous powder. $[\alpha]_D^{22}$ +41.2° (c=0.4, EtOH). IR ν_{max}^{KBr} cm^{-1}: 3445, 1757, 1718, 1644. UV λ_{max}^{MeOH} nm (log ε): 218 (4.2). HR-MS m/z: 380.1852 (M^+, Calcd for $C_{20}H_{28}O_7$; 380.1835). ^1H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

6β-(3′-Chloro-2′-hydroxy-2′-methylbutyloyloxy)-3β-hydroxyeremophil-7(11)-en-12,8β-olide (4) Colorless oil. $[\alpha]_D^{21}$ −55.6° (c=0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3447, 1750. UV λ_{max}^{MeOH} nm (log ε): 220 (4.1). HR-MS m/z: 400.1684 (M^+, Calcd for $C_{20}H_{29}{}^{35}ClO_6$; 400.1652), m/z: 402.1667 (M^++2, Calcd for $C_{20}H_{29}{}^{37}ClO_6$; 402.1624). ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

6β-Epoxyangeloyloxy-3β-hydroxyeremophil-7(11)-en-12,8β-oilde (5) Colorless oil. $[\alpha]_D^{20}$ −96.2° (c=0.05, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3423, 1750. UV λ_{max}^{MeOH} nm (log ε): 224 (3.9). HR-MS m/z: 364.1912 (M^+, Calcd for $C_{20}H_{28}O_6$; 364.1886). ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

3β-Hydroxy-6α-methoxyeremophil-7(11)-en-12,8β-olide (6) Colorless oil. $[\alpha]_D^{26}$ −54.4° (c=0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3475, 1751. UV λ_{max}^{MeOH} nm (log ε): 220 (4.0). HR-MS m/z: 280.1650 (M^+, Calcd for $C_{16}H_{24}O_4$; 280.1674). ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

3β-Hydroxy-6β-methoxyeremophil-7(11)-en-12,8α-olide (7) Coloress oil. $[\alpha]_D^{25}$ +95.7° (c=0.2, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3455, 1747. UV λ_{max}^{MeOH} nm (log ε): 220 (4.1). HR-MS m/z: 280.1632 (M^+, Calcd for $C_{16}H_{24}O_4$; 280.1674). ^{1}H-NMR: see Table 1. ^{13}C-NMR : see Table 2.

8β-Hydroxy-3-oxoeremophil-7(11)-en-12,8α-olide (8) Colorless needles (from AcOEt), mp 204—206 °C. $[\alpha]_D^{27}$ +48.4° (c=0.1, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3346, 1753, 1709. UV λ_{max}^{MeOH} nm (log ε): 219 (3.9). HR-MS m/z: 264.1351 (M^+, Calcd for $C_{15}H_{20}O_4$; 264.1361). ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

3β-Hydroxy-8-oxoeremophil-6-en-12-oic Acid Methyl Ester (9) Colorless oil. $[\alpha]_D^{24}$ −9.3° (c=0.2, MeOH). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3452, 1733, 1671. UV λ_{max}^{MeOH} nm (log ε): 231 (3.9). HR-MS m/z: 280.1637 (M^+, Calcd for $C_{16}H_{24}O_4$; 280.1675). ^{1}H-NMR: see Table 1. ^{13}C-NMR: see Table 2.

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