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GUAIANOLIDES FROM *ARTEMISIA MONTANA*

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Key Word Index—*Artemisia montana*, Compositae, sesquiterpene lactones, guaianolides, neoezoguaianin; ezoyomoginin

Abstract—From the aerial parts of *Artemisia montana* ('Ezo-yomogi' in Japanese), two new sesquiterpene lactones, neozeoguaianin and ezoyomoginin were isolated together with five known guaianolides, yomogiartemin, ezoartemin, yamayomoginin, ezomontanin, and 11,13-dihydroezomontanin The structures of new compounds were established mainly by high field ^1H NMR spectroscopic method.

INTRODUCTION

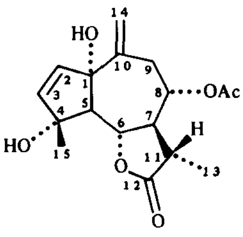
In continuation of our studies on sesquiterpene lactones from *Artemisia* species [1–5], we have now investigated the aerial parts of *A montana*, 'Ezo-yomogi' in Japanese. From the mature plants of *A montana*, two new sesquiterpene lactone guaianolides, neoezoguaianin (**1**) and ezoyomoginin (**2**) were isolated together with five known sesquiterpene lactones, yomogiartemin [1, 2], yamayomoginin [3], ezoartemin, and 11,13-dihydroezomontanin [4]. The structures of new compounds were elucidated mainly by high field ^1H NMR spectroscopic method and the known compounds were identified by direct compartion with authentic samples.

RESULTS AND DISCUSSION

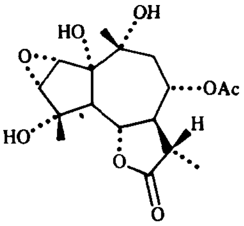
Neoezoguaianin (**1**) had a molecular formula of $C_{17}H_{22}O_6$ (m/z 322.1418 M^+) by HR-EIMS. Its IR spectrum showed the presence of hydroxy (3475 cm^{-1} and 3445 cm^{-1}), γ-lactone (1760 cm^{-1}) and acetate (1724 cm^{-1} and 1238 cm^{-1}) groups, and double bonds (1670–1625 cm^{-1}). The mass spectral fragmentation pattern suggested the presence of two hydroxy groups. Compound **1** was not acetylated with acetic anhydride–pyridine. This fact suggested the two hydroxy groups were tertiary.

The ^1H NMR spectrum (Table 1) of **1** contained signals for exomethylene protons at δ5.13 (*s*) and 4.88 (*d*) (H-14a, and 14b) and olefinic protons at δ5.62 (*d*) and 6.02 (*d*) (H-2, and H-3). The coupling patterns of the proton signals for H-2 to H-9a,b supported a guaianolide skeleton. All assignments were made by detailed ^1H homonuclear decoupling The magnitude of the coupling constants of H-5 to H-8 were in full agreement with an antiperiplanar disposition of H-5/H-6, H-6/H-7, and H-7/H-8. The tertiary hydroxy group at C-1 was assigned an α-orientation [6].

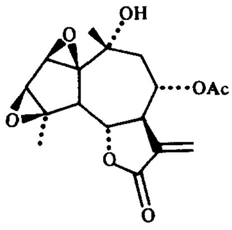
The ^{13}C NMR spectrum (Table 2) of **1** agree well with the structure. Finally, the stereochemistry of **1** was determined by NOE difference spectroscopy. Saturation of H-15 showed effects on H-3 (13.8%), H-6β (27.0%), and H-8β (4.6%). The position of tertiary hydroxy group at C-4 was confirmed as being in the α-orientation [7].



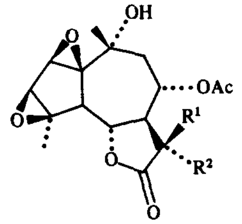
1



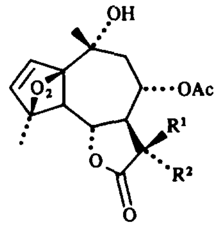
2



Yomogiartemin



Yamayomoginin
R^1 = Me, R^2 = H
Ezoartemin
R^1 = H, R^2 = Me



Ezomontanin
R^1 = R^2 = CH_2
Dihydyoezomontanin
R^1 = H, R^2 = Me

The second compound, ezoyomoginin (**2**), on mass spectral analysis showed a molecular ion peak at m/z 356, $C_{17}H_{24}O_8$, and an important fragment ion at m/z 340 $[M-16]^+$, which indicated the presence of an epoxy group. The fragmentation pattern suggested the presence of hydroxy groups and one acetate group. The IR spectrum of **2** showed the presence of hydroxy groups (3525, 3425

Table 1 ^{1}H NMR spectral data of neoezoguaianin (**1**) and ezoyomoginin (**2**) (270 MHz, $CDCl_3$ TMS as int. standard)

H	1	2
1 (OH)	2 06 *s*	1 58 *s*
2	5 62 *d*	4 07 *d*
3	6 02 *d*	3 87 *d*
4 (OH)	2 21 *s*	1 98 *s*
5α	2 41 *d*	2 83 *d*
6β	4 14 *dd*	4 37 *dd*
7α	2 74 *ddd*	3 09 *ddd*
8β	4 90 *ddd*	5 21 *ddd*
9α	2 92 *ddd*	2 34 *dd*
9β	2 61 *dd*	1 87 *dd*
11β	2 51 *dddd*	2 44 *dddd*
13	1 35 *d*	1 29 *d*
14a	5 13 *s*	1 19 *s*
14b	4 88 *d*	
15	1 33 *s*	1 54 *s*
17	2 12 *s*	2 12 *s*

Coupling constants compound **1** $J_{2\ 3} = 5\ 6$, $J_{5\ 6} = 10\ 9$, $J_{6,\ 7} = 9\ 6$, $J_{7,\ 8} = 10\ 4$, $J_{7,\ 11} = 10\ 6$, $J_{8\ 9\alpha} = 9\ 5$, $J_{8,9\beta} = 5\ 4$, $J_{9gem} = 12\ 4$, $J_{9\ 14} = 1\ 2$ $J_{11,13} = 7\ 2$ and H-8, $W_{1/2} = 13\ 4$ (Hz) Compound **2** $J_{2,\ 3} = 0\ 4$, $J_{5,\ 6} = J_{6,\ 7} = 10\ 7$, $J_{7,\ 8} = 10\ 4$, $J_{7,\ 11} = 11\ 4$, $J_{8,\ 9\alpha} = 7\ 2$, $J_{8,\ 9\beta} = 2.4$, $J_{9gem} = 16\ 7$, $J_{11\ 13} = 7\ 3$ and H-8, $W_{1/2} = 13\ 0$ (Hz)

Table 2 ^{13}C NMR spectral data of neoezoguaianin (**1**) and ezoyomoginin (**2**) (67 80 MHz, $CDCl_3$, TMS as int standard)

C	1	Off reson	2	Off reson.
1	85 44	*s*	79 89	*s*
2	140 82	*d*	63 57	*d*
3	136 36	*d*	63 77	*d*
4	82 42	*s*	72 36	*s*
5	67 33	*d*	41 69	*d*
6	78 00	*d*	74 74	*d*
7	40 34	*d*	49 42	*d*
8	76 37	*d*	73 14	*d*
9	36 32	*t*	43 18	*t*
10	143 33	*s*	71 03	*s*
11	50 55	*d*	50 78	*d*
12	177 97	*s*	177 37	*s*
13	15 97	*q*	15 02	*q*
14	117 18	*t*	24 14	*q*
15	24 61	*q*	27 64	*q*
16	170 15	*s*	170 08	*s*
17	21 11	*q*	21 30	*q*

Table 3 NOEDS of compound **2**

Proton irradiated	Proton affected (%)
H-5α	H-7α (5 95), H-6β (2 90), H-13α (6 02)
H-6β	H-8β (6 18), H-11β (3 57), H-14β (4 28), H-15β (4 06)
H-8β	H-6β (7 71), H-11β (8 68)
H-14β	H-2β (18 45), H-9β (5 57)
H-15β	H-3β (9 65), H-6β (4 07), H-5α (11 05)

and 3415 sh. cm^{-1}), a γ-lactone (1750 cm^{-1}), and an acetate (1725 and 1238 cm^{-1})

Compound **2** was not acetylated under the same conditions of **1**, which suggested that all hydroxy groups were tertiary. The ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2) contained the signals of an epoxy group at δ_H4 07 (*d*, H-2) and 3.87 (*d*), and at δ_C 63 57 (C-2) and 63 77 (C-3) The coupling patterns of the proton signals for H-2 to H-9a,b also supported a guaianolide The magnitude of coupling constants from H-5 to H-8 established an antiperiplanar arrangement The assignments were made by detailed ^{1}H-homonuclear decoupling. From the above data, three hydroxy groups were suggested, at C-1, C-4, and C-10

Finally, the stereochemistry of **2** (Table 3) was determined by NOE difference spectroscopy. Except for lactone moiety, all functional groups have α-orientation

EXPERIMENTAL

Mps uncorr IR KBr NMR 270 MHz, using TMS as int standard

The air-dried aerial parts (*ca* 5 kg) of *A montana* collected in the northern part (Hokkaido, north latitude 42°45′ Hakodate) of Japan, were extracted with $CHCl_3$ and treated in the usual manner [8] The crude extract (20 g) was subjected to CC (silica gel) and each fraction (100 ml) was collected and checked by TLC in $CHCl_3$-Me_2CO (4 1) Fractions 35–44 were rechromatographed on a silica gel column with $CHCl_3$, fractions 7-12 were combined and crystallized from Et_2O as colourless needles of sitosterol 7 mg, fractions 17–24 and 35–38 gave gums and crystallized from EtOAc as colourless orthorhombic yamayomoginin (7 mg) and yomogiartemin (10 mg), resp

Fractions 45–52 were rechromatographed on a silica gel column with EtOAc-C_6H_6 (1 1) fractions 8–15 gave colourless orthorhombic, ezomontanin (8 mg), fractions 17–19 gave 11,13-dihydroezomontanin (10 mg), and fractions 50–80 afforded a gum and crystallized from EtOAc colourless orthorhombic plates of ezoartemin *ca* (100 mg) Fractions 53–60 were rechromatographed on a silica gel column with $CHCl_3$-C_6H_6 (1 1), fractions 51–78 and 83–112 gave gums and crystallized from EtOAc as colourless orthorhombic of ezomontanin (10 mg), and dihydroezomontanin (20 mg), resp

Fractions 121–171 were combined and rechromatographed on a silica gel column with $CHCl_3$-C_6H_6 (1 1), fractions 162-180 afforded a colourless powder and crystallized from EtOAc as colourless orthorhombic ezoyomoginin **2** (10 mg)

Fractions 301–410 were rechromatographed on a silica gel column with *n*-hexane-EtOAc-$CHCl_3$ (3 7 10), fractions 75-87 were rechromatographed under the same condition, and fractions 59–64 gave colourless orthorhombic, neoezoguaianin (18 mg). The known compounds agreed in spectral properties, mmp and TLC with standard samples.

Neoezoguaianin (**1**) $C_{17}H_{22}O_6$, colourless orthorhombic, mp 180–181°, EIMS (70 eV) m/z (rel int.). 322 $[M]^+$ (3 3), 307 $[M-Me]^+$ (100), 304 $[M-H_2O]^+$ (2 1), 289 $[M-Me-H_2O]^+$ (0.9), 286 $[M-H_2O-H_2O]^+$ (0.3), 265 $[M-C_2H_5CO]^+$ (87 6), 262 $[M-HOAc]^+$ (24 9), 247 $[M-HOAc-Me]^+$ (30 7), 244 $[M-HOAc-H_2O]^+$ (58.0); HRMS (70 eV) m/z 322.1418 and 262. 1206 ($C_{17}H_{22}O_6$ and $[M-HOAc]^{+\cdot}$ calcd. for $C_{17}H_{22}O_6$. 322.1416, and $C_{15}H_{18}O_4$ 262.1205 resp.). IRν_{max}^{KBr} cm^{-1}. 3475 and 3445 (νOH), 1760 (lactone), 1724 and 1238 (acetyl), and 1670–1625 (ν C=C).

Ezoyomoginin (**2**). $C_{17}H_{24}O_8$, colourless orthorhombic, mp 192–194° EIMS (70 eV) (rel. int) m/z: 356 $[M]^+$ (3.43), 341 $[M-Me]^+$ (0 30), 340 $[M-O]^+$ (0 34), 338 $[M-H_2O]^+$ (0 42), 320 $[M-2H_2O]^+$ (14.83), 314 $[M-CH_2CO]^+$ (6.31), 296 $[M-HOAc]^+$ (6 02) HRMS (70 eV) m/z 356.1537 $C_{17}H_{24}O_8$ (calcd for $C_{17}H_{24}O_8$. 356 1471) IR ν_{max}^{KBr} cm^{-1}. 3525 sh, 3425, and 3415 sh. (ν OH), 1750 (lactone), 1725 and 1238 (acetyl) *Yomogiartemin*, $C_{17}H_{20}O_7$, mp 242–243° [2]; *yamayomoginin*, $C_{17}H_{22}O_7$, mp 260–261° [3], *ezoartemin*, $C_{17}H_{22}O_7$, mp 266–267° [4]; *ezomontanin*, $C_{17}H_{20}O_7$, mp 147–148° [4]; *11,13-dihydroezomontanin*, $C_{17}H_{22}O_7$, mp 163–164° [4]

REFERENCES

1 Matsueda, S , Nagaki, M and Koreeda, M. (1980) *Yakugaku Zasshi* **100**, 615

2. Koreeda, M., Matsueda, S , Satomi, T , Hirotsu, K and Clardy, J. (1979) *Chemistry Letters* 81

3 Matsueda, S and Nagaki, M (1984) *Yakugaku Zasshi* **104**, 753.

4. Koreeda, M , Nagaki, M , Hayami, K and Matsueda, S. (1988) *Yakugaku Zasshi* **108**, 434

5. Koreeda, M , Nagaki, M., Katsukura, Y., Hayami, K and Matsueda, S (1988) *Yakugaku Zasshi* **108**, 458.

6. Irwin, M. A and Geissman, T. A (1973) *Phytochemistry* **12**, 863

7. Bohlmann, F , Hartono, L , Jakupovic, J. and Huneck, S. (1985) *Phytochemistry* **24**, 1003.

8 Nagaki, M (1984) *Phytochemistry* **23**, 462