New 3,4-seco-Grayanane Diterpenoids from the Flowers of Pieris japonica

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Three new 3,4-seco-grayanane diterpenoids, neopierisoids D–F (2–4), and a new natural one, neopierisoid C (1), were isolated from the flowers of Pieris japonica. Their structures were elucidated on the basis of extensive spectroscopic analysis, including one and two dimensional (1- and 2D)-NMR, as well as high resolution-electron ionization (HR-EI)-MS.

Key words Pieris japonica; Ericaceae; 3,4-seco-grayanane diterpenoid; neopierisoid

Grayanane diterpenoids, possessing 5/7/6/5 ring system, have been found particularly in the genera Pieris, Rhododendron, Leucothoe of the family Ericaceae, which are responsible for the toxicity of these genera.1–2) Recently, a series of grayanane diterpenoids, including polyestersified 3,4-seco and 9,10-seco ones, have been isolated from the plants belonging to the genera Pieris, Rhododendron and Leucothoe by our group and other researchers,1–13) and some of them have shown significant physiological properties, including cAMP-decreasing17) and antifeedant5,12) activities. As a continuation of our search for more grayanane diterpenoids from the family Ericaceae, three new 3,4-seco-grayanane diterpenoids, neopierisoids D–F (2–4), and a new natural one, neopierisoid C (1) (Fig. 1), were isolated from the flowers of Pieris japonica. Herein, we describe the isolation and structural elucidation of 1–4.

Results and Discussion

The air-dried flowers of P. japonica were extracted with 75% aq. Me$_3$CO, and the concentrated extract was suspended in water and partitioned with ethyl acetate (EtOAc). The EtOAc fraction was subjected to repeated column chromatography (silica, MCIgel CHP-20P, Sephadex LH-20, and octadecylsilane (ODS)) and semi-preparative HPLC to yield three new 3,4-seco-grayanane diterpenoids, neopierisoids D–F (2–4), and a new natural one, neopierisoid C (1).

Compound 1 was obtained as white amorphous powder. The molecular formula C$_{20}$H$_{30}$O$_{8}$ was deduced from the quasi-molecular-ion peaks at $m/z$ 399 ([M+H]$^{+}$), 421 ([M+Na]$^{+}$) and 437 ([M+K]$^{+}$) in the positive electrospray ionization (ESI)-MS, and further confirmed by the high resolution-electron ionization (HR-EI)-MS ($m/z$ 398.1960, [M]$^{+}$), requiring six degrees of unsaturation. The IR spectrum indicated the presence of hydroxy (3443 cm$^{-1}$), ester carbonyl (1759 and 1747 cm$^{-1}$) and C=O (1631 cm$^{-1}$) functional groups. The $^1$H-NMR of 1 (Table 1) showed resonances attributed to three tertiary methyls at δ$_H$ 1.49 (3H, s, H-17), 2.19 (3H, s, H-19) and 1.42 (3H, s, H-20), a terminal double bond at δ$_H$ 5.03 (1H, s, H-18a) and 5.55 (1H, s, H-18b), as well as four O-methines at δ$_H$ 4.87 (1H, brs, H-5), 5.39 (1H, d, $J$=8.9 Hz, H-7), 5.58 (1H, s, H-14) and 4.10 (1H, s, H-15). The $^{13}$C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of 1 (Table 2) exhibited 20 carbon resonances, including three methyls, four methylenes (an olefinic one), seven methines (four oxygen-bearing ones) and six quaternary carbons (a carbonyl, an olefinic and three oxygen-bearing ones). The above NMR data indicated that compound 1 possessed a highly oxygenated 3,4-seco-grayanane diterpenoid skeleton with a lactone ring between C-3 and C-5. Detailed analysis of the one and two dimensional (1- and 2D)-NMR spectra of 1 and secorhodomollolide A$^{10}$ suggested structural similarity, except that four acetyl and a propionyl units in secorhodomollolide A were all replaced by hydroxyl groups in 1, which led to 224 mass units less than that of secorhodomollolide A. Therefore, compound 1 was finally identified as a new natural 3,4-seco-grayanane diterpenoid and named as neopierisoid C, since it had been reported as the basic hydrolyzed product of secorhodomollolide A$^{10}$ Furthermore, the similar rotating frame nuclear Overhauser enhancement spectroscopy (ROESY) spectra of 1 with that of secorhodomollolide A revealed that both compounds possessed the same relative configurations. And the absolute configuration was determined to be the same with that of secorhodomollolide A, which was confirmed by the single crystal X-ray diffraction analysis of secorhodomollolide A.$^{10}$

Compound 2 had the molecular formula C$_{20}$H$_{28}$O$_{7}$ with seven degrees of unsaturation, as inferred from the HR-EI-MS ($m/z$ 380.1856), which indicated 18 mass units less than com

Fig. 1. Chemical Structures of Compounds 1–4

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The 1H- and 13C-NMR data of 2 (Tables 1, 2) were closely comparable to those of 1. The main differences between them existed in the 13C-NMR data of C-4 [δC 149.4 (s) in 1 and 140.5 (s) in 2] and the signals in the seven-membered ring moiety [δC 55.7 (d, C-1), 92.8 (s, C-5), 73.2 (d, C-6), 71.0 (d, C-7), 58.0 (s, C-8), 45.9 (d, C-9) and 77.1 (s, C-10) in 1; 42.6 (d, C-1), 101.3 (s, C-5), 83.3 (d, C-6), 71.7 (d, C-7), 50.9 (s, C-8), 55.2 (d, C-9) and 85.4 (s, C-10) in 2], which suggested that 2 was different from 1 in the seven-membered ring. Additionally, in combination with the molecular formula of 2, it was reasonable to assume that 2 was a dehydrated derivative of 1. Heteronuclear multiple bond connectivity (HMBC) cross-peaks (Fig. 2) of H-6 (δH 4.76, d, J = 5.5 Hz) with C-1, C-5, C-7, C-8 and C-10 fully corroborated that OH-6 and OH-10 dehydrated by forming an oxygen bridge, which was responsible for the extra degree of unsaturation.

The relative configuration of compound 2 was established based on ROESY experiment (Fig. 3). Biogenetically, Me-17 and Me-20 were assigned to be β-directed and H-13 to be α-oriented.9) ROESY correlations of H-15/H-9, H-15/Me-17, and H-9/Me-20, indicated that H-9 and H-15 were co-facial with Me-17 and Me-20, and assigned to be β-oriented. And nuclear Overhauser effect (NOE) cross-peaks of H-7/H-9, H-7/H-15 and H-7/H-6, suggested that H-6 and H-7 were in the same β-orientations. Moreover, the cis relationship of H-7 and H-6 was confirmed by the coupling constant of 5.5 Hz for the vicinal protons.6,9,14) Thus, the oxygen bridge between C-6 and C-10 was unambiguously determined to be α-directed. In addition, NOE correlation between H-13 and H-14 revealed that H-14 possessed α-orientation. Accordingly, the structure of 2 was unequivocally determined and named as neopierisoid D.

Compound 3, which was named neopierisoid E, was obtained as white amorphous powder, and the molecular for-
of HMBC correlations of the olefinic protons at δC 151.1, s), of H-1 (δH 3.31, dd, J=10.8, 2.4 Hz) with C-2 (δC 33.3, t), C-3 (δC 176.4, s), C-4 (δC 147.7, s), C-9, C-10 and C-20 (δC 118.4, t), of H-9 (δH 2.40, overlapped) with C-1, C-10 and C-20. In the ROESY spectrum of 3, correlations of Me-17/H-15, Me-15/H-7 and H-7/H-9, indicated that H7, H-9, H-15, and Me-17 were in the same β-orientations. The cross-peaks observed between H-1 and H-18a, between both H-6 and H-18b with Me-19, between H-6 and H-14, and between H-13 and H-14 demonstrated that H-6, H-14, and H-13 were all α-oriented. Furthermore, H-7 was assigned to be opposite to that of H-6 based on a coupling constant of 9.5 Hz for the vicinal protons and no NOESY cross-peak was observed between them.6,9) Thus, the structure of 3 was finally determined as shown in Fig. 1.

The molecular formula of compound 4 was deduced as C_{20}H_{28}O_{7} by means of HR-EI-MS from the molecular ion peak at m/z 380.1836, indicating seven degrees of unsaturation. The 1H-NMR spectrum of 4 (Table 1) revealed the presence of four tertiary methyls at δH 1.44 (3H, s, H-17), 1.32 (3H, s, H-18), 1.22 (3H, s, H-19), and 1.72 (3H, s, H-20), and four O-bearing methines at δH 5.29 (1H, dd, J=10.6Hz, H-6), 5.51 (1H, d, J=10.6Hz, H-7), 4.15 (2H, overlapped, H-14, 15). The 13C-NMR and DEPT spectra (Table 2) exhibited 20 signals, including four methyls, three methenes, six methines (including four O-bearing ones), and seven quaternary C-atoms (including three O-bearing ones, two olefinic ones for a double bond, and one ester carbonyl), which suggested that compound 4 possessed the same 3,4-seco-grayanane diterpenoid skeleton as 3. A series of HMBC correlations (Fig. 2) of Me-20 (δH 1.72, s, C-20) with C-1 (δC 47.9, d), C-9 (δC 135.2, s) and C-10 (δC 121.0, s), of H-1 (δH 3.48, overlapped) with C-2 (δC 38.0, t), C-4 (δC 77.0, s), C-5 (δC 91.9, s), C-6 (δC 75.9, d), C-9 and C-10, and of H-11 (δH 1.85, m, H-11a; 2.53, dd, 20.6, 9.0, H-11b) with C-8 (δC 60.6, s), C-9, C-10, C-12 (δC 25.0, t) and C-13 (δC 51.6, d), revealed that the double bond was located between C-9 and C-10, rather than C-10 and C-20. In addition, the HMBC cross-peaks between Me-18 (δH 1.32, s) and C-4, C-5 and C-19 (δC 24.7, q), and between Me-19 (δH 1.22, s) and C-4, C-5 and C-18 (δC 26.3, q), revealed that the double bond between C-4 and C-18 in 3 was saturated in 4. Considering the same degrees of unsaturation of the two compounds, compound 4 should possess one more ring than 3. The HMBC correlations from H-7 (δH 5.51, d, J=10.6Hz) to C-4, C-5, C-6, C-8, C-9, C-14 (δC 84.0, d), C-15 (δC 83.4, d), C-18 and C-19, along with the oxygenated nature of C-4 and C-7 (δC 74.2, d), suggested the existence of an oxygen bridge between C-4 and C-7, which was confirmed by its HR-EI-MS and biogenetical pathway.

The relative configurations of all of the chiral centers of 4 and the conformation of each ring were elucidated by analysis of the ROESY data (Fig. 3), proton coupling constants, and analogy with 3 and secorhodomollolide A, of which the absolute configuration was determined by single-crystal X-ray crystallography.6) The same relative stereochemistry of C-1, C-5, C-6, C-8, C-13, C-14, C-15 and C-16, as well as the conformations of rings A–D in 4 as in 3, were deduced from the similar carbon chemical shifts, proton coupling constants, and ROESY correlations found in 4. Meanwhile, H-7 was assigned to β-orientation, ROESY correlations between H-1 with Me-19, H-6 with Me-18, and H-7 with Me-18 allowed
a 2,2-dimethyltetraphosphorurate (ring E) to be located at the underneath of B ring. Consequently, the structure of 4 was established as shown and named neoepiscos F.

**Experimental**

**General Experimental Procedures** Optical rotations were measured with a JASCO DIP-370 digital polarimeter (JASCO Corporation, Tokyo, Japan). UV data were obtained on a Shimadzu UV-2401A spectrophotometer (Shimadzu, Kyoto, Japan). A Bio-Rad FTS-135 spectrophotometer was used for scanning infrared spectroscopy with KBr pellets (Bio-Rad Corporation, CA, U.S.A.). 1- and 2D-NMR spectra were recorded on AM-400, DRX-500 and AVANCE III-600 instruments with tetratetramethylsilane (TMS) as an internal standard (Bruker BioSpin Group, Karlsruhe, Germany). ESI-MS were obtained with an API-Qstar TOF instrument (Allen-Bridge, Milwaukee, MI, U.S.A.). HR-EI-MS were measured on a VG 1200 liquid chromatograph with a ZORBAX SB-C18 (5 µm, U.K.). Semi-preparative HPLC was performed on an Agilent 1200 liquid chromatograph with a ZORBAX SB-C18 (5 µm, Agilent, U.S.A.) column. Column chromatography (CC) was performed with silica gel (100–200 or 200–300 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), MCIgel CHP-20P (CHP-20P, 75–150 mesh, Qingdao Haiyang Chemical Co., Ltd., Qingdao, China), and Sephadex LH-20 (30% MeOH) to give fraction C-1. Fraction D (CHCl3/MeOH 30 : 1–1 : 1), ODS (40% MeOH) and Sephadex C-1 (9 g) was repeatedly chromatographed on silica gel (CC) eluting with CHCl3/Me2CO (100% MeOH, 50% Me2CO) to a ff

**Plant Material** The flowers of *P. japonica* were collected from Xundian County, Yunnan province, China, in April 2012, and identified by Prof. Haizhou Li, Kunming University of Science and Technology. A voucher specimen (KMM20120405) was deposited at the Laboratory of Phytochemistry, Faculty of Life Science and Technology, Kunming University of Science and Technology.

**Extraction and Isolation** The air-dried and powdered flowers of *P. japonica* (3.9 kg) were extracted with 75% aq. Me2CO (3×15 L, 48 h, each) at room temperature, then concentrated *in vacuo* to yield an extract, which was partitioned between H2O and EtOAc. The EtOAc fraction (580 g) was chromatographed over silica gel CC eluting with CHCl3/Me2CO gradient system (1 : 0, 9 : 1, 7 : 3, 6 : 4, 10 L for each gradient) to afford five fractions, A–E. Fraction C (CHCl3/Me2CO 8 : 2, 20 g) was subjected to MCIgel CHP-20P (90% MeOH) to remove pigments and triterpenoids, and then separated by Sephadex LH-20 (30% MeOH) to give fraction C-1. Fraction C-1 (9 g) was repeatedly chromatographed on silica gel (CHCl3/MeOH 30 : 1–1 : 1), ODS (40% MeOH) and Sephadex LH-20 (CHCl3/MeOH 1 : 1) to get a mixture, which was then separated by semi-preparative HPLC (28% CH3CN/H2O) to yield compounds 2 (retention time (tR) = 8.5 min, 6.6 mg) and 4 (tR = 5.1 min, 3.2 mg). Fraction D (CHCl3/Me2CO 7 : 3, 100 g) was chromatographed over MCIgel CHP-20P (40, 60, 80, 100% MeOH, 50% Me2CO) to afford five fractions, fractions D-1–D-5. Fraction D-2 (60% MeOH/H2O, 15 g) was repeatedly purified on Sephadex LH-20 (MeOH/H2O 10–100%), silica gel (CHCl3/MeOH 30 : 1) and ODS (40–60% MeOH/H2O) to give compounds 1 (5.4 mg) and 3 (2.8 mg).

Neopierisoid C (1)

White amorphous powder; [α]D22 +7.8 (c = 0.22 MeOH); UV (MeOH) λmax (logε) 201.0 (3.31), 254.2 (3.37) nm; IR (KBr) νmax: 3443, 3424, 3268, 2977, 2932, 2871, 1759, 1747, 1631, 1590, 1451, 1437, 1231, 1039; 1H- and 13C-NMR data, see Tables 1 and 2; ESI-MS per os (p.o.) m/z 399 [M+H]+, 421 [M+Na]+, 437 [M+K]+; HR-EI-MS m/z 398.1960 [M]+ (Calcd for C29H36O9, 398.1941).

Neopierisoid D (2)

White amorphous powder; [α]D22 +5.5 +96.9 (c = 0.07 MeOH); 1H- and 13C-NMR data, see Tables 1 and 2; ESI-MS (p.o.) m/z 403 [M+Na]+; HR-EI-MS m/z 380.1856 [M]+ (Calcd for C20H36O7, 380.1835).

Neopierisoid E (3)

White amorphous powder; [α]D22 +25.3 (c = 0.08 MeOH); UV (MeOH) λmax (logε) 205.4 (2.55) nm; IR (KBr) νmax: 3456, 3424, 2956, 2933, 2914, 1770, 1727, 1631, 1213, 1121 cm−1; 1H- and 13C-NMR data, see Tables 1 and 2; ESI-MS (p.o.) m/z 403 [M+Na]+; HR-EI-MS m/z 380.1816 [M]+ (Calcd for C20H36O7, 380.1835).

Neopierisoid F (4)

White amorphous powder; [α]D22 +3.30 (c = 0.07 MeOH); UV (MeOH) λmax (logε) 202.8 (2.94) nm; IR (KBr) νmax: 3441, 2922, 2852, 1743, 1639, 1630, 1164, 1100, 1060, 1029 cm−1; 1H- and 13C-NMR data, see Tables 1 and 2; ESI-MS (p.o.) m/z 380 [M]+; HR-EI-MS m/z 380.1836 (Calcd for C20H36O7, 380.1835).

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**Conflict of Interest** The authors declare no conflict of interest.

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