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Logeracemin A, an Anti-HIV *Daphniphyllum* Alkaloid Dimer with a New Carbon Skeleton from *Daphniphyllum longeracemosum*

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Supporting Information

ABSTRACT: Logeracemin A (**1**), the first *Daphniphyllum* alkaloid dimer featuring an unprecedented carbon skeleton with a unique conjugated trispiro[4,5] decane backbone, was isolated from *Daphniphyllum longeracemosum*. Its structure and absolute configuration were established on the basis of spectroscopic data and X-ray crystallography. Logeracemin A showed significant anti-HIV activity with an EC_{50} of 4.5 ± 0.1 μM and a selectivity index of 6.2. The structure−activity relationship of the tested compounds was briefly discussed.

From the plants of Daphniphyllaceae family, *Daphniphyllum* alkaloids are a class of complex alkaloids with diverse polycylic skeletons. Their fascinating structures and important biological activities have attracted broad interests from both natural products and synthetic chemists over the last half century.[1,2] Recently, a number of excellent work on the total synthesis of *Daphniphyllum* alkaloids[2] have made this compound class a hot topic in the related scientific communities. In our continuing studies on *Daphniphyllum* alkaloids,[3] logeracemin A (**1**) (Figure 1), along with its possible biosynthetic precursors daphniyunnines B (**2**) and D (**3**) (Scheme 1),[3f] was isolated from a sample of *D. longeracemosum*, which is distributed mainly in the south and southwest of China.[4] Although about 280 *Daphniphyllum* alkaloids with diverse structures have been isolated,[1] logeracemin A represents the first dimeric and the most complex *Daphniphyllum* alkaloid identified hitherto.

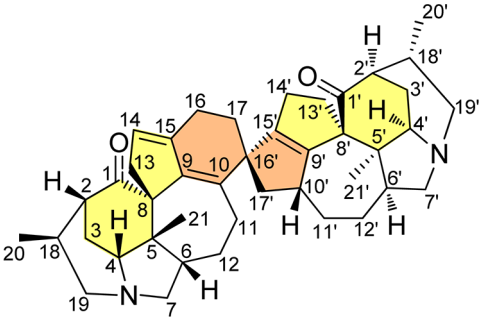
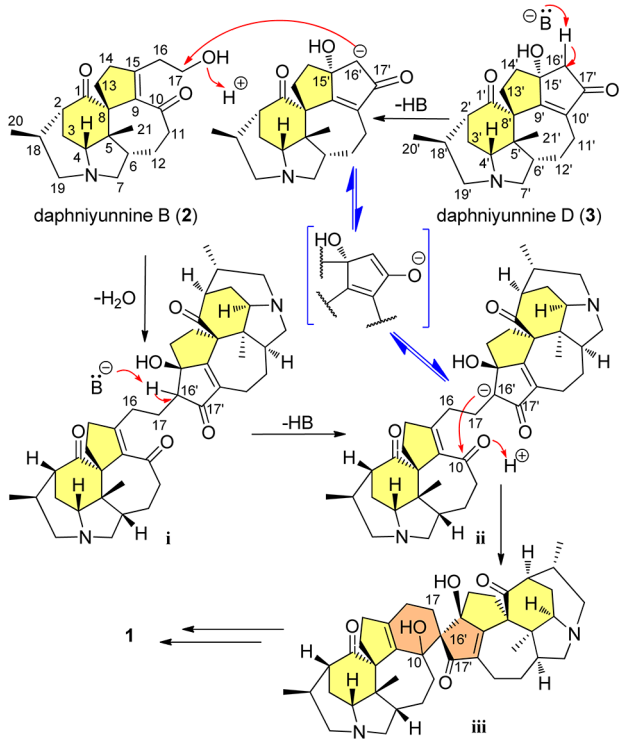


Figure 1. Structure of logeracemin A (**1**).

Scheme 1. Plausible Biosynthetic Pathway of Logercemin A (1)



Acquired immunodeficiency syndrome (AIDS) developed by the infection of human immunodeficiency virus (HIV) has become a serious risk to human lives worldwide, and the development of effective new drugs keeps a high priority. Natural products have been an important source of new anti-HIV agents with novel structures and/or new modes of action.[5] Logeracemin A (**1**) and its two possibly biosynthetic precursors, daphniyunnines B (**2**) and D (**3**), were evaluated

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for anti-HIV activity. Compound **1** showed potent anti-HIV activity (EC_{50} = 4.5 ± 0.1 μM; SI = 6.2), while **2** and **3** were inactive.

Herein, we describe the isolation, structural elucidation, biosynthetic consideration, anti-HIV evaluation, and brief structure−activity relationship (SAR) discussion of logeracemin A (**1**) as well as its two monomeric precursors **2** and **3**.

Compound **1** was obtained as a colorless crystal (in MeOH) with a $[\alpha]^{25}_{D}$ −148 (*c* 0.1, MeOH). Its molecular formula $C_{42}H_{54}N_2O_2$ with 17 double-bond equivalents (DBE) was determined by HRESI(+)MS at *m*/*z* 619.4269 $[M + H]^+$ (calcd 619.4264). The IR spectrum displayed absorption bands for carbonyl (at 1697 cm^{-1}) and vinyl (at 1656 cm^{-1}) functionalities. Analysis of the ^1H and ^{13}C NMR data (Table S1) with the aid of DEPT experiment revealed the presence of 4 methyls, 2 persubstituted double bonds, 1 trisubstituted double bond, 2 carbonyls (δ_C 214.8 and 221.2), 16 methylenes (four nitrogenated at δ_C 49.0, 49.6, 53.4, and 54.6), 9 methines (two nitrogenated at δ_C 64.1 and 65.4), and 5 quaternary carbons. The aforementioned spectroscopic data, and the fact of coexistence of daphniyunnines B (**2**) and D (**3**), suggested that **1** was probably a dimer of two C-21 *Daphniphyllum* alkaloids. The dimeric feature of **1** was further corroborated by the NMR observations, in which the characteristic proton and/or carbon signals of the calyciphylline A type alkaloids[1b,3c,e,f] appeared in pairs. Taking the ^{13}C NMR of **1** into accounts, the carbon signal pairs at δ_C 18.2/18.1 (C-20/20′), 22.4/21.1 (C-21/C-21′), 214.8/221.2 (C-1/C-1′), 50.8/50.0 (C-6/C-6′), 65.4/64.1 (C-4/C-4′), 49.6/49.0 (C-19/C-19′), and 54.6/53.4 (C-7/C-7′) assignable to the characteristic carbons of the A−C (or A′−C′) rings of a calyciphylline A type alkaloid were distinguished, suggesting that the A−C (or A′−C′) rings in the parts I and II of **1** were identical.

The planar structure of **1** was constructed by 2D NMR analysis (Figures 2; S6−S9), in which the proton-bearing

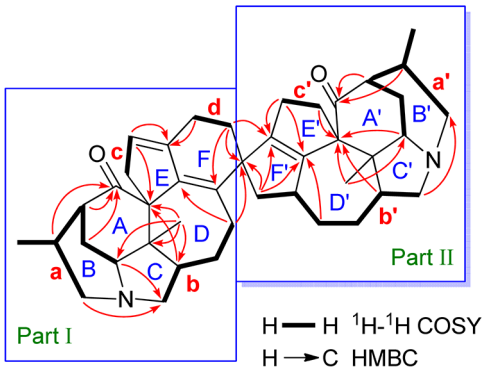

Figure 2. $^1H-^1H$ COSY and key HMBC correlations of **1**.

structural units **a**−**d** in part I and **a**′−**c**′ in part II as drawn in bold bonds were readily furnished by analysis of the $^1H-^1H$ COSY spectrum. The HMBC correlation networks of H-18, H-2 and H-3b/C-1; H_3-21/C-4, C-5, C-6, and C-8; H-4 and H_2-19/C-7; H-6/C-8; H_2-11/C-9; H-14/C-15 and C-8; H_2-16/C-15; and H-17b/C-10 as depicted with arrows from H to C, further secured the connection of A to F rings in part I and located the conjugated Δ^9 and Δ^{14} double bonds. Similarly, the structural part II of **1** was corroborated by the multiple HMBC correlations as shown, in which an $\Delta^{9'(15')}$ double bond was assigned by the key correlations from H-14′ and H-17′ to C-9′ and C-15′. The two monomeric parts I and II were finally linked via the quaternary C-16′ carbon by the key HMBC correlations of H_2-17/C-10, C-15′, and C-16′; H_2-17′/C-15′, and C-16′; and H_2-11/C-16′, which furnished an additional spiro[4,5] decane system and satisfied the DBE requirement of **1**.

The absolute configuration of **1** was finally determined as depicted via single crystal X-ray diffraction (Figure 3A) by using

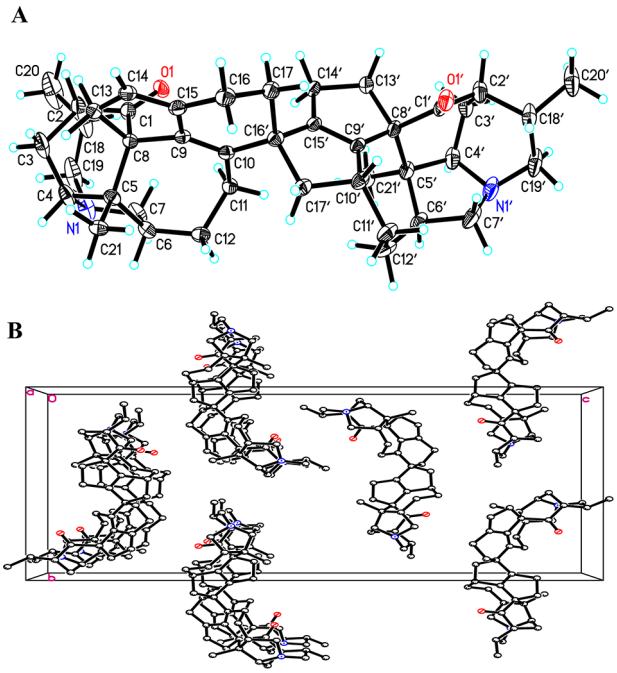

Figure 3. (A) X-ray structure of **1**. (B) Assembly of molecules of **1** in the crystals.

the anomalous dispersion of Cu Kα radiation. The absolute configuration of **1** was unambiguously determined by refinement of Hooft parameter [0.01(17)],[6] which has greatly boosted the value of X-ray crystallography in determining the absolute configuration of natural products recently.[7] This assignment is consistent with the result of a combined electronic circular dichroism (ECD) strategy of experimental and calculation, in which the experimental CD spectrum of **1** matched well with the calculated ECD curves (Figure S2), further corroborating the absolute configuration of **1**.

The conformation of **1** in the solution of CD_3OD envisaged by the NMR data, particularly the ROESY experiment (Figures S1 and S10), was consistent with that in the solid state obtained by X-ray diffraction study, suggesting that the polar solvent mediation did not substantially affect the conformation of this rigid molecule. It is quite interesting that the twisted dumbbell-shaped molecules of **1** in the crystal (Figure 3B) seemingly assembled in a way to yield a high density, in which one and/or two overlapping molecules are regularly arranged and are thought to be kept in balance mainly by intermolecular van der Waals forces.

A possible biosynthetic pathway for **1** was proposed, and two coexisting alkaloids, daphniyunnines B (**2**) and D (**3**), were considered as the biosynthetic monomeric precursors of **1** (Scheme 1). An enolate anion of **3** initiated by a base involved enzymatic reaction would attack the C-17 of **2** to form intermediate **i**.[8] Intermediate **i** would further be transformed into another enolate anion **ii** in a similar process, which after an

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intramolecular Aldol condensation would produce the key spiro-linked intermediate **iii**. Compound **1** could be finally produced from **iii** by a cascade of redox and aromatization chemistry.

While the discovery of logeracemin A (**1**) is of great interest, it also puts forward a question whether **1** is a natural product or a handling artifact. To prove this, we analyzed the crude extract with LC-ESIMS (Figure S13) which clearly revealed an ion peak at m/z 619.2 ($[M + H]^+$, t_R 4.8 min) in accord with that of **1** and confirmed the natural occurrence of **1**.

Logeracemin A (**1**) has a very complex polycyclic skeleton without precedent among the known *Daphniphyllum* alkaloid families. We thus proposed to name this dimeric scaffold logeracemin.

Logeracemin A (**1**) and its two biosynthetic precursors daphniyunnines B (**2**) and D (**3**) were evaluated *in vitro* for their anti-HIV activities on HIV-1 NL 4-3 infected MT4 cells.[9] Compound **1** exhibited significant anti-HIV activity with an EC_{50} of 4.5 ± 0.1 μM and a mild cytotoxicity against MT4 cell line (CC_{50} = 28.1 ± 1.2 μM), revealing a good selectivity index (SI) of 6.2, while two monomers daphniyunnines B (**2**) and D (**3**) were inactive. This finding implied a very simple and clear SAR, i.e., the dimerization is crucial for the anti-HIV activities, which is likely associated with the length and/or the conformation of the twisted dumbbell-shaped molecule of **1**.

Being the first dimeric *Daphniphyllum* alkaloid, logeracemin A features a unique architecture and decent anti-HIV activity, which represents a milestone work during decades of studies on this structure family. While the report of **1** will appeal to both chemists and biologists, further investigations such as synthetic effort and in-depth biological testing are also required.

■ ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR data, selected ROESY correlations, X-ray crystallographic data, experimental ECD spectrum and ECD calculation, ESI(+)MS and HR-ESI(+)MS, and 1D and 2D NMR spectra of **1** as well as general experimental procedures, plant materials, extraction and isolation, anti-HIV and cytotoxicity assays of **1**−**3**. 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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