ORGANIC LETTERS

2013
Vol. 15, No. 12
3074–3077

Mollolide A, a Diterpenoid with a New 1,10:2,3-Disecograyanane Skeleton from the Roots of *Rhododendron molle*

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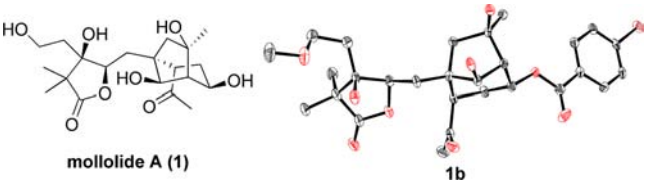
Received May 5, 2013

ABSTRACT



Mollolide A (1), a diterpenoid featuring a new 1,10:2,3-disecograyanane skeleton, was isolated from the roots of *Rhododendron molle*. Its structure was elucidated through extensive MS, IR, and NMR spectroscopy analyses. The absolute configuration was determined by single-crystal X-ray diffraction of its *p*-bromobenzoate derivative (1b). Compound 1 exhibits a significant analgesic effect at a dose of 20 mg/kg and antiviral activity against the Coxsackie B3 virus with an IC_{50} value of 27.7 μM.

Grayanoids, a special type of diterpenoid, are toxic compounds found exclusively in Ericaceae plants. Some members of the grayanoids exhibit potent sodium channel modulating,[1] analgesic, sedative,[2] and insect antifeedant activities,[3] which attracted great interest from both synthetic and biological perspectives.[4]

Rhododendron molle G. Don (Ericaceae), a toxic plant in China, has been used as an anodyne and an anesthetic since ancient times and is still used today to treat rheumatism and relieve pain.[5] Previous chemical studies of this plant have shown that it is a rich source of grayanoids.[6] However, the trace constituents have not yet been well studied. In our present research, a trace amount of a grayanoid (0.00008%) with a new 1,10:2,3-disecograyanane skeleton, designated as mollolide A (**1**), was isolated from the roots of *Rhododendron molle* (50 kg).[7] Mollolide A (**1**) represents the first example of a disecograyanane and was biosynthetically formed from multiple ring cleavage of a grayanane and an intramolecular esterification (Scheme 1). To the best of our knowledge, it is the first time either the 1,10-seco or 2,3-seco pattern of **1** has been discovered in grayanoids. We report herein the isolation, structure elucidation, postulated biogenetic formation, and biological activities of compound **1**.

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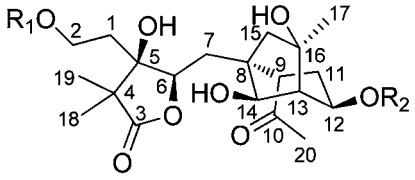
(7) For plant material, experimental procedures, and physical–chemical properties for compounds **1**, see the Supporting Information.

10.1021/ol401254e
Published on Web 05/31/2013



1 $R_1 = H, R_2 = H$
1a $R_1 = CH_3, R_2 = H$
1b $R_1 = CH_3, R_2 = 4\text{-Bromobenzoyl}$

Compound **1**, $[\alpha]^{20}_D$ + 41.0 (*c* 0.08, MeOH), was isolated as an amorphous powder. The molecular formula was established as $C_{20}H_{32}O_8$ by HRESI-MS with an *m*/*z* 423.2000 $[M + Na]^+$ (calcd 423.1989), corresponding to 5 degrees of unsaturation. The IR spectrum indicated the presence of an ester carboxyl group (1757 cm^{-1}) and a ketone moiety (1701 cm^{-1}). The ^{13}C and DEPT NMR spectra (Table 1) revealed four sp^3 quaternary carbons (two oxygenated at δ_C 81.4 and 80.6), five sp^3 methines (three oxygenated at δ_C 84.6, 79.3 and 68.7), five sp^3 methylenes (one oxygenated at δ_C 57.8), four tertiary methyls, and two sp^2 quaternary carbons (one ketone carbon at δ_C 211.5 and one ester carbonyl carbon at δ_C 181.9). Among the 20 carbons, two carbonyl carbons accounted for 2 degrees of unsaturation, which suggested that **1** is a diterpenoid possessing a tricyclic ring system.

The gross structure of **1** was initially deduced by comprehensive analysis of its 1D and 2D NMR spectra. According to the $^1H-^1H$ COSY and HSQC spectra, three spin systems [**a**: C(9)H−C(11)H_2−C(12)H−C(13)H−C(14)H; **b**: C(6)H−C(7)H_2; **c**: C(1)H_2−C(2)H_2] were established, as shown in Figure 1. In **a**, the protons of H-9 and H-14 showed significant HMBC correlations to an sp^3 quaternary carbon at δ_C 50.9 (C-8), which suggested that C-8, C-9, C-11, C-12, C-13, and C-14 constituted a six-membered carbon ring (ring B, Figure 1). In contrast, the protons of H_2-15 showed HMBC correlations to C-8, C-9, C-14, and C-16. Meanwhile, protons of H-13, H-14, and H-12 also showed critical HMBC correlations to an sp^3 quaternary carbon at δ_C 80.6 (C-16). These HMBC correlations indicated that a five-membered carbon ring (ring C, Figure 1) was formed by C-15, C-8, C-14, C-13, and C-16. Moreover, rings B and C comprised a bicyclo[3.2.1]octane ring system, and C-8 and C-13 were bridged by C-14.

Furthermore, three oxygenated carbons in rings B and C (δ_C 68.7, 79.3, and 80.6) were assigned to C-12, C-14, and C-16, respectively, by the HMBC correlations from H_2-11, H-9, and H-13 to C-12 (δ_C 68.7); from H_2-15 and H-13 to C-14 (δ_C 79.3); and from H_2-15, H-13, and H-12 to C-16 (δ_C 80.6). A tertiary methyl proton signal appearing at δ_H 2.34 showed strong HMBC correlations to the ketone carbon (δ_C 211.5) and C-9, which not only suggested the existence of an acetyl group but also placed it at C-9 of ring B. Another tertiary methyl at δ_H 2.05 was connected to C-16 of ring C as shown by HMBC correlations from H_3-17 to C-16, C-13, and C-15.

Table 1. NMR Data of Compound **1** in C_5D_5N (*J* in Hz)[8]

no.	δ_H	δ_C	no.	δ_H	δ_C
1	a 2.28 (dt, 14.5, 7.0) b 2.18 (dt, 14.5, 7.0)	35.7	11	2.12 (m)	30.2
2	4.30 (m)	57.8	12	4.31 (m)	68.7
3	–	181.9	13	2.89 (brs)	61.5
4	–	48.3	14	5.29 (d, 6.0)	79.3
5	–	81.4	15	a 2.44 (d, 14.5) b 2.00 (d, 14.5)	53.0
6	4.60 (brd, 10.0)	84.4	16	–	80.6
7	a 3.04 (brd, 15.5) b 2.66 (dd, 15.5, 10.0)	32.6	17	2.05 (s)	26.1
8	–	50.9	18	1.44 (s)	22.1
9	3.26 (brd, 7.0)	53.2	19	1.57 (s)	18.6
10	–	211.5	20	2.34 (s)	30.5

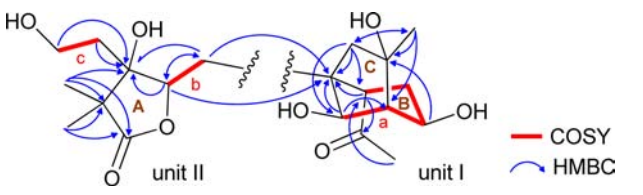


Figure 1. Selected $^1H-^1H$ COSY and HMBC correlations for compound **1**.

The above spectral data established unit I (Figure 1) of **1**, which resembles rings C/D of grayanane. However, the remaining part (unit II, Figure 1) of **1** was quite different from any known grayananes. The nine carbons of unit II showed signals at δ_C 181.9 (C), 84.4 (CH), 81.4 (C), 57.8 (CH_2), 48.3 (C), 35.7 (CH_2), 32.6 (CH_2), 22.1 (CH_3), and 18.6 (CH_3). The HMBC correlations from H_3-18 and H_3-19 to C-3 (δ_C 181.9), C-4 (δ_C 48.3), and C-5 (δ_C 81.4) indicated a fragment of −C(5)(O)−C(4)(CH_3)$_2$−COO−. Meanwhile, HMBC correlations from H_2-1 and H_2-2 to C-5 established the connection of C-1 to C-5; HMBC correlations from H-6 and H_2-7 to C-5 suggested the direct connection between C-6 and C-5. To fulfill the 5 degrees of unsaturation, an additional ring (ring A, Figure 1) was required in unit II of **1**. Considering that C-6 (CH) (δ_C 84.4) shifted dramatically downfield compared to a normal hydroxylated methine, a lactone was likely present at this position. The structure of unit II of **1** (Figure 1) was thereby constructed, in which the 5/7-fused ring system of grayanane was absent and replaced with a γ-lactone ring.

Finally, the connection of the two units (I and II) via C-7 and C-8 was confirmed by the key HMBC correlations from H-6 and H-7 to C-8 (δ_C 50.9). As a result, the planar structure of **1** was fully established, which is a new 1,10:2,3-disecograyanane skeleton.

The relative configuration of unit I was deduced by NOESY experiments (Figure 2). A chair conformation

of ring B was indicated by the correlations of H-14/H-12 and H-12/H-13, which also defined the 1,3-diaxial orientation of H-12 and H-14 and the equatorial orientation of H-13. Additionally, an NOE correlation between H-9 and H-15b suggested that H-9 was equatorial because C-15 and C-16 must adopt a 1,3-diaxial orientation to form a bicyclo[3.2.1]octane ring system. In contrast, H_3-17 correlated with H-11, which meant that C-17 was in the α-orientation. These findings led to the assignment of the relative configuration of unit I.

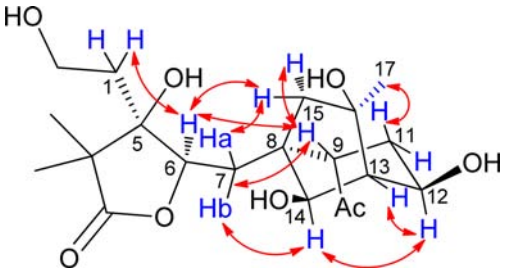


Figure 2. Key NOESY correlations for compound **1**.

However, the situation for C-5 and C-6 (unit II) is far more complex because they are located in a five-membered ring, which is known to exist as a combination of numerous puckered conformations. Each atom on this ring can assume many relative positions.[9] Thus, even though the correlation between H-6 and H-1b was observed in the NOESY spectrum (Figure 2), the relative configuration of C-5 and C-6 still remains uncertain.

In addition, units I and II of **1** were connected through a methylene, which should theoretically provide molecular flexibility and further complicate the stereochemical analysis. However, bulky substituents at C-5 and C-8 restrict the rotation of the C-6/C-7 bond and led to a preferential conformation in which C-5 and C-8 were in the *anti*-position (as shown in the Newman projections in Figure 3a). This explained how a small $^3J_{H6,H7a}$ ($J \approx 3$ Hz, measured by peak half-width) and a large $^3J_{H6,H7b}$ (J = 10.0 Hz) were observed in **1** (the ^{1}H NMR spectra of **1** did not change at 0, 20, and 60 °C; see Figure S21). Furthermore, the NOE correlations of H-7a/H-15a, H-7b/H-9, H-7b/H-14, H-6/H15a, and H-6/H-9 also suggested a restricted rotation of the C-7/C-8 bond and placed C-6 and C-14 in the *anti*-position (Figure 3b).

Based on the analyses described above, the relative stereochemistry of **1** could not be confirmed entirely due to the lack of evidence. To complete assignment, we attempted to obtain an X-ray crystal structure. However, we found it difficult to obtain a single crystal of **1** due to its polyhydric structure. To improve the crystallinity, a number of

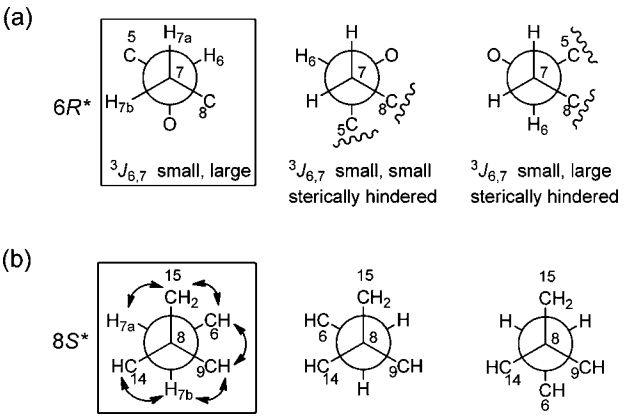


Figure 3. (a) Conformations of C-6 and C-7. (b) Conformations of C-7 and C-8. Boxes indicate conformations that agree with the measured data and are energetically favored. Arrows indicate the observed NOE correlations. For the designation of H-7a and H-7b, see Figure 2.

attempts were made to structurally modify **1**. Ultimately, the primary alcohol of **1** was selectively converted to methyl ether **1a**, and then **1a** was treated with *p*-bromobenzoyl chloride in pyridine to obtain **1b**. Crystallization of **1b** from 4:1 acetone/H_2O resulted in colorless crystals, which gave an X-ray crystal structure with a Flack parameter of −0.02(2). Thus, we were able to establish the relative stereochemistry at C-5 and C-6 of the five-membered ring as well as the absolute configuration of each chiral center (5*R*, 6*R*, 8*S*, 9*R*, 12*R*, 13*R*, 14*R*, 16*R*) (Figure 4).[10]

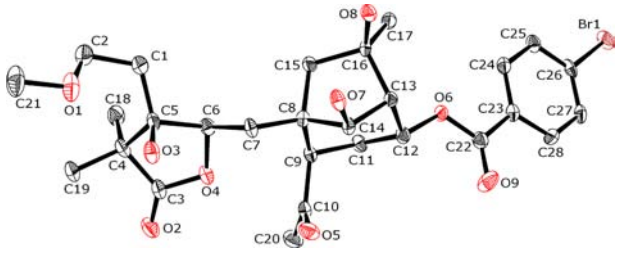


Figure 4. X-ray structure of **1b**.

(8) ^{1}H and ^{13}C NMR were measured at 500 and 125 MHz, repectively. Overlapped signals were reported without designating multiplicity.

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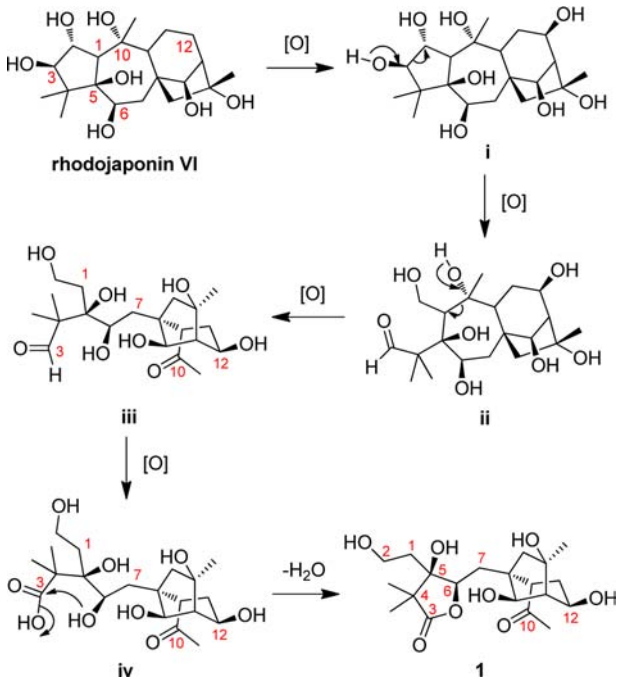
(10) For reaction conditions, see the Supporting Information. Upon crystallization from 4:1 acetone−H_2O using the vapor diffusion method, colorless crystals of **1b** were obtained. A crystal (2 mm × 0.03 mm × 0.02 mm) was separated from the sample and mounted on a glass fiber, and data were collected using a Gemini E X-ray single crystal diffractometer Eos CCD area detector with a graphite monochromator and Cu Kα radiation at 101.7 K, μ(Cu Kα) = 2.471 mm^{-1}; Crystal data: $C_{28}H_{37}BrO_9$, MW = 597.49, space group monoclinic, $P2_1$; unit cell dimensions were determined to be a = 13.3229 (4) Å, b = 6.1591 (2) Å, and c = 16.9032 (6) Å; V = 1382.46 (8) Å^3, Z = 2, ρ_{calcd} = 1.435 mg/mm^{-3}, F(000) = 624, 9233 reflections collected, 5308 independent (R_{int} = 0.0419) which were used in all calculations. The final refinement gave R_1 = 0.0555 and wR_2 = 0.1231. Crystallographic data have been deposited at The Cambridge Crystallographic Data Centre and allocated the deposition number CCDC 923268. The data can be obtained free of charge via www.ccdc.cam.ac.uk/products/csd/ request.

Scheme 1. Proposed Biogenetic Pathway of Compound **1**



Compound **1** represents the first example of a grayanoid with a new 1,10:2,3-disecograyanane skeleton. The biogenetic precursor of **1** could be plausibly traced back to rhodojaponin VI, a common grayanoid in this plant. Rhodojaponin VI could be initially transformed into 12-hydroxyl rhodojaponin VI (**i**) by enzyme-catalyzed oxidation. Intermediate **i** could undergo multiple ring cleavage (between C-2 and C-3 and between C-1 and C-10) to form intermediate **iii**. Then, the aldehyde group in **iii** could be further oxidized to carboxylic acid **iv**, which could trigger an intramolecular esterification to generate compound **1** (Scheme 1).

Compound **1** was evaluated for analgesic, antiviral, and cytotoxic activities.[11] In the acetic acid induced writhing test, at a dose of 20 mg/kg (i.p.), **1** exhibited an analgesic effect with a 73% reduction in writhes compared with the blank control. In the antiviral test, **1** demonstrated moderate antiviral activity against the Coxsackie B3 virus in African green monkey kidney cells (Vero cells) with an IC_{50} value of 27.7 μM, and in the cytotoxic test, no cytotoxicity against all of the assayed cell lines (HCT-8, Bel-7402, BGC-823, A549, and A2780) was observed at a concentration of 10 μM.

Acknowledgment. This work was supported by grants from the Natural Science Foundation of China (No. 21132009), the National Science and Technology Project of China (No. 2012ZX09301002-002), and PCSIRT (No. IRT1007). The authors are grateful to the Department of Instrumental Analysis of our institute for the UV, IR, NMR, and MS spectra measurements and to the Instrumental Analysis Center of Beijing University of Chemical Technology for X-ray crystallographic measurement and analyses.

Supporting Information Available. Detailed experimental procedures; 1D and 2D NMR, MS, UV, IR, CD spectra; and X-ray crystal data. This material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.