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Highly Rigid Labdane-Type Diterpenoids from a Chinese Liverwort and Light-Driven Structure Diversification

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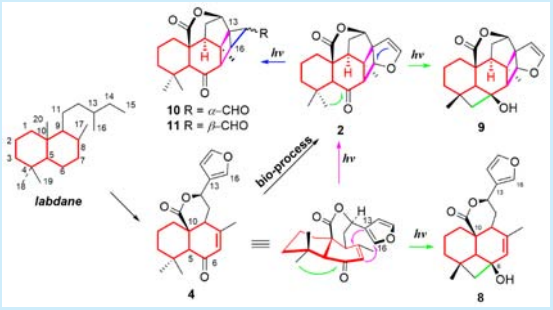
Supporting Information

ABSTRACT: Two unprecedented labdane-type diterpenoids haplomintrins A (**1**) and B (**2**) with six rings system were isolated from a Chinese liverwort *Haplomitrium mnioides*. Light-driven reaction of homologous haplomitrenonolides C (**6**), A (**4**), and D (**3**) afforded haplomintrins A−C (**1**, **2**, and **7**), respectively, while **4** was converted to more complex congeners haplomintrins D−G (**8**−**11**) through intramolecular cyclization. Formation of **1** and **2** from compounds **6** and **4**, respectively, helps us to postulate that a photochemical reaction is involved in the biosynthetic pathway. These structure features can be used as molecular markers of *H. mnioides*, and their allelopathic effects are also preliminarily tested.



As the most primitive group of terrestrial plants, liverworts are well-known natural resources for structurally diverse terpenoids and aromatic compounds that exhibit interesting biological properties, such as cytotoxic, antifungal, allelopathic, insect antifeedant, and antioxidantive effects.[1] The liverwort *Haplomitrium mnioides* (Lindb.) R. M. Schust is considered as a very primitive taxon,[2] with remote affinity to other liverwort groups.[3] Previous chemical investigation on this species produced several chain-type and labdane-type diterpenoids.[3b] Our continued study on the labdane-typediterpenoids from Chinese liverworts[4] led us to discover two novel compounds, haplomitrins A and B (**1** and **2**), possessing unprecedented scaffolds, one new biosynthetically related labdane haplomitrenolide D (**3**), as well as three known haplomitrenolides A−C (**4**−**6**)[3b] from *H. mnioides* (Figure 1). Light-driven reaction of compound **6** to **1** and **4** to **2**, respectively, enabled us to speculate that the photochemical reactions are involved in the late step of the biosynthetic pathway of these diterpenoids. The formation of more complex congeners haplomintrins C−G (**7**−**11**) during the photochemical reaction provides one useful way to diversify the natural products.

Haplomitrin A (**1**) was obtained as a colorless needle. Its molecular formula of $C_{21}H_{24}O_6$ was established by HRESIMS (*m*/*z* 373.1653 $[M + H]^+$, calcd $C_{21}H_{25}O_6$, 373.1646), requiring 10 indices of hydrogen deficiency. Analysis of the ^{1}H NMR data (Table 1) revealed the existence of two tertiary methyls at δ_H 1.72 (s) and 1.24 (s), an oxygenated methyl group at δ_H 3.69 (s), and one disubstituted double-bond group (δ_H 6.52, d, *J* = 2.8 and 4.77 d, *J* = 2.8). The ^{13}C NMR (Table 1) and HMQC data showed 21 carbons, including one ketone carbonyl (δ_C 204.8), two ester carbonyls (δ_C 172.3 and 179.4), four quaternary carbons, six methines (two oxygenated, two olefinic and two other), four methylenes, three methyls (one oxygenated). The aforementioned data suggested a modified labdane framework with a δ-lactone ring and a methyl ester carbonyl. Compared with the known structure of haplomitrenonolide C (**6**) isolated from the same plant,[3b] the disappearance of the two double bonds $\Delta^{7(8)}$ and $\Delta^{13(16)}$ in the ^{13}C NMR of **1** and the appearance of the coupling correlations between H-7 (δ_H 2.91) and H-16 (δ_H 4.72) in ^{1}H−^{1}H COSY demonstrated the formation of a new tetracycle.

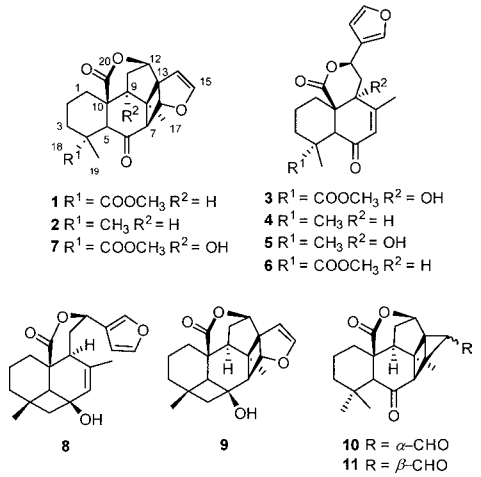


Figure 1. Structures of labdane-type diterpenoids **1**−**6** isolated from *H. mnioides* and congeners **7**−**11** formed by photochemistry reaction.

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Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Data (in ppm) for 1 and 2 in $CDCl_3$

	1		2	
no.	δ_C	δ_H	δ_C	δ_H
1α	38.6 t	1.39 m	39.5 t	1.38 td (13.6, 3.6)
1β		2.39 br d (13.6)		2.40 br d (13.6)
2α	17.6 t	1.62 m	18.4 t	1.49 m
2β		2.10 ddd (14.0, 3.2, 3.2)		2.00 m
3α	37.9 t	1.45 m	42.9 t	1.13 m
3β		1.82 m		1.50 m
4	43.5 s		32.7 s	
5	59.4 d	3.03 s	63.7 d	1.94 s
6	204.8 s		206.6 s	
7	56.4 d	2.91 d (3.2)	57.5 d	2.83 d (3.2)
8	50.1 s		50.7 s	
9	55.3 d	2.64 dd (4.8, 2.0)	56.0 d	2.04 dd (4.4, 1.2)
10	47.1 s		48.7 s	
11α	33.7 t	2.33 d (13.6)	33.9 t	2.30 d (13.2)
11β		1.86 m		1.79 ddd (13.2, 4.8, 2.8)
12	83.1 d	4.55 br s	83.2 d	4.54 br s
13	66.8 s		66.6 s	
14	100.2 d	4.77 d (2.8)	100.1 d	4.71 m
15	150.7 d	6.52 d (2.8)	150.9 d	6.52 d (2.8)
16	80.3 d	4.72 d (3.2)	81.7 d	4.72 m
17	23.0 q	1.24 s 3H	23.5 q	1.23 s 3H
18	179.4 s		34.2 q	1.08 s 3H
19	16.5 q	1.72 s 3H	21.1 q	1.47 s 3H
20	172.3 s		172.5 s	
COOMe	52.6 q	3.69 s 3H		

HMBC correlations from H-9 (δ_H 2.64) to C-13 (δ_C 66.8) and from H-12 (δ_H 4.55) to C-8 (δ_C 50.1) also confirmed the linkages (Figure 2A). Thus, compound **1** was elucidated as a highly rigid structure with a novel tetracyclo[7.4.1.0^{2,7}.0^{11,14}]-tetradecane skeleton.

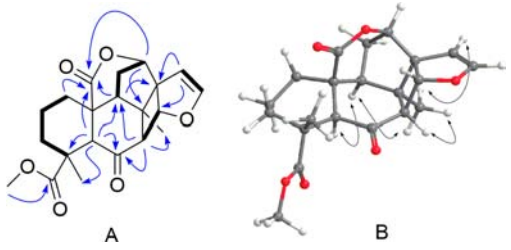

Figure 2. (A) Selected HMBC (H → C) and $^{1}H-^{1}H$ COSY (H–H) correlations of **1**. (B) Selected NOESY correlations (H↔H) of **1**.

NOESY correlations (Figure 2B) of H-5/H-9, H-9/H_3-17, H_3-17/H-14, and H-7/H_3-17 indicated the relative configuration of **1**. To determine the absolute configuration of **1**, a single-crystal X-ray diffraction analysis with Cu Kα radiation was performed (CCDC 1059512, Figure 3). Accordingly, the stereochemistry of **1** was determined as 4*R*,5*S*,7*S*,8*R*,9*S*,10*S*,-12*R*,13*S*,16*R*.

The same strategy was adopted for the elucidation of the structure of **2**, based on its molecular formula of $C_{20}H_{24}O_4$, as determined by HRESIMS (*m*/*z* 329.1752 [M + H]$^+$, calcd 329.1747) and comparison of its NMR data with those of **1** (Table 1), which showed the absence of resonances for the methyl ester carbonyl and the appearance of an additional tertiary methyl at C-18 (δ_H 1.08, δ_C 34.2) in **2**. This deduction was confirmed by the HMBC correlations from H_3-18/H_3-19 to C-3, C-4, and C-5. Therefore, the molecular structure of **2** was established as shown.

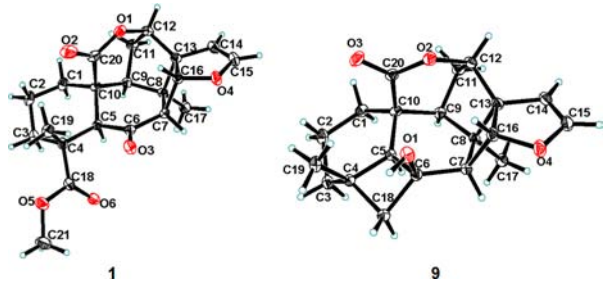

Figure 3. X-ray crystallographic structures of **1** and **9**.

Compound **3** was also a new compound which had a molecular formula of $C_{21}H_{24}O_7$, with 10 degrees of unsaturation, as established by HRESIMS data. Analysis of its 1D and 2D NMR data (Supporting Information) determined **3** to be the 9-hydroxyl derivative of haplomitrenonolide C (**6**). Compounds **4** and **5** were known and had never isolated from the same plant.[3b]

Topologically, the novel labdane-type diterpenoids show very special features. They have a six-ring system with an untouched six-membered latctone ring as well as a fused furan ring. Obviously, these unique structures are formed through intramolecular cyclization. Here, we outlined a plausible biosynthetic pathway of these novel compounds (Scheme 1A). Intramolecular [2 + 2] cycloaddition[5] of the precursor **6** enzymatically or nonenzymatically delivered the highly rigid structure of **1**, while compound **4** converted to **2** by a same mechanism. Also, an alternative way to form **1** from **2** was through C-18 methyl oxidation into a carboxylic acid followed by a methyl esterification.

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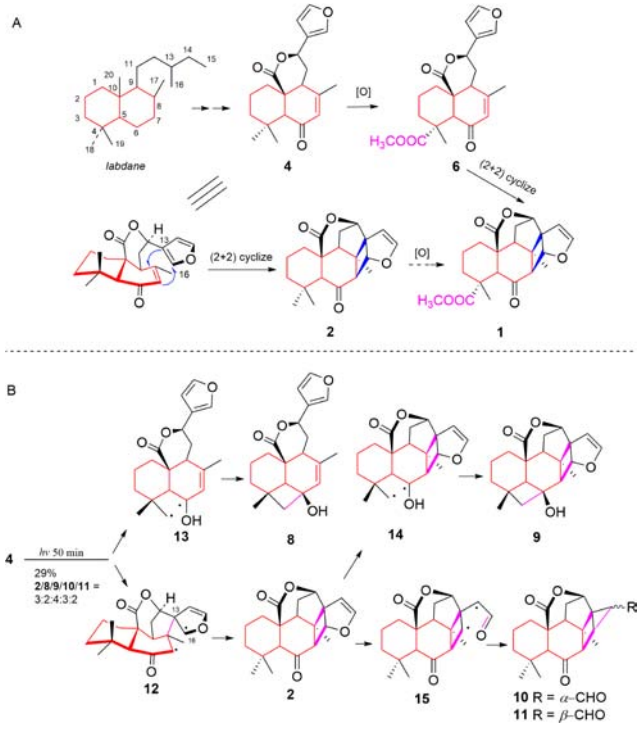
Scheme 1. (A) Proposed Biosynthetic Pathways of Compounds 1 and 2. (B) Proposed Mechanism of Photodriven Formation of Compounds 8−11



According to the above analysis, it is reasonable to speculate that a photochemical-catalyzed intramolecular cyclization was involved in the formation of **1** and **2** biosynthetically[6] as they are not artifacts as detected in the crude extract of the fresh material (Figure S33). As expected, irradiation of **6** with UV light (low pressure Hg lamps, 400 W, 254 nm) under nitrogen atmosphere at room temperature afforded compound **1**(31% yield), as detected by the time-lapse HPLC monitoring analysis (Figure S35). Applying the same protocol to **4** and **3** generated **2** (34% yield) and **7** (33% yield), respectively (Figure S36 and S37). The ^{1}H and ^{13}C NMR data (Table S2) of **7** suggested the disappearance of two double bonds. 2D NMR data (Supporting Information) confirmed that an intramolecular [2 + 2] photocycloaddition has arisen between the $\Delta^{7(8)}$ and $\Delta^{13(16)}$ double bonds with good diastereoselectivity. Compound **7** was determined to be the 9-OH derivative of **1**, which was not found in the crude plant extract by HPLC analysis (Figure S33). In addition, the increase in the contents of **1** and **2** when the plant species was exposed to prolonged daylight also supports the above speculation (Figure S34).

Surprisingly, these complex labdane-type diterpenoids were not the end products of the photochemical reaction cascade. In the light-driven reaction, several other photoadducts were detected by the HPLC traces. In order to establish the structures, the amplified reaction of **4** was subjected to the irradiation, and after all of the starting material was consumed (Figure S51), a mixture of compound **2** and compounds **8**−**11** (29% yield, **2**/**8**/**9**/**10**/**11** = 3:2:4:3:2) was obtained.

To determine the structures of these photochemical products, NMR spectroscopy and X-ray diffraction analysis were employed. Compound **8** was assigned the molecular formula of $C_{20}H_{24}O_4$ by HRESIMS data (Figure S60). The 2D NMR data (Table S3) of **8** were close to those of **4**, except for the absence of C-18 methyl and the C-6 ketone carbonyl in **4** and the presence of a methylene group (δ_H 2.20, m, 2H; δ_C 55.90) in **8**. Its molecular structure with a tricyclo[3.5.1.$0^{3,11}$]-undecane skeleton was confirmed on the basis of the HMBC data (Figure S52). The methyl at C-4 was determined to be β oriented, supported by the NOESY correlations (Figure S52) of H_3-19/H-1β and H-18/H-5. The hydroxyl group at C-6 revealed a β configuration from the stereoconsideration in which *cis* arrangement of the tetratomic ring was required.

Compound **9** was obtained as a colorless crystal with a molecular formula $C_{20}H_{24}O_4$, as established by HRESIMS (Figure S72). Compound **9** was confirmed to be the cagelike labdane possessing an unprecedented pentacyclic [7.4.1.$1^{2,4}$.$0^{8,15}$.$0^{12,14}$]pentadecane scaffolding bearing two heterocylic rings by comparing the 1D and 2D NMR data (Table S4 and Figure S64) with those of **2**. The relative configuration of **9** was verified by the NOESY correlations (Figure S65). Single-crystal X-ray diffraction study permitted the unambiguous assignment of its absolute configuration (CCDC 1059598, Figure 3).

Compounds **10** and **11** were determined to be a pair of diastereoisomers. Their identical molecular formula ($C_{20}H_{24}O_4$) was established by HRERMS (Figures S84 and S94). Aside from the absence of resonances for the $\Delta^{14(15)}$ double bond and the presence of aldehyde groups at C-15 (δ_H 9.90, δ_C 198.2 in **10** and δ_H 9.04, δ_C 196.5 in **11**), other assignments of NMR data (Table S5) resembled with those of **2**. Moreover, the newly formed cyclopropane ring was confirmed by the connectivity of −C-7(H)−C-16(H)−C-14(H)− established by the $^{1}H-^{1}H$ COSY spectrum and the HMBC correlation from H-14 to C-15 (Figure S76), elucidating the striking C-14/C-16 linkage. Therefore, the unique pentacyclic [7.4.1.$0^{2,7}$.$0^{10,12}$.$0^{12,15}$]pentadecane frameworks of **10** and **11** were determined. According to their NOESY data (Figure S77), the aldehyde groups of **10** and **11** were determined to be α and β oriented, respectively.

To investigate the formation of these unique carbon skeletons formed by a photochemical reaction, we proposed that the complexity was achieved through photoexcited diradical intermediates (Scheme 1B). Compound **8** arose from **4** and **9** from **2**, in which formation of the cyclobutane ring stereoselectively was through the Norrish−Yang cyclization[5a,7] with 1,4-diradical **13** and **14** as intermediates, respectively. In addition, the cleavage of the furan ring of **2** at C16−O forming **10** and **11** was postulated through a diradical intermediate **15**. This is the first report related to the light-induced furan ring opening to form a cyclopropyl ring attached with an aldehyde group.

Ecologically, besides the phenological responses, the chemicals metabolized by liverworts also help them to cope with their living conditions by combatting other organisms.[8] Here, we tested the allelopathic activity of the isolated compounds **2** and **4**, the representives of two skeletons, with the model of *Arabidopsis thaliana*. The results showed that compounds **2** and **4** could regulate the growth of *A. thaliana* by inhibiting the root elongation with the IC_{50} at 44.57 ± 0.78 and 19.08 ± 0.73 μg/mL respectively (Figure S107).

In conclusion, haplomitrins A and B (**1** and **2**) are rearranged labdane-type diterpenoids with unprecedented scaffolds isolated from Chinese liverwort. Their structures had been clarified, and the formation of **1** and **2** from **6** and **4** through light-driven [2 + 2] cycloaddition was demonstrated. Formation of haplomintrin C (**7**) from **3** and haplomintrins

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D−G (**8**−**11**) with more appealing labdane skeletons from **4** after an extended amount of time through light-driven conversion provides one intriguing way to diversify natural products. Finally, phytotoxic activities of typical compounds have been preliminarily studied. Taken together, our work shows the UV/vis light involving in the biosynthesis step in vivo or photodriven transformation in vitro will access to more complex structures.

■ ASSOCIATED CONTENT

Supporting Information

General experimental procedures, structure elucidation of **3** and **7**−**11**, 1D and 2D NMR data, HRESIMS, IR, UV, and CD spectra of the new compounds, 1D NMR data and ESIMS of the known compounds, crystal information for **1** and **9**, HPLC and UV analysis for the photoinduced reaction, and ecological biochemistry tests. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b01664.

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Author Contributions

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Notes

The authors declare no competing financial interest.

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