Daphnicyclidins A−H, Novel Hexa- or Pentacyclic Alkaloids from Two Species of *Daphniphyllum*

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Abstract: Eight highly modified *Daphniphyllum* alkaloids with unprecedented fused hexa- or pentacyclic skeletons, daphnicyclidins A−H (**1**−**8**), have been isolated from the stems of *Daphniphyllum humile* and *D. teijsmanni*, and their structures were elucidated on the basis of spectroscopic data and chemical means. The stereochemistry was elucidated by combination of NOESY correlations, X-ray crystallographic data, and CD analyses.

Introduction

Daphniphyllum alkaloids are a structurally diverse group of natural products, which are elaborated by trees of the genus *Daphniphyllum* and have attracted great interest from biogenetic and synthetic points of view.[1,2] A number of *Daphniphyllum* alkaloids have been isolated and classified into six different types of backbone skeletons.[1,2] Heathcock and co-workers have demonstrated a marvelous biomimetic transformation of a dialdehyde to a pentacyclic alkaloid due to formation of seven new σ bonds.[3] Recently, we have isolated two novel types of *Daphniphyllum* alkaloids named as daphnezomines A and B[4] with a unique aza-adamantane core, and daphnezomines F and G[5] with an 1-azabicyclo[5.2.2]undecane ring system as well as some new related alkaloids[6] from the leaves or stems of *D. humile*. In our continuing search for biogenetically interesting *Daphniphyllum* alkaloids, daphnicyclidins A−H (**1**−**8**), eight novel alkaloids with unprecedented fused hexa- and pentacyclic skeletons, were isolated from the stems of *D. humile* and *D. teijsmanni*.[7] This paper describes the isolation and structural elucidation of **1**−**8**.

Results and Discussion

Isolation of Daphnicyclidins A−H (1−8). The stems of *D. teijsmanni* were extracted with MeOH, and the extract was partitioned between EtOAc and 3% tartaric acid. Water-soluble materials, which were adjusted at pH 9 with saturated Na_2CO_3, were extracted with $CHCl_3$. $CHCl_3$-soluble materials were subjected to an amino silica gel column (Hex/EtOAc, 9:1 → 1:1, and then $CHCl_3$/MeOH, 1:0 → 0:1), in which a fraction eluted with MeOH was purified by C_{18} HPLC (25% CH_3CN/0.1%TFA) and then amino silica gel HPLC (15% CH_3CN) to afford daphnicyclidins A (**1**, 0.003% yield), B (**2**, 0.0003%), C (**3**, 0.001%), D (**4**, 0.002%), and F (**6**, 0.001%) as colorless solids. Alkaloidal fractions prepared from the stems of *D. humile* described in the previous paper[4,5] were separated by the same procedure as described above to give daphnicyclidins A (**1**, 0.003% yield), B (**2**, 0.0005%), C (**3**, 0.003%), D (**4**, 0.001%), E (**5**, 0.001%), F (**6**, 0.003%), G (**7**, 0.001%), and H (**8**, 0.004%).

Structural Elucidation of Daphnicyclidins A−C (1−3). The FABMS spectrum of daphnicyclidin A (**1**) showed the pseudomolecular ion peak at *m*/*z* 368 $(M + H)^+$, and the molecular formula, $C_{22}H_{25}NO_4$, was established by HRFABMS [*m*/*z* 368.1862, $(M + H)^+$, Δ +0.0 mmu]. IR absorptions implied the presence of OH or NH (3440 cm^{-1}) and conjugated carbonyl (1680 cm^{-1}) functionalities. ^1H and ^{13}C NMR data are presented in Tables 1 and 2, respectively. The ^{13}C NMR spectrum[8] revealed 22 carbon signals due to one sp^3 quaternary carbon,

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(1) For reviews of *Daphniphyllum* alkaloids, see: (a) Yamamura, S.; Hirata, Y. In *The Alkaloids*; Manske, R. H. F., Ed., Academic Press: New York, 1975; Vol. 15, p 41. (b) Yamamura, S. In *The Alkaloids*; Brossi, A., Ed.; Academic Press: New York, 1986, Vol. 29, p 265.

(2) (a) Hao, X.; Zhou, J.; Node, M.; Fuji, K. *Yunnan Zhiwu Yanjiu* **1993**, *15*, 205−207. (b) Arbain, D.; Byrne, L. T.; Cannon, J. R.; Patrick, V. A.; White, A. H. *Aust. J. Chem.* **1990**, *43*, 185−190. (c) Yamamura, S.; Lamberton, J. A.; Niwa, M.; Endo, K.; Hirata, Y. *Chem. Lett.* **1980**, 393−396. (d) Yamamura, S.; Lamberton, J. A.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Chem. Lett.* **1975**, 923−926. (e) Yamamura, S.; Irikawa, H.; Okumura, Y.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1975**, *48*, 2120−2123. (f) Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1974**, *42*, 3673−3676. (g) Yamamura, S.; Sasaki, K.; Toka, M.; Hirata, Y. *Tetrahedron Lett.* **1974**, 2023−2026 and references therein.

(3) (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 therein.

(4) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **1999**, *64*, 7208−7212.

(5) Morita, H.; Yoshida, N.; Kobayashi, J. *J. Org. Chem.* **2000**, *65*, 3558−3562.

(6) (a) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **1999**, *55*, 12549−12556. (b) Morita, H.; Yoshida, N.; Kobayashi, J. *Tetrahedron* **2000**, *56*, 2641−2646.

(7) In the previous study, daphnilactone B, daphniteijsmanine, daphnijsmine, desacetyldaphnijsmine, and zwitterionic alkaloid have been isolated from the seeds of *D. teijsmanni*. (a) Niwa, H.; Toda, M.; Hirata, Y.; Yamamura, S. *Tetrahedron Lett.* **1972**, 2697−2700. (b) Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1974**, 2849−2852. (c) Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1974**, 3673−3676. (d) Yamamura, S.; Toda, M.; Hirata, Y. *Bull. Chem. Soc. Jpn.* **1976**, *49*, 839.

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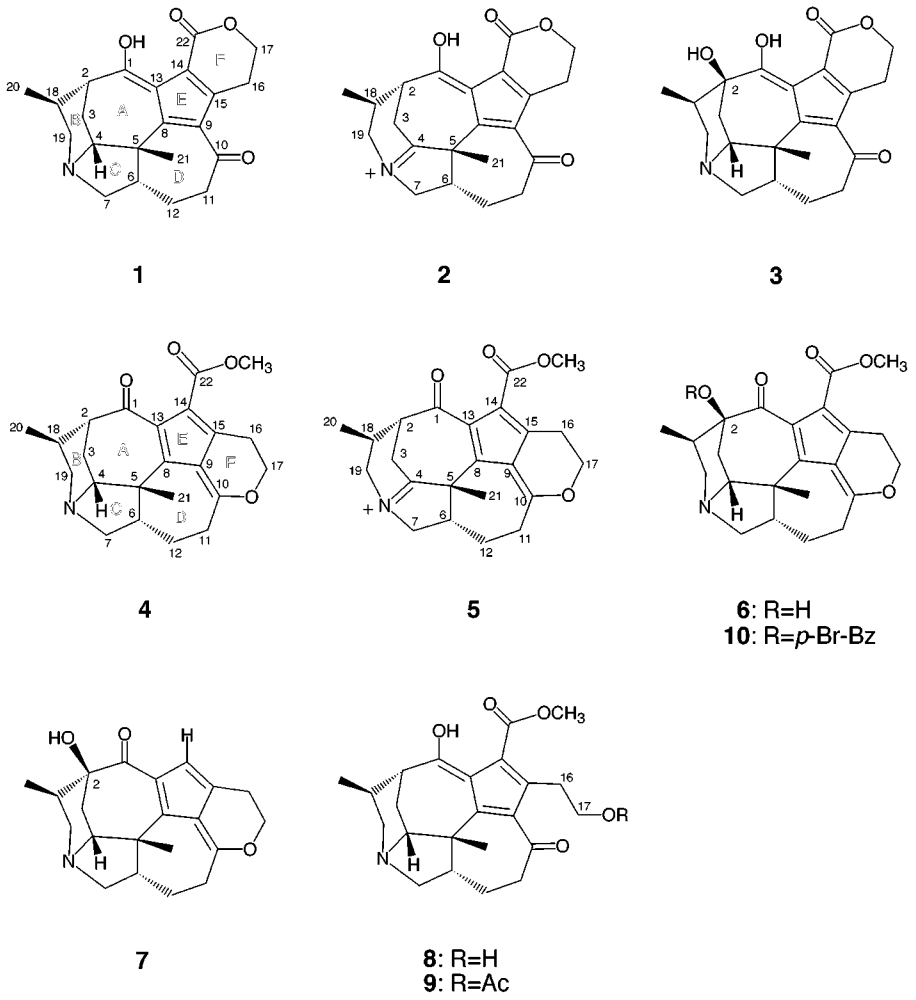


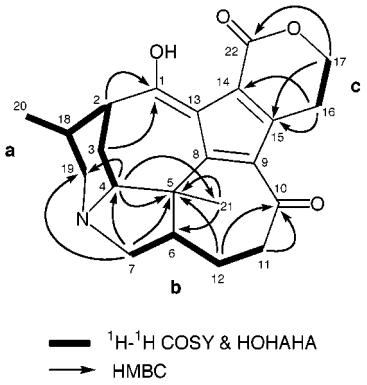


Figure 1. Selected 2D NMR Correlations for Daphnicyclidin A (**1**).

three tetra-substituted olefins, two carbonyls, four sp^3 methines, seven sp^3 methylenes, and two methyls. Among them, one methine (δ_C 65.0; δ_H 3.74) and two methylenes (δ_C 59.3; δ_H 2.52 and 4.07, δ_C 52.1; δ_H 3.05 and 3.24) were ascribed to those bearing a nitrogen.

Three partial structures **a** (from C-2 to C-4 and from C-18 to C-19 and C-20), **b** (from C-6 to C-7 and C-12 and from C-11 to C-12), and **c** (from C-16 to C-17) were deduced from extensive analyses of 2D NMR data of **1** including the $^1H-^1H$ COSY, HOHAHA,[9] HMQC, and HMBC[10,11] spectra in $CDCl_3-CD_3OD$ (9:1) (Figure 1). HMBC correlations for H_a-7 of C-4 (δ_C 65.0) and C-19 (δ_C 52.1) and for H-4 of C-19 gave rise to the connectivity of partial structures **a** and **b** through a nitrogen atom (N-1). In addition, HMBC correlations for H-4 to C-5 and C-21, H_3-21 to C-5 and C-6, and H_b-7 and H_b-12 to C-5 suggested that partial structures **a** and **b** were also connected through a quaternary carbon (C-5, δ_C 50.5) with a methyl group (C-21). HMBC correlations for H-2 and H_2-3 to C-1 (δ_C 187.0), and H_2-11 and H_2-12 to C-10 (δ_C 202.8) indicative of adjacent enolic carbon or ketone were observed.

The X-ray crystal structure (Figure 2) of daphnicyclidin A (**1**) TFA salt revealed a unique fused-hexacyclic ring system consisting of two of each five-, six-, and seven-membered rings containing a nitrogen atom and two methyls at C-5 and C-18, in which an intramolecular hydrogen bond was observed between C-1 hydroxyl proton and C-22 carbonyl oxygen.[12] The relative configurations at C-4, C-5, C-6, and C-18 were deduced from NOESY correlations of H_3-21/H-6, H_3-21/H-4, H-4/H-6, H_a-3/H_3-21, H_a-7/H_a-19, H-2/H_3-20, and H_b-3/H_3-20 together with a stable chair conformation of ring B[13] as depicted in the computer-generated 3D drawing (Figure 3).

(8) The ^{13}C NMR signals (C-1, C-8, C-9, C-13, C-14, C-15, and C-22) around ring E in **1** were detected by setting delay time to 8 s. These NMR anomalies seem to be due to long relaxation time around this ring.

(9) Edwards, M. W.; Bax, A. *J. Am. Chem. Soc.* **1986**, *108*, 918−923.

(10) (a) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093−2094. (b) Bax, A.; Aszalos, A.; Dinya, Z.; Sudo, K. *J. Am. Chem. Soc.* **1986**, *108*, 8056−8063.

(11) The HMBC correlations of **1**: H-2/C-1 and C-13, H-3/C-5, H-4/C-8, C-21, C-5, and C-19, H_3-21/C-5, C-4, C-6, and C-8, H-7/C-5, C-4, and C-19, H-19/C-7 and C-4, H-11, and H-12/C-10, H-12/C-5, H-16/C-15 and C-14, and H-17/C-15 and C-22.

(12) Distance and angle of the intramolecular hydrogen bond are followed: O1−H···O2, 1.377 Å, 166.322°.

(13) The piperidine ring (ring B) in the X-ray structure adopts a chairlike conformation: internal torsion angles in the ring B are −46.0(4), −52.1(4), −55.1(4), 45.9(4), 55.4(4), and 51.7(5)°.

Table 1. ^{1}H NMR Data (δ_H) of Daphnicyclidins A−H (**1**−**8**) at 300 K

proton	**1**[a]	**2**[c]	**3**[c]	**4**[a]
2	2.87 (1H, brs)	3.13 (1H, brs)		2.58 (1H, brs)
3a	2.26 (1H, d, 15.8)	3.69 (1H, dd, 2.4, 13.8)	2.45 (2H, m)	2.12 (1H, d, 15.4)
3b	2.45 (1H, dt, 15.8, 6.0)	2.77 (1H, m)		2.40 (1H, m)
4	3.74 (1H, d, 6.7)		3.95 (1H, d, 7.2)	3.77 (1H, m)
6	2.64 (1H, m)	2.97 (1H, m)	2.81 (1H, m)	2.58 (1H, m)
7a	2.52 (1H, dd, 8.7, 12.2)	3.61 (1H, brt, 11.4)	2.81 (1H, m)	2.53 (1H, m)
7b	4.07 (1H, brt, 11.3)	4.43 (1H, dd, 8.7, 13.6)	4.11 (1H, m)	3.96 (1H, m)
11a	2.61 (2H, m)	2.57 (1H, dd, 6.6, 18.0)	2.66 (1H, dd, 3.3, 19.7)	2.60 (1H, dd, 5.5, 18.6)
11b		2.79 (1H, m)	2.74 (1H, dd, 13.0, 19.7)	2.73 (1H, dt, 13.0, 18.6)
12a	1.67 (1H, m)	1.55 (1H, m)	1.79 (1H, m)	1.46 (1H, m)
12b	2.11 (1H, m)	2.18 (1H, m)	2.19 (1H, m)	2.24 (1H, m)
16a	2.69 (1H, dt, 18.8, 5.1)	2.81 (1H, m)	2.81 (1H, m)	2.67 (1H, dd, 4.9, 17.0)
16b	3.27 (1H, m)	2.89 (1H, ddd, 5.0, 13.8, 18.3)	3.28 (1H, ddd, 5.7, 9.2, 18.1)	2.96 (1H, dd, 3.0, 17.0)
17a	4.38 (1H, dt, 4.7, 9.8)	4.20 (1H, ddd, 3.7, 10.5, 13.8)	4.46 (1H, m)	4.04 (1H, ddd, 3.0, 11.0, 14.5)
17b	4.51 (1H, m)	4.39 (1H, dd, 5.0, 10.5)	4.59 (1H, m)	4.57 (1H, dd, 5.4, 11.0)
18	2.24 (1H, m)	2.56 (1H, m)	2.24 (1H, m)	2.24 (1H, m)
19a	3.05 (1H, dd, 3.4, 12.8)	4.10 (1H, dd, 7.7, 14.2)	3.36 (1H, dd, 2.9, 13.2)	3.13 (2H, m)
19b	3.24 (1H, brd, 13.3)	3.32 (1H, dd, 9.1, 14.2)	3.47 (1H, brd, 13.2)	
20	1.32 (3H, d, 7.3)	1.33 (3H, d, 6.9)	1.32 (3H, d, 7.3)	1.17 (3H, d, 6.7)
21	1.42 (3H, s)	1.82 (3H, s)	1.50 (3H, s)	1.34 (3H, s)
22-OMe				3.66 (3H, s)

proton	**5**[c]	**6**[a]	**7**[c]	**8**[b]
2	3.17 (1H, brs)		2.36 (1H, d, 15.5)	2.54 (1H, m)
3a	3.72 (1H, m)	2.06 (1H, d, 15.7)	2.43 (1H, dd, 7.1, 15.5)	2.35 (2H, m)
3b	2.90 (1H, m)	2.50 (1H, brd, 15.7)	3.93 (1H, d, 7.0)	
4		4.05 (1H, m)	2.77 (1H, m)	3.58 (1H, t, 3.8)
6	3.08 (1H, m)	2.73 (1H, m)	2.88 (1H, dd, 9.2, 12.6)	2.61 (1H, m)
7a	3.76 (1H, m)	2.57 (1H, m)	4.00 (1H, dd, 10.1, 12.6)	2.77 (1H, m)
7b	4.52 (1H, dd, 8.8, 13.7)	4.01 (1H, m)	2.71 (1H, dd, 5.5, 18.5)	3.97 (1H, dd, 10.7, 12.3)
11a	2.77 (1H, dd, 6.4, 18.9)	2.68 (1H, m)	2.94 (1H, dd, 12.2, 18.5)	2.58 (1H, m)
11b	3.12 (1H, m)	2.85 (1H, m)	1.65 (1H, m)	2.63 (1H, m)
12a	1.49 (1H, m)	1.51 (1H, m)	2.35 (1H, m)	1.84 (1H, m)
12b	2.42 (1H, m)	2.35 (1H, m)	6.51 (1H, br s)	2.10 (1H, m)
16a	2.91 (1H, m)	2.76 (1H, m)	2.81 (1H, m)	2.76 (1H, m)
16b	3.00 (1H, dd, 4.0, 18.7)	3.19 (1H, dd, 3.0, 17.6)	2.83 (1H, m)	2.84 (1H, m)
17a	4.27 (1H, ddd, 4.0, 10.9, 15.0)	4.15 (1H, ddd, 3.0, 11.0, 14.9)	4.16 (1H, ddd, 5.9, 10.8, 11.2)	3.70 (2H, m)
17b	4.80 (1H, dd, 5.3, 10.9)	4.67 (1H, dd, 5.3, 11.0)	4.68 (1H, ddd, 1.8, 4.8, 10.8)	
18	2.49 (1H, m)	2.29 (1H, m)	2.07 (1H, m)	2.26 (1H, m)
19a	4.11 (1H, dd, 7.1, 14.0)	3.28 (1H, m)	3.44 (1H, br d, 11.4)	3.20 (1H, dd, 1.6, 13.0)
19b	3.41 (1H, dd, 9.5, 14.0)	3.36 (1H, m)	3.37 (1H, dd, 4.5, 13.3)	3.62 (1H, dd, 4.1, 13.0)
20	1.33 (3H, d, 6.8)	1.15 (3H, d, 6.7)	1.21 (3H, d, 7.1)	1.29 (3H, d, 7.4)
21	1.84 (3H, s)	1.41 (3H, s)	1.49 (3H, s)	1.45 (3H, s)
22-OMe	3.73 (3H, s)	3.76 (3H, s)		3.72 (3H, s)

[a] $CDCl_3/CD_3OD$ (9:1). [b] $CDCl_3/CD_3OD$ (1:9). [c] CD_3OD

Table 2. ^{13}C NMR Data (δ_C) of Daphnicyclidins A−H (**1**−**8**) at 300 K

carbon	**1**[a]	**2**[c]	**3**[c]	**4**[a]	**5**[c]	**6**[a]	**7**[c]	**8**[b]
1	187.0	199.1	190.9	197.4	197.3	196.7	199.0	196.9
2	43.3	50.4	73.6	47.3	54.0	72.9	74.3	48.1
3	16.8	27.2	25.6	16.8	26.6	26.2	25.8	18.0
4	65.0	200.8	69.5	65.3	197.5	66.7	69.7	68.8
5	50.5	60.9	50.8	51.2	61.1	51.0	52.2	51.3
6	47.9	46.3	49.5	47.4	45.6	47.6	49.0	49.5
7	59.3	65.2	60.5	59.1	65.0	58.5	60.1	61.2
8	146.7	135.6	145.9	137.3	134.4	138.9	140.7	132.7
9	132.6	123.1	131.8	120.8	120.3	120.9	124.5	126.9
10	202.8	203.2	204.9	180.6	184.9	181.5	180.9	204.5
11	39.0	40.8	40.2	31.0	32.1	31.0	32.0	40.5
12	27.1	27.5	28.2	29.7	29.3	29.1	31.2	29.5
13	117.4	122.8	118.8	134.4	133.8	131.1	134.0	123.4
14	113.1	113.9	114.0	122.9	122.5	123.1	118.4	123.1
15	149.3	145.0	149.9	130.2	133.5	131.7	127.6	131.6
16	22.8	26.2	24.6	22.4	24.4	22.6	24.5	30.8
17	68.7	69.6	70.0	69.1	71.7	69.3	71.2	65.3
18	29.8	36.9	36.3	27.7	36.4	32.9	35.6	30.4
19	52.1	54.2	56.9	52.4	54.0	52.8	55.6	55.2
20	16.1	19.3	11.8	16.9	18.7	12.0	12.2	17.1
21	34.8	28.9	34.9	33.3	27.0	32.9	34.4	36.1
22	169.7	168.9	171.1	167.3	167.9	166.7		174.2
22-OMe				51.5	52.1	51.8		52.1

[a] $CDCl_3/CD_3OD$ (9:1). [b] $CDCl_3/CD_3OD$ (1:9). [c] CD_3OD

The FABMS spectrum of daphnicyclidin B (**2**) showed the molecular ion peak at *m/z* 366 (M^+), and the molecular formula was inferred as $C_{22}H_{24}NO_4$ by HRFABMS [*m/z* 366.1702 (M^+), Δ −0.3 mmu]. The IR spectrum was indicative of the presence of OH (3440 cm^{-1}), conjugated carbonyl and imine (1680 and 1630 cm^{-1}) functionalities. ^{13}C NMR data (Table 2) including

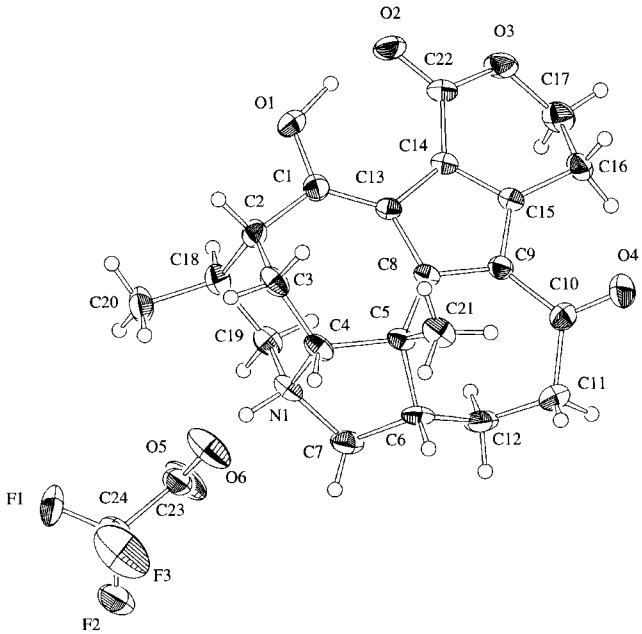


Figure 2. Molecular structure of daphnicyclidin A (**1**) TFA salt obtained by X-ray analysis (ORTEP drawing; ellipsoids are drawn at the 30% probability level).

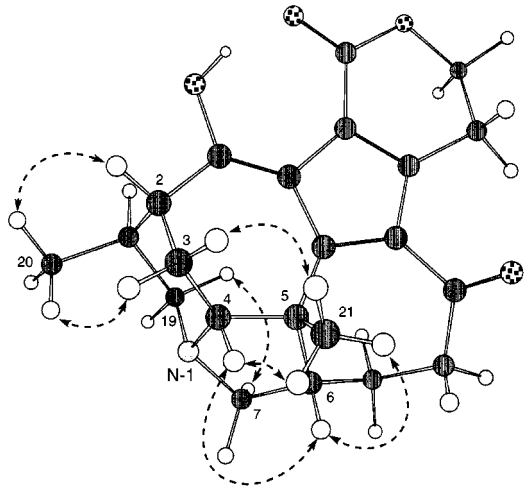


Figure 3. Selected NOESY correlations (dotted arrows) and relative configurations for daphnicyclidin A (**1**).

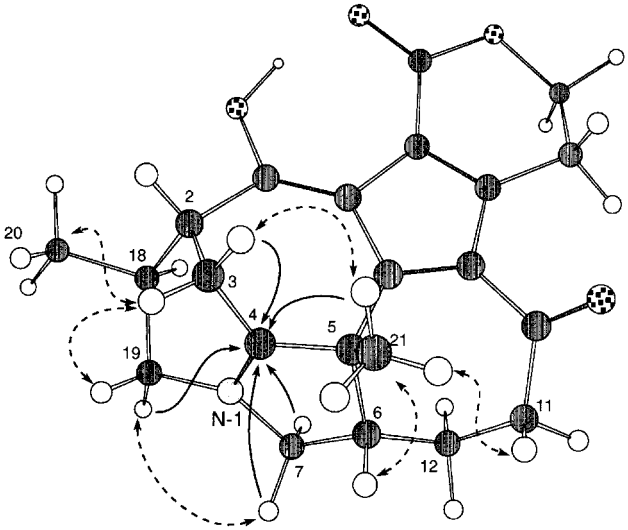


Figure 4. Key HMBC (arrows) and NOESY correlations (dotted arrows) for daphnicyclidin B (**2**).

DEPT experiments revealed that the chemical shift [δ_C 200.8 (s)] of C-4 was remarkably shifted to lower field as compared with that [δ_C 65.0 (d)] of **1**. The presence of an iminium carbon[14] (C-4) was elucidated by HMBC correlations for H_3-21 and H_b-3 to C-4, and H_2-7 and H_a-19 to C-4 though a nitrogen atom (Figure 4). In addition, the ^{1}H and ^{13}C signals at 3-, 5-, 7-, and 19-positions around the imine functionality were observed at lower field due to deshielding effects (Tables 1 and 2). 2D NMR data of **2** including the ^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC[15] spectra corroborated well with those of the imine (C-4 and N-1) form of **1**. The relative stereochemistry and ring conformation[16] of **2** elucidated by NOESY correlations (Figure 4) were almost same as those of **1**. Thus, daphnicyclidin B (**2**) was concluded to be the imine (C-4 and N-1) form of daphnicyclidin A (**1**).

The FABMS spectrum of daphnicyclidin C (**3**) showed the pseudomolecular ion peak at *m*/*z* 384, and the molecular formula

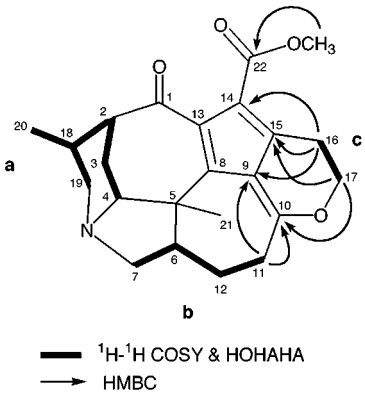


Figure 5. Selected 2D NMR correlations for daphnicyclidin D (**4**).

was indicated to be $C_{22}H_{25}NO_5$ by HRFABMS [*m*/*z* 384.1821, $(M + H)^+$, Δ +1.0 mmu], which was larger than that of daphnicyclidin A (**1**) by one oxygen atom. The ^{1}H and ^{13}C NMR (Tables 1 and 2) spectra of daphnicyclidin C (**3**) were almost the same as those of daphnicyclidin A (**1**), except for the downfield shift of the C-2 carbon (δ_C 73.6) and the lack of a methine signal assignable to H-2. HMBC correlations for H_2-2, H-4, H_3-20, and H_b-19 to C-2, and extensive 2D NMR data indicated that daphnicyclidin C (**3**) was the 2-hydroxy form of daphnicyclidin A (**1**).

Structural Elucidation of Daphnicyclidins D−G (4−7). HRFABMS data [*m*/*z* 382.2029, $(M + H)^+$, Δ +1.1 mmu] of daphnicyclidin D (**4**) established the molecular formula to be $C_{23}H_{27}NO_4$, which was larger than that of daphnicyclidin A (**1**) by a CH_2 unit. ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **4** were analogous to those of **1** in the left-hand part, consisting of rings A to C with a nitrogen atom, except that a methoxy signal (δ_H 3.76) lacking for **1** was observed for **4**. The structure of **4** was elucidated by 2D NMR (^{1}H−^{1}H COSY, HOHAHA, HMQC, and HMBC) data. HMBC cross-peaks of H_2-11 and H_b-17 to C-10 (δ_C 180.6) indicated that C-10 and C-17 (δ_C 69.1) were connected to each other through an oxygen atom to form a dihydropyran ring (Figure 5). The presence of a methoxy group at C-22 was suggested by the HMBC correlation for H_3−OCH_3 to C-22 (δ_C 167.3). In addition, the presence of a conjugated cyclopentadiene moiety (C-8∼C-9 and C-13∼C-15) like **1** was suggested by HMBC correlations for H_2-16 to C-9, C-14, and C-15, H_b-17 to C-15, and H_a-11 to C-9. Treatment of **1** with methanolic *p*-TsOH gave daphnicyclidin D (**4**) (Scheme 1). Thus, the structure of daphnicyclidin D was assigned as **4**.

HRFABMS data [*m*/*z* 380.1853, $(M)^+$, Δ −0.9 mmu] of daphnicyclidin E (**5**) established the molecular formula, $C_{23}H_{25}NO_4$. The IR spectrum was indicative of the presence of conjugated carbonyl and/or imine (1680 cm^{-1}) functionalities. ^{1}H and ^{13}C NMR data (Tables 1 and 2) of **5** were analogous to those of daphnicyclidin D (**4**). The presence of an iminium carbon [C-4, δ_C 197.5 (s)] was elucidated by HMBC correlations for H_3-21 and H_b-3 to C-4, and H_2-7 and H_a-19 to C-4 through a nitrogen atom. Treatment of **5** with $NaBH_4$ afforded daphnicyclidin D (**4**) (Scheme 1). Thus, daphnicyclidin E (**5**) was concluded to be the imine form at C-4 of daphnicyclidin D (**4**).

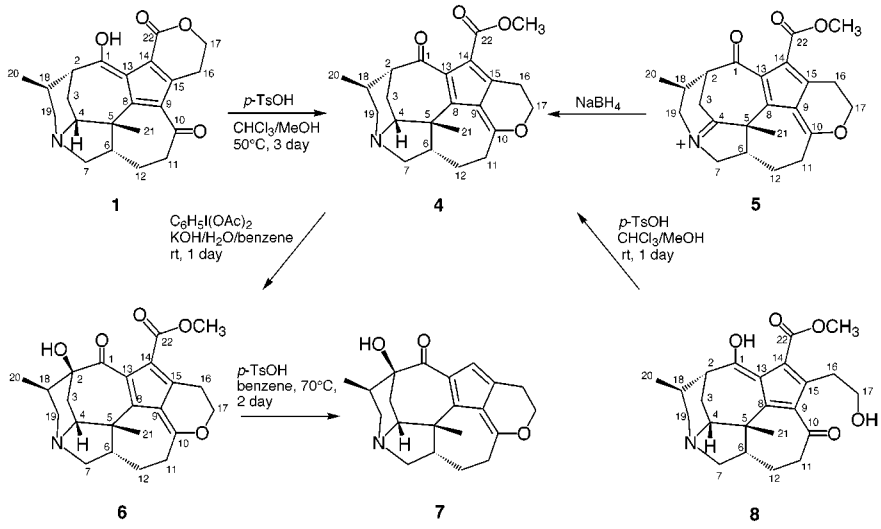
Daphnicyclidin F (**6**) had the molecular formula of $C_{23}H_{27}NO_5$ as revealed by HRFABMS data [*m*/*z* 398.1950, $(M + H)^+$, Δ −1.7 mmu]. IR absorptions at 3430 and 1680 cm^{-1} indicated

(14) Iminium carbons have been observed at ca. 200 ppm. Some examples; Uemura, D.; Chou, T.; Haino, T.; Nagatsu, A.; Fukuzawa, S.; Zheng, S.-z.; Chem, H.-s. *J. Am. Chem. Soc.* **1995**, *117*, 1155−1156: Chou, T.; Haino, T.; Kuramoto, M.; Uemura, D. *Tetrahedron Lett.* **1996**, *37*, 4027−4030.

(15) The HMBC correlations of **2**: H-2 and H-3/C-1, H-3, H-19, H-7, and H_3-21/C-4, H-7, H_3-21, and H-12/C-5, H_3-21/C-6, H-6 and H_3-21/C-8, H-11 and H-12/C-10, H-16 and H-17/C-15, and H-17/C-22.

(16) Conformational search and molecular mechanics calculations were conducted by the *Macromodel* program: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467. All conformers took a bort form in the ring A.

Scheme 1. Chemical Correlations for Daphnicyclidins A (**1**) and D−H (**4**−**8**)



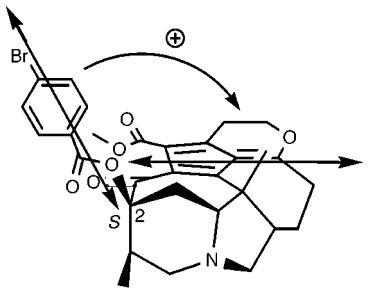


Figure 6. Stereostructure of *p*-bromo benzoate (**10**) of daphnicyclidin F (**6**). Arrows denote the electric transition dipole of the chromophore.

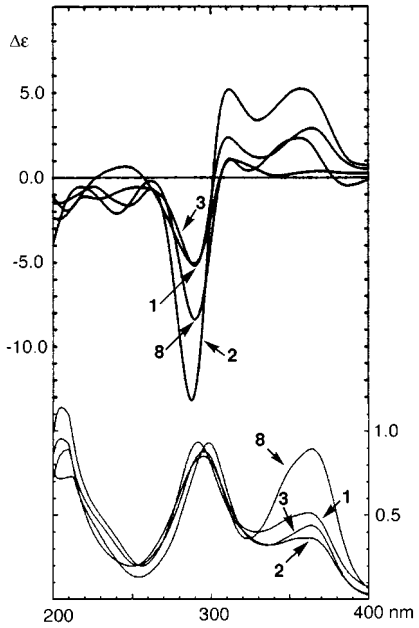


Figure 7. CD and UV spectra of daphnicyclidins A (**1**), B (**2**), C (**3**), and H (**8**).

the presence of hydroxyls and conjugated carbonyl groups, respectively. ^{1}H and ^{13}C NMR data (Tables 1 and 2) disclosed that **6** possessed a hydroxyl at C-2 (δ_C 72.9) as compared with those of daphnicyclidin D (**4**). HMBC and NOESY correlations[17] indicated the proposed structure for **6**. Treatment of **4** with iodosobenzene acetate[18] for 2 days afforded daphnicyclidin F (**6**) (Scheme 1). Thus, daphnicyclidin F (**6**) was assigned as the 2-hydroxy form of daphnicyclidin D (**4**).

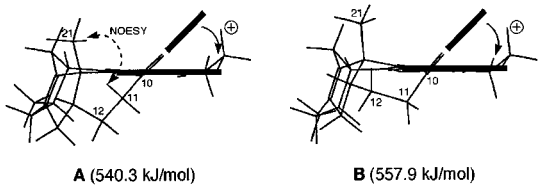


Figure 8. Two representative stable conformers (A and B) of daphnicyclidin B (**2**) analyzed by Monte Carlo simulation followed by minimization and clustering analysis. Conformer A indicated by a NOESY correlation of Hβ-11/H$_3$-21 in solution is energetically stabler than conformer B (more than 17 kJ/mol). Bold lines denote the electric transition dipole of the chromophore. The alternative conformation would provide the same positive chirality.

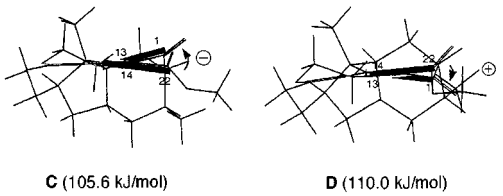


Figure 9. Two representative stable conformers (C and D) of daphnicyclidin D (**4**) analyzed by Monte Carlo simulation followed by minimization and clustering analysis. Conformer C is energetically stabler than conformer D (more than 4 kJ/mol). Bold lines denote the electric transition dipole of the chromophore.

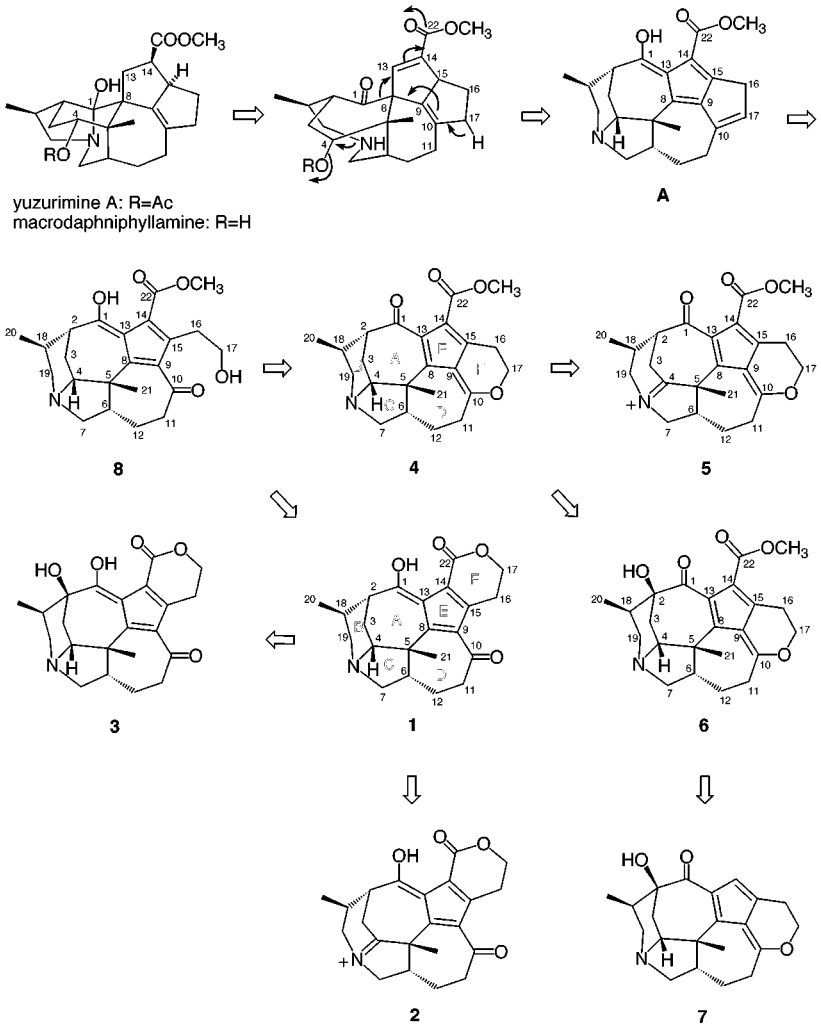
HRFABMS data [*m*/*z* 340.1920, $(M + H)^+$, Δ +0.7 mmu] of daphnicyclidin G (**7**) revealed the molecular formula, $C_{21}H_{25}NO_3$, which was smaller than that of daphnicyclidin F (**6**) by a $C_2H_2O_2$ unit. Though ^{1}H and ^{13}C NMR data (Tables 1 and 2) were close to those of daphnicyclidin F (**6**), differences were observed for the methoxy carbonyl moiety. Daphnicyclidin F (**6**) possessed a methoxy carbonyl moiety at C-14, while ^{1}H and ^{13}C NMR data of **7** showed signals due to an sp^2 methine [δ_H 6.51 (1H, s), δ_C 118.4 (d)]. Treatment of **6** with *p*-TsOH at 70 °C for 2 days gave daphnicyclidin G (**7**) (Scheme 1). Therefore, daphnicyclidin G (**7**) was elucidated to be the demethoxycarbonyl form at C-14 of daphnicyclidin F (**6**).

Structure of Daphnicyclidin H (8). Daphnicyclidin H (**8**) had the molecular formula of $C_{23}H_{29}NO_5$ as revealed by

(17) Key HMBC and NOESY correlations of **5** are followed: HMBC (H-3/C-1, H-3 and H$_3$-20/C-2, H$_3$-21/C-4, C-5, and C-6, H-11/C-6, H$_3$-21/C-8, H-16/C-9, H-11/C-9, C-10, and C-12, H-17/C-10, H-16/C-14, C-15, and C-17, H-17/C-15, H-3 and H$_3$-20/C-18, H$_3$-20/C-19, and H−OMe/C-22); NOESY (H-3/H$_3$-20, H-4 and H-6/H$_3$-21, and H-4/H-6).

(18) Moriarty, R. M.; Hu, H. *Tetrahedron Lett.* **1981**, *22*, 2747−2750.

Scheme 2. Plausible Biogenetic Path of Daphnicyclidins A−H (**1**−**8**)



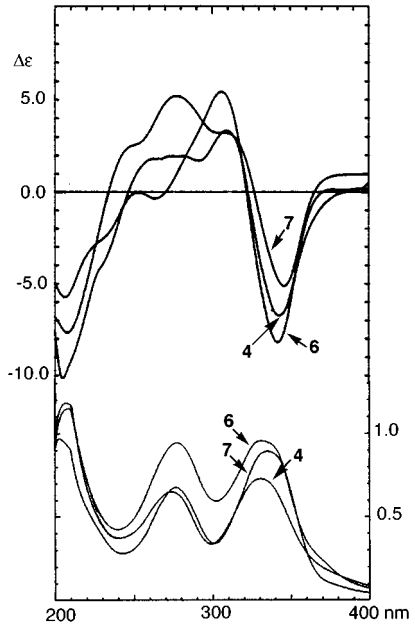


Figure 10. CD and UV spectra of daphnicyclidins D (**4**), F (**6**), and G (**7**).

HRFABMS data [*m*/*z* 400.2140, $(M + H)^+$, Δ +1.6 mmu]. IR absorptions at 3435 and 1680 cm^{-1} indicated the presence of hydroxyls and conjugated carbonyl groups, respectively. The ^1H NMR (Table 1) signals assignable to H_2-17 (δ_H 3.70) were observed equivalently as compared with those of **1**−**7**. Treatment of **8** with acetic anhydride afforded the monoacetate (**9**), in which the hydroxyl group at C-17 was acetylated. On the other hand, the presence of a methoxy carbonyl group at C-14 and rings A∼E with a ketone at C-10 (δ_C 204.5) was deduced from 2D NMR analysis.[19,20] The 2D NMR data indicated that the conjugated keto−enol moiety of **8** was the same as that of daphnicyclidin A (**1**). Treatment of daphnicyclidin H (**8**) with *p*-TsOH gave daphnicyclidin D (**4**) (Scheme 1).[21] Thus, the structure of **8** was assigned as shown.

Absolute Stereochemistry of Daphnicyclidins A−H (1−8). Absolute stereochemistry of daphnicyclidin F (**6**) was analyzed by applying exciton chirality method[22] after introduction of *p*-bromobenzoyl chromophore into the hydroxyl group at C-2. As the sign of the first Cotton effect [λ_{max} 280 (θ +20000) and 225 (−16000) nm] was positive, the chirality between the cyclopentene moiety and the benzoate group of the *p*-bromobenzoyl derivative (**10**) of **6** was assigned as shown

(19) The HMBC correlations of **6**: H-2 and H-3/C-1, H-3, H-4, and H-19/C-2, H-3 and H-7/C-5, H-7, and H-11/C-6, H-4/C-8, H-16/C-9, H-11, and H-12/C-10, H-6, and H-7/C-12, H-16/C-14 and C-15, H-17/C-15, H-3, H-19, and H_3-20/C-18, H-2, and H-7/C-19, H-19/C-20, and H−OMe/C-22.

(20) Acid-catalyzed chemical conversion of daphnicyclidin H (**8**) under this condition afforded daphnicyclidin D (**4**) but not daphnicyclidin A (**1**).

(21) The NOESY correlations of **8**: H-3 and H-2/H_3-20, H-7/H-19, H-3, H-4, and H-6/H_3-21 and H-4/H-6.

(22) Harada, N.; Nakanishi, K.; Tatsuoka, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896−5898.

in Figure 6 (right-handed screw), indicating the absolute stereochemistry at C-2 was *S*.

The absolute stereochemistry of dapnicyclidins A (**1**), B (**2**), C (**3**), D (**4**), G (**7**), and H (**8**) was examined by CD spectroscopy.[23] Daphnicyclidins A (**1**), B (**2**), C (**3**), and H (**8**) with a ketone at C-10 exhibited Cotton effects centered at 290 nm corresponding to the UV maximum of a cyclopentene group conjugated with a ketone at C-22. This transition was coupled with the ketone transition at C-10, and consequently the CD spectra had two Cotton effects at 320 and 285 nm (Figure 7). The positive sign of the first Cotton effect was coincident with the chirality between the long axis of the conjugate cyclopentene moiety and the ketone group at C-10. This helicity was essentially independent of their conformations as described (Figure 8) in the case of daphnicyclidin B (**2**). On the other hand, in daphnicyclidins D (**4**), F (**6**), and G (**7**), the two long axes of the fulvene moiety conjugated with the ketones at C-1 and C-22 were coupled to each other. The conformational space for **4** was searched using the MMFF force field implemented in the Macromodel program.[16] The lowest-energy conformers belonging to two separate clusters were represented as conformers C and D (Figure 9). Conformer C was energetically more stable than conformer D (>4 kJ/mol). As the sign of the first Cotton effect at 345 nm was negative (Figure 10), the long axis transitions of the two chromophores coupled as depicted in Figure 9 (negative chirality), and consequently the absolute configurations of **4**, **6**, and **7** were elucidated.

Plausible Biogenesis of Daphnicyclidins A−H (1−8). Daphnicyclidins A−G (**1**−**7**) and H (**8**) are novel types of natural products consisting of fused hexa- or pentacyclic ring system, respectively. A plausible biogenetic pathway for daphnicyclidins A−H (**1**−**8**) is proposed as shown in Scheme 2. The biogenetic origin of them seems to be yuzurimine-type alkaloids such as yuzurimine A[24] and macrodaphniphyllamine[25] with an appropriate leaving group at C-4 and a methyl group at C-21. Rings B and C might be constructed by loss of the leaving group at C-4 followed by N-1−C-4 bond formation. Subsequently, cleavage of C-1−C-8 bond followed by formation of C-1−C-13 bond would result in enlargement of ring A, and aromatization of ring E to generate an intermediate **A**. Furthermore, oxidative cleavage of C-10−C-17 bond could lead to daphnicyclidin H (**8**), followed by cyclization and dehydration to produce daphnicyclidin D (**4**), which may be oxidized to give daphnicyclidins E (**5**) and F (**6**). On the other hand, cyclization of 17-OH to C-22 in **8** to form ring F would generate daphnicyclidins A (**1**), B (**2**), and C (**3**).

Cytotoxicity of Daphnicyclidins A−H (1−8). Daphnicyclidins A−H (**1**−**8**) exhibited cytotoxicity against murine lymphoma L1210 cells (IC_{50}, 0.8, 0.1, 3.0, 1.7, 0.4, 4.3, 4.2, and 0.5 μg/mL, respectively) and human epidermoid carcinoma KB cells (IC_{50}, 6.0, 2.6, 7.2, 4.6, 5.2, 7.6, >10, and 0.9 μg/mL, respectively) in vitro.

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Supporting Information Available: Experimental procedures, 1D and 2D NMR spectra for compounds **1**−**8**, and X-ray crystallographic data of **1** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(23) The CD spectra of daphnicyclidins E (**5**) showed different Cotton curves (**5**, $\Delta\epsilon_{320}$ −2.5 and $\Delta\epsilon_{265}$ +2.0) from those of daphnicyclidins D (**4**), F (**6**), and G (**7**).

(24) Sakurai, H.; Irikawa, H.; Yamamura, S.; Hirata, Y. *Tetrahedron Lett.* **1967**, 2883−2888; Irikawa, H.; Yamamura, S.; Hirata, Y. *Tetrahedron* **1972**, *28*, 3727−3738.

(25) Nakano, T.; Saeki, Y. *Tetrahedron Lett.* **1967**, 4791−4797.