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Angustimine and Angustifolimine: Two New Alkaloids from *Daphniphyllum angustifolium*

Chuan-Rui Zhang, Cheng-Qi Fan, Shi-Hui Dong, Hong-Bing Liu, Wen-Bin Zhou, Yan Wu, and Jian-Min Yue*

State Key Laboratory of Drug Research, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Zhangjiang Hi-Tech Park, Shanghai 201203, People's Republic of China

jmyue@mail.shcnc.ac.cn

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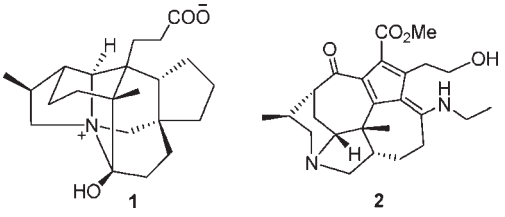
ABSTRACT



Two new alkaloids, angustimine (1) featuring an unprecedented skeleton, and angustifolimine (2) being a rare diamino *Daphniphyllum* alkaloid, were isolated from *Daphniphyllum angustifolium*. Their structures were elucidated by extensive spectroscopic analysis. The biosynthetic origin of 1 was postulated. Cytotoxic activities of 1 and 2 were tested, and all of them were inactive.

Daphniphyllum alkaloids are a class of structurally diverse and polycyclic natural compounds which have been attractive subjects of natural products and synthetic chemistry for decades.[1] In recent years, a series of novel *Daphniphyllum* alkaloids have been isolated, some of which exhibited cytotoxic activity against several tumor cell lines.[2]

Daphniphyllum angustifolium Hutch. is an evergreen shrub native to southwestern China.[3] Previous chemical studies on this plant have resulted in the isolation of a few flavonoids, iridoid glucosides, and phenyl glucosides.[4] In the current study, two novel alkaloids, angustimine (**1**) possessing an unprecedented hexacyclic fused skeleton that was likely derived biosynthetically from a bukittinggine-type alkaloid, caldaphnidine P,[1j] via the cleavage of a C-6−C-7 bond and the formation of a C-6−N bond as the key steps, and angustifolimine (**2**) representing the second diamino *Daphniphyllum* alkaloid,[1g] were isolated from the ethanolic extract of the *D. angustifolium* twigs. Their structures were elucidated by spectroscopic methods, especially two-dimensional NMR techniques. The isolation and structural elucidation of these alkaloids were herein presented.

(1) (a) Yamamura, S.; Hirata, Y. *Tetrahedron Lett*. **1974**, 3673–3676. (b) Heathcock, C. H. *Angew. Chem., Int. Ed. Engl*. **1992**, *31*, 665–681. (c) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A*. **1996**, *93*, 14323–14327. (d) Kobayashi, J.; Morita, H. In *The Alkaloids*; Cordell, G. A., Ed.; Academic Press: New York, 2003; Vol. 60, pp 165−205. (e) Yang, S. P.; Yue, J. M. *J. Org. Chem*. **2003**, *68*, 7961–7966. (f) Zhan, Z. J.; Yang, S. P.; Yue, J. M. *J. Org. Chem*. **2004**, *69*, 1726–1729. (g) Yang, S. P.; Yue, J. M. *Org. Lett*. **2004**, *6*, 1401–1404. (h) Zhan, Z. J.; Zhang, C. R.; Yue, J. M. *Tetrahedron* **2005**, *61*, 11038–11045. (i) Yang, S. P.; Zhang, H.; Zhang, C. R.; Cheng, H. D.; Yue, J. M. *J. Nat. Prod*. **2006**, *69*, 79–82. (j) Zhang, C. R.; Yang, S. P.; Yue, J. M. *J. Nat. Prod*. **2008**, *71*, 1663–1668. (k) Zhang, C. R.; Liu, H. B.; Dong, S. H.; Zhu, J. Y.; Wu, Y.; Yue, J. M. *Org. Lett*. **2009**, *11*, 4692–4695. (l) Dunn, T. B.; Ellis, J. M.; Kofink, C. C.; Manning, J. R; Overman, L. E. *Org. Lett*. **2009**, *11*, 5658–5661.

(2) (a) Jossang, A.; Bitar, H. E.; Pham, V. C.; Sévenet, T. *J. Org. Chem*. **2003**, *68*, 300–304. (b) Kobayashi, J.; Takatsu, H.; Shen, Y. C.; Morita, H. *Org. Lett*. **2003**, *5*, 1733–1736. (c) Morita, H.; Kobayashi, J. *Org. Lett*. **2003**, *5*, 2895–2898. (d) Morita, H.; Takatsu, H.; Kobayashi, J. *Tetrahedron* **2003**, *59*, 3575–3579. (e) Zhang, H.; Yang, S. P.; Fan, C. Q.; Ding, J.; Yue, J. M. *J. Nat. Prod*. **2006**, *69*, 553–557. (f) Zhang, C. R.; Liu, H. B.; Feng, T.; Zhu, J. Y.; Geng, M. Y.; Yue, J. M. *J. Nat. Prod*. **2009**, *72*, 1669–1672.

(3) Zhen, M.; Min, T. L. In *Flora Republicae Popularis Sinicae (Zhongguo Zhiwu Zhi)*; Science Press: Beijing, China, 1980; Vol. 45 (1), pp 1−11.

(4) (a) Bai, H. Y.; Hu, L. H. *Helv. Chim. Acta* **2006**, *89*, 884–894. (b) Xu, M. F.; Shen, L. Q.; Wang, K. W. *Fitoterapia* **2009**, *80*, 461–464. (c) Xu, M. F.; Shen, L. Q.; Wang, X. Y.; Wang, K. W. *Chem. Ind. For. Prod*. **2009**, *29*, 110–112.

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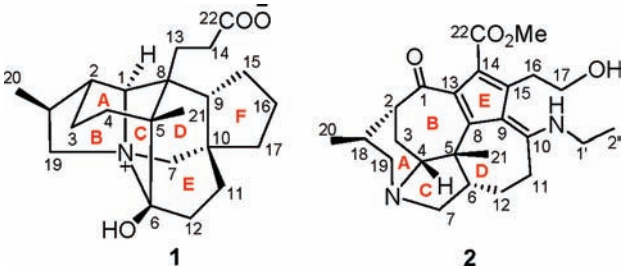
Table 1. ^{1}H and ^{13}C NMR Spectroscopic Data of **1** and **2**[a]

	1		**2**	
no.	δ_C	δ_H (multi, J in Hz)	δ_C	δ_H (multi, J in Hz)
1	81.4	3.72 (d, 5.0)	201.9	
2	38.0	2.46 (m)	51.4	2.42 (m)
3a	16.6	1.78 (m)	20.1	2.26 (m)
3b		1.48 (m)		2.05 (m)
4a	33.7	1.84 (m)	69.3	2.90 (brd, 6.3)
4b		1.80 (m)		
5	52.2		51.8	
6	111.8		53.2	2.26 (m)
7a	66.1	3.44 (d, 12.5)	62.3	3.38 (m)
7b		3.33 (d, 12.5)		2.30 (m)
8	46.9		140.2	
9	50.5	2.34 (m)	120.8	
10	43.9		179.4	
11a	34.0	1.86 (m)	32.0	2.26 (m)
11b		1.79 (m)		2.06 (m)
12a	32.4	2.20 (m, 2H)	30.8	2.08 (m)
12b				1.72 (m)
13a	29.3	2.05 (m)	128.3	
13b		1.83 (m)		
14a	32.0	2.38 (m)	124.6	
14b		2.28 (m)		
15a	28.8	2.00 (m)	130.9	
15b		1.92 (m)		
16a	23.0	1.78 (m, 2H)	30.3	2.75 (m)
16b				2.60 (m)
17a	39.5	1.64 (m)	66.0	3.92 (dd, 8.0, 2.7, 2H)
17b		1.58 (m)		
18	38.9	2.77 (m)	32.0	2.26 (m)
19a	59.8	3.54 (dd, 12.3, 11.2)	55.2	3.09 (dd, 11.5, 4.1)
19b		3.04 (dd, 12.3, 10.4)		2.50 (dd, 11.2, 2.7)
20	12.9	1.03 (d, 6.8, 3H)	19.5	1.25 (d, 7.9, 3H)
21	21.4	1.17 (s, 3H)	36.0	1.24 (s, 3H)
22	180.7		173.9	
23			52.5	3.68 (s, 3H)
1′			41.2	3.59 (m, 2H)
2′			14.4	1.38 (t, 7.4, 3H)

[a] Data were measured in CD_3OD at 400 MHz (^{1}H) and 100 MHz (^{13}C). Chemical shifts (δ) are in ppm being relative to TMS.



Angustimine (**1**),[5] obtained as a colorless solid, showed molecular formula $C_{22}H_{33}NO_3$ as determined by HREIMS at m/z 359.2449 $[M]^+$ (calcd 359.2460) with seven degrees of unsaturation, which was supported by the pseudomolecular ions at m/z 360 $[M + H]^+$ and 358 $[M - H]^-$ in the positive and negative modes of ESIMS, respectively. The IR spectrum implied the functionalities of hydroxy (3421 cm^{-1}) and carboxylate (1568, 1458, and 1385 cm^{-1}).

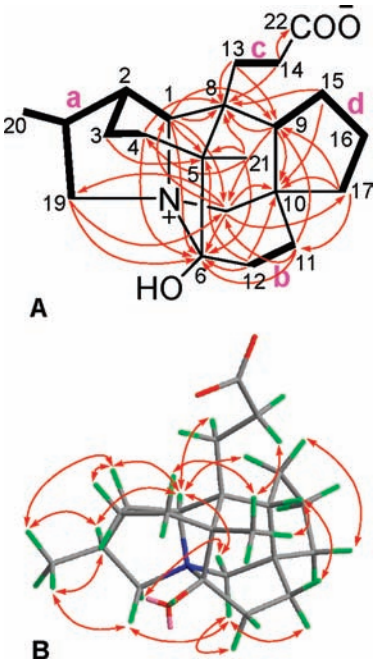


Figure 1. $^{1}H-^{1}H$ COSY (A: —), selected HMBC (A: →), and ROESY (B: ↔) correlations of **1**.

The ^{13}C NMR spectrum (Table 1) in combining with the DEPT experiments and 2D NMR spectra displayed 22 carbon resonances, including two methyls, eleven methylenes, four methines, and five quaternary carbons (including one carboxylate at δ_C 180.7, and one at δ_C 111.8). The only carbonyl group accounted for one out of seven degrees of unsaturation, and the remaining six degrees of unsaturation required alkaloid **1** being hexacyclic. Four proton-bearing structural fragments, **a** (C-1 to C-4, and C-18 to C-20), **b** (C-11 and C-12), **c** (C-13 and C-14), and **d** (C-9, and C-15 to C-17) as drawn with bold bonds, were readily established by $^{1}H-^{1}H$ COSY spectrum with the assistance of HMBC (Figure 1). The linkages of four fragments (**a**–**d**) with the quaternary carbons and heteroatoms were finally achieved by the examination of the HMBC spectrum (Figure 1). The CH-1 methine (δ_H 3.72, d, J = 5.0 Hz; δ_C 81.4) and CH_2-19 (δ_H 3.54, dd, J = 12.3, 11.2 Hz, δ_H 3.04, dd, J = 12.3, 10.4 Hz; δ_C 59.8) of fragment **a** were tentatively attributed to those attached to the N-atom by the chemical shifts to form the ring B of pyrrolidine; the CH_2-7 methylene (δ_H 3.44, d, J = 12.5 Hz, δ_H 3.33, d, J = 12.5 Hz; δ_C 66.1) and the quaternary carbon of C-6 (δ_C 111.8) were also attached to the N-atom on the basis of chemical shifts, which was confirmed by the mutual HMBC correlations of H-1/C-6 and C-7, H_2-19/C-6 and C-7, and H_2-7/C-1, C-6, and C-19. The linkage of

(5) Colorless solid; $[\alpha]^{20}_D$ −15 (c 0.155, $CHCl_3$); IR (KBr) ν_{max} 3421, 2956, 2928, 1705, 1568, 1458, 1385, 1259, 1223, 1136, 752 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS m/z 360 $[M + H]^+$, 358 $[M - H]^-$; EIMS m/z 359 $[M]^+$ (80), 330 (21), 317 (35), 300 (32), 286 (100), 258 (94), 97 (18), 58 (47); HREIMS m/z 359.2449 $[M]^+$ (calcd for $C_{22}H_{33}NO_3$ 359.2460).

C-12 to C-6 that bears a hydroxy forming the aminoketal was assigned by the HMBC correlations from H_2-11 and H_2-12 to C-6; the attachments of C-7, C-9, C-11, and C-17 to C-10 were made by the multiple HMBC correlations of H_2-7/C-10, H-9/C-10, H_2-12/C-10, H_2-15/C-10, H_2-17/C-10, H-9/C-7, H_2-11/C-7, H_2-7/C-17, H_2-17/C-9, and H_2-17/C-11, which constructed rings E and F. The C-1, C-5, C-9, and C-13 were linked to C-8 by the HMBC correlations of H-1/C-8, H_2-4/C-8, H-9/C-8, H_2-14/C-8, H_2-15/C-8, H_3-21/C-5 and C-8, H-1/C-5, H-1/C-9, and H_2-13/C-9; the Me-21, C-4, and C-6 were fixed to C-5 by the HMBC correlations from H-1, H_2-3, H_2-12, and H_3-21 to C-5, and from H_2-4 to C-6, which furnished rings A and D. The connection between C-14 and C-22 was established by the HMBC correlation from H_2-14 to C-22. The planar structure of **1** was thus established as an intramolecular salt with an unprecedented nitrogen-containing hexacyclic carbon skeleton.

The relative configuration of **1** was fixed by a ROESY experiment (Figure 1), in which the correlations of H_3-21/H-4a, H_3-21/H-9, H_3-21/H-14b, and H-9/H-11a indicated that H-9, H-11a, and H_3-21 were cofacial and randomly assigned in a β-configuration. In consequence, the ROESY correlations of H-1/H-2, H-1/H-7b, H-1/H-13b, H-1/H-15b, H-1/H-18, and H-7b/H-19b showed that H-1, H-2, H-7b, H-15b, H-18, and H-19b were α-oriented. The ROESY correlations of H_3-20/H-19a, H-19a/H-7a, H-7a/H-11b, H-7a/H-12b, and H-15a/H-17b were also observed to support the above assignment. The aforementioned ROESY correlations suggested that the six-membered rings A and D took chair conformation, the five-membered rings B, C, and F adopted envelope conformation, and the six-membered ring E was in a twisted boat conformation. Thus, the structure of **1** was finally elucidated.

Alkaloid **1** was an intramolecular salt featuring an unprecedented hexacyclic fused skeleton via the cleavage of a C-6−C-7 bond and the formation of a C-6−N bond.

Angustifolimine (**2**)[6] was obtained as a yellow solid. Its molecular formula was established as $C_{25}H_{34}N_2O_4$ by HREIMS at m/z 426.2529 $[M]^+$ (calcd 426.2519) requiring ten degrees of unsaturation, and this assignment was supported by the pseudomolecular ions at m/z 427 $[M + H]^+$ and 425 $[M - H]^-$ in the ESIMS. The ^{13}C NMR spectrum with DEPT experiments resolved 25 carbon signals (Table 1) comprising four methyls (one oxygenated-), eight methylenes (for those resonated at δ_C 66.0, 62.3, 55.2, and 41.2 being linked with O- or N-atoms), four methines (one resonated at δ_C 69.3 being likely linked with the N-atom), and nine quaternary carbons (six olefinic, one ketone at δ_C 201.9, and one ester carbonyl at δ_C 173.9). Two carbonyl groups and three double bonds accounted for five out of the ten degrees of unsaturation, and the remaining ones required that compound **2** was pentacyclic. Four structural fragments **a** (C-2 to C-4 and C-18 to C-20), **b** (C-6 and C-7, C-11 and C-12), **c** (C-16 and C-17), and **d** (C-1′ and C-2′) were deduced as drawn in bold lines (Figure 2) by using a combination of the 2D NMR spectra (HSQC, $^1H-^1H$ COSY, and HMBC). The linkages of the four structural fragments **a**−**d**, the quaternary carbons, and heteroatoms were mainly achieved by examination of the HMBC spectrum (Figure 2), in which the methoxyl group was readily located at C-22 by the correlation between OCH_3 at δ_H 3.68 and C-22 at δ_C 173.9. The only hydroxy was assigned to C-17 by the chemical shifts of CH_2-17 (δ_H 3.92, dd, J = 8.0, 2.7 Hz, 2H; δ_C 66.0). The Δ^{14} double bond and the linkage of C-15/C-9 were fixed by the multiple HMBC correlations of H_2-16/C-9, C-14 and C-15, and H_2-17/C-15, which also allowed the attachment of $-C_2H_5OH$ to C-15. The connectivity of C-11 and C-10 was revealed by the HMBC correlations from H_2-11 and H_2-12 to C-10. The linkage of C-1′ and C-10 via a nitrogen atom was furnished by the proton and carbon chemical shifts of CH_2-1′ (δ_H 3.59, m, 2H; δ_C 41.2) and C-10 (δ_C 179.4), and the key HMBC correlation between H_2-1′ and C-10. Two major fragments **a** and **b** were linked via a nitrogen and the quaternary carbon C-5 (δ_C 51.8) to form the piperidine of A ring and the pyrrolidine of C ring by the chemical shifts of C-4 (δ_C 69.3), C-7 (δ_C 62.3), and C-19 (δ_C 55.2), and by the mutual HMBC networks of H-4/C-5, C-7, and C-19; H_2-7/C-4, C-5, and C-19; H_2-19/C-4 and C-7; and H_2-3, H-6, and H_2-12/C-5. The HMBC correlations from H_3-21 to C-4, C-5, C-6, and C-8, and from H-4 and H-6 to C-8 allowed the linkage between C-5 and C-8, and also attached CH_3-21 to C-5. The ketone group was assigned to C-1, which built a bridge between C-2 and C-13 as judged from the HMBC correlations from H-2, H_2-3, and H-18 to C-1, and from H-2 to C-13. The connectivity

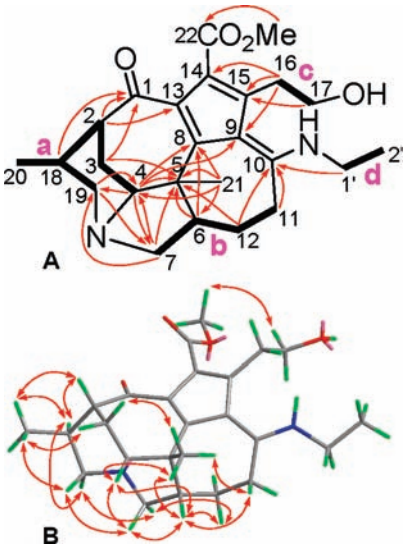
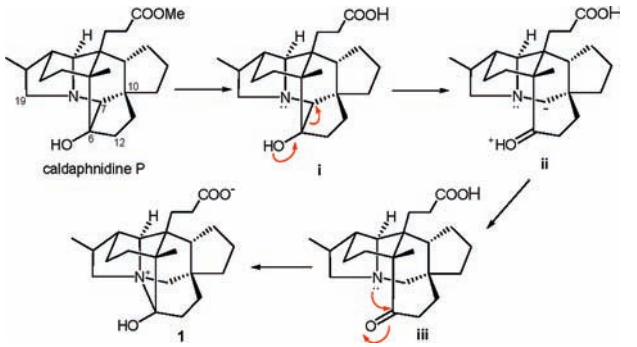


Figure 2. $^1H-^1H$ COSY (—), selected HMBC (A: →), and ROESY (B: ↔)correlations of **2**.

(6) Yellow solid; $[\alpha]^{20}_D$ −243.0 (*c* 0.070, MeOH); UV (MeOH) λ_{max} (log ε) 376 (4.28), 292 (4.02) nm; IR (KBr) ν_{max} 3425, 2929, 1699, 1589, 1423, 1352, 1242, 1158, 1076, 889, 731, 605 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 427 $[M + H]^+$, 425 $[M - H]^-$; EIMS m/z 426 $[M]^+$ (12), 394 (32), 381 (100), 376 (14), 349 (18), 294 (30), 175 (16), 109 (75), 111 (100), 107 (89), 94 (13), 77 (2), 55 (3); HREIMS m/z 426.2529 $[M]^+$ (calcd for $C_{25}H_{34}N_2O_4$ 426.2519).

Scheme 1. The Plausible Biosynthetic Origin of **1**



between C-8 and C-9 was tentatively assigned by the HMBC correlations from H-4 to C-8 and C-9. Although there were no direct HMBC correlations observed to furnish the linkages of C-8/C-13, C-13/C-14, and C-14/C-22, these linkages could be tentatively assigned by chemical shifts of relevant carbons to finally construct the B and E rings, and to attach the moiety of $-CO_2Me$ at C-14, and this assignment was supported by comparison of the ^{13}C NMR data of the E ring of **2** with those of daphnicyclidin F.[7] Thus, the planar structure of **2** was established.

The relative configuration of **2** was assigned by the performance of a ROESY experiment (Figure 2). The ROESY correlations of H_3-21/H-4, H_3-21/H-6, H_3-21/H-11a, H-4/H-6, H-6/H-11a, H-4/H-7a, and H-6/H-7a suggested that they were cofacial, and arbitrarily assigned in a β-configuration. Consequently, the ROESY correlations of H-18/H-19b and H-19b/H-7b indicated that they were α-oriented. The ROESY correlations of H_3-20/H-2, H-2/H-3b, and H-3b/H_3-21 showed that H-2 and H-3b were β-directed.

To the best of our knowledge, alkaloid **2** is the second diamino *Daphniphyllum* alkaloid with a unique structure, and the first one was isolated from *D. paxianum*.[1g]

The biosynthetic origin of **1** could be traced back to a bukittinggine-type alkaloid, caldaphnidine P,[1j] which after hydrolysis would give intermediate **i**. The intermediate **i** would yield **1** via a cascade of chemical reactions by involving the key steps of a C-6−C-7 bond cleavage (**ii**) and a C-6−N bond formation (**iii**) as shown in Scheme 1.

The in vitro cytotoxic activities of alkaloids **1** and **2** were evaluated against HL-60 (human leukemia) and A-549 (human lung adenocarcinoma) tumor cell lines by using the MTT[8] and SRB[9] methods, respectively. All of them were inactive.

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Supporting Information Available. Experimental details and ^1H and ^{13}C NMR, EIMS, ESIMS, IR, and 2D NMR spectra of angustimine (**1**) and angustifolimine (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

(7) Kobayashi, J.; Inaba, Y.; Shiro, M.; Yoshida, N.; Morita, H. *J. Am. Chem. Soc.* **2001**, *123*, 11402–11408.

(8) Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. G.; Shoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589–601.

(9) Skehan, P. A.; Storeng, R.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107–1112.