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Isolation and Complete Structural Assignment of *Lycopodium* Alkaloid Cernupalhine A: Theoretical Prediction and Total Synthesis Validation

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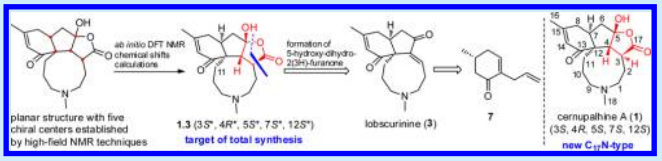
Supporting Information

ABSTRACT: Cernupalhine A (**1**) is a trace *Lycopodium* alkaloid (0.7 mg) possessing a new $C_{17}N$ skeleton with an unusual hydroxydihydrofuranone motif newly isolated from *Palhinhaea cernua* L. Its complete structural assignment, including absolute stereochemistry, was established through a combination of high-field NMR techniques and computational methods and further unequivocal confirmation by the first asymmetric total synthesis. Following the first total synthesis of lobscurinine (**3**), **1** was achieved via regio- and stereoselective cyanide ion addition and subsequent acid treatment.



The *Lycopodium* alkaloids are a family of structurally diverse natural products from the genus *Lycopodium* (Lycopodiaceae).[1] The isolation and synthesis of *Lycopodium* alkaloids have attracted tremendous attention recently due to their fascinating skeletons and a wide range of biological activities.[1,2] In this regard, our recent efforts in the isolation and synthesis of *Lycopodium* alkaloids led to the discovery of cernupalhine A (**1**), a new tetracyclic alkaloid isolated from *Palhinhaea cernua* L., which bears a $C_{17}N$ skeleton with an unusual hydroxydihydrofuranone motif (Figure 1).[3] Biosynthetically, cernupalhine A

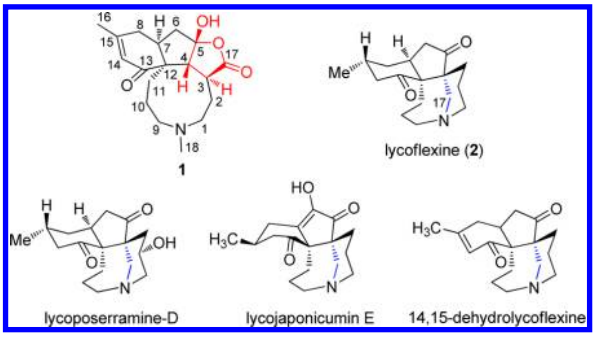


Figure 1. Structures of cernupalhine A (**1**) and representative reported $C_{17}N$-type *Lycopodium* alkaloids.

could probably be transformed from lycoflexine (**2**),[4] a known $C_{17}N$ skeleton of *Lycopodium* alkaloid (Scheme S1 in the Supporting Information). Lycoflexine (**2**) was reported by Ayer and co-workers in 1973, and it was believed to arise from fawcettimine via an intramolecular Mannich cyclization.[4] Recently, in our and other syntheses of lycoflexine (**2**), the same biomimetic cyclization strategies were successfully utilized.[3f,5] So far, only eight of the $C_{17}N$ skeleton compounds have been reported among more than 300 *Lycopodium* alkaloids.[1,4,6] The planar structure of **1** was readily elucidated by spectroscopic methods. However, its stereochemical assignment posed a significant challenge because of the minute amount of sample obtained from the natural source (0.7 mg isolated). Herein, we present the isolation and complete structural assignment of cernupalhine A (**1**) based on the combination of high-field NMR techniques, computational methods, and the first asymmetric total synthesis.

Cernupalhine A (**1**) was obtained as a white powder with an optical rotation of $[\alpha]^{18}_{D}$ +75.3 (*c* 0.07, MeOH). Its molecular formula, $C_{18}H_{25}NO_4$, was deduced from the positive HRESIMS at *m*/*z* 320.1855 $[M + H]^+$, indicating seven degrees of unsaturation. In the ^1H NMR spectrum (Table S1, Supporting Information), methyl (δ_H 1.93, s, $H_3$16 and δ_H 2.29, s, $H_3$18) and olefinic protons (δ_H 5.85, s, H14) were clearly apparent. The ^{13}C NMR and DEPT spectra (Table S1, Supporting Information) exhibited 18 carbon signals that were ascribable to an α,β-unsaturated carbonyl group (δ_C 200.5, 156.0, and 123.7), a carboxylic carbon (δ_C 179.6), a double-oxygenation carbon (δ_C 113.2), a methyl (δ_C 24.1), a *N*-methyl (δ_C 43.9), seven methylenes, three methines, and a quaternary carbon (δ_C 57.1). The characteristic chemical shift at δ_C 57.1 is typical for the quaternary carbon C12, which is present in most fawcettimine-type *Lycopodium* alkaloids.[1] These observations, together with the molecular formula, indicated that **1** should be a fawcettimine-type *Lycopodium* alkaloid derivative possessing a tetracyclic ring system.

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In the ^{1}H−^{1}H COSY spectrum, the cross-peaks of $H_2$1/$H_2$2/H3/H4, $H_2$9/$H_2$10/$H_2$11, and $H_2$6/$H_2$7/$H_2$8 suggested the presence of spin systems **a** (C1 to C4), **b** (C9 to C11), and **c** (C6 to C8). The HMBC correlations were observed from $H_3$18 to C1 (δ_C 47.7) and C9 (δ_C 54.7), and H1a (δ_H 2.67) to C9 indicated the connection of C1, C9, and C18 through a nitrogen atom. Key HMBC networks from H11 (δ_H 1.80, 2H) to C12 and C4 (δ_C 53.1) as well as H4 (δ_H 3.23) and H3 (δ_H 3.28) to C12 were observed. Thus, it could be deduced that units **a** and **b** were connected to C12, which then formed a 1-azacyclononane ring (ring A). The HMBC correlations from H14 (δ_H 5.85) to C12 and C8 (δ_C 29.9), together with H7 (δ_H 2.31) to C12 and C13 (δ_C 200.5), suggested the existence of a 2-cyclohexen-1-one ring (ring B). The methyl group of C16 (δ_C 24.1) was attached at C15 (δ_C 156.0) as evidenced by the HMBC correlations from $H_3$16 to C8, C14, and C15. In addition, a five-membered ring (ring C) was established by the HMBC cross-peaks from H6a (δ_H 2.21) to C4, C5 (δ_C 113.2), and C12, together with H4 to C5. The carboxylic carbon of C17 (δ_C 179.6) was linked to C3 (δ_C 39.2), which was supported by the HMBC correlations from H3 and H4 to C17. On the basis of the molecular formula, one more ring was needed to fulfill the degrees of unsaturation, and it was assigned to a hydroxydihydrofuranone motif (ring D) as evidenced by the chemical shift of C5 (δ_C 113.2). Thus, the planar structure of **1** containing a unique C_{17}N skeleton with a hydroxydihydrofuranone motif was established as shown in Figure 2.

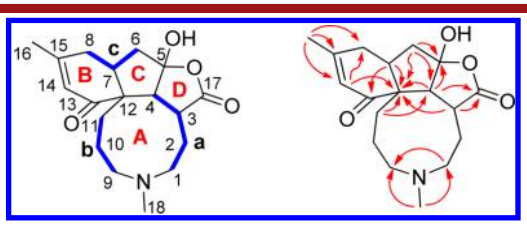


Figure 2. ^{1}H−^{1}H COSY (bold) and key HMBC (arrows) correlations of **1**.

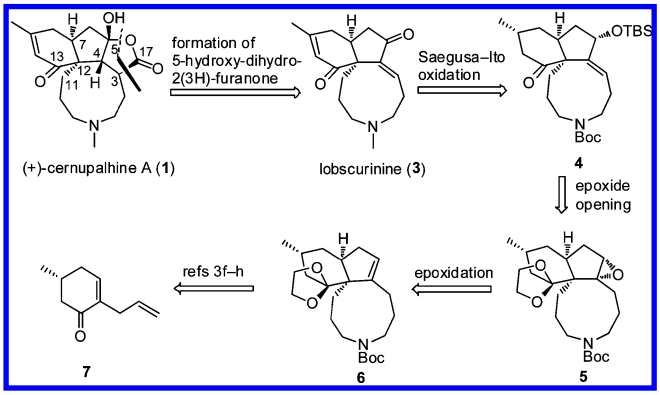
The relative stereochemistry of **1** was first investigated by a ROESY experiment (Figure S12, Supporting Information). In light of the biosynthesis point of view, the relative configurations of C7 and C12 were likely 7*S** and 12*S**. Therefore, the ROESY correlation of H3 or H4 with H7 would play a key role in the assignment of the relative configurations of C3, C4, and C5. However, unfortunately, the key signal of H7 was partially overlapped with H2a, H9a, and $H_3$18 in the ^{1}H NMR spectrum obtained from a 600 MHz spectrometer (Figure S2, Supporting Information); thus, it was hard to distinguish that H3 showed a ROESY correlation with H7 or those with other protons. The H4 showed ROESY correlation with H2a indicating that the two protons were cofacial, but, at the same time, both of the H3 and H4 exhibited equal strength of ROESY correlations with H1a, H10b, and H11. Taken together, these facts could not warrant a conclusion of the stereochemical structure. Although X-ray crystallographic analysis could provide the reliable structural information, unfortunately, since a limited amount of **1** was isolated, its single crystals could not be obtained.

For the above reasons and its potential bioactivity, we wondered if accumulating **1** would be a good way to serve our purposes. Because of the low content, reisolation from its mother plant is impractical. We thus decided to carry out an asymmetric total synthesis of **1**, which not only can unequivocally confirm the complete structure including the absolute configuration but also can provide us sufficient quantities for further bioactivity studies. Given the unassigned stereochemistry of the target, we first utilized the ab initio density functional theory (DFT) GIAO calculation of NMR chemical shifts to predict a likely correct structure before our total synthesis. Of note, this theoretical method has been proven as a powerful additional tool for the assignments of gross structure and stereochemistry of natural products in the case of only having one set of experimental data.[7]

Cernupalhine A (**1**) contains five stereocenters, in principle, and it has 16 possible diastereomers of different relative configuration (**1.1**−**1.16**, Figure S54, Supporting Information). All of the diastereomers were submitted to a conformational search using molecular mechanics calculations in Discovery Studio 3.1 Client.[3a,c,8] The corresponding minimum geometries were fully optimized at the B3LYP/6-31G(d) level and then followed at the B3LYP/6-31+G(d,p) level in the gas phase to get more accurate conformers. The ^{1}H and ^{13}C NMR shielding constants of the corresponding optimized configurations were computed using the GIAO technique at the mPW1PW91-SCRF/6-311+G(2d,p) level of theory in the PCM solvent continuum model with chloroform as a solvent.[7a] The calculated Boltzmann-calculated population-weighted chemical shifts of the 16 diastereomers were afforded after corrections using the slope and intercept obtained from linear regression analysis.[9] Comparisons of experimentally measured ^{1}H and ^{13}C NMR resonances for **1** and those of the Boltzmann-weighted DFT GIAO NMR calculations for the 16 possible diastereomers were carried out. The DP4 analysis, recently developed by Smith and Goodman,[9b] identified structure **1.3** as most likely, with probabilities of 99.98%, 100%, and 100% for the ^{1}H NMR, ^{13}C NMR, and combined ^{1}H and ^{13}C NMR chemical shifts, respectively (Table S3, Supporting Information). In addition, the lowest values both of largest deviations and the average errors (CMAD) of **1.3** (Tables S1 and S4, Supporting Information) further supported that the relative configuration of **1** was likely 3*S**,4*R**,5*S**,7*S**,12*S**.[7a]

Our retrosynthetic analysis of **1** is outlined in Scheme 1. We proposed that the unique hydroxydihydrofuranone motif in the target could be assembled at the final stage from cyclization of cyclopentanone and a carboxylic acid, which in turn should be attached to the tricyclic backbone via a carbon synthon. Tricyclic precursor lobscurinine (**3**) is a known alkaloid, which was obtained from the natural *Lycopodium* alkaloid lobscurinol

Scheme 1. Retrosynthetic Analysis of (+)-1



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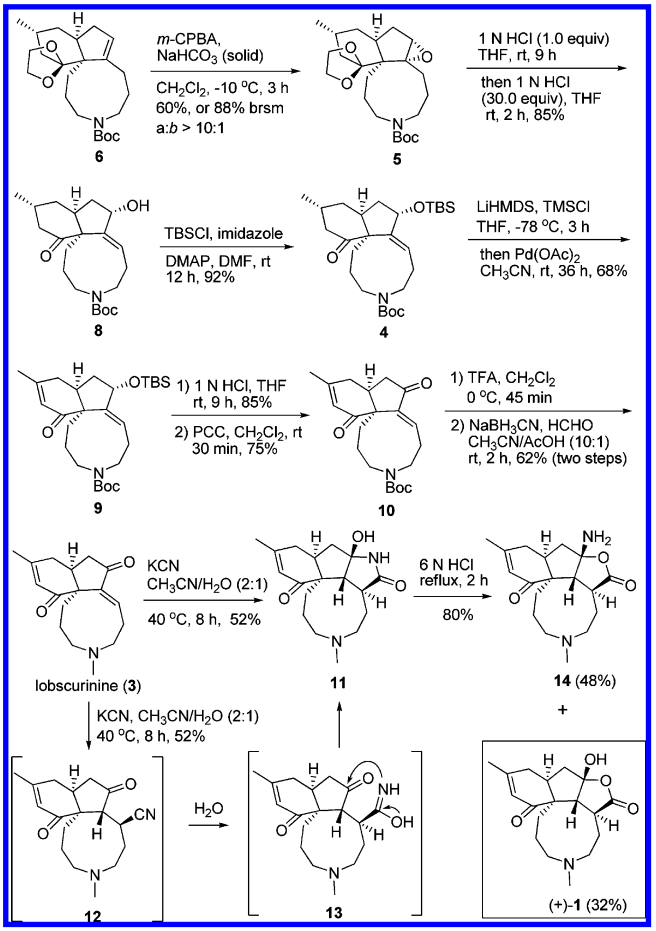
oxidation.[10] Two enones of **3** could be installed selectively at the different stages. Namely, Saegusa–Ito oxidation[11] of protected ketone **4** was expected to deliver cyclohexenone, and the epoxide opening of **5** was required to furnish the allyl alcohol functionality. The selected α-configured epoxide **5** was envisioned to arise from epoxidation of **6** with *m*-CPBA. Finally, the tetracycle **6** can be readily achieved from (−)-enone **7** through our previously established nine-step protocol involving key steps of Helquist annulation, Suzuki–Miyaura cross-coupling, and *N*-alkylation.[3f−h]

Our synthesis commenced with tetracycle **6**. By treatment with *m*-CPBA, **6** was converted into α-epoxide **5** (α/β > 10:1). Exposure of α-epoxide **5** in 1 N HCl (1.0 equiv) in THF at rt led to an almost quantitative yield of the (*E*)-allyl alcohol group in **8**.[12] The acetal group of cyclohexanone was found to be easily cleaved by further addition of excessive 1 N HCl (85% overall yield). Protection of the allyl alcohol group using TBSCl (92%) and then a Saegusa–Ito oxidation[11] was introduced to afford enone **9** in a good yield of 68%. Other methods like Nicolaou dehydrogenation[13] (2-iodoxybenzoic acid, IBX) only gave lower yields and decomposed the starting material. Desilylation of **9** (1 N HCl, 85%) and then oxidation using PCC yielded dienone **10** (75%).

After the removal of *N*-Boc group and then *N*-methylation, lobscurinine (**3**) was prepared successfully (62%, two steps). At this stage, we were pleased to find that **3** was identical with the literature compound of lobscurinine.[10] With key precursor **3** in hand, we were able to install the one carbon synthon on the azacyclononane ring through selective 1,4-addition of two enones, probably due to the methyl group at cyclohexenone being more steric. The choice of one carbon synthon is critical. Use of copper(I)-catalyzed vinyl group 1,4-addition of **3** turned out to be inefficient. Cyanide ion is a good choice because that cyanide can be hydrolyzed conveniently into the desired carboxylic acid. Indeed, after treatment of **3** with KCN in wet acetonitrile, the 1,4-addition reaction took place smoothly. Interestingly, the product was not identified as the expected cyanide **12** and turned out to be hydroxypyrrolidinone **11** as evidenced by the positive HREIMS at *m*/*z* 318.1947 $[M]^+$ together with the characteristic signals in the ^{1}H and ^{13}C NMR spectra of δ_H 7.25 (NH) and δ_C 178.8 and 93.6, respectively, in the solvent of pyridine-d_5. Cyanide **12** might be an intermediate that continued to be hydrolyzed to acetimidic acid **13**, which was terminated by cyclization with the cyclopentanone to deliver hydroxypyrrolidinone **11** (Scheme 2). Based on the ROESY correlations of H4 with H2b and H3 with H11, the absolute configurations of C3 and C4 were elucidated as 3*S* and 4*R*, respectively. Compound **11** closely matched our target molecule. Treatment of hydroxypyrrolidinone **11** with 6 N HCl under reflux yielded two products in the ca. 1.5:1 ratio. The minor one (32%) was easily identified as the desired natural product through TLC comparison with the authentic sample of cernupalhine A (**1**). The spectroscopic data (^{1}H and ^{13}C NMR, IR, OR, UV, CD, and HRMS analysis) for synthetic cernupalhine A (**1**) were identical to those acquired for the natural product. In order to characterize the key signal of H7, the ^{1}H NMR and ROESY experiments of synthetic **1** were carried out in a 800 MHz spectrometer. We were pleased to find that the signal of H7 was a distinguishable triple peak with a coupling constant 6.8 Hz from the adjacent proton signals and show obvious ROESY correlation with H3 (Figures S52 and S53, Supporting Information). This result and other key ROESY correlations of H3 with H11, H4 with H1a, H2b, and

Scheme 2. Completion of the Total Synthesis of (+)-1



H10a as well as H7 with H11 determined the stereochemistry of synthetic **1** (3*S*,4*R*,5*S*,7*S*,12*S*) in accordance with that of the theoretical prediction. Thus, our total synthesis of (+)-cernupalhine A (**1**) not only validated the corrected relative configuration predicted through DFT GIAO calculation of NMR chemical shifts but also unambiguously established its absolute configuration.

It is worth noting that characterization of the major product aminodihydrofuranone **14** was not trivial. Compound **14** was unstable and can be gradually transformed into **1**, and many of its spectroscopic data were close to that of **1**. Nevertheless, the distinctive signal of δ_C 103.2 in the ^{13}C NMR and 1759 cm^{-1} in the infrared spectrum helped us to assign it as **14**. The plausible pathway for the transformation from **11** to **14** and (+)-**1** is shown in Scheme 3. Hydroxypyrrolidinone **11** was dehydrated to form the pyrrolium **15** which was cleaved by water molecules, and then the resulting carboxylic acid iminium **16** underwent two pathways of reactions. In pathway **a**, intramolecular cyclization of carboxylic acid and the iminium forms amino dihydrofuranone **14**. In pathway **b**, iminium **16** is hydrolyzed to a ketone and then cyclized with carboxylic acid group affords the (+)-**1**. Aminodihydrofuranone **14** can be transformed into (+)-**1**, suggesting that the pathway **a** is a reversible and kinetic control process, while the pathway **b** is a thermodynamic one.

In summary, we have discovered a new type of $C_{17}N$ skeleton *Lycopodium* alkaloid with a unique hydroxydihydrofuranone motif named cernupalhine A (**1**) from *P. cernua*. Herein we have demonstrated that the power of the combination of high-

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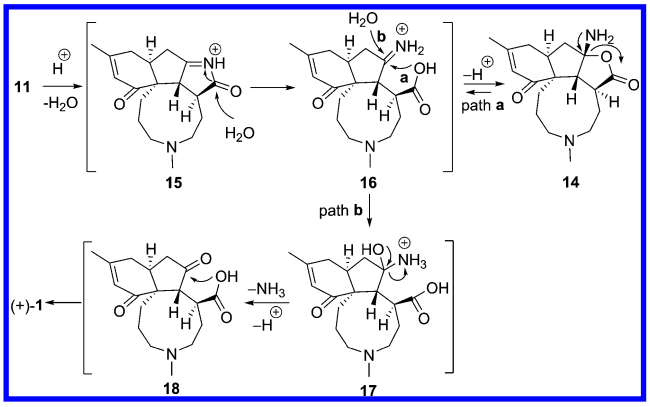
Scheme 3. Plausible Pathways for the Transformation of 11



field NMR techniques, computational methods, and asymmetric total synthesis in the complete structural assignment of natural products, in particular, when the natural source is extremely scarce and the X-ray information is unavailable. Our first total synthesis of cernupalhine A (**1**) features stereospecific α-epoxidation, facile epoxide ring-opening to construct an allyl alcohol group, and the regio- and stereoselective cyanide ion addition of lobscurinine (**3**), which is also synthesized in this study. The use of cyanide in the late stage to construct the unique hydroxydihydrofuranone motif and the intriguing mechanistic chemistry disclosed in the synthesis are striking.

■ ASSOCIATED CONTENT

Supporting Information

1D and 2D NMR and HRMS spectra of **1**, 1D NMR calculations, and total synthesis details. 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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