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Novel Polyesterified 3,4-*seco*-Grayanane Diterpenoids as Antifeedants from *Pieris formosa*

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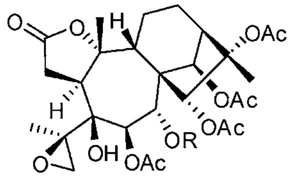
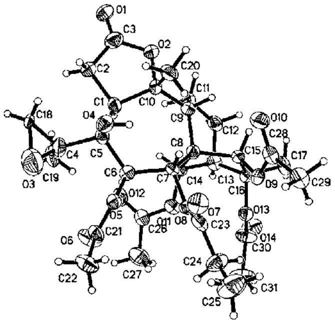
ABSTRACT



1 R = $COCH_2CH_3$
2 R = $COCH_3$



Pieris formosa **is a poisonous plant to livestock and is used as an insecticide in rural areas of China. Two novel polyesterified 3,4-*seco*-grayanane diterpenoids, pierisoids A and B (1 and 2), were isolated from its flowers and were identified by spectroscopic analysis and X-ray diffraction. Both compounds showed obvious antifeedant activity against cotton bollworm, indicating their toxic properties, suggesting a defensive role of polyesterified 3,4-*seco*-grayanane diterpenoids for *P. formosa* against herbivores.**

Pieris formosa D. Don (Ericaceae) is an evergreen shrub that has a rich distribution in hill and valley regions of southern and southwestern China especially the Yunnan province. The plant is well-known for poisoning livestock after accidentally feeding on its leaves and flowers, which can cause coma or even death of the animals. In rural areas of China, the juice of its fresh leaves and flowers are used as an insecticide and a lotion for treatment of ring worm and scabies.[1] Previous work by Qin et al. showed that the plant contains grayanane, leucothane, and 5,6-*seco*-kaurane diterpenoids and diphenylamine.[2−4] However, it is not clear yet if these components are responsible for the toxicity of the plant.

We investigated *P. formosa* to identify the toxic constituents of the flowers. Here we describe the isolation and structural elucidation of two novel polyesterified 3,4-*seco*-

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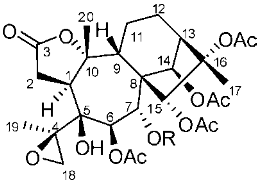
grayanane diterpenoids, pierisoids A (**1**) and B (**2**), and their antifeedant activities against cotton bollworm.



1 R = $COCH_2CH_3$
2 R = $COCH_3$

Air-dried *P. formosa* flowers (3.5 kg) were powdered and extracted with MeOH (3 × 5 L) at room temperature, which was partitioned between H_2O and EtOAc. The EtOAc part (594 g) was subjected to silica gel column chromatography with a gradient elution of $CHCl_3$−Me_2CO (1:0 to 0:1) to afford six fractions. Fraction 2 (45.0 g, $CHCl_3$−Me_2CO, 9:1) was repeatedly chromatographed on silica gel ($CHCl_3$−Me_2CO, 10:1; petroleum ether−Me_2CO, 4:1) and Sephadex LH-20 (MeOH; $CHCl_3$−MeOH, 1:1) columns to yield compound **1** (120 mg). Fraction 3 (4.2 g, $CHCl_3$−Me_2CO, 8:2) was repeatedly chromatographed on silica gel (petroleum ether−Me_2CO, 3:1) and Sephadex LH-20 ($CHCl_3$−MeOH, 1:1; Me_2CO) columns to afford compound **2** (13 mg).

Pierisoid A (**1**), $[\alpha]_D^{15.7} = +12.2$ (acetone, *c* 0.50), was obtained as colorless crystals from a mixture of acetone and methanol (5:1). Its molecular formula was determined to be $C_{31}H_{42}O_{14}$ by high-resolution ESI-MS (found: *m/z* 661.2477 $[M + Na]^+$, calcd: 661.2472). Its IR spectrum[7] displayed absorptions at 3436 cm^{-1} due to the hydroxyl group and at 1766 and 1743 cm^{-1} owing to ester carbonyl groups. The ^{1}H NMR spectrum (Table 1) of **1** displayed one primary methyl at δ_H 1.14 (3H, t, $J = 8.0$ Hz), three tertiary methyls at δ_H 1.32, 1.58, and 1.65 (each 3H of singlet), and four acetyl methyls at δ_H 1.98 (3H, s), 2.02 (6H, s), and 2.04 (3H, s). In the low-field region, three singlets (δ_H 3.89, 5.05, and 5.68) and a pair of AB doublets (δ_H 5.23 and 5.78, $J =$ 9.5 Hz) were also observed. Other signals centered between 1.6 and 3.3 ppm. The ^{13}C NMR spectrum (Table 1) of **1** demonstrated 31 carbon resonances (one was overlapped by acetone-d_6 solvent signals as recognized from an HSQC experiment), which were further classified by DEPT-90 and DEPT-135 experiments as eight methyls (δ_C 9.1, 18.3, 19.2, 20.6, 20.9, 21.8, 22.9, and 29.3), five methylenes with one of them occurring relatively downfield (δ_C 49.6), seven methines including four oxygen-occurring ones (δ_C 68.2, 74.0, 77.4, and 91.6), eleven quaternary carbons including three oxygenated ones (δ_C 73.0, 89.2, and 88.6), a likely oxygenated one (δ_C 60.1), and six carbonyl carbons (δ_C 169.8, 170.5, 171.2, 171.9, 173.9, and 174.1). Through analysis of the HSQC spectral data, all protons except for the one at δ_H 3.89 were assigned to their respective carbons unambiguously, indicating that the singlet at δ_H 3.89 was from the free hydroxyl group. In addition, a propionyl group was determined from analysis of ^{1}H−^{1}H COSY and HMBC spectra. All these data suggested a highly oxygenated diterpenoid for **1** possessing a hydroxyl group, a propionyloxy group, and four acetoxy groups as its substituents.

Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1** and **2**

	1[a]		**2**[b]	
position	^{13}C	^{1}H	^{13}C	^{1}H
1	41.7 d	3.12 dd (12.5, 7.5)	41.7 d	2.99 dd (12.8, 7.2)
2	30.9 t	2.52 dd (17.0, 7.5) 3.01 dd (17.5, 12.5)	30.8 t	2.40 dd (16.8, 7.2) 2.89 dd (16.8, 12.8)
3	174.1 s		174.1 s	
4	60.1 s		60.1 s	
5	73.0 s		72.9 s	
6	74.0 d	5.23 d (9.5)	73.8 d	5.11 d (9.6)
7	68.2 d	5.78 d (9.5)	67.9 d	5.68 d (9.6)
8	53.1 s		52.8 s	
9	57.5 d	2.97 m	57.1 d	2.78 m
10	89.2 s		89.1 s	
11	19.2 t	1.73 m 1.85 m	19.0 t	1.63 m 1.71 m
12	22.9 t	1.73 m 2.15 m	22.8 t	1.63 m 2.04 m
13	42.8 d	3.21 d (10.5)	42.8 d	3.06 d (10.4)
14	77.4 d	5.68 s	77.4 d	5.56 s
15	91.6 d	5.05 s	91.2 d	4.94 s
16	88.6 s		88.7 s	
17	19.2 q	1.58 s (3H)	19.4 q	1.49 s (3H)
18	49.6 t	2.31 d (5.0) 2.95 d (5.0)	49.6 t	2.21 d (5.2) 2.84 d (5.2)
19	18.3 q	1.32 s (3H)	18.2 q	1.21 s (3H)
20	29.3 q	1.65 s (3H)	29.1 q	1.54 s (3H)
5-OH		3.89 s		3.79 s
6-OAc	169.8 s		169.8 s	
	20.6 q	1.98 s (3H)	20.5 q	1.88 s (3H)
7-OAc			170.4 s	
			21.7 q	2.00 s (3H)
7-OPr	173.9 s			
	28.4 t	2.38 m 2.52 m		
	9.1 q	1.14 t (3H, 8.0)		
14-OAc	171.2 s		171.0 s	
	21.8 q	2.04 s (3H)	21.7 q	1.94 s (3H)
15-OAc	171.9 s		171.8 s	
	20.9 q	2.02 s (3H)	20.8 q	1.92 s (3H)
16-OAc	170.5 s		170.6 s	
	22.9 q	2.02 s (3H)	22.8 q	1.90 s (3H)

[a] Recorded in acetone-d_6 at 500 MHz for ^{1}H and 125 Hz for ^{13}C. [b] Recorded in acetone-d_6 at 400 MHz for ^{1}H and 100 Hz for ^{13}C.

Detailed analysis of the ^{1}H−^{1}H coupling relationships and ^{1}H−^{13}C long-range correlations (Figure 1) in the 2D spectra of **1** led to the establishment of a 7/6/5 ring system for its skeleton, indicating that **1** is a grayanane-type diterpenoid. However, the failure to construct an additional five-membered ring suggested that **1** is not a normal grayanane

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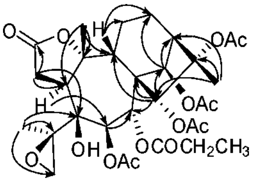

Figure 1. Selected HMBC correlations (H→C) of **1**.

diterpenoid as those previously reported from *P. formosa*.[1,4] Full assignments of all carbon signals revealed that the signals at δ_C 174.1 and 60.1 were ascribable to C-3 and C-4, respectively, which indicated a cleavage between C-3 and C-4. The former was further oxidized to the ester carbonyl carbon, while the latter was presumably also oxygenated. Considering the fact that Me-18 was replaced by a special methylene group at δ_C 49.6, it was thus concluded that **1** contains a 4,18-oxirane group.

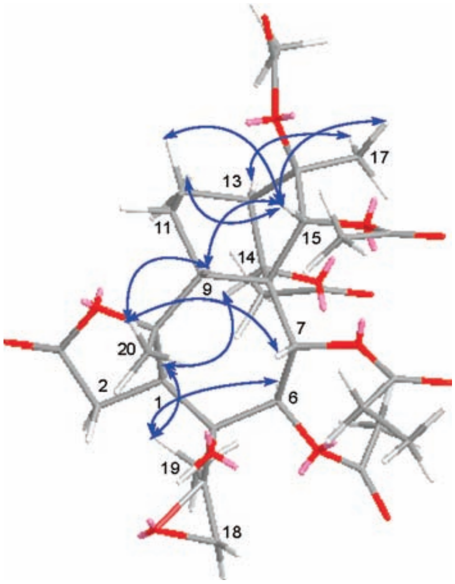

Figure 2. Key ROESY correlations (H↔H) of **1**.

The molecular formula of **1**, corresponding to 11 double bond equivalents, suggesed the existence of an additional oxygen bridge in the molecule. Considering that C-10 of **1** (δ_C 89.2) shifted dramatically downfield compared to hydroxylated C-10 of normal grayanane diterpenoids (ca. 77 ppm),[1,4] a lactone was very likely formed between C-3 and C-10. The propionyloxy group was assigned to C-7, and three acetoxy groups were located at C-6, C-14, and C-15, respectively, as evident from the long-range correlations of H-7, H-6, H-14, and H-15 with the corresponding carbonyl carbons. The acetoxy group is determined at C-16, due to its large downfield chemical shift (δ_C 88.6). The only hydroxyl group was accordingly assigned to C-5 (δ_C 73.0).

In the 2D ROESY spectrum (Figure 2), the ROE correlations of H-6/Me-19, Me-19/H-1, and H-1/H-14 indicated that H-1, H-6, and H-14 were in the same orientation (α) and 5-OH was in the opposite direction (β). Meanwhile, H-7, H-9, H-15, Me-17, and Me-20 were established as β-oriented in the light of the ROE interactions of H-7/Me-20, Me-20/H-9, H-9/H-15, and H-15/Me-17. However, the relative configuration of C-4 could not be determined by ROESY experiment due to the free rotation of the σ-bond between C-4 and C-5. However, we were able to obtain a single crystal of **1**, and its X-ray diffraction analysis[9] (Figure 3) determined the stereochemistry of C-4 and further confirmed the structure of **1**. Consequently, compound **1** was identified as a polyesterified 3,4-*seco*-grayanane diterpenoid as depicted and named pierisoid A.

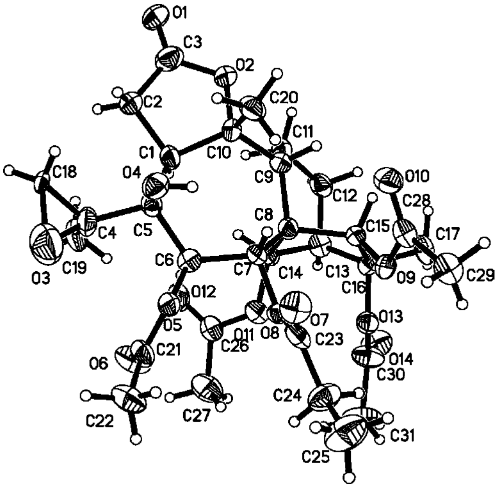

Figure 3. Single-crystal X-ray structure of **1**.

Pierisoid B (**2**), colorless crystals, showed a molecular formula of $C_{30}H_{40}O_{14}$ on the basis of the HR-ESI-MS (found: *m/z* 647.2306 [M + Na]$^+$, calcd: 647.2315). Comparison of the IR,[8] MS, 1D (^{1}H, ^{13}C, and DEPT) (Table 1), and 2D (^{1}H−^{1}H COSY, HSQC, HMBC, and ROESY) NMR spectral data of **2** with those of **1** clearly revealed that **2** was also a 3,4-*seco*-grayanane diterpenoid. The only difference between the two compounds was that the propionyloxy group at C-7 in **1** was replaced by an acetoxy group in **2**. Accordingly, **2** was characterized as 7-depropionyl-7-acetylpierisoid A and was named pierisoid B.

Compounds **1** and **2** were tested for their antifeedant effects against cotton bollworm (*Helicoverpa armigera*), a plant-feeding generalist insect herbivore, using an established method as described previously.[5] Both compounds showed

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(7) Pierisoid A (**1**): colorless crystals (from acetone and methanol), $[\alpha]_D^{15.7}$ +12.2 (acetone, *c* 0.50); UV (MeOH) λ_{max} (log ε) 196 (2.86) nm; IR (KBr) ν_{max} 3517, 3436, 2986, 2943, 1766, 1743, 1714, 1374, 1243, 1180, 1061, 1046, 933 cm^{-1}; ^{1}H and ^{13C} NMR data: see Table 1; ESIMS *m/z* 661 [M + Na]$^+$, HR-ESI-MS *m/z* 661.2477 (calcd for $C_{31}H_{42}O_{14}Na$, 661.2472).

(8) Pierisoid B (**2**): colorless crystals (from acetone and methanol), $[\alpha]_D^{14.7}$ +10.1 (acetone, *c* 0.37); UV (MeOH) λ_{max} (log ε) 210 (2.53) nm; IR (KBr) ν_{max} 3566, 3484, 2993, 1773, 1753, 1699, 1372, 1273, 1237, 1061, 1044, 960, 936 cm^{-1}; ^{1}H and ^{13}C NMR data: see Table 1; ESIMS *m/z* 647 [M + Na]$^+$; HR-ESI-MS *m/z* 647.2306 (calcd for $C_{30}H_{40}O_{14}Na$, 647.2315).

obvious antifeedant activities, with antifeedant index (AI%) of 54.9% at $5.54 \times 10^{-3}\ \mu g/cm^2$ for **1** and 40.7% at $5.67 \times 10^{-3}\ \mu g/cm^2$ for **2**, indicating toxic properties of **1** and **2**, which suggests a defensive role of 3,4-*seco*-grayanane diterpenoids for *P. formosa* against herbivores. Recently, four similar compounds were also reported[6] from the flower buds of another poisonous plant *Rhododendron molle* which belongs to the same family as *P. formosa*. Thus, polyesterified 3,4-*seco*-grayanane diterpenoids may belong to a new class of constitutive defense compounds typical of the fragile part such as flowers in Ericaceae plants, which warrant further investigation.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of compounds **1** and **2** and 2D NMR spectra of compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Crystal Data of **1**: $C_{31}H_{42}O_{14}$, $M = 638.65$, colorless block, size $0.23 \times 0.14 \times 0.08$ mm^3, Orthorhombic, space group $P2(1)$, $a = 11.4793(10)$ Å, $b = 16.7755(15)$ Å, $c = 17.5200(16)$ Å, $\alpha = 90°$, $\beta = 90°$, $\gamma = 90°$, $V = 3373.8(5)$ Å^3, $T = 25$ °C, $Z = 2$, $d = 1.257$ g/cm^3, μ(Mo Kα) = 0.71073 Å, $F(000) = 1360$, 31 101 reflections in $h(-14/14)$, $k(-21/21)$, $l(-22/22)$, measured in the range $1.68° \le \theta \le 28.36°$, completeness $\theta_{max} = 96.0\%$, 14 659 independent reflections, $R_{int} = 0.0653$, 7787 reflections with $F_O > 4\sigma(F_O)$, 290 parameters, 0 restraint, $R1_{obs} = 0.1390$, $wR2_{obs} = 0.3381$, $R1_{all} = 0.\ 2022$, $wR2_{all} = 0.3861$, GOF = 1.091. Flack structure parameter 0.9(19), largest difference peak and hole = 0.742 and −0.478 e/Å^3. The crystal structure of **1** was solved by direct method using the program SHELXS-97 (Sheldrick, G. M. *SHELXS97 and SHELXL97*; University of Göttingen: Germany, 1997.) and subsequent Fourier difference techniques and refined anisotropically by full matrix least-squares on $F2$ using SHELXL-97 (Sheldrick, G. M., *SHELXTL*, Version 6.10; Bruker AXS Inc.: Madison, Wisconsin, USA, 2000). Crystallagraphic data for the structure of **1** have been deposited in the Cambridge Crystallographic Data Centre (deposition number: CCDC 771351). Copies of this data can be obtained, free of charge, on application to the CCDC via www.ccdc.com.ac.uk/conts/retrieving.html (or 12 Union Road, Cambridge CB2 1EZ, UK, fax: +44 1223 336033, e-mail: deposit@ccdc.cam.ac.uk).