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Daphnioldhamine A, a novel alkaloid from *Daphniphyllum oldhami*

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ABSTRACT

Daphnioldhamine A, the first *Daphniphyllum* alkaloid with transannular effect, easily tautomerized under acidic or alkaline conditions, was isolated from the fruits of *Daphniphyllum oldhami*. The structure was elucidated by spectroscopic and computational approaches and chemical transformation. A plausible biosynthetic pathway of daphnioldhamine A was also proposed.

Daphniphyllum alkaloids are a family of diversified and complex polycyclic natural products elaborated by the trees of the genus *Daphniphyllum*.[1] Previous investigation led to oldhamine A, a structure-variable *Daphniphyllum* alkaloid.[2] In our continuing search for new alkaloids of this genus, daphnioldhamine A, an alkaloid with transannular effect, tautomerized on acidic or basic conditions, was isolated from the extract of the fruits of *D. oldhami* collected in Songtao County of Guizhou Province, People's Republic of China. Herein, we reported its isolation, structural elucidation, chemical transformation, and biological activity.

The powdered fruits (12 kg) of *D. oldhami* were percolated three times with 95% EtOH to give a crude extract (1000 g). The extract was concentrated to dryness under reduced pressure, followed by partitioning between EtOAc and 2% HCl. The aqueous phase was then adjusted to pH 10 with 3% NaOH and extracted with $CHCl_3$ to give crude alkaloids (80 g). The crude alkaloids were subjected to a silica gel column eluted with $CHCl_3$/MeOH (1:0–0:1), in which a fraction eluted with $CHCl_3$/MeOH (10:1) was further chromatographed over a silica gel column with $CHCl_3$/MeOH (20:1) and then RP-18 (30% MeOH) to afford daphnioldhamine A (12 mg) (Fig. 1).

Daphnioldhamine A (**1**)[3] was isolated as an optically active ($[\alpha]_D^{19}$ = −44.2, *c* 0.27 $CHCl_3$) white powder. The molecular formula was established as $C_{23}H_{33}NO_5$ by HR-ESI-MS (*m/z* 404.2432, $[M+H]^+$, calcd 404.2436), indicating 8 degrees of unsaturation.

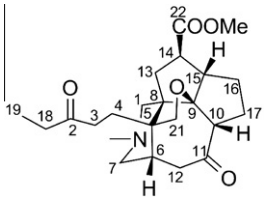

Figure 1. Molecular structure of **1**.

The ^{13}C NMR (DEPT) spectra (Table 1) resolved 22 carbon signals corresponding to three methyls, ten methylenes, four methines, three sp^3 quaternary carbons, and two carbonyls (one isolated ketone at δ_C 210.5 and one ester at δ_C 174.9). Obviously, the speculated molecular formula ($C_{22}H_{33}NO_4$) from NMR spectra differed from that inferred by HR-ESI-MS.

Previous experiment suggested that the presence of TFA could recover the unexhibited NMR signal.[4] Thus, 0.05 mL TFA was added to CD_3OD solution of daphnioldhamine A to yield **2**. In its ^{13}C NMR spectra (Table 1), 23 carbon signals were clearly observed, including two sp^2 carbon atoms (one isolated ketone at δ_C 212.8 and one ester at δ_C 176.0) and 21 sp^3 carbon atoms (4 × C, 4 × CH, 10 × CH_2, and 3 × CH_3), involved one more quaternary carbon signal (δ_C 109.2) than that mentioned above in daphnioldhamine A (**1**).

The ^1H–^1H COSY spectrum of **2** indicated connectivities of three protonated fragments: ***a*** (C-19–C-18), ***b*** (C-3–C-4), ***c*** (C-7–C-6–C-12), and ***d*** (C-13–C-14–C-15–C-16–C-17–C-10) drawn with bold lines in Figure 2. Comprehensive analyses of the HMBC spectrum

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Table 1
^{1}H and ^{13}C NMR data of **1** and **2**

No.	1					2	
	δ_H (pattern, J (Hz))[a]	δ_C[a]	δ_C[b]	δ_C[c]	δ_C[d]	δ_H (pattern, J (Hz))[e]	δ_C[e]
1α	2.86 (d, 12.0)	55.4	57.4	54.4	55.4	3.80 (d, 11.0)	59.7
1β	2.48 (d, 12.0)					2.50 (d, 11.0)	
2		210.5	213.2	210.3	210.6		212.8
3α	2.39 (m)	37.5	38.2	37.2	38.3	2.53 (m)	38.0
3β	2.20 (m)					2.37 (m)	
4α	1.55 (m)	23.6	24.8	23.4	24.5	1.68 (m)	23.8
4β	2.03 (m)					2.03 (m)	
5		46.2	47.6	45.7	46.9		47.4
6	1.95 (br s)	38.1	40.0	37.7	38.5	2.51 (m)	39.5
7α	2.59 (m)	55.4	56.3	54.8	55.4	3.70 (m)	67.6
7β	2.38 (m)					3.40 (m)	
8		56.7	57.7	56.5	57.7		55.9
9		102.5	103.5	102.2	103.2		101.8
10	2.66 (m)	59.5	60.0	59.5	60.8	2.56 (m)	55.9
11		Disappeared	Disappeared	203.0	203.0		109.2
12α	2.38 (m)	45.8	46.6	45.7	47.1	2.32 (m)	44.1
12β	2.80 (m)					2.90 (m)	
13α	2.17 (t, 16.0)	37.2	38.0	37.0	38.1	2.27 (dd, 8.0, 15.5)	36.2
13β	1.63 (dd, 8.0, 16.0)					1.80 (dd, 8.0, 15.5)	
14	2.63 (m)	51.0	51.9	50.7	51.7	2.75 (m)	50.7
15	2.80 (m)	53.4	55.0	53.1	54.2	2.75 (m)	55.8
16α	1.55 (m)	28.7	29.5	28.5	29.6	1.77 (m)	26.7
16β	2.09 (m)					2.15 (m)	
17α	1.47 (m)	26.0	27.1	25.8	27.3	1.76 (m)	28.6
17β	2.21 (m)					1.99 (m)	
18	2.45 (m)	36.0	36.5	35.5	36.3	2.50 (m)	36.4
19	1.06 (t, 8.0)	7.8	8.1	7.4	8.4	1.02 (t, 9.0)	7.4
21α	3.86 (d, 11.0)	73.7	74.8	76.7	74.2	3.96 (d, 11.0)	75.1
21β	3.80 (d, 11.0)					3.80 (d, 11.0)	
22		174.9	176.9	174.7	174.7		176.0
NCH_3	2.15 (s)	43.8	43.8	43.3	44.2	2.93 (s)	45.7
$COOCH_3$	3.70 (s)	51.9	52.4	51.5	52.2	3.70 (s)	52.3

[a] $CDCl_3$.
[b] CD_3OD.
[c] $CDCl_3 + (C_2H_5)_2NH$.
[d] CD_5N.
[e] $CDCl_3$ + TFA.

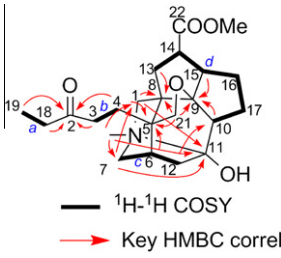


Figure 2. ^{1}H–^{1}H COSY and selected HMBC of **2**.

connected the above-mentioned fragments (***a***–***d***), as well as heteroatoms and quaternary carbon atoms. The linkage of fragments ***a*** and ***b*** through a ketone moiety was assigned by the HMBC correlations of H_3-19, H_2-18, H-3α, H-3β, and H-4β–C-2 (δ_C 212.8). HMBC correlations of H-4α, H-4β, H-21α, H-21β and H-6–C-5 (δ_C 47.4) indicated that C-4, C-6, and C-21 were attached to C-5, thus inferring the connection of fragments ***b*** and ***c*** through C-5. The connectivity of C-1 and C-7 via a nitrogen atom was established by the HMBC correlations of CH_3-*N*–C-1 (δ_C 59.7) and C-7 (δ_C 67.6), and H-7α–C-1. HMBC correlations of H-21α, H-13α, and H-1α–C-8, and H-10–C-9 and C-8 not only confirmed the attachment of C-1 and C-5–C-8, but also the linkage of fragment ***d*** to C-8 and C-9, respectively. The ether linkage between C-21 (δ_C 75.1) and C-9 (δ_C 101.8) was illustrated by the key HMBC correlation from H-21β–C-9. The connections of C-12 and C-10–C-11 were proved by the HMBC correlations of H-12β and H-17α–C-11. Furthermore, the HMBC correlation between H-1β, CH_3-*N* and H-7α–C-11 set up the linkage of C-11 to nitrogen atom, which assigned the quaternary carbon (δ_C 109.2) as C-11. Thus, the gross planar structure of **2** was assigned as shown in Figure 2.

Careful inspection of NMR data for **1** (δ_C[a]) and **2** revealed great discrepancy in chemical shifts of C-1, C-7, C-10, and C-12, all in a neighborhood with C-11, which implied that **1** was converted into **2** on the treatment of TFA. Additionally, compound **2** can be transformed into **1** when **2** was concentrated to dryness and then solved in $CDCl_3$ followed by the addition of 0.05 mL diethylamine. In the ^{13}C NMR spectrum of **1** (δ_C[c]) (Table 1), 23 carbon signals were well exhibited, which displayed one more ketone carbon signal (δ_C 203.0) and one less sp^3 quaternary carbon signal than that of **2**. Analysis of 1D and 2D NMR spectrum further confirmed the structure of **1** as shown in Figure 3A.

The interconversion between quaternary salt of **2** and C-11 ketone of **1** (Scheme 1) and the IR absorption in 1680 cm^{-1} demonstrated that compound **1** has transannular effect,[5] which was caused by the influence of the lone pair of nitrogen on the C-11 ketone group.[5] This phenomenon could happen when the lone pair of nitrogen in alkaloid is spatially close to ketone group, which is the characteristic of special protonation involved in the reaction between proton and ketone group, and the formation of C–N bond handled with acidification of this class of alkaloids.[5] The ^{13}C NMR signal of C-11 disappeared in $CDCl_3$ and CD_3OD, probably owing to the inadequate alkalization of **1** by the trace acid in $CDCl_3$ or $CHCl_3$ used to isolate. When **1** was solved in CD_5N or $CDCl_3 + (C_2H_5)_2NH$, all the carbons including C-11 (both in δ_C

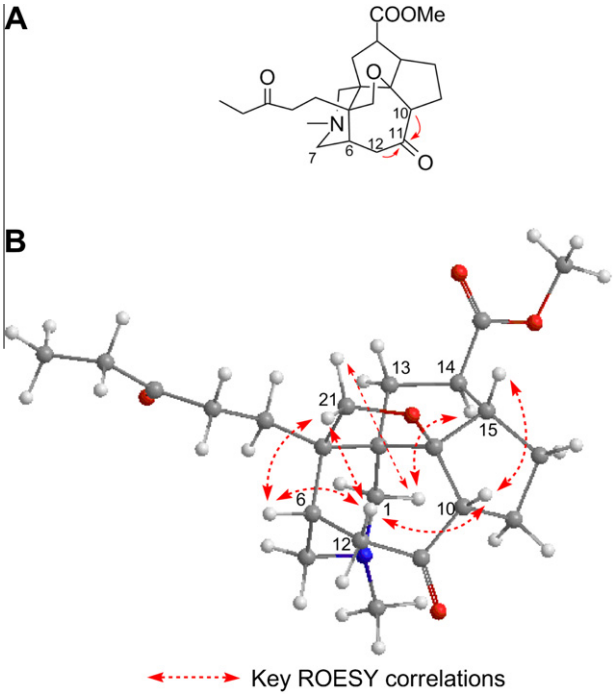
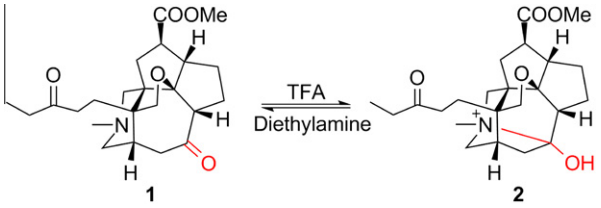


Figure 3. (A) Selected HMBC correlations and (B) stereoconfiguration of **1**.

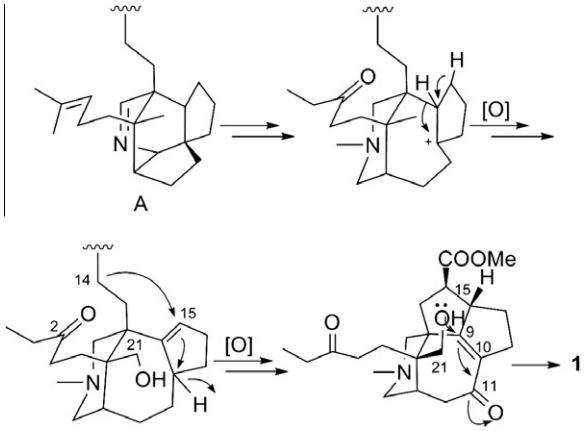


Scheme 1. Chemical correlations between **1** and **2**.

203.0) were well resolved, which verified the inference mentioned above. In addition, replacing the solvent with DMSO-d_6 and CD_5N, or prolonging the relaxation time usually helps recover the unexhibited NMR signal involved in transannular effect.[6] The chemical shifts of $\delta_C{}^a$ and $\delta_C{}^c$ showed resemblance except in C-1 and C-21, probably originating from the partial protonation of $\delta_C{}^a$. The discrepancy between $\delta_C{}^a$ and $\delta_C{}^d$ in resonances of C-8, C-12, and C-17 was due to the different degree of alkalization and solvent effect. The solvent effect also led to the discrepancy in some chemical shifts of $\delta_C{}^c$ and $\delta_C{}^d$ (Table 1).

The relative configuration of **1** was deduced by the ROESY spectrum as shown in the computer-generated three-dimensional drawing (Fig. 3B). The ROESY correlations of H-21β/H-6, H-21β/H-12β, H-12β/H-10, H-10/H-15, and H-15/H-13β indicated H-6, H-10, and H-15 were β-oriented. H-14 was assigned in α-configuration on the basis of the correlations of H-13α/H-1α and H-1α/H-14.

To determine its absolute configuration, chemical computation was employed. The optical rotation (OR) value of **1** was calculated using density functional theory (DFT) methods[1c,d,7] in the Gaussian 03 program package.[8] The 'self-consistent reaction field' method (SCRF) was employed to perform the OR value (−42.03°) in $CHCl_3$, which is close to its experimental value (−44.2°), and thus the absolute configuration of **1** was assigned as (5*R*, 6*S*, 8*R*, 9*R*, 10*R*, 14*R*, and 15*S*), as shown in Figure 3B.



Scheme 2. Biogenetic pathway proposed of daphnioldhamine A.

To the best of our knowledge, daphnioldhamine A (**1**) is the first example of *Daphniphyllum* alkaloids with such transannular effect, and it is also the second compound of (14*R*, 15*S*) series among *Daphniphyllum* alkaloids.[7]

From a biogenetic point of view, daphnioldhamine A might be derived from a common intermediate A, which involved oxidative reaction to form the ether linkage between C-21 and C-9, and the C=O group at C-11,[7] as proposed in Scheme 2.

The cytotoxicity assays showed that daphnioldhamine A was not active against the acute myelogenous leukemia (HL-60) or human lung cancer (A-549) cell line (ED_{50} > 10 μg/ml).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.03.021.

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3. Daphnioldhamine A (**1**): White powder; $[\alpha]_D^{19}$=−44.2 (*c* 0.27, $CHCl_3$); UV ($CHCl_3$) λ_{max} (logε) 240 nm (3.70), 298 nm (3.45), 415 nm (2.78); IR (KBr) υ_{max} 3432, 2937, 2873, 1733, 1713 and 1680 cm^{-1}; ^1H and ^{13}C NMR data (Table 1); ESIMS *m*/*z* 404 $[M+H]^+$; HRESIMS *m*/*z* 404.2432 $[M+H]^+$ (calcd for $C_{23}H_{33}NO_5$, 404.2436).
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