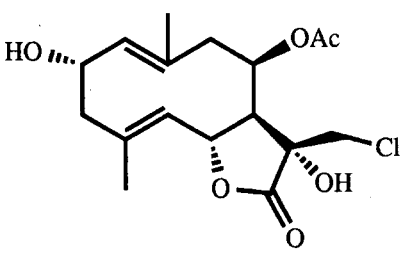
TWO NEW CHLORINE-CONTAINING GERMACRANOLIDES, EUPAGLEHNINS E AND F FROM *EUPATORIUM GLEHNI*

Motoo Tori,* Yoshiko Takeichi, Hiroe Kuga, Katsuyuki Nakashima, and Masakazu Sono

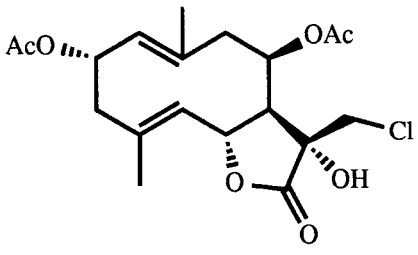
Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Yamashiro cho, Tokushima 770-8514, Japan

Abstract—Two new chlorine-containing germacrane-type sesquiterpenoids, eupaglehnins E and F, have been isolated from the MeOH extract of *Eupatorium glehni* (Compositae) and their structures have been determined on the basis of high resolution 2D NMR analyses.

Eupatorium glehni is found through Hokkaido, Honshu, and Shikoku Island in Japan, especially at higher altitude in the south part normally between 1000 and 1800 m. Kupchan and his group isolated eupaserrin and deacetyleupaserrin as antiluekemic sesquiterpenes from *E. semiserratum* in 1973.[1] Later Takahashi and his group reported isolation and their biological activities of hiyodorilactones A - F from *E. sachalinense*.[2,3] We have been interested in biologically active terpenoids from Compositae[4] and collected *E. glehni*[5,6] in Tokushima Prefecture. From the ethyl acetate soluble fraction of the methanol extract, we have found two new chlorine-containing germacranolides, named eupaglehnins E (**1**) and F (**2**). This communication describes structure elucidation of these terpenoids mainly based on 2D NMR techniques.



eupaglehnin E (**1**)



eupaglehnin F (**2**)

This paper is dedicated to Professor Teruaki Mukaiyama on the occasion of his 73rd birthday.

The ethyl acetate soluble fraction of the MeOH extract was subjected to silica gel column chromatography followed by Sephadex LH-20 ($CHCl_3$-MeOH, 1:1) and HPLC (Nucleosil 50-5, $CHCl_3$-EtOAc) to yield eupaglehnins E (**1**) and F (**2**).[7]

Eupaglehnin E (**1**)[8] exhibited the quasi molecular ion peaks at *m/z* 361 $[M+2+H]^+$ and 359 $[M+H]^+$ (CI) whose molecular formula was determined as $C_{17}H_{23}O_6Cl$ by CI-HRMS. The IR spectrum showed the presence of a lactone (1775 cm^{-1}) and an acetate (1740 cm^{-1}) as well as a hydroxyl group (3450 cm^{-1}). The ^{13}C NMR spectrum clearly exhibited 17 peaks, three methyl (18.1, 20.0, and 21.3), three methylene (44.0, 44.3, and 48.4), six methine (58.5, 68.8, 69.8, 74.5, 129.5, and 134.5), and five quaternary carbons (77.0, 133.8, 141.3, 169.4, and 174.7). Two carbonyl (a lactone and an acetate) and two olefins are obvious from ^1H and ^{13}C NMR data. These data suggested that this molecule contains two rings calculated from six degrees of unsaturation. In the HMBC spectrum the methyl group at δ_H 1.59 (3H, d, J=1.1 Hz, H-14) had correlations between carbons at δ_C 134.5 (C-1), 133.8 (C-10), and 44.3 (C-9). The methine proton at δ_H 5.58 (dd, J=6, 1), which attached to C-8 (δ_C 69.8), had correlation peaks between those at δ_C 133.8 (C-10), 44.3 (C-9), 58.5 (C-7), 74.5 (C-6), 77.0 (C-11), and 169.4 (OAc). The proton at δ_H 2.58 (H-7) correlated to C-5 (δ_C 129.5), C-6 (δ_C 74.5), and C-11 (δ_C 77.0). The methyl group at 1.75 (3H, d, J=1.4 Hz, H-15) had correlations between C-3 (δ_C 48.4), C-4 (δ_C 141.3), and C-5 (δ_C 129.5). These results indicated a partial structure through C-3 to C-10 and C-1 as well as C-11 from C-7. The linkage from C-1 to C-3 was revealed by the ^1H-^1H COSY spectrum. Therefore, a ten-membered carbocycle fused with a butyrolactone at C-6 and C-7 was thus established. An acetyl group was attached to C-8. The chlorine atom must be at either C-11 or C-13. The chemical shift at C-11 was δ_C 77.0, while that at C-13 was δ_C 44.0, which indicated the chlorine atom should be at C-13, although the protons at C-13 were found at δ_H 3.54 and 3.65 (each 1H, J=11 Hz). The stereochemistry was analyzed by the NOESY spectrum. The methyl proton at δ_H 1.59 (H-14) had NOE's into H-2β (δ_H 4.80) and H-9β (δ_H 2.73). The methyl group at δ_H 1.75 (H-15) had correlations between H-3β (δ_H 2.73) and H-6β (δ_H 5.33). The NOE's between H-5 (δ_H 4.92) and H-7α (δ_H 2.58), H-5 and H-3α (δ_H 2.13), H-7α and H-8α (δ_H 5.58), H-2β (δ_H 4.80) and H-3β (δ_H 2.73), and H-1 (δ_H 5.02) and H-9α (δ_H 2.21) were observed. Therefore, the configurations as indicated in Figure 1 was suggested for the ten-membered ring. The conformation was also indicated by the NOESY spectrum and the analysis of coupling constants for each proton (Table 1). The chloromethyl group at C-11 must have β-orientation, because the NOE between H-6 and H-13 was observed in the NOESY spectrum. Thus, the total structure was established as depicted in the formula (**1**).

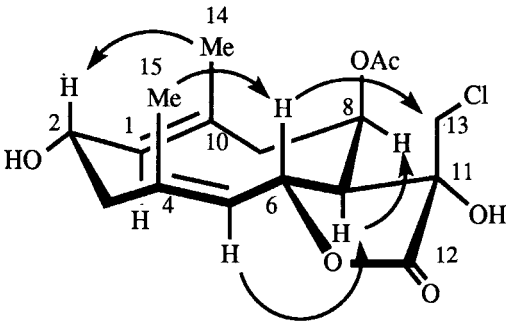


Figure 1. Conformation and selected NOE's for eupaglehnin E (**1**).

Table 1. ^{1}H and ^{13}C NMR Data (in $CDCl_3$) for Eupaglehnins E (**1**) and F (**2**).

	1		**2**	
1	5.02 (br d, 11)	134.5	5.00 (br d, 10)	130.4
2	4.80 (td, 11, 6)	68.8	5.69 (td,10, 6)	70.7
3α	2.13 (t, 11)	48.4	2.22 (t, 11)	44.7
3β	2.73 (dd, 11, 6)	-	2.73 (dd, 11, 6)	-
4	-	141.3	-	140.1
5	4.92 (br d, 10)	129.5	4.96 (br d, 10)	130.1
6	5.33 (t, 10)	74.5	5.34 (t, 10)	74.4
7	2.58 (d, 10)	58.5	2.58 (d, 10)	58.5
8	5.58 (dd, 6, 1)	69.8	5.58 (br d, 6)	69.5
9α	2.21 (dd, 11, 1)	44.3	2.21 (br d, 11)	44.2
9β	2.73 (dd, 11, 6)	-	2.85 (dd, 11, 6)	-
10	-	133.8	-	135.7
11	-	77.0	-	77.0
12	-	174.7	-	174.7
13a	3.54 (d, 11)	44.0	3.55 (d, 11.5)	43.9
13b	3.65 (d, 11)	-	3.65 (d, 11.5)	-
14	1.59 (d, 1)	20.0	1.68 (d, 1)	19.9
15	1.75 (d, 1.4)	18.1	1.78 (d, 1)	17.9
2-OAc	-	-	2.06 (s)	21.2
	-	-	-	170.6
8-OAc	2.14 (s)	21.3	2.14 (s)	21.2
	-	169.4	-	169.4

Eupaglehnin F (**2**)[9] exhibited the molecular ion peak at *m/z* 402 $(M+2)^+$ and 400 $(M)^+$, $C_{19}H_{25}O_7Cl$ (by HRMS). The ^{1}H NMR spectrum is very similar to that of eupaglehnin E (**1**) except that compound (**2**) had two acetyl groups and that one proton assignable to H-2 shifted down to δ 5.69. Therefore it was very easy to assume that compound (**2**) is 2-OAc derivative of compound (**1**), which was confirmed by 2D NMR spectra. The NMR data are listed in the Table 1. The stereochemistry of **2** was also established as depicted in the formula.

These two compounds are chlorine atom-containing substances. Examples do exist but not very often in terrestrial plants.[10] These compounds did not show cytotoxic activity, which is understandable because they have no exomethylene group. We have not isolated compounds having an epoxide at C-11 and 13. Hiyodorilactones[2] have not been found yet, although the plant species should be the same as studied before.[2,5,6] These points are currently under study and will be published in due course.

ACKNOWLEDGEMENTS

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5. 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 work, the plant name should be changed to *E. glehni* based on gene identification methodology.[6]
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7. Isolation: The terrestrial part of of *E. glehni* was collected in Tokushima Prefecture in June, 1995. The voucher specimen was deposited in the herbarium of Tokushima Bunri University. The half-dried (overnight) plant (2.6 kg) was extracted with MeOH (21 L) at rt for three weeks to afford a residue (145 g). The EtOAc soluble fraction (56 g) was subjected to silica gel column chromatography (Hexane:EtOAc, in gradient to EtOAc:MeOH, in gradient) to give frs. Frs. were further separated by silica gel CC (Hexane:EtOAc, gradient) and Sephadex LH-20 ($CHCl_3$:MeOH = 1:1) followed by HPLC ($CHCl_3$: EtOAc) to afford eupaglehnins E (**1**) (7 mg) and F (**2**) (11.7 mg).
8. Eupaglehnin E (**1**); $[\alpha]_D^{24}$+63.8° (c 0.5, EtOH); IR 3450, 1770, 1740 cm^{-1}; MS (CI) *m/z* 361 $(M+2+H)^+$, 359 $(M+H)^+$, 323, 301, 299, 283, 281 (Base), 265, 263, 245, 227, 199, 177, 159, 95, 61; CI-HRMS Found 359.1237 $(M+H)^+$. Calcd for $C_{17}H_{24}O_6Cl$ 359.1261.
9. Eupaglehnin F (**2**); $[\alpha]_D^{20}$ +40.0° (*c* 0.3, $CHCl_3$); IR (KBr) 3450, 1780, 1740 cm^{-1}; MS (EI) *m/z* 402 $(M+2)^+$, 400 (M^+), 358, 342, 340, 300, 298, 282, 280, 231, 217, 175 (base), 157; HRMS (EI) Found 400.1287. Calcd for $C_{19}H_{25}O_7Cl$ 400.1289.
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