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Grayanane Diterpenoids from *Pieris formosa*[1]

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Abstract – Three grayanane diterpenoids (**1-3**) were isolated from *Pieris formosa*. **1** was identified as a new natural product and **2** and **3** as known grayanoside C and grayanotoxin XVIII on the basis of spectral analysis.

Key words – *Pieris formosa*; Ericaceae; diterpenoids; pierisformosin A.

Introduction

The grayanoids are a relatively unknown class of diterpenoids, which possess a 5/7/6/5 (*trans* or *cis*/*cis*/*cis*) ring system, formed pro- bably by rearrangement of kaurane skeleton in biogenetic pathway. Its existence was found especially in the genera *Pieris, Rhododendron, Lyonia* and *Leucothoe* of Er- ica- ceae family. Up to now more than 50 grayan- oids have been reported in literatures, am- ong which the majority exhibited remark- able toxicities, and significant antifeedant and insecticidal activities (Wang *et al.*, 1997). In order to search the natural products which have potential to be developed as insecticide, pesticide and herbicide, we have initiated the chemical studies on grayanane diterpenoids from Chinese Ericaceae plants.

Pieris formosa (Wall) D. Don (Ericaceae) is an evergreen shrub or tree, growing mainly in hilly and valley regions of south and southwest China. It is a well known poisonous plant, which Chinese name "Mei-Li-Ma-Zui-Mu" means the nice wood that horse would get drunk after eating them. The monograph concerned describes that the poultry would fall into coma after taking its leaves or stems accidentally. The symptoms including dyspnea, motion imbalance, spreading the four limbs would appear if mice were administrated with the chloroform extracts. As folk practice, the juice of the fresh leaves can be used as insecticide and as lotion for treatment of tinea and scabies in clinic (Chen *et al.*, 1987). Previously some steroids have been isolated from this plant (Puri *et al.*, 1982), but no any grayanoids were reported. In the course of searching bioactive natural products, we found that ethyl acetate and *n*-BuOH fractions of the plant extracts were effective in brine shrimp tests, which encouraged us to do its chemical investigation. This paper describes the isolation and structural elucidation of three grayanane diterpenoids (**1-3**) from the ethyl acetate fraction of the plant. Compound **1** was identified as a new natural product, named pierisformosin A and **2** and **3** as known grayanoside C and grayanotoxin XVIII (Sakakibara *et al.*, 1979, 1980), respectively, which were first discovered in the plant.

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Experimental

General – $[\alpha]_D$: JASCO, DIP-181, polarimeter. IR: Perkin-Elmer 599B spectrometer. ^1H and ^{13}C NMR spectra: Bruker AM-400. Chemical shifts are reported in ppm with solvent signal as int. standards. MS: MAT-95.

Plant material – The leaves of the plant were collected from Kaihua county of Zhejiang Province in November, 1996 and identified by Prof. Bing-Yang Ding of Hangzhou University. A voucher specimen was deposited in the Herbarium of Shanghai Institute of Materia Medica.

Extraction and isolation – The leaves of *P. formosa* (20 kg) were airdried, ground and extracted with 95% ethanol under reflux. After removal of the solvent by evaporation, the residue was adjusted to about 15% ethanol solution and stored in refrigerator overnight to precipitate chlorophyll. The supernatant was extracted with $CHCl_3$, EtOAc and *n*-BuOH, respectively. The EtOAc extract was evaporated to give a red mass (200 g), which was applied to a silica gel column, eluting with EtOAc containing increasing amounts of MeOH. Repeated column chromatography of the fraction, eluting with $CHCl_3$:MeOH (15:1) led to yield **1** (12 mg) and **2** (10 mg). And **3** (19 mg) was obtained from a fraction of the column chromatography and purified by RP-8 column chromatography, eluting with MeOH:H_2O (6:4).

1, *pierisformosin A*: amorphous powder. $[\alpha]_D^{15}$ 18.33° (MeOH, c 0.36); IR: ν_{max}^{KBr} 3419, 1637, 1456, 1036 cm^{-1}; ^1H NMR (C_5D_5N), see Table 2. ^{13}C NMR (C_5D_5N), see Table 1. EIMS m/z: 318(75, $[M-H_2O]^+$), 282(32), 271(35), 257(38), 239(28), 229(22), 211(25), 159(31), 147(32), 145(40), 135(45), 119(43), 109(100), 91(62), 69(56), 55(54).

2, *grayanoside C*: viscous syrup, $[\alpha]_D^{15}$ 0.66° (MeOH, c 0.35); IR: ν_{max}^{KBr} 3100-3500, 1635, 1448, 1080, 916, 883 cm^{-1}; FABMS m/z: 522 $[M+Na+H]^+$, 537 $[M+K]^+$; ^1H NMR (C_5D_5N): δ 1.31, 1.48, 1.91 (each 3H, s), δ 3.17 (1H, t, J=9.0 Hz), δ 3.28 (1H, dd, J=13.8, 10.3), δ 5.14 (1H, d, J=7.8 Hz), δ 5.26, 5.28 (2H, each s). ^{13}C NMR (C_5D_5N), see Table 1.

3, *grayanotoxin XVIII*: oil. $[\alpha]_D^{15}$ -4.23° (MeOH, c 1.50); ^1H NMR (C_5D_5N), see Table 2. ^{13}C NMR (C_5D_5N), see Table 1. EIMS m/z: 318 (28, $[M-H_2O]^+$, 300(41), 282(31), 267(15), 275(18), 257(35), 239(41), 229(21), 211(30), 185(32), 159(37), 135(53), 119(86), 109(86), 93(73), 69(100), 55(80).

Results and Discussion

Compound **1**, $[\alpha]_D^{15}$ 18.33° (MeOH, c 0.36), had a molecular formula $C_{20}H_{32}O_4$ from its EIMS (m/z 318, $[M-H_2O]^+$) and the ^1H and ^{13}C NMR spectra. The IR spectrum showed

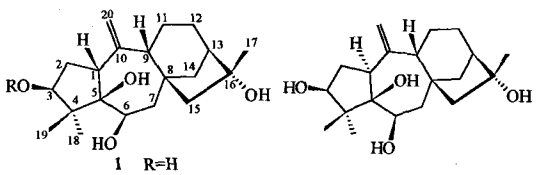
Table 1. ^{13}C NMR data of **1-3**

C	1	2	3	C	1	2	3
1	58.7d	59.3d	44.0d	14	35.5t	35.8t	36.1t
2	36.7t	35.4t	39.0t	15	57.2t	57.5t	62.1t
3	83.5d	92.2d	80.9d	16	78.5s	78.7s	79.2s
4	50.7s	51.7s	50.2s	17	24.7q	24.9q	25.0q
5	86.7s	86.3s	83.2s	18	22.7q	23.5q	23.9q
6	71.0d	71.4d	70.4d	19	20.9q	21.1q	18.9q
7	48.7t	49.0t	46.3t	20	110.5t	110.4t	112.0t
8	45.6s	45.9s	44.3s	glu-1'		105.2d	
9	55.6d	55.5d	52.1d	2'		75.9d	
10	153.9s	154.2s	152.6s	3'		78.8d	
11	26.5t	26.8t	23.7t	4'		71.9d	
12	26.7t	26.9t	25.5t	5'		78.8d	
13	49.5d	49.8d	47.5d	6'		63.0t	

Table 2. ^{1}H NMR data of **1** and **3**

H	1	3	H	1	3
1	3.01 dd(9.8, 3.4)	3.15t(9.4)	12	1.38 m	1.57-1.80 m
2	1.99 m	2.64 m	13	2.02 m	2.27 m
	2.19 m	2.75 m	14	1.83 d(11.0)	1.85 d(11.1)
3	3.85 d(4.0)	3.96 dd(6,6, 2.5)		2.09 dd(11.0, 4.0)	2.37 dd(11.1, 4.5)
6	3.80 dd(10.0, 2.0)	4.20 dd(9.4, 2.3)	15	1.63 d(15.0) 1.77 d(15.0)	2.07 d(14.2), 2.17 d(14.2)
7	1.67 m	1.92 dd(14.0, 2.0)	17	1.3 ls	1.55 s
	2.99 m	2.57 dd(14.0, 9.4)	18	1.0 ls	1.11 s
9	2.01 m	2.82 m	19	1.51 s	1.57 s
11	1.43 m, 1.55 m	1.57-1.80 m	20	5.04 s, 5.09 s	5.17 s, 5.19 s

absorption band of hydroxyl group (3419 cm^{-1}) and double bond (1637 cm^{-1}). The ^{1}H and ^{13}C NMR spectra (Table 1 and 2) indicated the presence of three singlet methyls (δ_H 1.01, 1.31, 1.51; δ_C 24.7, 22.7, 20.9), two oxygenated methines (δ_H 3.84, 3.80; δ_C 83.5, 78.5), two oxygenated quaternary carbons (δ_C 86.7, 78.5) and one terminal double bond (δ_H 5.04, 5.09; δ_C 153.9, 110.5). The ^{1}H-^{1}H COSY revealed the existence of the following fragments: H_aC=C-CH-CH_2-CH_2-CH(OH)-, H_bC=C-CH-CH_2-CH_2-CH- and -CH(OH)-CH_2-, each of which was connected to quaternary carbon atoms at one or both ends. The all above data confirmed to structural requirement of grayanane diterpenoids. Further investigation exhibited that the ^{13}C NMR data of **1** were in good agreement with that of the aglycone of grayanoside C (**2**), obtained by its enzymatic hydrolysis (Sakakibara *et al.*, 1980). Considering other spectral evidences, **1** was determined as the aglycone of **2**. Due to complicated stereochemistry, the structure of **2** and its conformation was finally determined by X-ray crystallography of its derivative (Sakaki- bara *et al.*, 1980). However, after our literature investigation, **1** was determined as a new natural product and named as pierisformosin A.



1 R=H
2 R=glu

3

In our NMR study, three methyls and four quaternary carbons were differentiated by NOESY and HMBC experiments. The NOE's between one methyl (δ 1.01) and both H-3 and H-6 were observed, which indicated the signal at δ 1.01 should be assigned as C_{18} methyl. The signal at δ 1.51 was assigned as C_{19} methyl from correlation with the signal at δ 1.01 in NOESY spectrum. The HMBC spectrum revealed cross-peaks between the signals at δ_C 50.7/H-18, 19; δ_C 86.7/H-1, 2, 7, 18, 19; δ 45.6/H-6, 7, 9, 11 and δ78.5/H-14, 17, which indicated C_4, C_5, C_8 and C_{16} respectively. From above analyses together with ^{1}H-^{1}H COSY results, ^{1}H and ^{13}C NMR data of **1** were assigned unambiguously as shown in Table 1 and 2.

Comparing general *trans*-fused ring A and B, **1** and **2** were found to be a rare exception among the grayanoids, possessing the *cis*-conjunctive ring A/B system with 1β-H. It was confirmed by the fact that H-1 and H-9 showed correlation in the NOESY spectrum. The conformation of **1** was elucidated as shown in Fig. 1 by previous study on X-ray crystallography of its derivative (Sakakibara,

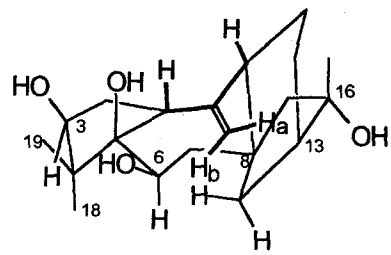


Fig. 1. The conformation of **1**.

et al., 1980). According to this conformation, it could be rationalized that H-20a/H-1 and H-20b/H-9 appeared allylic coupling combined with W-type long-range coupling in ^{1}H-^{1}H COSY due to taking the same plane. It also could be explained that only C_{18} methyl had correlation with both H-3 and H-6 in NOESY spectrum.

The second compound isolated from the plant had a molecular formula $C_{26}H_{42}O_9$ by its FABMS and ^{1}H and ^{13}C NMR data. It showed positive reaction for Molisch test. On acidic hydrolysis, it afforded glucose as sugar constituent, detected by co-TLC. Further spectral study revealed that all ^{1}H and ^{13}C NMR data of this compound were identical with that of grayanoside C. Thus it was determined as known grayanoside C, **2**.

Compound **3** had the molecular formula $C_{20}H_{32}O_4$ by EIMS and NMR spectra. The ^{1}H and ^{13}C NMR spectra of **3** were very similar with that of **1**, except little differences around C-1. The further investigation suggested that **3** should be grayanotoxin XVIII, the C_1 epimer of **1** with α-orientation of H-1, which was isolated from *Leucothoe grayana* (Sakakibara *et al.*, 1979). The ^{1}H and ^{13}C NMR data of **3** could be assigned by ^{1}H-^{1}H COSY and comparison with **1** and **2**. The compounds **2** and **3** were known grayanane diterpenoids, but first isolated from *Pieris formosa*. The biological tests of compounds **1-3** are in progress.

References

Chen, J. S. and Zheng, S., *Chinese Poisonous Plants*, Science Press, Beijing, 1987.

Puri, H. S. and Jain, S. C., Steroids in some plants from Sikkim Himalayas, *Pharmazie* **37**(1), 77-78 (1982).

Sakakibara, J., Shirai, N., Kaiya, T. and Iitaka, Y., Grayanoside C, A new diterpene glucoside from *Leucothoe grayana, Phytochemistry* **18**, 135-137 (1979).

Sakakibara, J., Shirai, N., Kaiya, T. and Iitaka, Y., Grayanotoxin-XVIII and Grayanoside B, a new A-nor-B-home-ent-kaurane and its glucoside from *Leuthoe grayana, Phytochemistry* **19**, 1495-1497 (1980).

Wang, L. Q. and Qin, G. W., Progress on chemistry and bioactivity of grayanoids in Ericaceae plants. *Natural Product Research and Development* **9**(4), 82-90 (1997).

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