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Isolation, X-ray Crystallography, and Computational Studies of Calydaphninone, a New Alkaloid from *Daphniphyllum calycillum*

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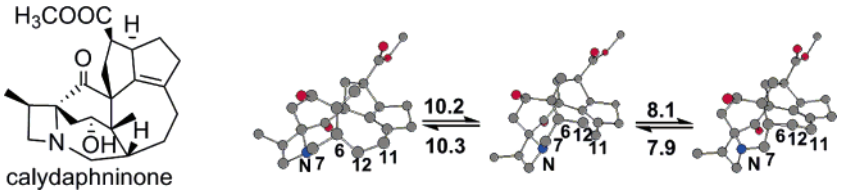
ABSTRACT



A new hexacyclic alkaloid, calydaphninone (1), containing a 4-azatricyclo[5.2.2.0^{1,4}]undecan ring system, has been isolated from the leaves and twigs of *Daphniphyllum calycillum* (Daphniphyllaceae). Its structure was elucidated by spectral and crystallographic means. Furthermore, conformations of calydaphninone (1) in solution are discussed on the basis of NMR spectral analysis and computational (B3LYP/6-31G*) results.

Daphniphyllum alkaloids are a group of structurally diverse natural products with unusual polycyclic skeletons that were isolated from plants of the genus *Daphniphyllum*.[1] In recent years, many new alkaloids with novel skeleton have been still obtained from this genus.[2] Their unique ring systems challenged chemists as targets for total synthesis[3] and biogenetic studies.[4] In our search for structrually interesting alkaloids,[5] a novel one, calydaphninone (**1**), containing a 4-azatricyclo[5.2.2.0^{1,4}] undecan moiety, was isolated from *Daphniphyllum calycillum* Benth (Daphniphyllaceae), and its chemical structure was elucidated by spectral and crystallographic means. Furthermore, the conformation of **1** in solution was also investigated by a synergic experimental and computational (B3LYP/6-31G*) study.

Leaves and twigs of *D. calycillum* were extracted with 95% EtOH. The extract was partitioned between EtOAc and

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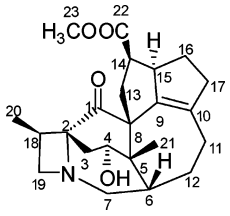
tartaric acid. The aqueous layer was then basified to pH 10, followed by exhaustive extraction with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column ($CHCl_3$/MeOH 1:0 → 0:1), from which a fraction, eluted with $CHCl_3$/MeOH (20:1), was purified on normal H silica gel ($CHCl_3$/MeOH, 50:1 → 20:1) to afford compound **1** (0.0001%).



Calydaphninone (**1**) was isolated as white powder, and a part crystalial could be obtained in acetone.[6] Its molecular formula, $C_{23}H_{31}NO_4$, was established by HRESIMS, indicating nine degrees of unsaturation. The ^{13}C NMR spectra of **1** showed partial broadening signals at 298 K in NMR solvents, e.g., $CDCl_3$ or a mixture of $CDCl_3$ and Pyr-d_5 (3:1). Twenty-three signals were revealed, as shown in Table 1, due to four tetrasubstituted sp^2 carbon atoms at lower field and 19 sp^3 carbon atoms (3 × C, 5 × CH, 8 × CH_2, 2 × CH_3, 1 × OCH_3) at higher field (Table 1). According to the molecular formula and relative NMR data, only one hydroxyl group was considered to be present in the structure (IR at 3431 cm^{-1}). Additionally, four sp^2 quaternary carbons were attributable to one ketone group (δ_C 212.9), one ester group (δ_C 175.3), and one tetrasubstituted double bond (δ_C 141.8, 134.2), while taking into account the three degrees of unsaturation. The remaining six degrees of unsaturation were assumed for the presence of the hexacyclic system of **1**.

Detailed 2D NMR ($^1H-^1H$ COSY, TOCSY, HMQC, and HMBC experiments) studies revealed that alkaloid **1** was composed of two moieties (Figure 1). The HMBC correlations suggested that the right moiety (in red) contained three fused ring systems (two five-, one seven-membered rings) with a double bond between C-9 and C-10, a carbomethoxy group at C-14, a methyl at C-5, and a methylene at C-6, which was the same as that of yunnandaphnine A.[5a] In the left moiety (in black), two protonated partial structures of C-19-C-18-C-20 and C-3-C-4 were established by the $^1H-^1H$ COSY and TOCSY spectra. In HMBC spectrum, cross-peaks of H-7b to C-2 and C-19 and of H_2-19 to C-7 showed that a quaternary carbon assigned to C-2 and two methylenes assigned to C-7 and C-19 were attributable to those attached to the nitrogen atom. HMBC correlations of H-18 with C-1,

Table 1. ^1H (500 MHz) and ^{13}C (100 MHz) NMR Spectral Data for Calydaphninone (**1**) at 298 K in $CDCl_3$ and $CDCl_3$/Pyr-d_5 (3:1)

no.	δ_C[a]	δ_C[b]	δ_H[b]
1	213.5	212.9 (s)	
2	74.5[c]	73.9 (s)	
3α	35.4	34.5 (t)	1.80 (1H, m)
3β			2.20 (1H, m)
4	74.5[c]	73.1 (d)	3.81 (1H, br d, 9.0)
5	44.9	44.8 (s)	
6	38.4	38.9 (d)	2.58 (1H, m)
7a	55.7	55.2 (t)	2.47 (1H, m)
7b			2.78 (1H, m)
8	58.7	58.5 (s)	
9	142.0	141.8 (s)	
10	134.2	134.2 (s)	
11	26.0	26.4 (t)	1.98 (2H, m)
12α	25.8	25.6 (t)	1.55 (1H, m)
12β			1.87 (1H, m)
13α	39.5	38.5 (t)	1.70 (1H, dd, 9.0, 14.0)
13β			2.61 (1H, dd, 6.5, 14.0)
14	42.0	41.6 (d)	2.98 (1H, m)
15	56.2	55.5 (d)	3.86 (1H, m)
16α	29.2	29.1 (t)	1.83 (1H, m)
16β			1.18 (1H, m)
17α	42.6	41.9 (t)	2.48 (1H, m)
17β			2.14 (1H, m)
18	25.7	25.7 (d)	2.86 (1H, m)
19a	57.7	57.2 (t)	2.81 (1H, m)
19b			2.92 (1H, m)
20	14.8	14.2 (q)	0.96 (3H, d, 7.0)
21	24.4	24.2 (q)	1.23 (3H, s)
22	175.6	175.3 (s)	
23	51.3	50.9 (q)	3.51 (3H, s)

[a] In $CDCl_3$. [b] In $CDCl_3$/Pyr-d_5 (3:1). [c] Overlapped.

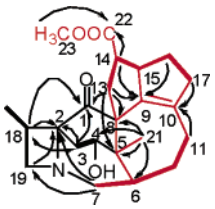


Figure 1. Key $^1H-^1H$ COSY (—) and HMBC (→) correlations of calydaphninone (**1**).

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(6) Calydaphninone (**1**): white powder; mp 202−204 °C; $[\alpha]^{20}_D = -66.7$ (*c* 0.38 $CHCl_3$); IR (KBr) 3431, 2919, 1729, 1705, 1634, 1438, 1169 cm^{-1}; ^1H and ^{13}C NMR data, see table **1**; ESIMS *m/z* 386 $[M + H]^+$; HRESIMS *m/z* calcd for $C_{23}H_{31}NO_4$ $[M + H]^+$ 386.2331, found 386.2333.

C-2, and C-3, as well as H_2-3 with C-1, C-2, and C-18 showed the linkage of both partial structures to C-2 and C-1. Furthermore, HMBC correlations of H_2-13/C-1, H-4/C-5 suggested that both moieties were also connected by bonds C-1−C-8 and C-4−C-5. Thus, the planar structure of calydaphninone was established as shown in Figure 1.

The relative configuration of **1** was subsequently elucidated by using ROESY and single-crystal diffraction experiments.[7] In ROESY spectrum (Figure 2A), NOE correlations

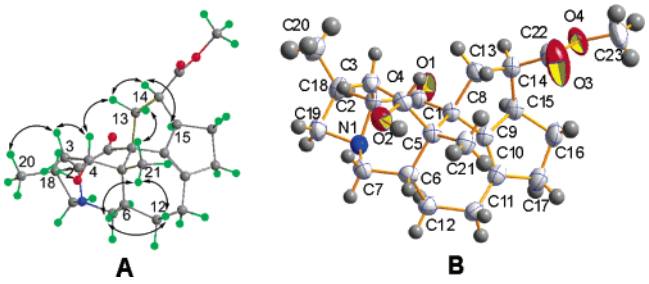


Figure 2. (A) Key ROESY correlations of *1*. (B) Single-crystal X-ray structure of **1**, in which C-11 and C-12 took upside and downside orientation, respectively, and the nitrogen lone pair pointed downside.

indicated that relative configuration of Me-21, C-13, H-14, H-15 in **1** was identical to that in yunnandaphnine A.[5a] H-4 took a β-orientation due to NOE correlation of H-13α/H-4. β-Configuration of the C-12 methylene group was deduced from the NOE effect between Me-21 and H-12β, and consequently, the α-orientation of C-11 was established.[8,9] The single-crystal structure of **1**, shown in Figure 2B, confirmed the planar structure and relative configuration of **1**. However, the conformer of **1** in crystal was not in accordance with that in solution as assigned by the ROESY spectrum. Specifically, configuration of C-11 and C-12 in the solution was opposite to that in crystal. Meanwhile, the broadening peaks in the ^{13}C NMR spectrum were partially coalesced by measuring it at 333 K.[10] All data suggested that several interconversional conformations of **1** coexist in solution.[11]

To model its conformational constitution and corresponding interconversion process in solution, DFT calculations at the B3LYP/6-31G(d) level[12] were carried out on calydaphninone (**1**). The calculations showed three low-energy conformations of **1** (Figure 3), which were roughly

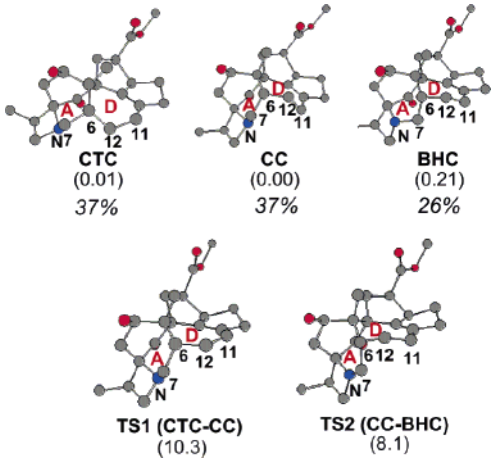


Figure 3. Three DFT-calculated minimum energy conformers (**CTC**, **CC**, and **BHC**) and two connecting TS (**TS1** and **TS2**) found for calydaphninone (**1**). Free energies in kcal/mol relative to the stablest conformer **CC** are given in parentheses. Populations of three conformers (in italics) are also given.

distinguished as chair,twist-chair (**CTC**), chair,chair (**CC**), and boat,half-chair (**BHC**) according to conformational difference in seven-membered rings A (C-1, C-2, C-5−C-8, and nitrogen atom) and D (C-5, C-6, C-8−C-12). Among them, conformer **CTC**, the only one with C-12 taking α-configuration, is in good agreement with the X-ray crystal structure of **1**,[13] while **CC**, characterized with the nitrogen lone pair taking an α-configuration and C-12 taking a β-configuration, is the lowest energy conformer, and **BHC**, with both the nitrogen lone pair and C-12 taking a β-orientation, is the highest energy conformer.

The calculations also showed two transition states (TS1 and TS2) (Figure 3)[11] which corresponded to **CTC**−**CC** conversion and **CC**−**BHC** conversion, and their free energies against the stablest conformer **CC** were 10.3 and 8.1 kcal/mol, respectively. Therefore, the occurrence of comparatively rapid interconversion of **CC** to **CTC** and **BHC** is a logical process in solution at room temperature, which leads to some broad peaks in NMR spectra.

The calculated distance between Me-21 and H-12β of **CC** (2.2 Å) and **BHC** (2.4 Å) implied that the NOE correlation

(7) Crystallographic data for calydaphninone (**1**) have been deposited at the Cambridge Crystallographic Data center (deposition no. CCDC-618286). Copies of data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html.

(8) The same conformation of **1** was indicated by NOE correlations, in NMR solvent, $CDCl_3$ or mixture of $CDCl_3$ and Pyr-d_5; for more details see Supporting Information.

(9) Configuration of C-11 or C-12 is relative to a reference plane, which is determined by C-5, C-9, and C-10.

(10) Broad signals of C-1, C-2, C-3, C-6, C-7, C-10, C-11, C-17, C-19, and C-20 located at or near the substructure 2,7-dimethyl-11-hydroxy-6,8,8-trisubstituted-4-azatricyclo [5.2.2.0^{1,4}]undecan-9-one ring system at 298 K were slightly changed sharply at 333 K in a mixture of $CDCl_3$ and Pyr-d_5 (3:1). For more details, see Supporting Information.

(11) Rapid interconversion between confromers at room temperature was also observed in other *Daphniphyllum* alkaloids: (a) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2000**, *65*, 3553−3563. (b) Morita, H.; Ishioka, N.; Takatsu, H.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459−462.

(12) (a) Gaussian 03 was used to search for the stable conformations of **1** and transition state structures. The crystal structure of **1** was used in the stable conformations search of **1**. B3LYP/6-31G(d) was used to optimize all conformations and transition states. Only one negative eigenvalue and one imaginary frequency were obtained for each TS in computations. The frequency calculations and eigenvalues for transition state structures were passed the analyses. The free energy magnitudes were used throughout the theoretical studies. (b) Frisch, M. J. et al. *Gaussian 03*, revision B.04; Gaussian, Inc.: Pittsburgh, PA, 2003. Full references for Gaussian programs are provided in Supporting Information.

(13) The calculated bond lengthes, bond angels, and dihedral angels of **CTC** are in good agreement with the crystallography results. The average rms differences are 0.012 Å, 0.97°, 2.7°, respectively. For more details, see Supporting Information.

signal between Me-21 and H-12β should be observed in ROESY spectrum. This is in agreement with the experimental results.

Furthermore, the calculated orientation of the nitrogen lone pair could be comfirmed by experiments. It is clear in Figure 4A that the ^{1}H NMR spectrum of **1a** (TFA salt of calydaphninone) showed two sets of signals with a ratio of 4:1, which suggested the existence of two types of conformational isomers, with different nitrogen configuration. This was further confirmed by ^{13}C NMR experiment. In this case, the contribution of both of the TFA salts of **CC** and **CTC**, with the same configuration of the nitrogen atom, should be the cause of one set of signals in NMR spectra.

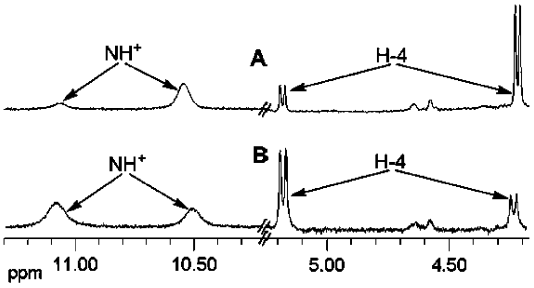


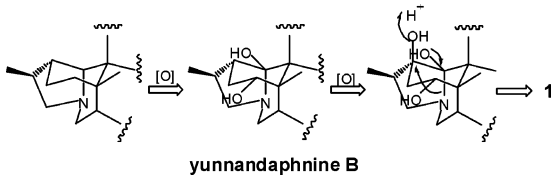
Figure 4. Partial ^{1}H NMR spectrum, including two sets of distinct protons at nitrogen and C-4, of TFA salt (**1a**) of calydaphninone in $CDCl_3$. (A) Spectrum recorded at 298 K soon after TFA was added. (B) Spectrum recorded at 298 K after the solution was heated at 333 K for 20 min.

As shown in Figure 4B, heating the solution at 333 K for 20 min led to (equilibrium) a change of the ratio from 4:1 to 1:2 in the ^{1}H NMR spectrum,[14] implying the occurrence of a transition from a higher energy isomer to a lower energy isomer. The most stable isomer was suggested to be the TFA salt of **BHC**, which was indicated by the ROESY correlations of H-12β/H$_3$-21, H-18/H-19α, and H-7α/H-19α of the corresponding major one in Figure 4B. Meanwhile, the calculation results also indicated that the **BHC-HCl** was the stablest one in the model of the HCl salt of **1**.[15] Therefore, the calculation was supported by experiment results.

(14) The ratio of two set of peaks remained almost unchanged after heating at 333 K for 1 h.

Biogenetically, **1** might be derived from Yuzurimine-type alkaloids such as yunandaphnine B, which may be converted to intermediate A followed by rearrangement to produce a new 4-azatricyclo[5.2.2.0^{1,4}]undecan ring system, calydaphninone (**1**) (Scheme 1).

Scheme 1. Biogenetic Pathway Proposed for Calydaphninone (**1**)



The cytotoxic activities of **1** against the growth of tumor cell lines [P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma)] were evaluated. The results indicated that **1** was inactive against the above cancer cells (50% effective dose of clonal inhibition (ED_{50}) >10 μg/mL).

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Supporting Information Available: Detailed 2D-NMR correlations of TFA salt of **1** (table and figures); experimental procedures and computational methods; detailed 1D-, 2D-NMR spectra of **1** and corresponding TFA salt; and calculation results (including free energy and standard orientation of three conformers, two TSs and HCl salts of three comformers). This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) DFT calculations at the B3LYP/6-31G(d) level were carried out on a model of the HCl salt of calydaphninone (**1**). Three stable conformers were found. Among them, **BHC-HCl** is the stablest conformer. The other two, **CC-HCl** and **CTC-HCl**, are 2.36 and 1.65 kcal/mol higher in free energy than the stablest conformer **CC**, respectively. For details, see Supporting Information.