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THREE NEW EREMOPHILENOLIDES FROM *LIGULARIA HIBERNIFLORUM* (MAKINO) KITAM.

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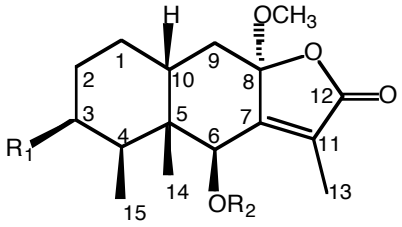
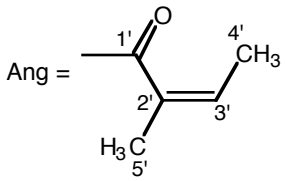
Abstract- Three new eremophilenolides, 3□-angeloyloxy-6□,8□-dimethoxy-eremophil-7(11)-en-12,8□-olide, 3□-angeloyloxy-6□-hydroxy-8□-methoxy-eremophil-7(11)-en-12,8□-olide and 3□-angeloyloxy-6□-hydroxy-8□-methoxy-eremophil-7(11)-en-12,8□-olide, were isolated from the rhizomes of *Ligularia hiberniflorum* (Makino) Kitam. These structures were elucidated on the basis of spectroscopic evidence. The CD spectra of the compounds are also briefly discussed.

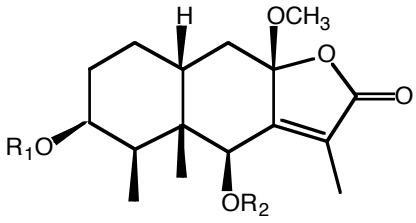
Many rhizomes from genus *Petasites* in Compositae have been used in folk medicine,[1,2] and their constituents have been well investigated and shown to contain eremophilane types of sesquiterpenes, eremophilenolides[3-5] and bakkanolides.[6] *Ligularia hiberniflorum* (Makino) Kitam. is a native Compositae plant closely related to *Petasites*, growing only at two islands of Yakushima and Tanegashima in Kagoshima.[7] A rearranged eremophilenolide, bakkenolide A, has been isolated from the aerial part collected at Tanegashima.[8] We studied the constituents of *L. hiberniflorum* collected at Yakushima and isolated three new eremophilenolides of 3□-angeloyloxy-6□,8□-dimethoxyeremophil-7(11)-en-12,8□-olide (**1**), 3□-angeloyloxy-6□-hydroxy-8□-methoxyeremophil-7(11)-en-12,8□-olide (**2**) and 3□-angeloyloxy-6□-hydroxy-8□-methoxyeremophil-7(11)-en-12,8□-olide (**3**) from the rhizomes, and four known compounds of □-sitosterol, stigmasterol, methyl 3,4-dihydroxy-*trans*-cinnamate and 2-hydroxy-4-methoxybenzoic acid from the leaves. The known compounds were identified by spectroscopic studies and comparison of their spectral data with those reported. We wish to report the isolation and characterization of the new compounds. In the connection with the structure elucidation, we also show the effectiveness of CD spectra in the stereochemical studies of rings A/B *cis*-fused elemophilenolides. Extraction and isolation were separately carried out on the rhizomes and leaves as described in the EXPERIMENTAL section.

Compound (**1**), an oil, $[\alpha]_D$ -96° (MeOH), was assigned the molecular formula $C_{22}H_{32}O_6$ by the HREIMS. The IR and UV spectra showed the presence of □,□-unsaturated □-lactone and ester groups (λ_{max} 1769,



1: R_1 = OAng, R_2 = CH_3
2: R_1 = OAng, R_2 = H
4: R_1 = OAng, R_2 = Ac
5: R_1 = OAng, R_2 = *p*-Br-Bz
6: R_1 = H, R_2 = H

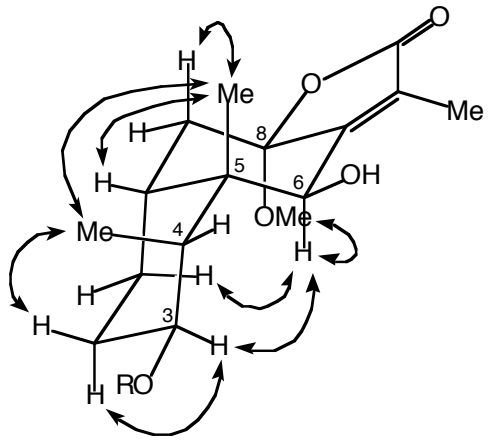




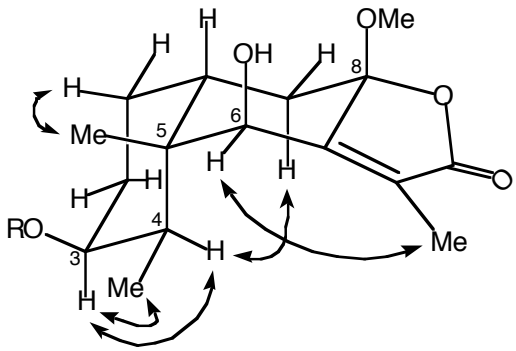
3: R_1 = Ang, R_2 = H
7: R_1 = H, R_2 = $COCH^{c}$=CHSMe

1713 and 1646 cm^{-1} and λ_{max} 221 nm: ε12000). The ^{13}C and ^{1}H NMR spectra (Table 1) indicated that **1** contained seven CH_3 (2 methoxy), three CH_2, four CH (one alkoxy and one acyloxy), four carbons not bonded to hydrogen and each one of tri- and tetra-substituted double bond. The NMR spectra also revealed the presence of an olefinic methyl group and a typical angeloyl substituent. These data suggested that this compound was an eremophilenolide similar to rings A/B *cis*-fused 3β-hydroxy-6β,8β-dimethoxyeremophil-7(11)-en-12,8α-olide (**4**),[3] isolated from a Japanese butterbur *Petastes japonicus*, except for the presence of an additional angeloyl group at C-3 in **1**. Stereochemistry of rings A/B *cis*-fused 8-methoxyelemophilenolides has been well studied and reported that the 8α- and 8β-methoxy isomers had non-steroidal (A) and steroidal (B) chair/chair conformations as shown in Figure 1.[9] Together with a homoallylic coupling of the 6-H with the olefinic 11-Me (13-H, δ2.01), the NOE correlations of 5-Me (14-H, δ0.82) signal with the 4-Me (15-H, δ 0.98) doublet and the 6α-H (δ 4.38) quartet with the 3β-δ (δ 5.22) and 8β-OMe (δ 3.20) signals, elucidated the proposed stereochemistry. Therefore, the structure of compound (**1**) was identified as 3β-angeloyloxy-6β,8β-dimethoxyeremophil-7(11)-en-12,8α-olide.

Compound (**2**), $[\alpha]_D$ -137°, $C_{21}H_{30}O_6$, showed similar spectral data with **1** including the presence of an angeloyloxy group at C-3 except for the change of the 6-methoxy group in **1** to a hydroxyl group in **2**, although some chemical shifts and NOE correlations were different from **1**. The 6$\alpha\alpha$ signal was



A: 8α-methoxy isomer



B: 8β-methoxy isomer

Figure 1. Conformations and NOEs for 8α- and 8β-methoxy isomers of eremophilenolides.

Table 1. ^{1}H- and ^{13}C- NMR spectral data of compounds (**1**, **2** and **3**)

	1		**2**		**3**	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1α	2.16 m	26.9	2.14 m	26.7	2.00 m	21.3
β	1.55 dq (14.0, 3.7)		1.56 dq (13.7, 2.9)		1.33 m	
2α	1.70 m	25.5	1.88 m	25.3	1.69 m	24.8
β	1.75 m		1.73 m		1.55 m	
3	5.22 dt (11.3, 4.3)	71.2	5.16 ddd (9.0, 7.1, 4.5)	71.4	5.04 br q (2.8)	70.3
4	2.50 dq (4.3, 7.3)	35.4	2.43 dq (4.5, 7.3)	35.2	1.48 dq (3.7, 7.3)	32.8
5		47.3		47.4		42.9
6	4.38 q (1.5)	79.7	5.01 br d (3.3)	70.0	4.49 d (9.9)	69.9
7		157.0		157.6		153.6
8		106.8		106.5		106.1
9α	2.15 br d (13.0)	37.5	2.15 m	37.1	1.88 t (13.8)	36.1
β	1.79 dd (13.0, 5.8)		1.90 m		2.24 dd (13.9, 3.9)	
10	1.82 (m)	35.0	1.85 m	34.8	2.08 ddt (13.8, 3.2, 3.9)	33.1
11		126.5		126.5		126.5
12		167.5		171.7		170.8
13	2.01 br d (1.5)	8.4	2.09 d (1.5)	8.9	1.93 s	8.7
14	0.82 s	18.7	0.86 s	18.4	1.35 s	12.2
15	0.98 d (7.3)	8.9	0.98 d (7.3)	8.7	0.91 d (7.3)	
1'		167.5		167.9		167.3
2'		128.3		128.0		127.8
3'	6.07 qq (7.3, 1.5)	137.6	6.18 qq (7.0, 1.5)	138.4	6.08 qq (7.3, 1.5)	138.7
4'	2.00 dq (7.3, 1.5)	15.8	1.99 dq (7.0, 1.4)	15.8	2.08 dq (7.3, 1.5)	15.7
5'	1.91 quint (1.5)	20.7	1.89 dq (1.5, 1.4)	20.6	1.93 quint (1.5)	20.9
OH			2.86 d (3.3)		2.72 d (9.9)	
6-OMe	3.47 s	59.8				
8-OMe	3.20 s	50.0	3.17 s	50.0	3.34 s	51.2

Measured in $CDCl_3$.

Chemical shift values are in ppm from TMS, and *J* values (in Hz) are presented in parentheses.

particularly observed at a lower field of □ 5.01 and it showed NOE correlation with the 8□-methoxy (□ 3.17) and 3□-H (□ 5.16) signals. The *cis*-fusion of rings A/B in **2** was confirmed from the NOEs of the 5□-Me (14-H, □ 0.86) signal with the 4□-Me (15-H, □ 0.98) and 10□□□ (□ 1.85) signals. The stereochemistry at C-6 and C-8 was clarified from the spectral studies on the acetate (**4**) and *p*-Br-benzoate (**5**). In **5**, the NOE correlations were also observed between the 6-H signal and the 3-H and 8-OMe signals. On the other hand, the up-field shifts of the 4□-H and 11-Me signals in **5** supported the presence of 6□-OH group in **2**.[9] By the way, the 6□-H signal is usually observed at a lower field in 6□-hydroxy-8□-methoxyeremophilenolides; □ 4.95[10] and 4.99[11] in **6**. These observations established the structure of **2** as depicted. The stereochemistry of **2** was also provided from similar CD spectra (Figure 2) of **2** and **5** to

that of **1**. As predicted from the Dreiding model, **6** did not show any split Cotton effect.[12]

Compound (**3**) having the same molecular formula of $C_{21}H_{30}O_6$ as **2**, showed the presence of methoxy and angeloyl groups from the NMR spectra (Table 1). Although **3** showed similar ^1H and ^{13}C NMR data to those of **2**, it showed the positive specific rotation of $[\alpha]_D$ +37° opposite in the sign from **1** and **2** to suggest **3** being a 8α-methoxyeremophilenolide.[9] The spectral features of the eremophilane skeleton in **3** were, in fact, almost the same as reported for an 8α-isomer, eremopetasitenin D1 (**7**),[5] except for the differences due to ester substitution.

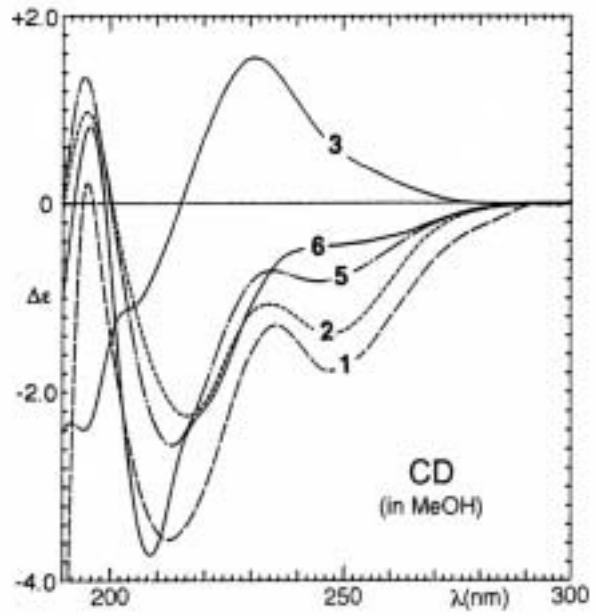


Figure 2. CD spectra of compounds **1-3**, **5** and **6**.

The NOE correlations between 4- and 9α-H signals and 6α-H and 13-H_3 signals, also showed **3** to have the same steroidal conformation and stereochemistry as the structure B shown in Figure 1. The CD spectrum of **3** showed a positive Cotton effect at 231 nm ($\Delta\varepsilon$ +1.6) different from negative ones of compounds (**1** and **2**), which also suggested strongly **3** to be present not in non-steroidal but in steroidal chair/chair conformation.[11]

EXPERIMENTAL

^1H and ^{13}C NMR spectra were measured at 400 and 100 MHz at 45° on a JEOL FX-400 spectrometer. IR and UV spectra were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers. Specific rotations and CD spectra were measured using JASCO DIP-370S and JASCO J-720 spectropolarimeters. HPLC was performed on a Waters μBondapak C_{18} column.

Plant Material. The plant samples were collected in March 1997 at Yakushima in Kagoshima Prefecture and identified by Prof. M. Hotta of Kagoshima University.

Extraction and isolation. i) The fresh rhizomes (1.7 kg) were extracted with MeOH (3 L) at rt for 1 week to yield the extract (72 g), which was partitioned by successive extraction with ether (500 mL). 7.3 g of the ether soluble part (43 g) was flash chromatographed on SiO_2 with MeOH-CH_2Cl_2 solvent system to give 9 frs. Fr. 2 eluted with 1% MeOH-CH_2Cl_2 was purified by a combination of prep TLC (5% MeOH-CH_2Cl_2) and HPLC (20-35% H_2O/MeOH) to give compounds (**1**) (3 mg) and (**3**) (3.5 mg). Fr. 3 eluted with 2% MeOH-CH_2Cl_2 was similarly purified by prep TLC followed HPLC to give compound (**2**) (25 mg). From the fr. 4 eluted with 3% MeOH-CH_2Cl_2, methyl 3,4-dihydroxy-*trans*-cinnamate (61 mg) was afforded. ii) The fresh leaves (1.9 kg) were extracted with MeOH (10 L) at rt for 1 week to give the extract (43 g). 1.5 g of the ether soluble part of the MeOH extract was flash chromatographed on SiO_2

followed by HPLC to give β-sitosterol (6 mg), stigmasterol (2 mg), methyl 3,4-dihydroxy-*trans*-cinnamate (15 mg) and 2-hydroxy-4-methoxybenzoic acid (6 mg).

3β-Angeloyloxy-6β,8β-dimethoxyeremophil-7(11)-en-12,8β-olide (1). An oil, $C_{22}H_{32}O_6$; HREIMS m/z 392.2191 $[M]^+$, Δ -0.8 mmu; $[\alpha]_D$ –96° (c 0.5, MeOH); IR (Film) ν_{max} cm^{-1}: 1769, 1713, 1646; UV (MeOH) λ_{max} nm (ε): 221 (12000); CD (MeOH) : $\Delta\varepsilon_{195}$ +0.2, $\Delta\varepsilon_{212}$ –3.6, $\Delta\varepsilon_{246}$ –1.8.

3β-Angeloyloxy-6β-hydroxy-8β-methoxyeremophil-7(11)-en-12,8β-olide (2). An oil, $C_{21}H_{30}O_6$; HREIMS m/z 378.2054 $[M]^+$, Δ +1.1 mmu; $[\alpha]_D$ –137° (c 0.5, MeOH); IR (Film) ν_{max} cm^{-1}: 3447, 1765, 1745 (sh), 1713, 1649; UV (MeOH) λ_{max} nm (ε): 219 (ε 12000); CD (MeOH): $\Delta\varepsilon_{195}$ +1.0, $\Delta\varepsilon_{216}$ –2.2, $\Delta\varepsilon_{247}$ –1.4.

3β-Angeloyloxy-6α-hydroxy-8β-methoxyeremophil-7(11)-en-12,8β-olide (3). An oil, $C_{21}H_{30}O_6$; HREIMS m/z 378.2044 $[M]^+$, Δ +0.1 mmu; $[\alpha]_D$ +37° (c 0.5, MeOH); IR (Film) ν_{max} cm^{-1}: 3391, 1767, 1755(sh), 1715, 1653; UV (MeOH) λ_{max} nm (ε): 217 (ε 10000); CD (MeOH): $\Delta\varepsilon_{195}$ –2.4, $\Delta\varepsilon_{204}$ -1.1, $\Delta\varepsilon_{231}$ +1.6.

3β-Angeloyloxy-6α-acetoxy-8β-methoxyeremophil-7(11)-en-12,8β-olide (4). Acetylation of **2** (5 mg) with acetic anhydride in pyridine for 1 day at rt gave the acetate (**4**: 3 mg). $C_{23}H_{32}0_7$; EIMS m/z 420 $[M]^+$; IR (Film) ν_{max} cm^{-1}: 1768, 1730, 1720, 1650; UV (MeOH) λ_{max} nm (ε): 221 (12000); ^{1}HNMR ($CDCl_3$); δ 0.93 (3H, s, 14-H), 0.95 (3H, d, J = 7.3 Hz, 15-H), 1.58 (1H, m, 1β-H), 1.70 (1H, m, 2β-H), 1.75(1H, m, 2α-H), 1.84 (1H, m, 10β-H), 1.87 (3H, quint, J = 1.5 Hz, 5'-H), 1.89 (1H, br s, 13-H), 1.90 (1H, m, 9β-H), 1.97 (3H, dq, J = 7.2, 1.5 Hz, 4'-H), 2.17 (1H, d, J = 1.5 Hz, 9α-H), 2.24 (1H, m, 1α-H), 2.25 (3H, s, Ac), 2.32 (1H, dq, J = 4.5, 7.3 Hz, 4-H), 3.22 (3H, s, OMe), 5.00 (1H, dt, J = 11.5, 4.5 Hz, 3-H), 5.92 (1H, br s, 6-H), 6.03 (1H, qq, J = 7.2, 1.5 Hz, 3'-H); ^{13}C NMR ($CDCl_3$): δ 8.2 (q, C-15), 8.7 (q, C-13), 15.7 (q, C-4'), 19.3 (q, C-14), 20.6 (q, C-5'), 20.6 (q, Ac), 25.1 (t, C-2), 26.7 (t, C-1), 35.0 (d, C-10), 35.4 (d, C-4), 37.9 (t, C-9), 45.9 (s, C-5), 50.3 (q, OMe), 71.1 (d, C-3), 71.1 (d, C-6), 106.7 (s, C-8), 126.5 (s, C-11), 128.0 (s, C-2'), 137.5 (s, C-3'), 155.0 (s, C-7), 167.1 (s, C-1'), 170.7 (s, Ac), 171.0 (s, C-12); CD (MeOH): $\Delta\varepsilon_{195}$ +1.4, $\Delta\varepsilon_{213}$ –2.6, $\Delta\varepsilon_{245}$ –0.8.

3β-Angeloyloxy-6α-*p*-bromobenzoyl-8β-methoxyeremophil-7(11)-en-12,8β-olide (5). Benzoylation of **2** (5 mg) with *p*-bromobenzoyl chloride (10 mg) in pyridine for 1 day at rt gave the benzoate (**5**: 3.5 mg). $C_{28}H_{33}O_7Br$; FABMS m/z 561, 563 $[M+1]^+$; IR (Film) ν_{max} cm^{-1}: 1769, 1726, 1656; UV (MeOH) λ_{max} nm (ε): 220 (ε11000), 245 (e 8000); ^{1}H NMR ($CDCl_3$): δ 1.03 (3H, d, J = 7.0 Hz, 15-H), 1.08 (3H, s, 14-H), 1.78 (3H, d, J = 1.5 Hz, 13-H), 1.81 (3H, dq, J = 1.5, 1.4 Hz, 5'-H), 1.85 (3H, dq, J = 7.0, 1.4 Hz, 4'-H), 2.23 (1H, d, J = 12.4 Hz, 9α-H), 2.29 (1H, m, 4α-H), 3.20 (3H, s, OMe), 5.08 (1H, dq, J = 12.0, 4.3 Hz, 3-H), 5.99 (1H, qq, J = 7.0, 1.5 Hz, 3'-H), 6.17 (1H, br s, 6-H), 7.50 (2H, br d, J = 8.6 Hz, 4''-H, 6''-H), 7.97 (2H, br d, J = 8.6 Hz, 3''-H, 7''-H); CD (MeOH): $\Delta\varepsilon_{196}$ +0.8, $\Delta\varepsilon_{208}$ –3.8, $\Delta\varepsilon_{246}$ –0.4.

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