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Diterpene glucosides from *Pieris formosa*[☆]

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Abstract

Five diterpene glucosides, pierisformosides B–F were isolated from *Pieris formosa* D. Don (Ericaceae). Their structures were elucidated on the basis of spectral analysis, including ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC and NOESY experiments. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: *Pieris formosa*; Ericaceae; Diterpene glucosides; Pierisformosides B–F; Structure elucidation

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1. Introduction

Pieris formosa D. Don (Ericaceae) is a poisonous plant, distributed mainly in the hill and valley regions of south and southwest China (Chen and Zheng, 1987). In previous studies on the chemical constituents of this plant, we have reported the isolation of several grayanane diterpenoids (Wang et al., 1998a,b,c). Our continuing studies on the plant led to the isolation of five new diterpene glucosides, pierisformosides B–F, from the *n*-BuOH fraction of the ethanol extracts of the plant leaves. In this paper, we describe the isolation and structural elucidation of these diterpene glucosides, and the assignment of NMR spectral data by a combination of NMR techniques, including ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC and NOESY experiments.

2. Results and discussion

The *n*-BuOH fraction of the ethanol extract of the leaves of *P. formosa* was subjected to repeated column chromatography on silica gel, RP-18 and Sephadex LH-20 to give **1** (10 mg), **2** (13 mg), **3** (10 mg), **4** (15 mg) and **5** (10 mg).

Pierisformoside B (**1**), a viscous syrup, has the molecular formula $C_{26}H_{42}O_8$ (M_r 482) based on FABMS (m/z 505 $[M+Na]^+$, 521 $[M+K]^+$) and NMR data. It showed a positive reaction to α-naphthol. Acid hydrolysis afforded glucose, as detected by TLC. The ^{1}H-NMR signals (δ 3.94–4.97) (Table 1) and ^{13}C-NMR signals (δ 106.3, 78.7, 78.2, 75.7, 71.8, 62.9) (Table 2), showed great similarity to those of the glucose part of known diterpene glucosides (Sakakibara et al., 1980), confirming that **1** possessed a glucose unit. Additionally, the ^{1}H-NMR spectrum showed signals for three tertiary methyls (δ 1.23, 1.38, 1.54, each *s*) and one olefinic exocyclic methylene (δ 5.21, 5.25, each *s*). Furthermore, the ^{13}C-NMR and DEPT data disclosed the presence of three methyl, eight methylene (one olefinic exocyclic methylene), four methine (one oxygenated), and five quaternary carbons (two oxygenated) for the aglycone, consistent with a grayanane diterpene skeleton.

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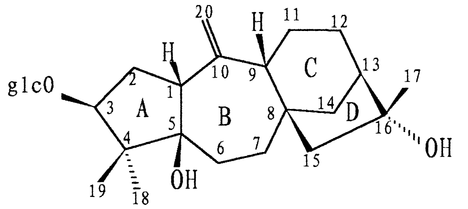

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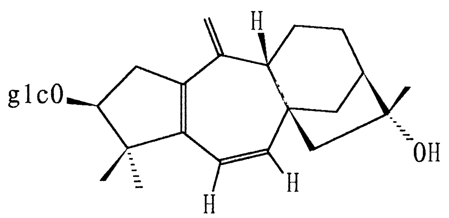

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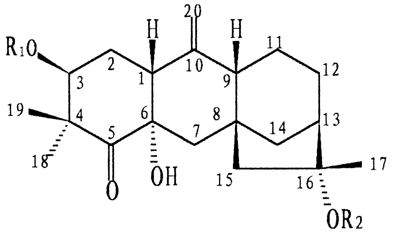
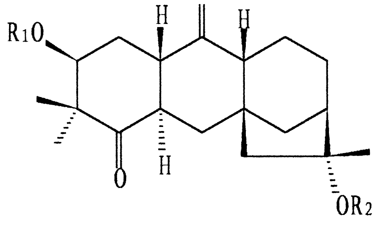


3 R1=glc R2=H
4 R1=H R2=glc



5 R1=H R2=glc
6 R1=glc R2=H

The placement of the exomethylene at C-10/C-20 was based on the following evidence observed in HMBC spectrum: (a) ^{13}C-NMR signal at δ 111.0 correlated with the ^{1}H-NMR signal at δ 2.03 (H-9) and 3.11 (H-1); and (b) δ 151.1 showed correlation with δ 3.11 (H-1), 2.60 (H-2), 2.03 (H-9) and 1.76 (H-11).

Analysis of the ^{1}H–^{1}H COSY, HSQC and HMBC spectra (Table 3) of **1** allowed its structural fragments to be determined. In ^{1}H–^{1}H COSY, signals at δ 2.25 and 2.60 (H_2-2) correlated with those at δ 3.11 (H-1) and 4.76 (H-3), and the signal at δ 1.55 and 2.03 (H_2-6) correlated with the signals at δ 1.67 and 1.80 (H_2-7). In the HMBC spectrum, the correlations of H-3 (δ 4.76) with C-18 (δ 21.0) and C-19 (δ 19.2), and H_3-18 (δ 1.38) and H_3-19 (δ 1.23) with C-3 (δ 88.5), C-4 (δ 51.2) and C-5 (δ 81.8) were observed, respectively. The above data identified the fragment from C-1 to C-7. In addition, the H-3 signal at δ 4.76 showed long range correlation with the anomeric carbon of glucose at δ 106.3 (C-1′), indicating that the C-3 hydroxyl is glucosylated. The anomeric configuration of the glucose unit was deduced as β-orientation by $J_{1',2'} = 7.8$ Hz in the ^{1}H-NMR spectrum (Sakakibara et al., 1980).

After considering a grayanane structure with *trans*-fused rings A and B, **1** was found to be a rare exception, possessing a *cis*-fused A/B ring system with H-1 in the β-position. In the *trans*-fused A/B ring system, H-1 has the α-orientation, resulting in a strong NOE to H-14α due to their close proximity (Wang and Qin, 1997). However, in **1** the NOE was between H-1 and H-9, instead of between H-1 and H-14. In addition, a NOESY correlation was observed between H-3 and H_3-18, indicating α-orientation for H-3. The NOESY correlation between H_3-18 and H-6α supported the positioning of C_5-OH in the β-orientation. From the above data and biogenetic considerations, the structure of pierisformoside B was established as **1**. To our knowledge, almost all grayanane diterpenoids have an oxygenated function at C-6 (Wang and Qin, 1997). **1** is a rare example lacking an oxygenated group at C-6.

Pierisformoside C (**2**) was obtained as a viscous syrup; its FABMS spectrum ($[M+1]^+$ at m/z 463) and ^{13}C-NMR data were consistent with a molecular formula of $C_{26}H_{38}O_7$. It showed a positive reaction to α-naphthol and IR absorption characteristic for hydroxyl groups. Acid hydrolysis afforded glucose, as detected by TLC. In addition to its glucose unit, the ^{1}H-NMR spectrum (Table 1) showed signals for three methyls (δ 1.17, 1.50, 1.62, each *s*), one oxygenated methine (δ 4.30, *dd*, J = 6.5, 4.0 Hz), an AB*q* methylene (δ 2.14, 2.43, each *d*, J = 14.3 Hz) and four olefinic hydrogens (δ 5.37, 5.40, each *s*; δ 5.78, 5.87, each *d*, J = 12.0 Hz). Furthermore, the ^{13}C-NMR data (Table 2) revealed the presence of three tertiary methyl, six methylene, five methine (one oxygenated) and six quaternary carbons (one oxygenated) in its aglycone. These structural features strongly suggested a grayanane skeleton, possessing a 5/7/6/5-membered ring system.

The molecular formula showed eight degrees of unsaturation, four of which were accounted for by a tetracyclic ring system and one by a glucose unit. The ^{1}H- and ^{13}C-NMR spectra of **2** revealed the presence of an exomethylene (δ_H 5.37, 5.40, each *s*; δ_C 149.3 *s*, 114.7 *t*), a tetrasubstituted double bond (δ_C 137.6 *s*, 139.5 *s*) and a *cis*-disubstituted double bond (δ_H 5.78,

Table 1
^{1}H-NMR spectral data of **1**–**5** in pyridine-d_5

H	**1**[a]	H	**2**[b]	H	**3**[b]	**4**[a]	H	**5**[b]
1	3.11 (*t*, 10.0)	1		1	3.11 (*d*, 12.2)	3.22 (*d*, 12.2)	1	2.93 (*m*)
2α	2.60 (*m*)	2	2.99 (*dd*, 16.7, 4.0), 3.37 (*dd*, 16.7, 6.5)	2 α	2.86 (*ddd*, 13.4, 12.2, 2.0)	2.86 (*ddd*, 13.4, 12.2, 2.0)	2	2.24 (*m*), 2.41 (*m*)
2β	2.25 (*m*)			2 β	2.50 (*ddd*, 13.4, 3.0, 3.0)	2.50 (*ddd*, 13.4, 3.0, 3.3)	3	4.23 (*br s*)
3	4.76 (*dd*, 9.6, 5.0)	3	4.30(*dd*, 6.5, 4.0)	3	4.33 (*m*)	4.33 (*m*)	6	2.54 (*m*)
6α	1.55 (*m*)	6	5.78 (*d*, 12.0)	7α	2.14 (*d*, 13.8)	2.48 (*d*, 14.7)	7	1.91 (*m*), 2.24 (*m*)
6β	2.03 (*m*)			7β	1.94 (*d*, 13.8)	2.10 (*d*, 14.7)	9	1.96 (*m*)
7α	1.67 (*m*)	7	5.87 (*d*, 12.0)	9	2.04 (*d*, 4.7)	2.05 (*d*, 4.5)	11	1.68 (*m*), 1.82 (*m*)
7β	1.80 (*m*)			11α	1.82 (*m*)	1.83 (*m*)	12	1.50 (*m*), 1.68 (*m*)
9	2.03 (*m*)	9	2.81 (*br s*)	11β	1.62 (*m*)	1.68 (*m*)	13	2.54 (*m*)
11α	1.55 (*m*)	11	1.87 (*m*)	12α	1.78 (*m*)	1.69 (*m*)	14	1.62 (*d*, 11.2), 2.12 (*dd*, 11.2, 4.9)
11β	1.63 (*m*)			12β	1.62 (*m*)	1.47 (*m*)	15	1.70 (*d*, 14.3), 2.42 (*d*, 14.3)
12	1.76 (*m*)	12	1.67 (*m*), 1.87 (*br s*)	13	2.22 (*br s*)	2.46 (*br s*)	17	1.65 (*s*)
13	2.13 (*m*)	13	2.30 (*br s*)	14	2.36 (*dd*, 11.4, 5.0)	2.35 (*br d*, 11.4)	18	1.23 (*s*)
14	1.60 (*m*), 2.11 (*m*)	14	1.77 (*d*, 11.0), 2.58 (*dd*, 11.0, 5.1)		2.45 (*d*, 11.4)	2.44 (*d*, 11.4)	19	1.55 (*s*)
15	1.95 (*br s*)	15	2.14 (*d*, 14.3), 2.41 (*d*, 14.3)	15α	2.38 (*d*, 14.4)	2.32 (*d*, 14.5)	20	4.95 (*s*), 5.13 (*s*)
17	1.54 (*s*)	17	1.62 (*s*)	15β	1.97 (*d*, 14.4)	1.64 (*d*, 14.5)	glc-1′	5.05 (*d*, 7.5)
18	1.38 (*s*)	18	1.17 (*s*)	17	1.62 (*s*)	1.56 (*s*)	glc-2′	4.04 (*m*)
19	1.23 (*s*)	19	1.50 (*s*)	18	1.65 (*s*)	1.64 (*s*)	glc-3′	4.34 (*m*)
20	5.21 (*s*), 5.25 (*s*)	20	5.37 (*s*), 5.40 (*s*)	19	1.72 (*s*)	1.53 (*s*)	glc-4′	4.34 (*m*)
glc-1′	4.97 (*d*, 7.8)	glc-1′	5.05 (*d*, 7.6)	20	5.13 (*s*), 5.19 (*s*)	5.09 (*s*), 5.20 (*s*)	glc-5′	3.98 (*m*)
glc-2′	4.07 (*dd*, 8.8, 7.8)	glc-2′	4.07 (*m*)	glc-1′	5.05 (*d*, 7.7)	4.91 (*d*, 7.7)	glc-6′	4.44 (*dd*, 11.7, 5.2), 4.58 (*dd*, 11.7, 2.5)
glc-3′	4.25 (*t*, 8.8)	glc-3′	4.32 (*m*)	glc-2′	4.09 (*dd*, 8.8, 7.7)	3.85 (*m*)		
glc-4′	4.31 (*t*, 8.8)	glc-4′	4.32 (*m*)	glc-3′	4.26 (*t*, 8.8)	4.25 (*m*)		
glc-5′	3.94 (*ddd*, 8.8, 6.0, 2.4)	glc-5′	4.07 (*m*)	glc-4′	4.32 (*t*, 8.8)	4.15 (*t*, 8.8)		
glc-6′	4.43 (*br d*, 11.9)	glc-6′	4.50 (*dd*, 11.7, 6.5), 4.67 (*dd*, 11.7, 2.4)	glc-5′	4.06 (*ddd*, 8.8, 5.4, 2.6)	3.85 (*m*)		
	4.53 (*br d*, 11.9)			glc-6′	4.45 (*dd*, 11.6, 5.4)	4.25 (*dd*, 11.6, 5.4)		
					4.63 (*dd*, 11.6, 2.6)	4.43 (*dd*, 11.6, 2.4)		

[a] 500 MHz.
[b] 400 MHz.

5.87, each *d*, J = 12.0 Hz; δ_C 119.1 *d*, 143.3 *d*). To date, we have found olefinic bond(s) in natural grayanane diterpenoids generally located at the positions of C_9–C_{10}, C_{10}–C_{20}, C_{15}–C_{16} and C_{16}–C_{17}, and exomethylene groups at C_{10}–C_{20} and/or C_{16}–C_{17} (Wang and Qin, 1997). In compound **2**, the exomethylene group should be at C_{10}–C_{20} since its characteristic carbon signal at δ 114.7(C-20) correlated with the proton signal at δ 2.81 (H-9) in the HMBC spectrum (Table 3). The tetrasubstituted double bond of **2** was determined to be at C_1–C_5 according to HMBC evidence that the signal at δ 137.6 (C-1) correlated with signals at δ 4.30 (*dd*, H-3) and δ 5.37, 5.40 (each *s*, H-20), respectively; the signal at δ 139.5 (C-5) correlated with signal at δ 4.30 (*dd*, H-3), δ 1.17 (*s*, H-18) and δ 1.50 (*s*, H-19), respectively. Analysis in the same way permitted a *cis*-disubstituted double bond to be assigned at C_6–C_7 due to the correlation of δ 137.6 (C-1)/δ 5.78 (H-6) and δ 139.5 (C-5)/δ 5.87 (H-7). From the above evidence, the three double bonds were determined to be conjugated, which was supported by a UV absorption at 217 and 303 nm.

In the HMBC spectrum, the anomeric carbon signal at δ 106.2 (C-1′) showed correlation with the signal at

Table 2
^{13}C-NMR spectral data of 1–5 in pyridine-d_5

C	1	2	3	4	5
1	50.6 *d*	137.6 *s*	45.8 *d*	45.4 *d*	43.4 *d*
2	35.2 *t*	42.3 *q*	27.3 *t*	28.6 *t*	32.6 *q*
3	88.5 *d*	87.4 *d*	86.0 *d*	76.4 *d*	77.4 *d*
4	52.1 *s*	51.1 *s*	49.5 *s*	49.6 *s*	50.3 *s*
5	81.8 *s*	139.5 *s*	214.0 *s*	214.6 *s*	215.0 *s*
6	32.8 *t*	119.1 *d*	78.5 *s*	78.9 *s*	48.5 *d*
7	40.2 *t*	143.3 *d*	45.3 *t*	45.1 *t*	38.9 *q*
8	47.1 *s*	50.9 *s*	46.1 *s*	46.2 *s*	46.2 *s*
9	54.3 *d*	46.6 *d*	50.5 *d*	50.8 *d*	49.6 *d*
10	151.1 *s*	149.3 *s*	150.0 *s*	150.1 *s*	152.5 *s*
11	26.0 *t*	25.0 *q*	21.8 *t*	22.0 *t*	22.0 *q*
12	24.7 *t*	26.2 *q*	25.4 *t*	25.2 *t*	24.7 *q*
13	49.3 *d*	50.7 *d*	50.2 *d*	47.5 *d*	47.5 *d*
14	36.3 *t*	41.4 *q*	38.7 *t*	38.1 *t*	35.8 *q*
15	58.9 *t*	57.7 *q*	56.5 *t*	53.9 *t*	52.8 *q*
16	79.6 *s*	79.8 *s*	77.9 *s*	86.9 *s*	87.6 *s*
17	24.6 *q*	24.5 *q*	24.5 *q*	20.8 *q*	20.9 *q*
18	21.0 *q*	25.7 *q*	27.9 *q*	27.5 *q*	25.4 *q*
19	19.2 *q*	21.7 *q*	23.4 *q*	23.9 *q*	23.4 *q*
20	111.0 *t*	114.7 *t*	107.1 *t*	107.0 *t*	105.5 *t*
glc-1′	106.3 *d*	106.2 *d*	106.2 *d*	99.3 *d*	99.5 *d*
glc-2′	75.7 *d*	75.7 *d*	75.6 *d*	75.4 *d*	75.5 *d*
glc-3′	78.7 *d*	78.7 *d*	78.5 *d*	78.8 *d*	79.0 *d*
glc-4′	71.8 *d*	71.9 *d*	71.8 *d*	72.2 *d*	72.0 *d*
glc-5′	78.2 *d*	78.6 *d*	78.3 *d*	77.8 *d*	78.3 *d*
glc-6′	62.9 *t*	63.1 *t*	63.1 *t*	63.2 *t*	63.1 *t*

δ 4.30 (*dd*, J = 6.5, 4.0, H-3), indicating that C-3 hydroxyl group should be glucosylated. The anomeric configuration of the glucose unit was deduced as β-orientation by $J_{1',2'} = 7.6$ Hz in the ^{1}H-NMR spectrum. However, comparing the C-3 chemical shift (δ 85.7) of **2** with that of other grayanane C-3 glucosides, an upfield shift of *Ca* 5.0 ppm was observed, probably caused by the existence of a C_1–C_5 double bond.

In the NOESY spectrum, the H-3 signal at δ 4.30 correlated with the α-methyl signal at δ 1.17 (H-18), suggesting that H-3 was α-orientated. From all of the above evidence and biogenetic considerations, the structure of **2** was established, which was the first example possessing three double bonds in a grayanane skeleton.

Table 3
Principle HMBC correlations of **1** and **2**

H	**1** (H → C)	**2** (H → C)
1	–	–
2	C-4	C-1
3	C-1, C-1′, C-5, C-18, C-19	C-1, C-5
6	C-1, C-4, C-7	C-1, C-5
7	C-4, C-5, C-9, C-15	C-6, C-8, C-14
9	–	C-10, C-11, C-12
11	–	C-9, C-10, C-12, C-13
12	–	C-11, C-13
13	–	–
14	C-7, C-8, C-9, C-12, C-15, C-16	C-7, C-8, C-9, C-12
15	C-8, C-9, C-14, C-16, C-17	C-7, C-8, C-9, C-14, C-16
17	C-13, C-15, C-16	C-13, C-15, C-16
18	C-3, C-4, C-5, C-19	C-3, C-4, C-5, C-19
19	C-3, C-4, C-5, C-18	C-3, C-4, C-5, C-18
20	C-1, C-9	C-1, C-9
glc-1′	C-3	C-16
glc-2′	C-1′, C-3′	C-1′, C-3′
glc-3′	C-2′, C-4′	C-2′, C-4′
glc-4′	C-6′	C-6′
glc-5′	–	–
glc-6′	C-5′	C-5′

Pierisformoside D (**3**), a viscous syrup, was shown to have the molecular formula $C_{26}H_{40}O_9$ (M_r 496) from FABMS (*m*/*z* 497 $[M+H]^+$) and NMR data. It also showed a positive reaction to α-naphthol. Its IR spectrum showed the presence of hydroxyl (3377 cm^{-1}), ketone carbonyl (1697 cm^{-1}) and double bond (1641 cm^{-1}) functions. The ^{1}H-NMR (δ 4.06–5.05) (Table 1) and ^{13}C-NMR signals (δ 106.2, 78.5, 78.3, 75.6, 71.8, 63.1) (Table 2), similar to those of **1**, disclosed that **3** also has a glucose unit. Acidic hydrolysis of **3** gave glucose as detected by TLC. The ^{1}H-NMR spectrum of **3** also showed the signals for three methyls (δ 1.62, 1.65, 1.72, each *s*), one exocyclic methylene (δ 5.13, 5.19, each *s*) and one oxygenated methine (δ 4.33, *m*). The placement of the exomethylene at C-10/C-20 was based on the long-range correlation: δ 111.0 (C-20)/δ 3.11 (H-1), 2.03 (H-9); δ 151.1 (C-10)/δ 3.11 (H-1), 2.60 (H-2), 2.03 (H-9), and 1.76 (H-11). From H-20, the ^{1}H–^{1}H COSY correlation of **3** revealed the existence of the following fragments: $CH_2{=}C{-}CH{-}CH_2{-}CH(OR){-}$ and $CH_2{=}C{-}CH{-}CH_2{-}CH_2{-}CH{-}CH_2{-}$. In combination with HSQC and HMBC spectrum (Table 3), the above two fragments were assigned as: C_{20}–C_{10}–C_1–C_2–C_3– and C_{20}–C_{10}–C_9–C_{11}–C_{12}–C_{13}–C_{14}–, respectively. Furthermore, there was a pair of isolated methylene protons (δ 1.94, *d*, J = 13.8 and 2.14, *d*, J = 13.8, each 1H), assigned to H-7, instead of the $CH_2(6)$–$CH_2(7)$– fragment in **1**. In addition, the ^{13}C-NMR and DEPT data revealed the 20 carbons of the aglycone as three methyl, seven methylene, four methine (one oxygenated) and six quaternary carbons (one carbonyl carbon). The above evidence suggested that **3** had a leucothane skeleton with 6/6-membered A/B ring system, which was derived probably from rearrangement of 5/7-membered A/B ring of the grayanane skeleton.

Presently, only four leucothane diterpenoids and one leucothane glucoside have been isolated from Ericaceae (Furusaki et al., 1972a,b; Hikino et al., 1972; Sakakibara et al., 1981). From literature investigation, these compounds possess in general, an exomethylene group at C-10/C-20, a carbonyl at C-5, and hydroxyls at C-3, C-6, C-14 and/or C-16, and the H-1 and H-9 protons are β-orientated. The ^{1}H- and ^{13}C-NMR spectra of **3** revealed that its aglycone possessed one ketone, one secondary hydroxyl, two tertiary hydroxyls and one exomethylene group in the skeleton. Besides, one of the oxygenated quaternary carbons was assigned to C-6 for it showed 2-bond coupling between C-16 and H-15, H_3-17. The secondary glucosylated hydroxyl was determined to be at C-3 by the long-range correlation of an oxygenated methine proton at δ 4.33 (H-3) with C-5 (δ 214.0) and two gem methyls (H_3-18 and H_3-19) with C-3 (δ 86.0). The observation of an NOE effect of H-3 to both, H-18 and H-19 confirmed the β-configuration of C_3-hydroxyl group due to equatorial H-3. Furthermore, the glucosylation at C-3 was concluded from the correlation between H-3/C-1′ and H-1′/C-3 in the HMBC spectrum and the β-orientation of glucose was deduced from the coupling constant $J_{1',2'} = 7.7$ Hz (Sakakibara et al., 1980).

The reported leucothane diterpenoids all have a *trans/cis/cis* conjunction for the A/B, B/C, and C/D ring systems. The stereochemistry of leucothol B and C, isolated from an Ericaceae plant and possessing a leucothane skeleton, has been confirmed by X-ray crystallographic analysis (Furusaki et al., 1972a). In the NOESY spectrum of **3**, H-1 showed a correlation with H-9, indicating that H-1 and H-9 are on the same side of the molecule. Comparing the NMR data of **3** with those of known leucothane diterpenoids and considering its biogenetic origin, the structure of pierisformoside D was established as **3**.

Pierisformoside E (**4**), a viscous syrup, had the same molecular formula $C_{26}H_{40}O_9$ (M_r 496) as **3**. The NMR data of **4** were similar to those of **3**, indicating that **4** also possessed a leucothane skeleton. The differences between **3** and **4** were observed as follows: (a) C-3 signal at δ 86.0 of **3** was shifted upfield to δ 76.4 in **4** and (b) C-16 signal at δ 77.9 of **3** was shifted downfield to δ 86.9 in **4**. This indicated that glucosylation was at C-16 hydroxyl instead of C-3 hydroxyl in **3**. The suggestion was confirmed by long-range correlation of C-16 signal at δ 86.9 with the anomeric proton of glucose at δ 4.91 and the C-17 methyl protons at δ 1.56 in the HMBC spectrum (Table 3). The anomeric configuration of the glucose unit was deduced as β-orientation by $J_{1',2'} = 7.7$ Hz (Sakakibara et al., 1980).

Pierisformoside F (**5**), a viscous syrup, had a molecular formula of $C_{26}H_{40}O_8$ from the FABMS ($[M+1]^+$ at *m/z* 481) and ^{13}C-NMR data. It showed positive reaction to α-naphthol test. Acidic hydrolysis of **5** afforded glucose, as detected by TLC. The IR spectrum showed absorption for hydroxyl (3400 cm^{-1}), keton carbonyl (1701 cm^{-1}) and double bonds (1643, 1446 cm^{-1}). In addition to its glucose unit, the ^{1}H-NMR spectrum of **1** (Table 1) showed signals for three methyls (δ 1.23, 1.55, 1.65, each *s*), one exomethylene (δ 4.95, 5.13, each *s*) and one oxygenated methine (δ 4.23, *br s*). Furthermore, the ^{13}C-NMR and DEPT data (Table 2) revealed the presence of three methyl, seven methylene, five methine (one oxygenated) and five quaternary carbons (one carbonyl carbon). The ^{1}H–^{1}H COSY spectrum of **5** revealed the existence of following fragments: $CH_2{=}C{-}CH(CH{-}CH_2){-}CH_2CH(OH){-}$ and $CH_2{=}C{-}CH{-}CH_2{-}CH_2{-}CH{-}CH_2{-}$, each of which was connected to a quaternary carbon at one or both ends. In the NOESY spectrum, the signal at δ 4.23 (*br s*, H-3) correlated with the signal at δ 1.23 (*s*, H-18) and δ 1.55 (*s*, H-19); and the signal at δ 2.93 (*m*, H-1) correlated with the signal at δ 1.96 (*br s*, H-9), suggesting that H-1 and H-9 took the same orientation. The above evidence suggested that **5** had a leucothane skeleton, with a 6/6-membered A/B ring system, presumably derived from rearrangement of 5/7-membered A/B ring of a grayanane skeleton. The ^{13}C-NMR spectrum of compound **5** showed a close resemblance to that of the acetate of pieroside B (**6**), isolated from *Pieris japonica* (Sakakibara et al., 1981). The only difference was a reversed assignment for two oxygen-bearing carbon signals. In **5**, the methine carbon signal was at δ 77.4 and quaternary carbon signal at δ 87.6, while in the acetate of **3**, the quaternary carbon and methine signals were at δ 87.8 and 78.6, respectively. Thus, it was suggested that **5** should be glucosylated at the C-16 tertiary hydroxyl instead of C-3 as in **6**, leaving the C_3-hydroxyl group free. This suggestion was further confirmed by an HMBC experiment (Table 3), in which the correlation between C-16 (δ 87.6) and H-1′ (δ 5.05) was observed. The glucose unit was in β-glycosidic linkage according to the coupling constant of $J_{1',2'} = 7.5$ Hz, observed for the H-1′ signal at δ 5.05. Since the NMR data of **5** closely resembled that of the aglycone of **6**, whose stereostructure was determined by X-ray crystallographic analysis (Furusaki et al., 1972b), the structure of **5** was established as shown.

Leucothane has an unprecedented diterpene skeleton, formed by possible rearrangement of a grayanane skeleton. It was reported that the aglycone of grayanoside C can be transformed to the aglycone of **6** by treatment with toluenesulfonyl chloride (Sakakibara et al., 1980). Compound **5** is the second leucothane diterpene glucoside, and the first which is glucosylated at the C-16 tertiary hydroxyl. Glucosylation at different positions, either C-3 or C-16, results in different chemical shifts for Glu-C-1′ in the grayanane and leucothane diterpene glucosides. For the grayanane glucosides, when the C-3 hydroxyl is glucosylated, the Glu-C-1′ signal is *Ca* δ 105, but when glucosylated at the C-6 hydroxyl, the Glu-C-1′ signal shifts upfield to around δ 100 (Wang et al., 1998c). For the leucothane glucosides, the Glu-C-1′ signal is near δ 106 when the glucose is at the C-3 hydroxyl, and δ 99.0 when at the C-16 hydroxyl (Sakakibara et al., 1980; Hikino et al., 1972). This observation could be useful for determination of the glucosylation position in grayanane and leucothane diterpenoids.

3. Experimetal

3.1. General

$[\alpha]_D$: JASCO DIP-181 polarimeter. IR: Nicolet Magna FTIR-750; ^{1}H and ^{13}C NMR spectra: Bruker DRX-500 and Bruker AM-400. Chemical shifts are reported in ppm with solvent signal as internal standard. MS: MAT-95.

3.2. Plant materials

The leaves of *Pieris formosa* were collected from Kaihua County of Zhejiang Province in November, 1996, and identified by Prof. Bing-Yang Ding of Department of Plant Sciences, Hangzhou University. A voucher specimen (No. SIMM96111001) was deposited in the Herbarium of Shanghai Institute of Materia Medica.

3.3. Extraction and isolation

The leaves of *P. formosa* (20 kg) were air-dried, 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 a refrigerator overnight to precipitate chlorophyll. The supernatant was extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. The *n*-BuOH extract was evaporated to give a red mass (300 g), which contained flavonoid compounds. It was applied to a silica gel column, eluting with $CHCl_3$ containing increasing amounts of MeOH. Repeated Sephadex LH-20 and RP-18 column chromatography led to yield **1** (10 mg), **2** (13 mg), **3** (10 mg), **4** (15 mg) and **5** (10 mg).

3.3.1. Pierisformoside B (1)

Viscous syrup, $[\alpha]_D^{18} - 2.31$ (*c* 0.17, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3388, 2930, 1635, 1080, 1030; FABMS *m*/*z*: 505 $[M+Na]^+$, 521 $[M+K]^+$; ^{1}H-NMR, spectral data (pyridine-d_5), see Table 1; ^{13}C-NMR spectral data (pyridine-d_5), see Table 2.

3.3.2. Pierisformoside C (2)

Viscous syrup, $[\alpha]_D^{23} + 87.81$ (*c* 0.11, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1639, 1448, 1362, 1165, 1076, 887, 756 cm^{-1}; UV λ_{max}^{MeOH} nm (log ε): 217 (0.28), 303 (0.14); FAB MS *m*/*z*: 463($[M+H]^+$); ^{1}H-NMR, spectral data (pyridine-d_5), see Table 1, ^{13}C-NMR spectral data (pyridine-d_5), see Table 2.

3.3.3. Pierisformoside D (3)

Viscous syrup. $[\alpha]_D^{18} - 4.35$ (*c* 0.15, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3377, 2929, 1697, 1640, 1080, 1028, 900; FABMS *m*/*z*: 497 $[M+H]^+$; ^{1}H-NMR, spectral data (pyridine-d_5), see Table 1; ^{13}C-NMR spectral data (pyridine-d_5), see Table 2.

3.3.4. Pierisformoside E (4)

Viscous syrup. $[\alpha]_D^{18} - 6.09$ (*c* 0.20, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 2930, 1701, 1647, 1261, 1078, 1030, 754; FABMS *m*/*z*: 535 $[M+K]^+$; ^{1}H-NMR spectral data (pyridine-d_5), see Table 1, ^{13}C-NMR spectral data (pyridine-d_5), see Table 2.

3.3.5. Pierisformoside C (2)

Viscous syrup, $[\alpha]_D^{16} - 13.78$ (*c* 0.13, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 1701, 1643, 1383, 1074, 1031 cm^{-1}; FAB MS *m*/*z*: 481 $[M+H]^+$; ^{1}H NMR spectral data (pyridine-d_5), see Table 1; ^{13}C-NMR spectral data (pyridine-d_5), see Table 2.

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