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# Calyciphylline D, a Novel Alkaloid with an Unprecedented Fused-Pentacyclic Skeleton from *Daphniphyllum calycinum*

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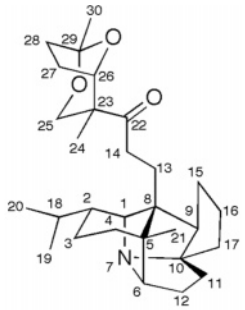
## ABSTRACT



**calyciphylline D (1)**

**Calyciphylline D (1), a novel *Daphniphyllum* alkaloid with an unprecedented fused-pentacyclic skeleton containing a 8-azatricyclo[4.2.1.0.$^{4,8}$]-nonane ring system, has been isolated from the leaves of *Daphniphyllum calycinum* (Daphniphyllaceae), and the structure and relative stereochemistry were elucidated on the basis of spectroscopic data.**

*Daphniphyllum* alkaloids are a family of fused-heterocyclic natural products elaborated by trees of the genus *Daphniphyllum* (Daphniphyllaceae).[1−7] These ring systems have attracted great interest as challenging targets for total synthesis as well as biosynthetic studies.[8] In our search for structurally unique and biogenetically interesting *Daphniphyllum* alkaloids,[2] we have isolated novel types of *Daphniphyllum* alkaloids such as calyciphyllines A−C[2a,g]

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(1) For a review of *Daphniphyllum* alkaloids, see: Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; Vol. 60, pp 165−205 and references therein.

(2) (a) Saito, S.; Kubota, T.; Fukushi, E.; Kawabata, J.; Zhang, H.; Kobayashi, J. *Tetrahedron Lett.* **2007**, *48*, 1587−1589. (b) Morita, H.; Ishioka, N.; Takatsu, H.; Iizuka, T.; Kobayashi, J. *J. Nat. Prod.* **2006**, *69*, 418−420. (c) Kubota, T.; Matsuno, Y.; Morita, H.; Shinzato, T.; Sekiguchi, M.; Kobayashi, J. *Tetrahedron* **2006**, *62*, 4743−4748. (d) Morita, H.; Ishioka, N.; Takatsu, H.; Shinzato, T.; Obara, Y.; Nakahata, N.; Kobayashi, J. *Org. Lett.* **2005**, *7*, 459−462. (e) Takatsu, H.; Morita, H.; Shen, Y. C.; Kobayashi, J. *Tetrahedron* **2004**, *60*, 6279−6284. (f) Morita, H.; Takatsu, H.; Shen, Y. C.; Kobayashi, J. *Tetrahedron Lett.* **2004**, *45*, 901−904. (g) Morita, H.; Kobayashi, J. *Org. Lett.* **2003**, *5*, 2895−2898. (h) Kobayashi, J.; Takatsu, H.; Shen, Y. C.; Morita, H. *Org. Lett.* **2003**, *5*, 1733−1736. (i) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575−3579.

(3) (a) Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B. *Tetrahedron Lett.* **2004**, *45*, 515−518. (b) Bitar, H. E.; Nguyen, V. H.; Gramain, A.; Sévenet, T.; Bodo, B. *J. Nat. Prod.* **2004**, *67*, 1094−1099.

(4) Zhang, W.; Guo, Y.-W.; Krohn, K. *Chem.−Eur. J.* **2006**, *12*, 5122−5127.

(5) Jossang, A.; Bitar, H. E.; Pham, V. C.; Sévenet, T. *J. Org. Chem.* **2003**, *68*, 300−304.

(6) (a) Di, Y. T.; He, H. P.; Lu, Y.; Yi, P.; Li, L.; Wu, L.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1074−1076. (b) Mu, S. Z.; Wang, Y.; He, H. P.; Yang, X. W.; Wang, Y. H.; Di, Y. T.; Lu, Y.; Chang, Y.; Hao, X. J. *J. Nat. Prod.* **2006**, *69*, 1065−1069. (c) Li, L.; He, H. P.; Di, Y. T.; Tian, J. M.; Hao, X. J. *Helv. Chim. Acta* **2006**, *89*, 1457−1462. (d) Li, L.; He, H. P.; Di, Y. T.; Gao, S.; Hao, X.-J. *Tetrahedron Lett.* **2006**, *47*, 6259−6262. (e) Di, Y. T.; He, H. P.; Liu, H. Y.; Du, Z. Z.; Tian, J. M.; Yang, X. W.; Wang, Y. H.; Hao, X. J. *Tetrahedron Lett.* **2006**, *47*, 5329−5331.

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**Table 1.** $^1$H and $^{13}$C NMR Data of Calyciphylline D (**1**) in $CD_3OD$

| | $\delta_H$ | $\delta_C$ | |
|---|---|---|---|
| 1 | 3.64 (1H, m) | 69.7 | d |
| 2 | 1.55 (1H, m) | 39.9 | d |
| 3a | 1.99 (1H, m) | 27.3[a] | t |
| 3b | 1.63 (1H, m) | | |
| 4a | 2.06 (1H, m) | 35.1[b] | t |
| 4b | 1.55 (1H, m) | | |
| 5 | | 44.7 | s |
| 6 | 4.15 (1H, m) | 70.6 | d |
| 7 | | | |
| 8 | | 53.4 | s |
| 9 | 2.42 (1H, m) | 52.6 | d |
| 10 | | 85.0 | s |
| 11a | 2.31 (1H, m) | 35.2[b] | t |
| 11b | 2.08 (1H, m) | | |
| 12 | 2.19 (2H, m) | 23.9 | t |
| 13a | 2.04 (1H, m) | 19.7 | t |
| 13b | 1.84 (1H, m) | | |
| 14a | 3.04 (1H, ddd, 18.6, 11.2, 5.6) | 35.8 | t |
| 14b | 2.81 (1H, ddd, 18.6, 10.4, 4.5) | | |
| 15a | 2.05 (1H, m) | 30.3 | t |
| 15b | 1.63 (1H, m) | | |
| 16a | 1.99 (1H, m) | 27.5[a] | t |
| 16b | 1.63 (1H, m) | | |
| 17a | 2.17 (1H, m) | 37.7 | t |
| 17b | 1.89 (1H, m) | | |
| 18 | 1.53 (1H, m) | 34.0 | d |
| 19 | 1.07 (3H, d, 6.0) | 21.9[c] | q |
| 20 | 1.05 (3H, d, 6.0) | 22.0[c] | q |
| 21 | 1.13 (3H, s) | 19.6 | q |
| 22 | | 214.5 | s |
| 23 | | 51.9 | s |
| 24 | 0.85 (3H, s) | 18.4 | q |
| 25a | 4.30 (1H, dd, 12.3, 1.9) | 67.0 | t |
| 25b | 3.65 (1H, d, 12.3) | | |
| 26 | 4.73 (1H, brd, 6.3) | 83.2 | d |
| 27 | 2.09 (2H, m) | 26.3 | t |
| 28a | 2.13 (1H, m) | 35.5 | t |
| 28b | 1.92 (1H, m) | | |
| 29 | | 107.5 | s |
| 30 | 1.40 (3H, s) | 24.8 | q |

[a]−[c]Assignments are interchangeable.

with unique fused-hexacyclic ring systems from the leaves of *Daphniphyllum calycinum*. Recently, we have isolated a novel alkaloid, calyciphylline D (**1**), with an unprecedented fused-pentacyclic skeleton containing an 8-azatricyclo-[4.2.1.0.$^{4,8}$]nonane ring system from *D. calycinum*. In this paper, we describe the isolation and structure elucidation of **1**.

The leaves of *D. calycinum* were extracted with MeOH, and the MeOH extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted to pH 10 with saturated $Na_2CO_3$, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (hexane/EtOAc, 1:0 → 4:6, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with hexane/EtOAc, 3:2 was purified repeatedly by silica gel columns ($CHCl_3$/MeOH, 1:0 → 0:1) to afford calyciphylline D (**1**, 0.00013% yield).

Calyciphylline D (**1**) showed the pseudomolecular ion peak at *m/z* 456 $(M + H)^+$ in the ESIMS, and the molecular formula, $C_{29}H_{45}NO_3$, was established by HRESIMS [*m/z* 456.3452 $(M + H)^+$, Δ-2.6 mmu]. IR absorptions implied the presence of keto carbonyl (1680 $cm^{-1}$) functionality. The $^{13}$C NMR (Table 1) spectrum of **1** gave signals including one ketone carbonyl, five sp$^3$ quaternary carbons, six sp$^3$ methines, 12 sp$^3$ methylenes, and five methyls. Among them, two methines ($\delta_C$ 69.7 and 70.6) and one quaternary carbon ($\delta_C$ 85.0) were ascribed to those bearing a nitrogen atom.

The $^1$H−$^1$H COSY and TOCSY spectra of **1** revealed connectivities of five partial structures **a** (C-1 to C-4, C-2 to C-18, and C-18 to C-19 and C-20), **b** (C-6 to C-12, and C-11 to C-12), **c** (C-13 to C-14), **d** (C-9 to C-15 and C-15 to C-17), and **e** (C-26 to C-28) as shown in Figure 1. HMBC

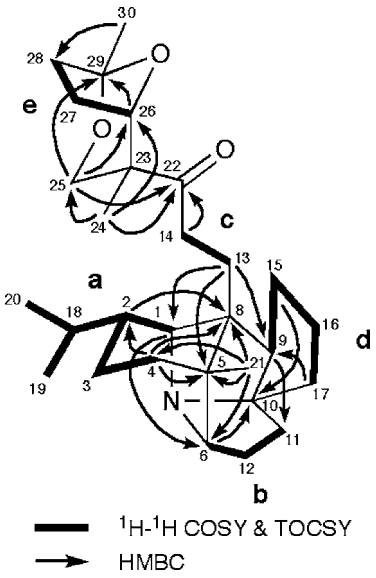



**Figure 1.** Selected 2D NMR correlations for calyciphylline D (**1**).

correlations of H-1 to C-6 ($\delta_C$ 70.6) and H-6 to C-10 ($\delta_C$ 85.0) suggested that C-1, C-6, and C-10 were connected to each other through a nitrogen atom. Connections between

(7) (a) Fan, C. Q.; Yin, S.; Xue, J. J.; Yue, J. M. *Tetrahedron*, **2007**, *63*, 115−119. (b) Zhang, H.; Yang, S. P.; Fan, C. Q.; Ding, J.; Yue, J. M. *J. Nat. Prod.* **2006**, *69*, 553−557. (c) Yang, S. P.; Zhang, H.; Zhang, C. R.; Cheng, H. D.; Yue, J. M. *J. Nat. Prod.* **2006**, *69*, 79−82. (d) Zhan, Z. J.; Zhang, C. R.; Yue, J. M. *Tetrahedron* **2005**, *61*, 11038−11045. (e) Chen, X.; Zhan, Z. J.; Yue, J. M. *Helv. Chim. Acta* **2005**, *88*, 854−860. (f) Chen, H.; Zhang, Z. J.; Yue, J. M. *Chem. Biodivers.* **2004**, *1*, 1513−1518. (g) Zhan, Z. J.; Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2004**, *69*, 1726−1729. (h) Yang, S. P.; Yue, J. M. *Org. Lett.* **2004**, *6*, 1401−1404. (i) Yang, S. P.; Yue, J. M. *J. Org. Chem.* **2003**, *68*, 7961−7966.

(8) (a) Wallace, G. A.; Heathcock, C. H. *J. Org. Chem.* **2001**, *66*, 450−454. (b) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323−14327. (c) Heathcock, C. H.; Joe, D. *J. Org. Chem.* **1995**, *60*, 1131−1142. (d) Heathcock, C. H.; Kath, J. C.; Ruggeri, R. B. *J. Org. Chem.* **1995**, *60*, 1120−1130. (e) Heathcock, C. H. *Angew. Chem.* **1992**, *104*, 675−691. (f) Heathcock, C. H. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 665−681 and references cited therein.

C-4, C-6, and C-21 via C-5 were implied by HMBC cross-peaks for $H_2$-4 to C-5 ($\delta_C$ 44.7) and $H_3$-21 to C-4 ($\delta_C$ 35.1), C-5, and C-6. On the other hand, connections among C-9, C-11, and C-17 via C-10 were indicated by HMBC cross-peaks for H-9 to C-11 ($\delta_C$ 35.2), H-17 to C-9 ($\delta_C$ 52.6), and H-15 to C-10. HMBC cross-peaks for H-2, $H_2$-4 and $H_3$-21 to C-8 ($\delta_C$ 53.4), H-9 to C-1 ($\delta_C$ 69.7), and $H_2$-13 to C-1, C-5, and C-9 suggested connectivities among units **a**, **b**, **c**, and **d** to form a nitrogen-containing fused-pentacyclic skeleton consisting of a 8-azatricyclo[4.2.1.0.$^{4,8}$]nonane ring, a cyclohexane ring, and a cyclopentane ring. The presence of a 2,8-dioxabicyclo[3.2.1]octane moiety including unit **e** was deduced from HMBC cross-peaks of $H_3$-24 to C-25 and C-26, $H_2$-25 to C-26 and C-29, H-26 to C-29, and $H_3$-30 to C-28. HMBC cross-peaks for H-14, $H_3$-24, and $H_2$-25 to C-22 provided the connection between C-14 and C-23 via C-22.

Thus, the gross structure of calycipylline D was elucidated to be **1**.

The relative stereochemistry of **1** was deduced from NOESY correlations as shown in Figure 2. NOESY correlations of H-1 to H-17b, H-2 to H-13a, H-9 to $H_3$-21, and H-12 to $H_3$-21 indicated the relative stereochemistry of the fused-pentacyclic ring and a chair form of the cyclohexane ring (C-1 to C-5 and C-8). The relative configurations at C-23, C-26, C-29 and a chair form of the 6-membered ring in the 2,8-dioxabicyclo[3.2.1]octane moiety were verified by NOESY correlations of $H_3$-24 to H-26 and H-25a, and H-25b to H-27 and H-28a.

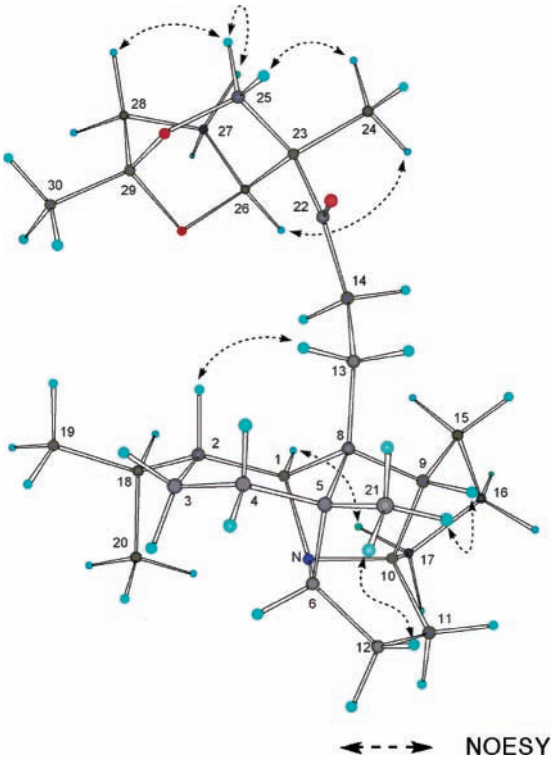
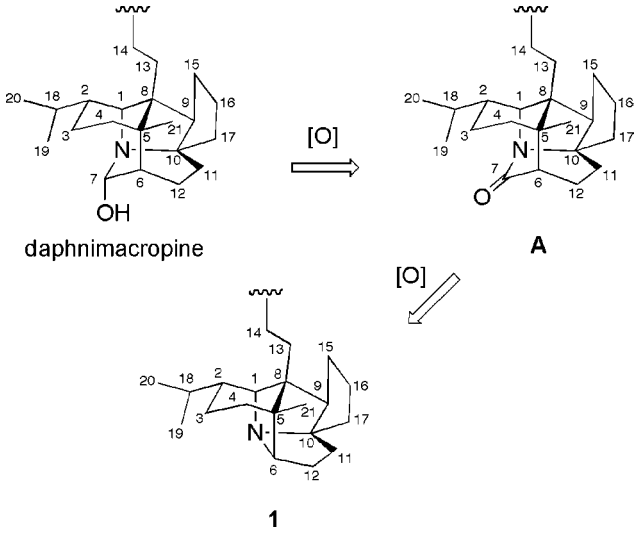



**Figure 2.** Selected NOESY correlations and relative stereochemistry for calyciphylline D (**1**).

Calyciphylline D (**1**) is a novel *Daphniphyllum* alkaloid having an unprecedented fused-pentacyclic skeleton containing a 8-azatricyclo[4.2.1.0.$^{4,8}$]nonane ring system.

Biogenetically, calyciphylline D (**1**) might be generated from intermediate **A**, which could be derived from oxidation of daphnimacropine,[10] through oxidative degradation of C-7[7h] followed by formation of N-7 to C-6 bond (Scheme 1).

**Scheme 1.** Plausible Biogenetic Path of Calyciphylline D (**1**)

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**Supporting Information Available:** One- and two-dimensional NMR spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Calyciphylline D (**1**): colorless amorphous solid; $[\alpha]^{24}_D$ −25.6 (*c* 0.5, MeOH); IR (neat) $\nu_{max}$ 1680 cm$^{-1}$; $^1$H and $^{13}$C NMR, see Table 1; ESIMS *m/z* 456 (M + H)$^+$; HRESIMS (*m/z* 456.3452 [(M + H)$^+$; calcd for $C_{29}H_{46}NO_3$, 456.3478]).

(10) Kamijo, N.; Nakano, T.; Terao, S.; Osaki, K. *Tetrahedron Lett.* **1966**, 2889−2892.