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Mollanol A, a Diterpenoid with a New C-Nor-D-homograyanane Skeleton from the Fruits of *Rhododendron molle*

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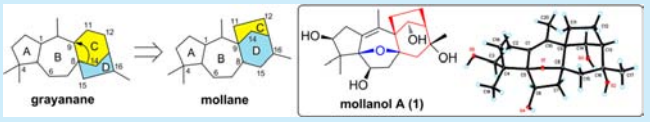
Supporting Information

ABSTRACT: Two new grayanoids, mollanol A (**1**) and rhodomollein XXV (**2**), were isolated from the fruits of *Rhododendron molle*. Their structures were elucidated by spectroscopic methods and X-ray diffraction analyses. Mollanol A (**1**) possesses a new C-nor-D-homograyanane carbon skeleton, while rhodomollein XXV (**2**) is the first example of an 11,16-epoxygrayanane and features a caged oxa-tricyclo[3.3.1.0^{3,7}]nonane ring system. Plausible biogenetic pathways for **1** were proposed. Compound **1** exhibited transcriptional activation effects on the *xbp1* upstream promoter in IEC-6, 293T, and RAW264.7 cells.



Grayanoids represent a special type of diterpenoid that exists exclusively in Ericaceae plants. Some grayanoids exhibited potent sodium-channel-modulating,[1] analgesic, sedative,[2] and insect antifeedant activities,[3] which have attracted great interest from both synthetic and biological perspectives.[4] The biogenetic precursor for grayanane is assumed to be *ent*-kaurene.[5] To date, nine types of grayanane-related carbon skeletons have been reported, including grayanane (A-nor-B-homo-*ent*-kaurane),[6] 1,5-secograyanane,[7] 3,4-secograyanane,[8] 9,10-secograyanane,[9] 1,10:2,3-disecograyanane,[10] leucothane (A-homo-B-norgrayanane),[11] kalmane (B-homo-C-nor-grayanane),[12] 1,5-secokalmane[13] and micranthane (C-homo-grayanane).[14] Seven types of skeletons have been found in plants of the Rhododendron genera, the largest genera of the Ericaceae family.[10,13−15] As a representative plant of the Rhododendron genera, *Rhododendron molle* G. Don has historically been used as an anodyne and anesthetic in China.[16] Previously, we reported a trace grayanoid, mollolide A, with a new 1,10:2,3-disecograyanane skeleton, that was isolated from the roots of *R. molle*.[10] In our continuing efforts to identify structurally unique and biologically interesting grayanoids, the fruits of *R. molle* were collected from the Guangxi province, and two new grayanoids were isolated: mollanol A (**1**), a diterpenoid possessing a new C-nor-D-homograyanane carbon skeleton, and rhodomollein XXV (**2**), the first example of an 11,16-epoxygrayanane, featuring a caged oxa-tricyclo[3.3.1.0^{3,7}]nonane ring system (Figure 1). Herein, we report the isolation, structural elucidation, and bioactivity of **1** and **2** as well as the likely biosynthetic pathway for **1**.

Mollanol A (**1**), $[\alpha]_D^{20}$ +25.3 (*c* 0.08, MeOH), was obtained as a colorless crystal. The HRESIMS data established the molecular formula of **1** as $C_{20}H_{30}O_5$ (*m*/*z* 373.1988 $[M + Na]^+$, calcd 373.1985), indicating six degrees of unsaturation. The IR spectrum suggested the presence of hydroxy group(s) (3362 cm^{-1}). Analysis of the ^{13}C NMR (DEPT) data indicated four

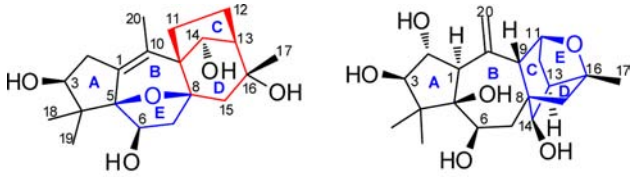


Figure 1. Structures of compounds **1** and **2**.

methyls, five methylenes, four methines (three oxygenated), and seven quaternary carbons (two olefinic and three oxygenated) (see Table 1). These data accounted for only one degree of unsaturation; thus, five rings should be present. The analysis of the $^1H-^1H$ COSY spectrum of **1**, aided by an HSQC experiment, revealed three spin-coupling systems. These spin-coupling systems (bold in Figure 2) were identified as follows: (a) C(2)H_2−C(3)H; (b) C(6)H−C(7)H_2; and (c) C(11)H_2−C(12)H_2−C(13)H−C(14)H.

Fragment a and the HMBC correlations (see Figures 2 and S13−S16 in the Supporting Information) from two *gem*-dimethyl singlets (H_3-18 and H_3-19) to carbons C-3, C-4, and C-5 from H_2-2 to C-5 and from H-3 to C-1 allowed the five-membered carbon ring (ring A in Figure 1) to be defined. The HMBC correlations from H-6 to C-1 and from H_2-7 to C-5 connected C-5 directly to fragment b via C-6. The HMBC correlations from methyl singlet H_3-20 to C-1 and C-10 indicated the connectivities of C-1 and C-10 and of C-20 and C-10. The above spectral data revealed a partial structure that resembles the 5/7-fused ring system of grayanane. However, unlike a typical grayanane, H_3-20 exhibited a strong HMBC correlation to an sp^3 quaternary carbon at δ_C 50.4 (C-9), which

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Table 1. NMR Data for Compound 1 in C_5D_5N (J in Hz)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
1		138.1	12	a 1.62 (m) b 1.33 (m)	22.8
2	a 2.90 (dd, 16.0, 8.0) b 2.54 (dd, 16.0, 8.0)	34.6	13	2.24 (t-like, 4.8)	48.1
3	4.69 (t, 8.0)	76.6	14	4.55 (dd, 7.0, 4.8)	78.4
4		46.3	15	a 2.35 (d, 13.6) b 2.21 (d, 13.6)	47.0
5		91.2	16		76.6
6	4.59 (brd, 7.2)	77.2	17	1.31 (s)	29.7
7	a 4.13 (dd, 13.6, 6.4) b 2.42 (brd, 13.6)	48.9	18	1.87 (s)	22.8
8		82.2	19	1.45 (s)	19.6
9		50.4	20	1.72 (s)	13.0
10		127.7	14-OH	7.02 (d, 7.0)	
11	a 2.14 (m) b 1.58 (m)	29.8	16-OH	6.78 (s)	

[a]Measured at 800 (^{1}H) and 200 (^{13}C) MHz. Overlapping signals are reported without designating multiplicity.

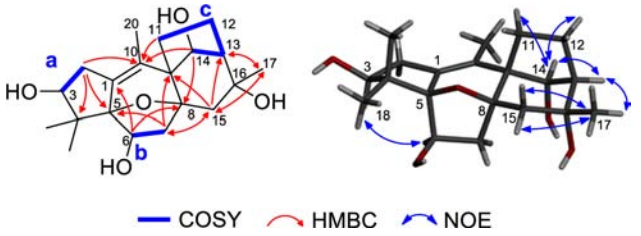

Figure 2. Selected ^{1}H−^{1}H COSY, HMBC, and NOESY correlations for compound **1**.

established the connection of CH_3-20 and C-9 through C-10. Moreover, in fragment c, the HMBC correlations from H_2-11 and H-14 to C-10 linked both C-11 and C-14 to C-9. Thus, C-9, C-11, C-12, C-13, and C-14 formed another five-membered carbon ring (ring C). In addition, the presence of a six-membered carbon ring (ring D) was deduced based on the HMBC correlations from H-14 to C-8 and C-16 as well as the correlations from H_2-15 to C-9 and C-13. Rings C and D composed a bicyclo[3.2.1]octane ring system, in which C-9 and C-13 were bridged by C-14. Meanwhile, the HMBC correlations from CH_3-17 to C-13, C-15, and C-16 unambiguously placed CH_3-17 on C-16. To fulfill the six degrees of unsaturation, an additional ring was required in the structure of **1**. Given that five oxygen atoms in the molecular formula accounted for six oxygenated carbons in the ^{13}C NMR spectrum and that C-5 (δ_C 91.2) was dramatically shifted downfield compared to a typical hydroxylated quaternary carbon, a furan ring (ring E) that connected C-5 to C-8 through an oxygen bridge was likely present. The planar structure of **1** was thus determined to possess a new C-nor-D-homograyanane carbon skeleton.

The NOESY correlations (see Figures 2 and S17−S19) of H-14/H-11b, H-14/H-12a, and H-14/H-13 suggested that H-14 and H-13 were equatorial because C-11 and C-12 must adopt a 1,3-diaxial orientation to form a bicyclo[3.2.1]octane ring system. The NOESY correlations of H_3-17/H-15a and H_3-17/H-15b confirmed the equatorial orientation of CH_3-17. In addition, the strong NOESY correlations of H_3-18/H-6 suggested that they were cofacial. However, due to a lack of evidence, the relative stereochemistry of **1** could not be completely confirmed by NOESY.

To complete the structural assignment, we resorted to an X-ray diffraction (Cu Kα radiation), performed on a high-quality single crystal of **1** that was obtained from a mixture of methanol/water. The X-ray crystallographic data corroborated the planar structure and the relative configuration of **1** and further allowed the assignment of its absolute configuration as 3*S*,5*R*,6*R*,8*R*,9*R*,13*R*,14*R*,16*R* [with a Flack parameter of 0.04(14)] (see Figure 3). Crystallographic data of **1** have been deposited at the Cambridge Crystallographic Data Centre (CCDC) and allocated the deposition number 1013958.

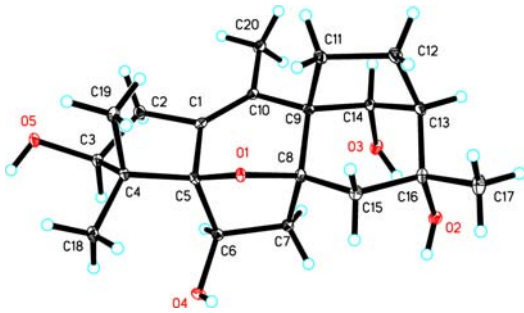

Figure 3. X-ray structure of compound **1**.

Rhodomollein XXV (**2**) was isolated as a colorless crystal. The molecular formula, $C_{20}H_{30}O_6$, was established by HRESIMS: m/z 367.2114, calcd for $[M + H]^+$, 367.2115. From this formula, **2** was determined to possess six degrees of unsaturation, of which, according to the ^{1}H and ^{13}C NMR data (see Table 2), one was due to a C═C double bond. Given that

Table 2. NMR Data for Compound 2 in C_5D_5N (J in Hz)[a]

no.	δ_H	δ_C	no.	δ_H	δ_C
1	3.57 (d, 8.0)	55.7	13	2.42 (m)	54.9
2	5.07 (dd, 8.0, 4.0)	80.2	14	4.90 (m)	81.2
3	4.18 (dd, 6.0, 4.0)	88.6	15	a 2.69 (d, 11.0) b 1.77 (d, 11.0)	51.9
4		49.3	16		86.7
5		85.2	17	1.57 (s)	24.6
6	4.88 (m)	72.2	18	1.63 (s)	27.0
7	a 2.44 (m) b 2.33 (dd, 13.5, 11.0)	40.3	19	1.78 (s)	21.5
8		50.2	20	a 5.73 (s) b 5.38 (s)	116.9
9	3.18 (brs)	57.8	2-OH	6.74 (m)	
10		146.6	3-OH	6.40 (d, 6.0)	
11	4.39 (t, 3.0)	84.4	6-OH	5.52 (d, 7.5)	
12	a 2.06 (brd, 11.0) b 1.94 (m)	38.7	14-OH	6.74 (m)	

[a]Measured at 500 (^{1}H) and 125 (^{13}C) MHz. Overlapping signals are reported without designating multiplicity.

six oxygen atoms in the molecule accounted for seven oxygenated carbons in ^{13}C NMR spectrum, compound **2** should be pentacyclic and possess an ether ring. The ^{1}H and ^{13}C NMR spectra of **2** displayed resonances for three quaternary methyls at δ_H 1.57/δ_C 24.6 (CH_3-17), δ_H 1.63/δ_C 27.0 (CH_3-18), and δ_H 1.78/δ_C 21.5 (CH_3-19); an exocyclic double bond at δ_H 5.73 (s, H-20a) and 5.38 (s, H-20b)/δ_C 116.9 (CH_2−20); five oxygenated methines at δ_H 5.07/δ_C 80.2

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(CH-2), δ_H 4.18/δ_C 88.6 (CH-3), δ_H 4.88/δ_C 72.2 (CH-6), δ_H 4.39/δ_C 84.4 (CH-11), and δ_H 4.90/δ_C 81.2 (CH-14); and two oxygenated quaternary carbons at δ_C 85.2 (C-5) and 86.7 (C-16).

The above data for **2** were similar to those of 2,3,5,6,14,16-hexahydroxygrayan-10(20)-enes such as rhodomollein I,[17] except for the presence of an additional oxygenated methine group (δ_H 4.39/δ_C 84.4) and an additional ether ring. The HMBC correlations (see Figures 4 and S27–S29) from H_2-20

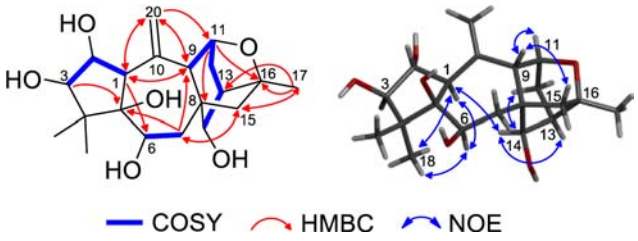


Figure 4. Selected ^{1}H–^{1}H COSY, HMBC, and NOESY correlations of compound **2**.

to the carbon at δ_C 84.4 and from a proton at δ_H 4.39 to C-8 indicated that the additional oxygenated methine was CH-11, as corroborated by its ^{1}H–^{1}H COSY interactions with H-9 and H_2-12. A key HMBC correlation from H-11 to C-16 further supported the presence of the C11–O–C16 oxygen bridge. Thus, the planar structure of **2** was identified as that shown in Figure 4.

Key NOESY correlations (see Figures 4 and S30 and S31) of H-1/H_3-18, H-1/H-14, H-1/H-6, and H-6/H_3-18 indicated that all of these protons were on the same face of the carbon skeleton. A chair conformation for ring C (see Figure 1) was indicated by the correlations of H-14/H-12a and H-14/H-13, which also indicated the 1,3-diaxial orientation of H-12a and H-14 and the equatorial orientation of H-13. The correlation of H-9/H-15b confirmed that they were on the same side of the molecule. Nevertheless, the relative stereochemistry at C-2 and C-3 could not be resolved unambiguously because of the uncertain conformation of the five-membered ring (ring A in Figure 1).

Ultimately, a single crystal was obtained from methanol, which allowed us to fully determine the relative configuration of **2** by X-ray diffraction (shown in Figure 5, data deposited at CCDC, no. 1013959). Compound **2** is the first example of an 11,16-epoxygrayanane and features a caged oxa-tricyclo-[3.3.1.0^{3,7}]nonane ring system.

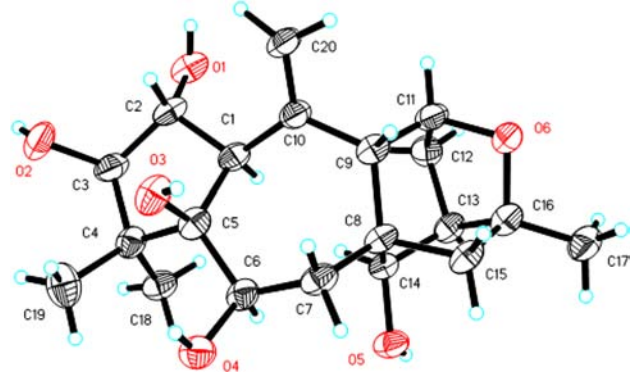


Figure 5. X-ray structure of compound **2**.

To determine the absolute configuration of **2**, the ECD spectra for **2a** (1*R*,2*R*,3*R*,5*R*,6*R*,8*S*,9*S*,11*S*,13*R*,14*R*,16*R*) and its enantiomer **2b** were calculated using time-dependent density functional theory calculations at the B3LYP/6-31G(d) level in methanol. The measured CD spectrum of **2** agreed well with the calculated ECD of **2a** and is the opposite of that of **2b** (see Figure 6). Thus, the absolute configuration of **2** was established.

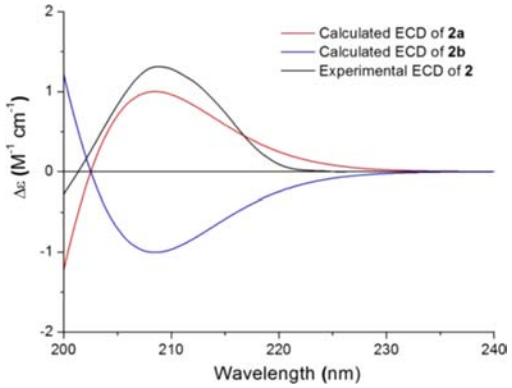
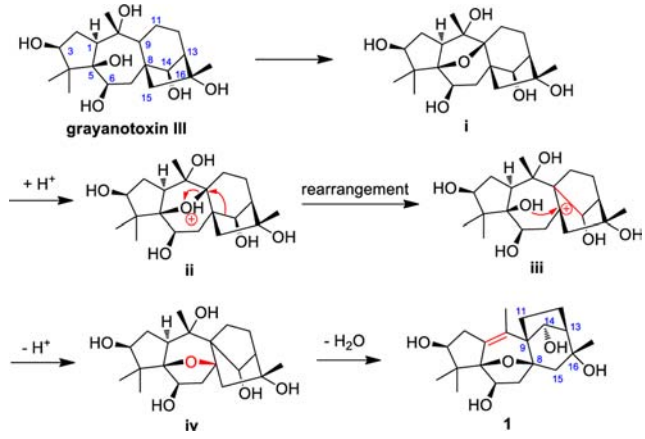


Figure 6. Calculated and experimental ECD spectra of **2**.

Mollanol A (**1**) represents a new tetracyclic diterpene carbon skeleton with an unprecedented C-nor-D-homograyanane ring system, and we named this new skeleton "mollane". The likely biosynthetic origin of the mollane skeleton is a grayanane precursor (grayanotoxin III),[3] as shown in Scheme 1. The

Scheme 1. Proposed Biosynthetic Pathway for 1



rearrangement could be initiated by cleavage of the oxygen bridge between C-5 and C-9, which would trigger an enzymatic Wagner–Meerwein rearrangement and result in the migration of the C-14 alkyl group to C-9.[12,14] The carbocation center created at C-8 could then be neutralized by the formation of a new oxygen bridge between C-5 and C-8.

Inflammatory bowel disease (IBD) is a complex disease that results from the interaction of environmental and genetic factors. Recent studies have indicated that IBD could originate from the dysfunction of the transcription factor XBP1 in intestinal epithelial cells (IECs), which thus serves as a potential therapeutic target for IBD.[18] In a dual luciferase report gene assay targeting *xbp1* (see S42 in the Supporting Information), compound **1** demonstrated transcriptional activation effects on the *xbp1* upstream promoter in different cell types (112, 148,

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and 207% activation in IEC-6, 293T, and RAW264.7 cells, respectively, compared with the untreated controls) at 10 μM.

■ ASSOCIATED CONTENT

Supporting Information

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

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Notes

The authors declare no competing financial interest.

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