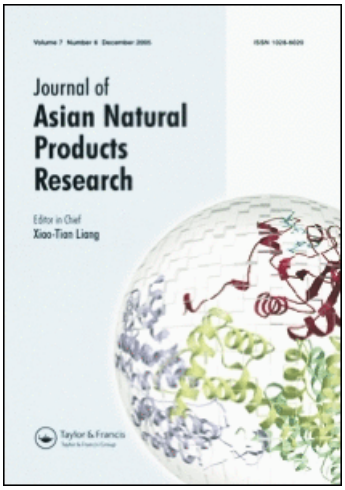

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ORIGINAL ARTICLE

New grayanol diterpenoid and new phenolic glucoside from the flowers of *Pieris formosa*

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A new grayanol diterpenoid, grayanotoxin XXII (**1**), and a new phenolic glucoside, benzyl 2-hydroxy-4-*O*-[β-xylopyranosyl($1'' \rightarrow 6'$)-β-glucopyranosyl]-benzoate (**2**), were isolated from the flowers of *Pieris formosa*. Their structures were determined on the basis of spectroscopic analysis and chemical methods.

Keywords: grayanol diterpenoid; phenolic glucoside; *Pieris formosa*; Ericaceae

1. Introduction

Many species of the family Ericaceae contain diterpenoids possessing several specialized carbon skeletons with highly oxygenated functionalities, which results in the toxicity of such species. These diterpenoids occur mainly in the genera *Kalmia*, *Leucothoe*, *Lyonia*, *Pieris*, and *Rhododendron*. Their structures include four types: (1) the grayanane-type with a 5/7/6/5 ring system; (2) the leucothane-type with a 6/6/6/5 consecutive carbocycle; (3) the 10/6/5 carbon ring grayanol-type; and (4) the kalmane-type with a 5/8/5/5 ring system [1]. It seems that all structural types were biosynthetically related to each other and derived from the *ent*-kaurane diterpenoids [2]. Some of these diterpenoids possess significant biological properties, such as potent acute toxicity in mammals [3,4], antifeedant, growth inhibitory, and insecticidal activities [5,6].

Pieris formosa (Wall) D. Don, a well-known poisonous plant, is distributed mainly in hilly and valley regions of south and southwest China. Poultry have been reported to go into coma after accidentally eating leaves or stems of this plant. In folk practice, the juice of the fresh leaves of *P. formosa* can be used as insecticide or lotion for the treatment of ring worm and scabies [7]. In previous investigations, a number of grayanane and leucothane diterpenes have been isolated and identified from this plant [8–12].

In the course of searching for diterpenoids with structural diversity and biological importance, a new grayanol diterpenoid, grayanotoxin XXII (**1**), and a new phenolic glucoside, benzyl 2-hydroxy-4-*O*-[β-xylopyranosyl($1'' \rightarrow 6'$)-β-glucopyranosyl]-benzoate (**2**), were isolated from the flowers of *P. formosa*. The grayanol-type was considered as the intermediate of all the structural types

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mentioned above [13], and up to the present, there are only four diterpenoids of this kind found in natural products. Details of the isolation and structural elucidation of these two new compounds are reported in this paper.

2. Results and discussion

Compound **1** was obtained as a colorless solid. The FAB-MS indicated a molecular ion peak at *m/z* 408, and the HR-ESI-MS at *m/z* 407.2432 $[M-H]^-$ supported the molecular formula of $C_{23}H_{36}O_6$, with six sites of unsaturation. The IR spectrum indicated the presence of hydroxyl (3432 cm^{-1}), ester carbonyl (1733 cm^{-1}), and C=C double bond (1657 cm^{-1}) groups. The ^{13}C NMR spectrum showed resonances for 23 carbons differentiated by DEPT experiment into five methyls, six methylenes, six methines, and six quaternary carbons, including three oxymethine functionalities at δ_C 82.7, 69.3, and 81.1, and one oxygenated quaternary carbon at δ_C 79.3.

The ^1H and ^{13}C NMR spectral data of **1** were quite similar to those of 3(*S*), 6(*R*),14(*R*),16(*R*)-tetrahydroxy-5-oxo-5, 10-seco-ent-kaur-1(10)-ene (**1a**, Figure 1), a known grayanotoxin isolated from *Leucothoe grayana* Max. [14]. The only difference was that the NMR spectra of **1** displayed resonances due to an additional propionyl group at δ_H 2.42 (q, $J = 7.8$ Hz), 1.16 (t, $J = 7.8$ Hz); δ_C 9.0 (q), 28.0 (t), and 173.6 (s), where the ester carbonyl carbon at δ_C 173.6 of the propionyl group was correlated with H-14 at δ_H 5.70 (br s) in the HMBC spectrum, demonstrating the C-14 location of the propionyl group. This assignment was in agreement with the observation of the H-14 signal being shifted downfield by 1.44 ppm compared to the signal from **1a** (δ_H 4.26, d, $J = 6.4$ Hz). Above deduction was further verified by $^1H-^1H$ COSY, HSQC, and HMBC (Figure 2) experiments and the unambiguous assignment of all ^1H and ^{13}C NMR signals (Table 1) was finished.

The relative configurations of the stereocenters of **1** were assigned the same as those of **1a** on the basis of the similarity of all the proton and carbon chemical shifts and proton multiplicities for both compounds. The NOE correlations from H-1 to H-2β, H-9, and Me-20, as well as, H-7β correlated with H-9 and Me-19 suggested that H-9, Me-19 were β-oriented. Additional NOE correlations from H-3 to H-6 and Me-18, H-6 to H-2α, H-3, H-14, and Me-18 indicated that H-3, H-6, H-14, Me-18 were in α-disposition. Thus, the relative configuration of compound **1** was determined as 3(*S*),6(*R*),16(*R*)-trihydroxy-5-oxo-14(*R*)-propionyloxy-5,10-seco-ent-kaur-1(10)-ene and named as grayanotoxin XXII.

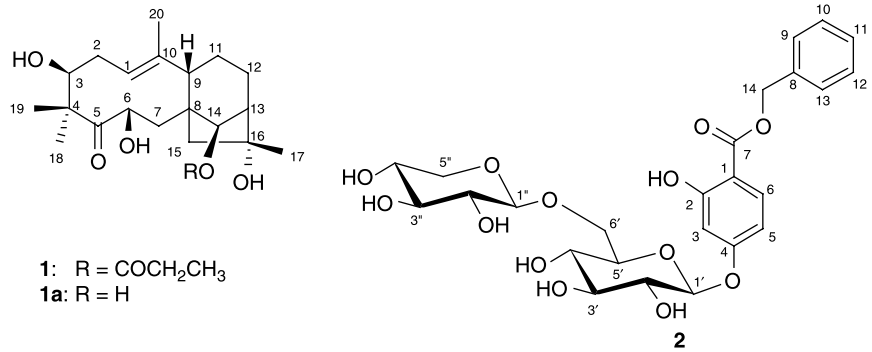
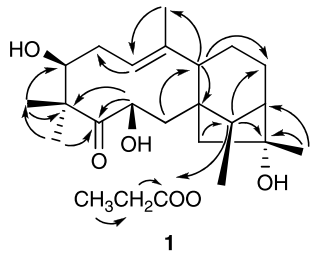


Figure 1. Structures of **1** and **2**.



1

Figure 2. Key HMBC correlations of compound **1**.

Compound **2** was assigned the molecular formula of $C_{25}H_{30}O_{13}$ on the basis of HR-ESI-MS analysis at *m/z* 537.1621 $[M-H]^-$. Its UV absorption maximum at λ_{max} 280 nm was characteristic of phenolic compounds. At the same time, its IR spectrum showed specific absorptions at 3442 cm^{-1}, which could be ascribed to the hydroxyl group. The intense IR absorption band at 1724 cm^{-1} revealed the presence of ester functionality, while the broad C—O stretching band in the region of 1076 cm^{-1} suggested its glycosidic nature [15].

Comparison of the ^1H and ^{13}C NMR spectral data of aglycone of **2** with those of trichocarpine [16] showed that the aglycone of both compounds was similar except that the aglycone of **2** was benzyl 2,4-dihydroxybenzoate while trichocarpine was benzyl 2,5-dihydroxybenzoate. The ^1H NMR spectrum in the aromatic region exhibited three aromatic protons (δ_H 7.31, d, $J = 2.5$ Hz; 6.60, br d, $J = 8.5$ Hz, and 6.79, d, $J = 8.0$ Hz), suggesting the existence of 2,4-bisubstituted phenyl. The ^1H NMR signal in another aromatic region exhibited five aromatic protons, including two pairs of symmetrical ones, which appeared at δ_H 7.49 (2H, d, $J = 7.5$ Hz), 7.38 (2H, t, $J = 7.5$ Hz), 7.29 (1H, m) with the data at δ_H 5.37 (2H, br s) could be ascribed to benzyl alcohol. The HMBC correlations (Figure 3) of H-14 with C-7, C-8, and C-9, of H-9 with C-10, C-11, and C-14, and of H-6 with C-2, C-4, C-5, and C-7 provided the position of the benzoyl residue.

The complete acid hydrolysis of **2** yielded various products, and in the hydrolysate separated from the aglycone parts, two sugars identified by the TLC comparison were found to be glucose and xylose, respectively. The HMBC correlations from H-6′ to C-4′, C-5′, and C-1″, from H-1″ to C-6′, and from H-1′ to C-4 and C-3′, suggested the position of the glycosidic nature. The signals of the anomeric doublets at δ_H 4.90 (H-1′) and δ_H 4.29 (H-1″) presumably belonged to

Table 1. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data ($CDCl_3$) of compound **1** (δ in ppm, J in Hz).

No.	^1H	^{13}C	No.	^1H	^{13}C
1	5.03 (dd, $J = 12.0, 1.5$)	128.4	12α	1.91 (m)	27.0 t
2α	2.56 (ddd, $J = 15.0, 12.0, 3.0$)	32.7 t	12β	1.33 (m)	
2β	2.17 (dd, $J = 15.0, 1.5$)		13	2.17 (overlap)	52.8 d
3	3.78 (br s)	82.7 d	14	5.70 (br s)	81.1 d
4		48.9 s	15	1.97 (br s)	57.3 t
5		215.8	16		79.3 s
6	4.28 (br s)	69.3 d	17	1.37 (s)	22.7 q
7α	1.60 (d, $J = 16.0$)	39.7 t	18	1.33 (s)	21.5 q
7β	2.71 (dd, $J = 16.0, 6.0$)		19	1.32 (s)	27.0 q
8		54.4 s	20	1.97 (s)	16.5 q
9	2.25 (d, $J = 7.5$)	57.8 d	*CH*$_3$CH$_2$	1.16 (t, $J = 7.8$)	9.0 q
10		139.9	CH$_3$*CH*$_2$	2.42 (q, $J = 7.8$)	28.0 t
11	1.38 (m)	22.5 t	CH$_3$CH$_2$*C*O		173.6 s
11β	1.80 (m)				

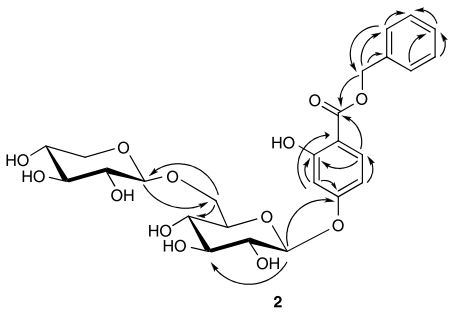


Figure 3. Key HMBC correlations of compound **2**.

two sugar moieties and the evidence for the β-configuration of these sugars was drawn from the coupling constants of 7.3 and 7.4 Hz for H-1′ and H-1″, respectively.

Therefore, based upon the above cumulative evidences, compound **2** was elucidated as benzyl 2-hydroxy-4-*O*-[β-xylopyranosyl(1″ → 6′)-β-glucopyranosyl]-benzoate.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured with a Jasco DIP-370 digital polarimeter (JASCO Corporation, Tokyo, Japan). UV spectra were run on a UV 210A spectrophotometer (Shimadzu, Kyoto, Japan). IR spectra were recorded on a Bio-Rad FtS-135 spectrophotometer with KBr pellets (Bio-Rad Corporation, Hercules, CA, USA). 1D and 2D NMR spectra were recorded using Bruker AM-400 and DRX-500 instruments with tetramethylsilane (TMS) as an internal standard (Bruker BioSpin Group, Bremen, German). FAB-MS were measured on a VG Auto Spec-3000 spectrometer (VG PRIMA, Birmingham, UK), and HR-ESI-MS were taken on an API Qstar Pulsar instrument (Applied Biosystem Corporation, Foster City, CA, USA). Semipreparative HPLC was performed on an Agilent 1200 liquid chromatograph with a ZORBAX SB-C18 (5 μm, 9.4 × 250 mm; Agilent, San Francisco, CA, USA) column. Column chromatography (CC) was carried out on silica gel (200–300 mesh; Qingdao Marine Chemical Factory, Qingdao, China), Lichroprep RP-18 (43–63 μm; Merck, Darmstadt, Germany), Sephadex LH-20 (Amersham Biosciences AB, Uppsala, Sweden), and MCI (MCI-gel CHP-20P, 75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Japan). TLC was performed on TLC plates (Si gel GF_{254}; Qingdao Marine Chemical Factory), and detected by spraying with 5% H_2SO_4–EtOH, followed by heating on a hot plate. The standard samples of D- and L-glucose, D- and L-xylose were purchased from the company of the Sigma (St Louis, MO, USA).

3.2 Plant material

The flowers of *P. formosa* were collected in Jindian, Kunming, China, in May 2008. The sample was identified by Dr Yong-Peng Ma, Kunming Institute of Botany, Chinese Academy of Sciences, and a voucher specimen (KMUST 2008050701) is deposited at the Laboratory of Phytochemistry, Biotechnology Research Center, Kunming University of Science and Technology.

3.3 Extraction and isolation

The air-dried and powdered plant material (5.5 kg) was extracted with acetone–water (7:3, 3× 25 liters, each 2 days) at room temperature and filtered. The filtrate was evaporated *in vacuo* to afford a residue, which was dissolved in H_2O (2 liters), and then extracted successively with petroleum ether (3× 4 liters), EtOAc (4× 4 liters), and *n*-BuOH (4× 4 liters), respectively. The EtOAc extract (450.0 g) was decolorized on MCI gel, eluted with 90% MeOH–H_2O to yield a yellow gum (427.5 g). The gum was subjected to CC over a silica gel, eluted with a $CHCl_3$–Me_2CO gradient system (1:0, 9:1, 8:2, 7:3, 6:4, 1:1), to yield

six fractions, A–F. Fraction B (15.8 g, $CHCl_3$–Me_2CO, 8:2) was separated into three subfractions B1–B3 by a silica gel column eluting with petroleum ether–isopropyl alcohol (30:1, 20:1, 10:1). Subfraction B2 (3.7 g, petroleum ether–isopropyl alcohol 20:1) was purified by a silica gel column, eluted with petroleum ether–EtOAc (5:1), and then followed by Sephadex LH-20 $CHCl_3$–MeOH (1:1), to afford **1** (7 mg). The *n*-BuOH extract (425 g) was purified by Sephadex LH-20, eluted with MeOH–H_2O (3:7, 6:4, 9:1 gradient system), to give fractions G1–G3. Fraction G1 (MeOH–H_2O 3:7, 15 g) was subjected to an RP-18 (MeOH–H_2O 45:55), semipreparative HPLC (50% MeOH–H_2O, 3 ml/min), to give **2** (6 mg).

3.3.1 *Acid hydrolysis of* **2**

A solution of **2** (3 mg) in 1 M HCl (0.5 ml) was heated at 90–100°C in a screw-capped vial for 5 h. The mixture was partitioned with EtOAc (0.5 ml), and the EtOAc layer was compared with the standard samples of glucose and xylose on TLC (EtOAc–MeOH–AcOH–H_2O 11:2:2:2) by visualizing the spots with $R_f = 0.5$ and 0.6, respectively.

3.3.2 *Compound* ***1***

White powder. $[\alpha]_D^{28}$ +2.00 ($c = 0.35$, $CHCl_3$); IR (KBr) ν_{max}: 3432, 3095, 2924, 2852, 1733, 1657, 1463, 1421, 1378, 1324, 1276, 1196, 1082, 1053, 1032, 943, 888 cm^{-1}. ^1H and ^{13}C NMR spectral data, see Table 1. FAB-MS (neg.): *m/z* (%) 408 ($[M]^-$, 38), 376 ($[M-CH_3O-H]^-$, 18), 367

Table 2. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectral data (CD_3OD) of compound **2** (δ in ppm, J in Hz).

No.	^1H	^{13}C	HMBC
1		110.8 s	
2		159.9 s	
3	7.31 (d, $J = 2.5$)	108.1 d	C-1, 2, 4
4		158.2 s	
5	6.60 (br d, $J = 8.5$)	111.7 d	C-1, 3, 6
6	6.79 (d, $J = 8.0$)	134.3 d	C-2, 4, 5, 7
7		170.0 s	
8		137.4 s	
9	7.49 (d, $J = 7.5$)	129.5 d	C-10, 11, 14
10	7.38 (t, $J = 7.5$)	129.2 d	C-8, 9, 11
11	7.29 (m)	129.2 d	C-9, 10
12	7.38 (t, $J = 7.5$)	129.2 d	C-8, 11, 13
13	7.49 (d, $J = 7.5$)	129.5 d	C-11, 12, 14
14	5.37 (br s)	68.2 t	C-7, 8, 9
1′	4.90 (d, $J = 7.5$)	102.7 d	C-4, 3′
2′	3.37 (m)	74.8 d	C-1′, 4′
3′	3.25 (br t, $J = 8.5$)	77.8 d	C-2′, 4′
4′	3.31 (m)	71.2 d	C-5′, 6′
5′	3.61 (ddd, $J = 1.5$, 6.0, 11.5)	77.6 d	C-1′, 3′, 6′
6′	4.08 (dd, $J = 1.5$, 11.5)	69.7 t	C-4′, 1″
	3.73 (dd, $J = 6.5$, 11.5)		C-5′, 1″
1″	4.29 (d, $J = 7.5$)	105.3 d	C-6′
2″	3.10 (d, $J = 8.8$)	75.0 d	C-1″, 3″, 4″
3″	3.43 (br t, $J = 7.8$)	77.7 d	C-2″, 4″, 5″
4″	3.11 (m)	71.2 d	C-3″
5″	3.81 (dd, $J = 6.0$, 11.0)	66.9 t	C-1″, 3″, 4″
	3.16 (m)		C-1″, 3″

([M−CH_2CHCH_2]$^-$, 15), 351 ([M−CH_3-CH_2CO]$^-$, 5), 346 (10), 326 (5). Negative HR-ESI-MS: *m/z* 407.2432 [M−H]$^-$ (calcd for $C_{23}H_{35}O_6$, 407.2433).

3.3.3 Compound **2**

White powder. $[\alpha]_D^{26}$ −65.39 ($c = 0.21$, MeOH). IR (KBr) ν_{max}: 3443, 2926, 1724, 1639, 1510, 1453, 1271, 1223, 1076, 991, 898, 670 cm^{-1}. ^{1}H and ^{13}C NMR spectral data, see Table 2. FAB-MS (neg.): *m/z* (%) 537 [M−H]$^-$, 509 ([M–CO]$^-$, 10), 491 (25), 447 ([M–C_7H_6]$^-$, 100), 339 (55), 325 (22), 294 ([β-xylopyranosyl(1″ → 6′)-β-glucopyranosyl-18]$^-$, 5), 153 (15). Negative HR-ESI-MS: *m/z* 537.1621 [M−H]$^-$ (calcd for $C_{25}H_{29}O_{13}$, 537.1608).

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