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Hapmnioides A–C, Rearranged Labdane-Type Diterpenoids from the Chinese Liverwort *Haplomitrium mnioides*

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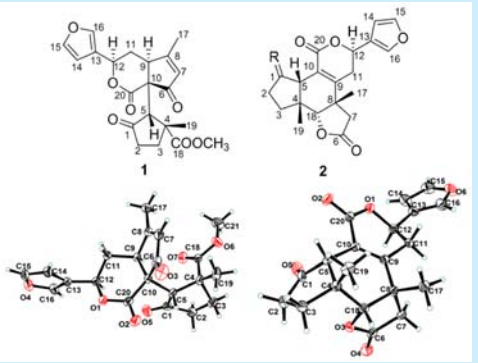
Supporting Information

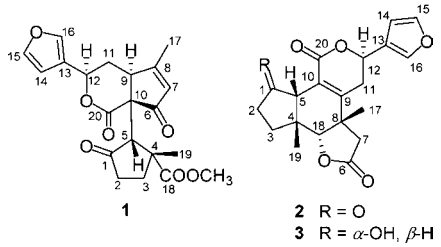
ABSTRACT: Many exceptional labdane-type diterpenoids have been exclusively found in liverworts, which serve as taxonomic molecules or play important ecological roles in interactions among organisms. Three unprecedented labdane-type diterpenoids hapmnioides A (**1**), B (**2**), and C (**3**) formed through cascade rearrangement from the Chinese liverwort *Haplomitrium mnioides* are reported. Their structures were established by comprehensive spectroscopic analysis coupled with single-crystal X-ray diffraction, and their anti-inflammatory activities were also preliminarily tested.



Labdane-type diterpenoids are a large class of natural metabolites with over 1000 members[1] that are biosynthetically derived via enzymatic or nonenzymatic pathways[2,3] and can be divided into five subclasses according to their ring fusions (Figure S1). Labdanes have been found to date in land plants, microbes, marine organisms, and insects, exhibit a wide range of biological activities, and play significant ecological roles in the interactions among organisms.[4−10] Liverworts, a rich source of bioactive substances,[11] produce many exceptional labdane-type diterpenoids, such as 7,8-*seco*-labdanes (e.g., pallavicinin, neopallavicinin, and pallambins A–D) from the genus of *Pallavicinia*,[12] 8,9-*seco*-labdanes (e.g., *seco*-infuscadione and infuscadiol) from *Jungermannia infusca*,[13] and the manoyl oxide-type labdanes (e.g., hamachilobenes A–E, ptychantins A–O, heteroscyphins A–D) from *Frullania hamachiloba*, *F. inouei*, *Ptychanthus striatus*, and *Heterpscyphus tener*,[14,15] respectively, which can be considered as taxomomic markers in Hepaticae.[16]

Previous chemical investigations on *Haplomitrium mnioides* (Lindb.) R. M. Schust, collected from Guizhou Province in China, which led to the isolation of two highly rigid labdane-type diterpenoids haplomintrins A and B, together with four analogues haplomitrenonolides A–D.[17] In order to enrich the diversity of the architecturally intriguing diterpenoids, our further research on the same species led to the discovery of three novel labdanes, hapmnioides A–C (**1**–**3**) (Figure 1), possessing three unprecedented scaffoldings.

Hapmnioide A (**1**) was a highly mutated labdane-type diterpenoid with a molecular formula $C_{21}H_{22}O_7$ as determined by HRESIMS (*m*/*z* 387.1440 $[M + H]^+$, calcd 387.1438) and



1 **2** R = O **3** R = α-OH, β-H

Figure 1. Structures of hapmnioides A–C (**1**–**3**) isolated form *H. mnioides*.

^{13}C NMR data, indicative of 11 degrees of unsaturation. The IR absorption bands at 1739 and 1623 cm^{-1} suggested the presence of carbonyl and olefinic functionalities, respectively. Analysis of the 1D (Table 1) NMR data of **1** revealed 21 carbon signals that were attributable to three methyls, three methylenes, four sp^3 methines, three sp^2 methines, and eight quaternary ones (two olefinic and four carbonyls), as evidenced by HSQC experiments (Figure S4). Further analysis of the 2D (Figure 2A) NMR data was carried out to establish the planar structure of **1**. The $^1H-^1H$ COSY spectrum (Figure 2A) indicated the presence of three independent spin systems of H_2-2/H_2-3, H-9/H_2-11/H-12, and H-14/H-15. The HMBC spectrum (Figure 2A) showed the correlations from H_3-19 to C-3, C-4, C-5, and C-18 from H-5 to C-1, C-6, C-9, and C-20, from H_3-17 to C-7, C-8, and C-9, from H-12 to C-13, C-14,

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data (ppm) for 1−3

	1[a]		2[b]		3[a]	
no.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	213.6 s		213.0 s		74.5 d	4.55 m
2	32.8 t	2.41 m	35.9 t	α 2.21 m	34.8 t	2.21 m
				β 2.37 m		1.82 dddd (14, 9.0, 5.0, 2.0)
3	36.3 t	1.94 m	32.8 t	α 2.03 m	36.4 t	α 2.31 ddd (13.6, 9.0, 7.3)
		2.17 m		β 2.19 m		β 1.62 m
4	48.7 s		39.2 s		42.2 s	
5	68.1 d	3.62 s	51.0 d	3.45 s	50.5 d	2.98 dd (2.0, 4.4)
6	199.2 s		173.2 s		172.9 s	
7	127.2 d	5.88 s	40.9 t	2.93 m	43.0 t	2.82 2.69 ABq (18.0)
8	180.9 s		44.5 s		44.9 s	
9	45.6 d	3.41 dd (11.2, 6.0)	152.0 s		149.0 s	
10	58.0 s		119.1 s		123.6 s	
11	36.6 t	2.57 ddd (14.0, 4.0, 2.0)	29.4 t	2.81 m	31.0 t	2.73 ddd (16.0, 12.0, 3.0)
		1.52 dt (13.6, 11.2)				2.46 dd (16.0, 3.0)
12	72.6 d	5.92 m	70.9 d	5.33 dd (12.4, 3.2)	71.2 d	5.38 dd (3.2, 12.6)
13	124.4 s		123.8 s		124.1 s	
14	108.5 d	6.39 d (0.8)	109.2 d	6.69 d (0.8)	108.6 d	6.48 dd (0.8, 1.8)
15	143.8 d	7.39 t like (1.6)	143.7 d	7.69 t (1.6)	144.0 d	7.45 t (1.6)
16	140.0 d	7.46 br s	140.9 d	7.82 br s	140.4 d	7.54 br s
17	17.4 q	2.06 s	21.4 q	1.30 s	23.8 q	1.41 s
18	175.1 s		89.5 d	4.40 s	89.8 d	4.28 s
19	24.1 q	1.34 s	23.5 q	1.17 s	27.5 q	1.17 s
20	168.8 s		163.7 s		164.5 s	
COOMe	52.6 q	3.65 s				

[a]Recorded in $CDCl_3$, [b]Recorded in methanol-d_4.

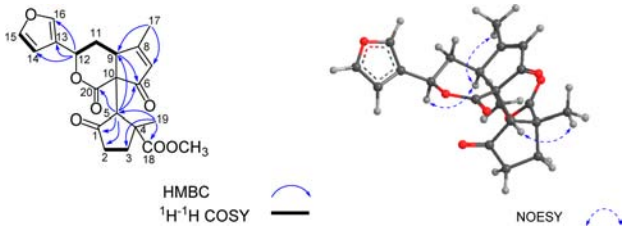

Figure 2. (A) Selected HMBC (H → C) and $^{1}H-^{1}H$ COSY (H−H) correlations of **1**. (B) Selected NOE correlations (H↔H) of **1**.

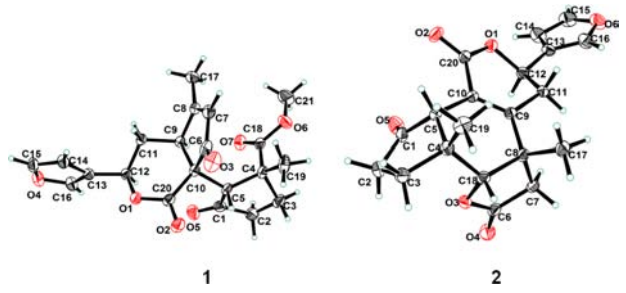

Figure 3. X-ray crystallographic structures of **1** and **2**.

and C-16. The above information indicated the same structural moieties of a δ-lactone ring and a monosubstituted furan ring with those of haplomitrenonolide C.[17] However, the downfield shift of C-8 (δ_C 180.9) and the unexpected HMBC correlations of H-9/C-6, H-7/C-10 and H-5/C-2 suggested the cleavages at C-1/C-10 and C-5/C-6 and linkages at C-1/C-5 and C-6/C-10.

The relative configuration of this reconstructive labdane structure was deduced by the NOE correlations (Figure 2B). The cross-peaks of H_3-17/H-9/H-12 revealed that they were cofacial and were assigned to be α-oriented. The NOE correlation of H-5/H_3-19 suggested they were cofacial. The absolute structure of **1** was finally confirmed by a single-crystal X-ray diffraction using Cu Kα radiation (CCDC 1059391, Figure 3), which indicated the absolute stereochemistry of **1** to be 4*R*,5*S*,9*S*,10*R*,12*R*. The structure of hapmnioide A (**1**) was thus unambiguously characterized to be a highly rearranged labdane-type diterpenoid with a novel 1,10:5,6-di-*seco*-12,20-olide-1,5:6,10-dicyclolabdane skeleton.

Hapmnioide B (**2**) was assigned the molecular formula of $C_{20}H_{20}O_6$ by HRESIMS (m/z 357.1336 $[M + H]^+$, calcd 357.1333) requiring 11 degrees of unsaturation. The ^{1}H and ^{13}C NMR data (Table 1) revealed a typical monosubstituted furan ring (δ_H 6.69, 7.66 and 7.82; δ_C 123.8, 109.2, 143.7 and 140.9), one double bond (δ_C 152.0 and 119.1), two ester carbonyls (δ_C 173.2 for γ-lactone ring and 163.7 for δ-lactone ring), as well as a ketone carbonyl (δ_C 213.0). The HMBC correlations (Figure S12) from two methyls [H_3-19 (δ_H 1.17) and H_3-17 (δ_H 1.30)] to the same oxygenated methine C-18 (δ_C 89.5) suggested a unique rearranged labdane skeleton of **2**, which was finally defined to be a 1,10:5,6-di-*seco*-12,20:6,18-diolide-1,5:18,8-dicyclolabdane carbon scaffold on the basis of the detailed 2D NMR data analysis. NOE correlations of H-18/H_3-17/H_3-19/H-5 (Figure S13) were supportive of their cofacial relationship, and they were determined to be β-oriented. Finally, the Cu Kα single-crystal X-ray diffraction analysis (CCDC 1059506, Figure 1) established the absolute structure of compound **2**.

The same strategy was adopted for the elucidation of the structure of **3**, based on its molecular formula of $C_{20}H_{22}O_6$, as determined by HRESIMS (m/z 381.1309 $[M + Na]^+$, calcd 381.1309) and comparison of its NMR data with those of **2**

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(Table 1), which showed the absence of resonances for the ketone carbonyl and the appearance of an additional methine at C-1 (δ_H 4.54, δ_C 74.5) in **3**. This deduction was confirmed by the HMBC correlations from H-1 to C-3 and C-4 and $^1H-^1H$ COSY correlations of H-5/H-1/H_2-2/H_2-3 (Figure S24). NOE correlations of H-18/H_3-19/H_3-17/H-5/H-3β disclosed their cofacial relationship. The relative configuration of H-1 was determined by the NOE correlation between H-1 and H-3β (Figure S25). Therefore, the molecular structure of compound **3** was established as shown and named as hapmnioide C.

Enlightened by the common δ-lactone ring and furan ring of these novel labdanes (**1**−**3**) and haplomitrenonolides A−C (**4**−**6**),[17] a plausible biosynthetic pathway of compounds **1**−**3** was proposed (Scheme S1). Initiated by the oxidation at C-1, compounds **1**−**3** might be formed from haplomitrenonolide C or B (**6** or **5**) through a cascade of diradical rearrangement reaction.

Anti-inflammatory activities of compounds **1**, **2**, and haplomitrenonolides A−C (**4**−**6**) (Scheme S1), were evaluated by BV2 cells. The results indicated that these chemicals differentially affect the secretory of IL-6, compounds **1** and **4** greatly suppressed the expression of IL-6 in LPS-induced cells, showing a potential anti-inflammatory activity (Figure S36). Compound **6**, to some extent, also displayed an inhibitory effect on the IL6 product. Since nuclear factor-kappa B (NF-κB) is critical in the acceleration of inflammation response and plays an important role in transcriptional activation of IL-6 expression,[18] the effect of the compounds on NF-κB were evaluated in prostate cancer PC3 cells.[19,20] Immunofluorescence analysis revealed that compounds **1** and **4** obviously inhibited phosphorylation and nuclear translocation of p65 in PC3 cells following 2 h treatments (Figure S37), while the others displayed less inhibitory effects. These results identified compounds **1** and **4** as potential anti-inflammatory agents by inactivation of NF-κB/IL-6 signaling.

In conclusion, hapmnioides A−C (**1**−**3**) are rearranged labdane-type diterpenoids with unprecedented scaffoldings isolated from the Chinese liverwort *H. mnioides*. Their structures had been determined, and the formation of **1** and **2** from **6** and **5** was deduced, respectively. Anti-inflammatory activities of compounds **1**, **2**, and **4**−**6** have been preliminarily studied.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01854.

General experimental procedures, 1D and 2D NMR data, HRESIMS, IR, UV, and CD spectra of the new compounds, crystal information for compounds **1** and **2**, and plausible biosynthetic pathways of compounds **1**−**3**, together with anti-inflammatory activities of compounds **1**, **2**, and haplomitrenonolides A−C (**4**−**6**) (PDF)

X-ray data for compound **1** (CIF)

X-ray data for compound **2** (CIF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Hanson, J. R. *Nat. Prod. Rep.* **2015**, *32*, 76−87.
(2) Peters, R. J. *Nat. Prod. Rep.* **2010**, *27*, 1521−1530.
(3) Zhang, J. Z.; Zhu, R. X.; Li, G.; Wang, L. N.; Sun, B.; Chen, W. F.; Liu, L.; Lou, H. X. *Org. Lett.* **2012**, *14*, 5624−5627.
(4) Frija, L. M.; Frade, R. F.; Afonso, C. A. *Chem. Rev.* **2011**, *111*, 4418−4452.
(5) Chinou, I. *Curr. Med. Chem.* **2005**, *12*, 1295−1317.
(6) Xu, M.; Hillwig, M. L.; Prisic, S.; Coates, R. M.; Peters, R. J. *Plant J.* **2004**, *39*, 309−318.
(7) Islam, A. K.; Ohno, O.; Suenaga, K.; Kato-Noguchi, H. *J. Plant Physiol.* **2014**, *171*, 877−883.
(8) Rivero-Cruz, I.; Trejo, J. L.; Aguilar, M. I.; Bye, R.; Mata, R. *Planta Med.* **2000**, *66*, 734−739.
(9) DellaGreca, M.; Fiorentino, A.; Isidori, M.; Monaco, P.; Zarrelli, A. *Phytochemistry* **2000**, *55*, 909−913.
(10) Langenheim, J. H. *J. Chem. Ecol.* **1994**, *20*, 1223−1280.
(11) Asakawa, Y.; Ludwiczuk, A.; Nagashima, F. *Phytochemistry* **2013**, *91*, 52−80.
(12) Wang, L. N.; Zhang, J. Z.; Li, X.; Wang, X. N.; Xie, C. F.; Zhou, J. C.; Lou, H. X. *Org. Lett.* **2012**, *14*, 1102−1105.
(13) Nagashima, F.; Suzuki, M.; Takaoka, S.; Asakawa, Y. *Tetrahedron* **1999**, *55*, 9117−9132.
(14) Guo, D. X.; Xiang, F.; Wang, X. N.; Yuan, H. Q.; Xi, G. M.; Wang, Y. Y.; Yu, W. T.; Lou, H. X. *Phytochemistry* **2010**, *71*, 1573−1578.
(15) Lin, Z. M.; Guo, Y. X.; Wang, S. Q.; Wang, X. N.; Chang, W. Q.; Zhou, J. C.; Yuan, H.; Lou, H. *J. Nat. Prod.* **2014**, *77*, 1336−1344.
(16) Asakawa, Y. *Phytochemistry* **2004**, *65*, 623−669.
(17) Zhou, J. C.; Zhang, J. Z.; Cheng, A. X.; Xiong, Y. X.; Liu, L.; Lou, H. X. *Org. Lett.* **2015**, *17*, 3560−3563.
(18) Karin, M.; Lin, A. *Nat. Immunol.* **2002**, *3*, 221−227.
(19) Siegall, C. B.; Schwab, G.; Nordan, R. P.; FitzGerald, D. J.; Pastan, I. *Cancer Res.* **1990**, *50*, 7786−7788.
(20) Domingo-Domenech, J.; Oliva, C.; Rovira, A.; Codony-Servat, J.; Bosch, M.; Filella, X.; Montagut, C.; Tapia, M.; Campas, C.; Dang, L.; Rolfe, M.; Ross, J. S.; Gascon, P.; Albanell, J.; Mellado, B. *Clin. Cancer Res.* **2006**, *12*, 5578−5586.

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