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Daphmacromines A−J, Alkaloids from *Daphniphyllum macropodum*

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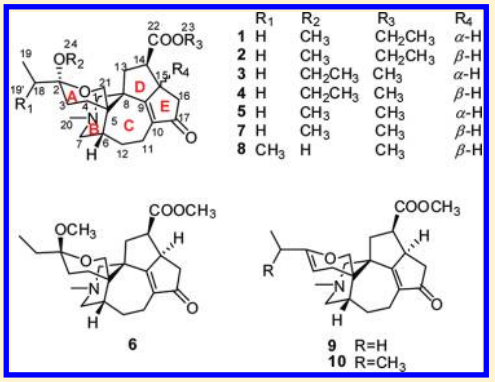
Supporting Information

ABSTRACT: Ten new yuzurine-type *Daphniphyllum* alkaloids, daphmacromines A−J (**1**−**10**), along with seven known alkaloids were isolated from the leaves and stems of *Daphniphyllum macropodum*. Their structures were elucidated by extensive spectroscopic techniques, including 2D NMR spectroscopy and mass spectrometry, and the structure of **1** was confirmed by single-crystal X-ray diffraction. The pesticidal and cytotoxic activities of the isolated alkaloids were evaluated in vitro against brine shrimp (*Artemia salina*) and five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480), respectively. This study also suggested structural revisions of oxodaphnigracine, oxodaphnigraciline, and epioxodaphnigraciline.



Daphniphyllum alkaloids are a family of structurally diverse natural products with complex polycyclic systems elaborated by plants of the genus *Daphniphyllum*.[1] Their unique structural features have attracted great interest as challenging targets for total synthesis[2] and biosynthetic research[3] for several decades. In recent years, quite a number of new *Daphniphyllum* alkaloids have been isolated and identified, and some of them possessed novel skeletons.[4] Our previous studies on *Daphniphyllum macropodum* have resulted in the isolation of a series of novel alkaloids and a new oxa-caged compound.[5] In our further search for structurally unique *Daphniphyllum* alkaloids, 10 new *Daphniphyllum* alkaloids, daphmacromines A−J (**1**−**10**), together with seven known ones were isolated from the leaves and stems of *D. macropodum*. We report herein the structural elucidation and the pesticidal and cytoxic activities of these alkaloids.

RESULTS AND DISCUSSION

Daphmacromine A (**1**) was obtained as colorless tetragonal crystals (acetone) with $[\alpha]_D^{22}$ −61 (*c* 0.2, MeOH). The molecular formula, $C_{25}H_{37}NO_5$, was established by positive HRESIMS (*m*/*z* 432.2758 $[M + H]^+$, calcd 432.2749), corresponding to eight degrees of unsaturation. IR absorptions indicated the presence of ester carbonyl (1729 cm^{-1}) and α,β-unsaturated carbonyl (1694 and 1662 cm^{-1}) groups. The ^{13}C NMR and DEPT data (Table 1) revealed 25 carbon signals including one tetrasubstituted double bond, one ketocarbonyl group, one ester carbonyl, three sp^3 quaternary carbons, four methines, 11 methylenes, and four methyls. Among them, two methylene groups (δ_C 62.4 and 55.6) and one methyl group (δ_C 46.1) were typical of nitrogenated carbons.

Inspection of the NMR data of **1** (Tables 1 and 2) indicated that its structure was related to yuzurine-type alkaloids.[1d] Analysis of 2D NMR spectra (HSQC, $^1H-^1H$ COSY, and HMBC) (Supporting Information) confirmed the above deduction and established the linkages of subunits **a**−**e** (Figure 2) with the quaternary carbon atoms and heteroatoms. The location of the α,β-unsaturated carbonyl moiety was determined by HMBC correlations of H_2-16 (δ_H 2.48, m, 2H) with C-9 (δ_C 183.7), C-10 (138.2), and C-17 (209.2). The HMBC cross-peaks of H_2-23 (δ_H 4.08, q, *J* = 7.1, 2H) to C-22 (δ_C 173.2) and a methyl carbon (δ_C 14.2) indicated the presence of an ethyl ester in **1**. Moreover, an O-methyl group (δ_C 98.9) was part of the acetal functionality, assigned as C-2 by HMBC correlation of H_3-OMe (δ_H 3.11, s, 3H) with C-2. The gross structure of daphmacromine A (**1**) was thus elucidated as indicated.

The relative configuration of **1** was defined by ROESY experiments (Supporting Information) and was consistent with yuzurine-type *Daphniphyllum* alkaloids. The ROESY cross-peaks of H-21b/H-4a, H-21b/H-13a, H-21a/H-12a, H-7a/H-1b, and H-6/H-3a indicated a chair conformation for rings A and B and a half-chair conformation for ring C, as well as the relative configuration shown in Figure 1. To confirm the structure and determine its absolute configuration, **1** was crystallized from acetone to afford a crystal of the tetragonal space group $P4_1$, which was analyzed by X-ray crystallography. The final refinement of the Cu Kα data resulted in a Flack[6] parameter of 0.12(19) and the Hooft[7] parameter of 0.06(8),

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Table 1. ^{13}C NMR Spectroscopic Data for Daphmacromines A–J (1–10)

position	1[a,c]	2[b,c]	3[a,c]	4[b,d]	5[a,d]	6[a,c]	7[b,d]	8[b,c]	9[b,e]	10[b,c]
1	62.4	61.3	62.3	60.9	62.4	63.3	61.3	61.9	63.2	62.7
2	98.9	99.4	99.9	99.4	98.9	102.1	99.3	98.0	156.3	159.5
3	27.6	27.6	27.7	27.6	27.7	27.5	27.5	25.3	93.1	91.0
4	22.6	21.8	22.6	21.8	22.5	26.0	21.7	22.0	28.4	27.9
5	36.8	35.7	36.8	35.7	36.8	39.0	35.6	35.9	37.3	37.4
6	32.7	33.0	32.7	33.0	32.7	37.9	33.0	32.9	35.2	34.9
7	55.6	55.7	55.5	55.8	55.5	55.6	55.7	55.8	56.6	56.1
8	50.1	50.1	50.2	50.0	50.0	52.1	50.0	50.1	50.3	50.5
9	183.7	184.9	183.6	184.9	183.4	185.2	184.4	184.8	183.7	183.7
10	138.2	139.5	138.2	139.4	138.2	138.2	139.5	135.3	138.3	138.3
11	21.0	22.4	21.0	22.5	20.9	20.7	22.4	22.4	22.2	21.7
12	26.1	27.9	26.1	27.9	26.1	26.3	27.9	27.9	27.0	26.6
13	37.9	38.7	38.0	38.8	38.0	39.6	38.8	39.0	38.6	38.2
14	42.4	45.9	42.5	46.0	42.4	41.7	45.9	45.7	43.1	42.8
15	44.7	50.3	44.6	50.4	44.5	44.6	50.3	50.3	45.5	45.3
16	38.3	42.5	38.4	42.4	38.2	37.9	42.3	42.2	39.1	38.7
17	209.2	207.7	209.2	207.7	209.1	210.0	207.3	207.3	208.1	208.0
18	28.6	29.0	29.4	29.1	28.4	26.0	29.0	39.2	27.3	32.3
19	7.8	7.9	7.7	7.9	7.7	7.6	7.9	17.6[f]	11.6	20.4[f]
19′								17.1[f]		20.6[f]
20	46.1	45.7	46.1	45.7	46.0	45.9	45.6	45.9	46.0	46.1
21	62.5	62.1	62.3	62.0	62.4	65.2	62.0	61.3	69.6	69.4
22	173.2	174.0	173.7	174.3	173.5	173.8	174.2	174.2	174.2	174.2
23	60.4									
14.2	60.9									
14.6	51. 2	51.7	51.5	51.4	51.8	51.8	51.3	51.2		
24	47.0	47.1	54.4							
15.2	54.9									
15.8	47.0	47.8	47.1							

[a]Recorded in $CDCl_3$. [b]Recorded in pyridine-d_5. [c]Recorded at 100 MHz. [d]Recorded at 125 MHz. [e]Recorded at 150 MHz. [f]Assignments are interchangeable.

which allowed unambiguous assignment of the absolute configuration of **1** as (2*R*, 5*R*, 6*R*, 8*S*, 14*R*, 15*R*) (Figure 3).

Daphmacromine B (**2**) has the same molecular formula as **1** ($C_{25}H_{37}NO_5$), as deduced from HRESIMS (*m*/*z* 432.2743 [M + H]$^+$, calcd 432.2750) and NMR data. The ^1H and ^{13}C NMR data (Tables 2 and 1) of **2** were closely related to those of **1**, implying that they likely shared the same gross structure, which was confirmed by HSQC, $^1H-^1H$ COSY, and HMBC spectra. The key ROESY correlations of **2** indicated that its relative configuration was similar to that of **1** except for C-15, implying that alkaloids **1** and **2** were a pair of C-15 epimers. ROESY correlations of H-15/H-13b suggested a β-orientation for H-15, which is consistent with daphmalenine A.[4c] Thus, the structure of daphmacromine B was established as **2**.

Daphmacromine C (**3**) has a molecular formula of $C_{25}H_{37}NO_5$, as deduced from the HRESIMS (*m*/*z* 432.2743 [M + H]$^+$, calcd 432.2750) and NMR data. The ^1H and ^{13}C NMR data (Tables 2 and 1) showed that alkaloid **3** was an analogue of **1**, and the only difference was the presence of an O-ethyl rather than an O-methyl group at C-2 (δ_C 99.9) in **3**, as judged by HMBC (Supporting Information) correlations from C-25 (δ_H 3.15, m, 2H) to C-2. The structure of **3** was thus confirmed by $^1H-^1H$ COSY, HMBC, and ROESY data.

Daphmacromine D (**4**) has a molecular formula of $C_{25}H_{37}NO_5$, as deduced from the HRESIMS data. Compared with **3**, the ^1H and ^{13}C NMR data of **4** (Tables 2 and 1) showed that **4** and **3** likely shared the same gross structure, which was confirmed by HSQC, $^1H-^1H$ COSY, and HMBC spectra (Supporting Information). Analysis of ROESY spectra, particularly the key ROESY correlations of H-15/H-13b, indicated that **4** and **3** were a pair of C-15 epimers.

The HRESIMS of daphmacromine E (**5**) suggested a molecular formula of $C_{24}H_{35}NO_5$ (*m*/*z* 418.2583 [M + H]$^+$, calcd 418.2593). The NMR spectra of **5** (Tables 1 and 2) showed that alkaloid **5** was an analogue of **1**. Compared with **1**, the major difference was the presence of a methyl ester group in **5**. The replacement of the ethyl with a methyl in **5** was further supported by the HMBC correlations of an O-methyl group (δ_H 3.60, s, 3H) with the ester carbonyl (δ_C 173.5) of C-22. Analysis of the HSQC, $^1H-^1H$ COSY, HMBC, and ROESY data confirmed that the rest of the molecule was analogous to **1**.

Daphmacromine F (**6**) has the same molecular formula as **5**, $C_{24}H_{35}NO_5$, as deduced from the HRESIMS data (*m*/*z* 418.2603, [M + H]$^+$, calcd 418.2593). The NMR features of **6** (Tables 1 and 3) closely resembled those of **5** except for the resonances in the vicinity of C-2. HSQC, $^1H-^1H$ COSY, and HMBC spectra (Supporting Information) implied that alkaloids **6** and **5** shared the same gross structure. The correlations of 2-OMe with H-3b and H-4b in the ROESY spectrum indicated that the methoxy group at C-2 was β-oriented. The structure of daphmacromine F (**6**) was thus established as the C-2 epimer of **5** (Figure 1).

Daphmacromine G (**7**) was assigned as $C_{24}H_{35}NO_5$, as deduced from the HRESIMS data (*m*/*z* 418.2593 [M + H]$^+$, calcd 418.2593). The ^{13}C NMR and DEPT data (Table 1) indicated that **7** had the same gross structure as **5**, which was

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Table 2. ^{1}H NMR Spectroscopic Data for Daphmacromines A–E (1–5)

position	1[a,c]	2[b,c]	3[a,c]	4[b,d]	5[a,d]
1a	2.38, d (12.0)	2.23, d (11.5)	2.23, m	2.24, d (11.4)	2.36, m
1b	2.47, d (12.0)	2.53, d (11.5)	2.29, m	2.53, m	2.46, m
3a	1.62, m	1.59, m	1.42, m	1.55, m	1.58, m
3b	1.62, m	1.59, m	1.42, m	1.64, m	1.58, m
4a	1.59, m	1.61, m	1.42, m	1.66, m	1.58, m
4b	2.02, m	1.95, m	1.79, m	1.95, m	1.98, m
6	2.35, m	2.35, m	2.16, m	2.36, m	2.31, m
7a	2.59, d (12.0)	2.47, d (12.0)	2.42, m	2.49, m	2.56, m
7b	2.71, d (12.0)	2.64, d (12.0)	2.54, d (12.0)	2.64, d (11.5)	2.68, m
11a	2.43, m	2.57, m	2.22, m	2.57, m	2.40, m
11b	2.53, m	3.02, m	2.35, m	3.03, m	2.49, m
12a	1.76, m	1.77, m	1.56, m	1.78, m	1.73, m
12b	2.14, m	2.10, m	1.94, m	2.12, m	2.11, m
13a	1.77, dd (15.1, 9.3)	1.73, m	1.58, m	1.73, m	1.74, m
13b	2.55, m	2.42, m	2.34, m	2.45, m	2.54, m
14	3.05, t (9.3)	2.73, m	2.89, t (8.5)	2.70, m	3.05, t (10.0)
15	3.21, m	3.08, t m	3.03, td (9.9, 5.0)	3.04, m	3.17, m
16a	2.46, m	2.32, m	2.24, m	2.27, m	2.43, m
16b	2.55, m	2.75, m	2.24, m	2.69, m	2.53, m
18a	1.41, dq (14.9, 7.5)	1.43, m	1.22, dd (14.3, 7.5)	1.45, dd (14.4, 7.5)	1.38, dq (14.9, 7.6)
18b	1.70, dq (14.9, 7.5)	1.76, m	1.50, dd (14.3, 7.5)	1.76, m	1.66, m
19	0.84, t (7.5)	0.82, t (7.5)	0.63, t (7.5)	0.83, t (7.5)	0.80, t (7.6)
20	2.14, s	2.06, s	1.96, s	2.03, s	2.11, s
21a	3.51, d (16.0)	3.63, d (12.0)	3.33, m	3.61, m	3.40, m
21b	3.55, d (16.0)	3.79, d (12.0)	3.33, m	3.80, d (11.4)	3.50, m
23	4.08, q (7.1, 2H)	4.13, q (7.1, 2H)			
	1.25, t (7.1, 3H)	1.14, t (7.1, 3H)	3.41, s	3.63, s	3.60, s
24	3.11, s	3.09, s	0.97, t (7.0, 3H)	1.17, t (7.0, 3H)	3.08, s
			3.15, m	3.33, m	
			3.15, m	3.46, m	

[a]Recorded in $CDCl_3$. [b]Recorded in pyridine-d_5. [c]Recorded at 400 MHz. [d]Recorded at 500 MHz.

verified by HSQC, ^{1}H–^{1}H COSY, and HMBC data (Supporting Information). Analysis of the ROESY spectra, particularly the key correlation of H-15/H-13b, indicated that **7** and **5** were a pair of C-15 epimers.

Daphmacromine H (**8**) gave a molecular formula of $C_{24}H_{35}NO_5$, as deduced from the HREIMS data (*m/z* 417.2509 M^+, calcd 417.2515). The ^{13}C NMR data (Table 1) showed that alkaloid **8** was an analogue of daphnigracine,[8] with the only difference being the presence of a ketocarbonyl group at C-17 (δ_C 207.3) in **8**. This structural assignment was supported by the HMBC (Supporting Information) correlations from H-16a (δ_H 2.26, m) and H-16b (δ_H 2.64, m) to C-17. The structure of **8** was further verified by a combination of HSQC, HMBC, ^{1}H–^{1}H COSY, and ROESY spectra (Supporting Information).

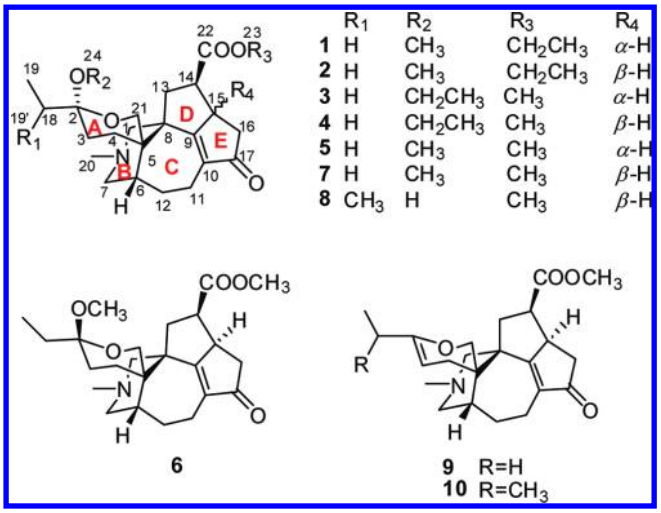

Figure 1. Structures of **1**–**10**.

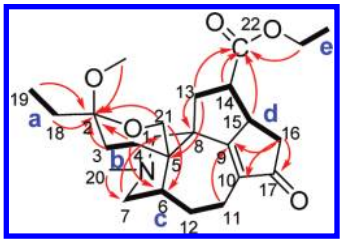

Figure 2. ^{1}H–^{1}H COSY (bold) and key HMBC correlations of **1**.

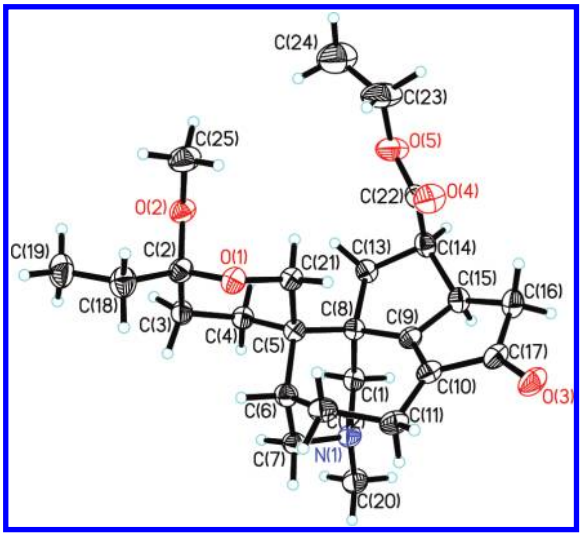

Figure 3. Single-crystal X-ray structure of **1**.

Daphmacromine I (**9**) was assigned as $C_{23}H_{31}NO_4$, as deduced from the HRESIMS data (*m/z* 386.2331 $[M + H]^+$, calcd 386.2331), which is 32 mass units (MeOH) less than that of **5**. One additional ethylenic bond assigned to C-2 and C-3 was included based on the HMBC spectrum (Supporting Information). The above analysis indicated **9** to be an elimination product of **5**, and this conclusion was confirmed by further analysis of the 2D NMR spectra. The assignment of the C-17 cabonyl carbon of **9** was deduced from the HMBC correlations of H-16a (δ_H 2.44, m) and H-16b (δ_H 2.58, m) with C-17. The structure of **9** was thus determined to be as shown in Figure 1. In addition, further comparison of the ^{1}H and ^{13}C NMR data of **9** and the dehydration product of

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Table 3. ^{1}H NMR Spectroscopic Data for Daphmacromines F–J (6–10)

position	6[a,c]	7[b,d]	8[b,c]	9[b,e]	10[b,c]
1a	2.28, d (11.1)	2.26, d (11.5)	2.30, m	2.23, m	2.24, m
1b	2.37, d (11.1)	2.54, d (11.5)	2.55, m	2.45, m	2.46, m
3a	1.38, m	1.60, m	1.71, m	4.42, br d (5.4)	4.44, dd (6.4, 2.4)
3b	1.78, m	1.60, m	1.71, m		
4a	1.13, m	1.61, m	1.87, m	1.83, br d (15.6)	1.87, br d (13.8)
4b	1.79, m	1.96, m	2.08, m	2.22, dd (15.6, 5.4)	2.25, dd (13.8, 6.4)
6	1.88, m	2.37, m	2.46, m	2.09, m	2.09, m
7a	2.63, m	2.47, d (11.7)	2.56, d (11.8)	2.46, m	2.48, m
7b	2.63, m	2.64, d (11.7)	2.68, d (11.8)	2.62, m	2.63, m
11a	2.40, m	2.57, m	2.56, m	2.60, m	2.60, m
11b	2.40, m	3.02, m	3.04, m	2.75, m	2.75, m
12a	1.66, m	1.78, m	1.81, m	1.64, m	1.65, m
12b	1.79, m	2.11, m	2.17, m	2.04, m	2.18, m
13a	1.84, m	1.72, m	1.74, m	1.72, dd (14.9, 9.2)	1.73, dd (14.9, 9.2)
13b	2.94, m	2.41, m	2.50, m	2.27, m	2.29, m
14	3.02, t (8.7)	2.74, m	2.72, m	3.09, t (8.4)	3.10, t (8.5)
15	3.14, m	3.07, m	3.03, m	3.27, m	3.28, m
16a	2.43, m	2.30, dd (16.4, 4.4)	2.26, m; 2.64, m	2.44, m; 2.58, m	2.43, m; 2.58, m
16b	2.87, m	2.73, m			
18a	1.13, m	1.45, dq (14.7, 7.4)		2.04, m	2.26, m
18b	1.79, m	1.78, m	2.02, m	2.20, m	2.26, m
19	0.83, t (7.5)	0.83, t (7.4)	1.18, d (6.9)[f]	1.00, t (7.5)	1.04, d (2.9)[f]
19′			1.11, d (6.9)[f]		1.06, d (2.9)[f]
20	2.12, s	2.06, s	2.07, s	2.10, s	2.11, s
21a	3.40, d (12.5)	3.64, m	3.8, m	3.76, d (11.4)	3.73, d (11.3)
21b	3.55, d (12.5)	3.80, d (11.4)	4.44, d (11.5)	4.36, m	4.35, m
$COOCH_3$	3.62, s	3.64, s	3.61, s	3.50, s	3.51, s
OCH_3	3.08, s	3.10, s			

[a]Recorded in $CDCl_3$. [b]Recorded in pyridine-d_5. [c]Recorded at 400 MHz. [d]Recorded at 500 MHz. [e]Recorded at 600 MHz. [f]Assignments are interchangeable.

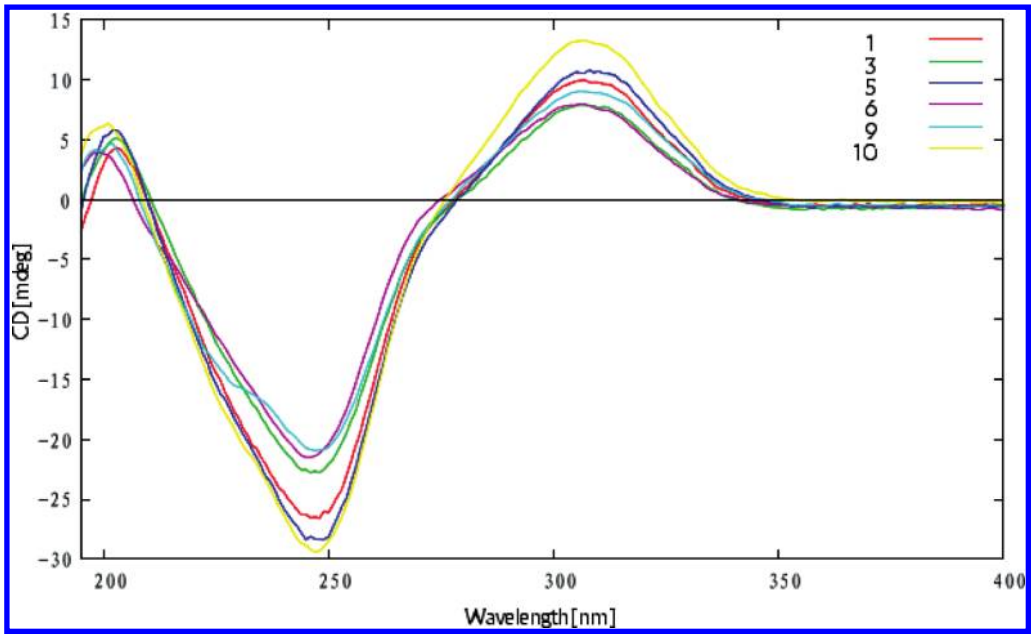

Figure 4. ECD spectra of **1**, **3**, **5**, **6**, **9**, and **10** (in MeOH).

oxodaphnigraciline[8,9] indicated these two compounds had the same NMR spectra, which compelled us to revise the structure of the dehydration product of oxodaphnigraciline, especially the location of the carbonyl group. Consequently, the carbonyl group in oxodaphnigracine, oxodaphnigraciline, and epioxodaphnigraciline[8,9] should also be located at C-17 rather than C-11.

Daphmacromine J (**10**) gave the molecular formula $C_{24}H_{33}NO_4$, as deduced from the HRESIMS data, amounting to an additional methylene group compared with **9**. The ^{1}H

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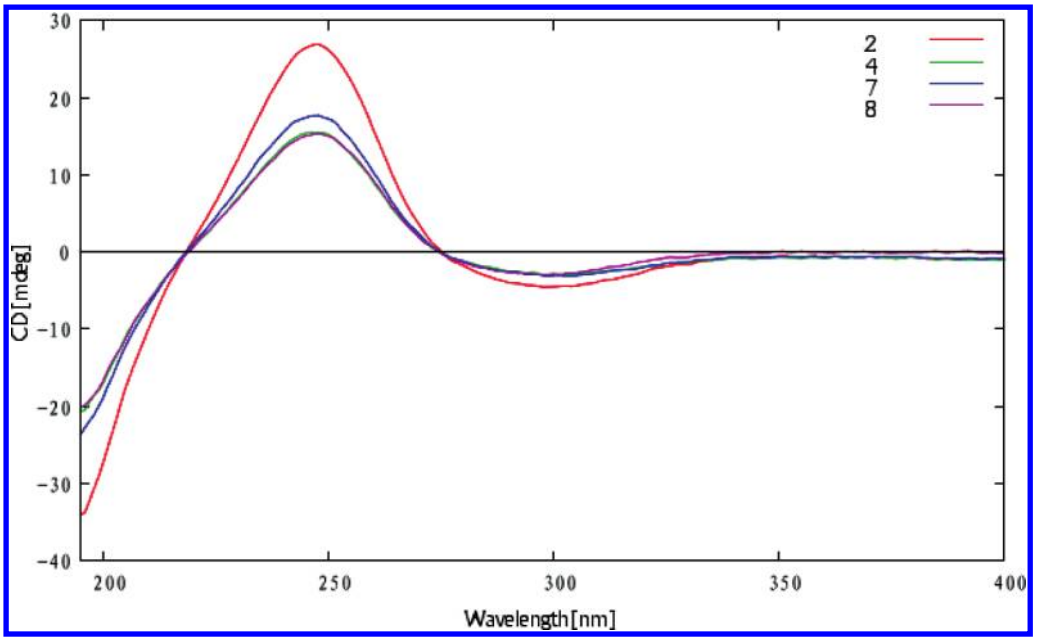

Figure 5. ECD spectra of **2**, **4**, **7**, and **8** (in MeOH).

and ^{13}C NMR data (Tables 3 and 1) of **10** showed the presence of one isopropyl group, suggesting the replacement of the C-2 ethyl group in **9** with an isopropyl group in **10**. This assignment was deduced by the observation of two doublet methyl signals [H-19 (δ_C 1.04, d, J = 2.9, 3H) and H-19′ (δ_C 1.06, d, J = 2.9, 3H)], which was further supported by the HMBC correlations from H-19, H-19′, and H-18 (δ_H 2.26, m) to C-2 (δ_C 159.5). The relative configuration of **10** was assigned to be the same as in **9** by ROESY data (Supporting Information).

The similar patterns of Cotton effects in the ECD spectra corresponding to the UV absorption maxima of alkaloids **1**, **3**, **5**, **6**, **9**, and **10** (Figure 4) indicated that the absolute configurations of **3**, **5**, **6**, **9**, and **10** were identical to that of **1**. Similarly, the stereogenic centers of alkaloids **2**, **4**, **7**, and **8** had the same absolute configurations, as determined by their similar ECD curves (Figure 5) and nearly identical specific rotation values.

In addition to the 10 new alkaloids, daphnezomine K (**11**),[10] deoxyyuzurimine (**12**),[5c] daphhimalenine B (**13**),[4b] yuzurimine C (**14**),[1a] daphtenidine C (**15**),[11] yuzurimine (**16**),[5c] and daphnezomine U (**17**)[12] were identified by comparing the experimental and reported physical data.

Selected compounds were assayed in vitro for pesticidal activity against brine shrimp (*Artemia salina*) by the microwell method.[13] Selected new compounds displayed activities at 100 mg/L, and the corrected lethality of the known compounds ranged from 0.00% to 81.81% at 100 mg/L (Table 4). Of note, compounds **12** and **14** showed higher corrected lethality values of 81.81% and 80.56%, respectively, while compound **11** had the lowest corrected lethality at 0.00%.

All 17 alkaloids were assayed for their cytotoxicity against five human cancer cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) using the MTT method[14] with cisplatin and paclitaxel as positive controls. None of the 17 alkaloids showed cytotoxic activity in this assay (IC_{50} > 40 μM).

Table 4. Corrected Mortality[a,b] of Selected Compounds against Brine Shrimp at 100 mg/L

compound	corrected mortality	compound	corrected mortality
1	40.98%	**10**	55.22%
2	49.92%	**11**	0.00%
3	50.87%	**12**	81.81%
4	53.83%	**13**	64.88%
5	70.90%	**14**	80.56%
6	44.99%	**15**	31.82%
7	67.70%	**16**	70.78%
9	64.56%	**17**	38.26%

[a]Corrected mortality = $(M_t - M_c)/(1 - M_c) \times 100\%$. [b]The mean mortality of the control group was 4.73%. M_t: mortality of treatment group; M_c: mortality of control group.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were measured on a Yuhua X-4 digital microdisplaying melting point apparatus. Optical rotations were measured with a Jasco P-1020 polarimeter. UV spectra were obtained using a Shimadzu UV-2401A spectrophotometer. ECD spectra were recorded with an Applied Photophysics Chirascan spectrometer. A Tenor 27 spectrophotometer was used for IR spectra as KBr pellets. 1D and 2D NMR spectra were recorded on Bruker AM-400, DRX-500, and AVANCEIII-600 spectrometers with TMS as internal standard. HRESIMS was performed on an API QSTAR time-of-flight spectrometer. X-ray data were collected using a Bruker APEX DUO instrument. Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with a Waters XBridge C18 (4.6 × 250 mm) column. Column chromatography (CC) was performed using silica gel (200−300 mesh and 300−400 mesh, Qingdao Marine Chemical, Inc., Qingdao, P. R. China) and Sephadex LH-20 (40−70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden).

Plant Material. The leaves and stems of *D. macropodum* were collected from Sichuan Province, People's Republic of China, in October 2010. The plant samples were identified by Prof. Liangke Song of the School of Life Science and Engineering, Southwest Jiaotong University. A voucher specimen (KIB H20101011) was deposited at the State Key Laboratory of Phytochemistry and Plant Resource in West China, Kunming Institute of Botany, Chinese Academy of Science.

Extraction and Isolation. The air-dried, powdered leaves and stems (34 kg) of *D. macropodum* were extracted three times with 95% EtOH. The extract was adjusted with saturated tartaric acid to pH 2−3

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and then defatted with petroleum ether (PE). Next, the aqueous phase was adjusted to pH 10 with saturated Na_2CO_3 and extracted with $CHCl_3$ to obtain the crude alkaloid fraction (260 g). The total alkaloid was subjected to normal-phase Si gel (200−300 mesh; $CHCl_3$/MeOH, 1:0 → 0:1) to obtain five major fractions (Fr 1−5). Compound **16** (4 g) was crystallized in acetone from fraction 1. Fraction 1 (30 g) was further chromatographed over a reversed-phase medium-pressure column (MeOH/H_2O, 1:1 → 1:0) to give four fractions (Fr 1A−1D). Fraction 1B gave compound **14** (0.8 g) as acicular crystals in acetone.

Fraction 1C (1.1 g) was subjected to normal-phase Si gel (300−400 mesh; PE/EtOAc, 9:1) to obtain four fractions (Fr 1C1−1C4). Fraction 1C4 (800 mg) was purified by Sephadex LH-20 CC eluted with $CHCl_3$/MeOH (1:1), followed by HPLC with 50% MeCN/H_2O, to obtain compounds **17** (30 mg), **5** (40 mg), **3** (27 mg), **6** (28 mg), and **1** (11 mg). Compounds **11** (5 mg), **10** (3 mg), **13** (15 mg), and **7** (26 mg) were separated from fraction 1C3 by HPLC with 50% MeCN/H_2O. Compounds **2** (5 mg) and **4** (20 mg) were obtained from fraction 1C2 (63 mg) by HPLC with 70% MeOH/H_2O. Fraction 1D (6.1 g) was subjected to normal-phase Si gel (300−400 mesh; petroleum ether/EtOAc, 9:1) to obtain three fractions (Fr 1D1−1D3). Fraction 1D1 was separated to give compounds **15** (10 mg), **8** (6 mg), and **12** (7 mg) by HPLC with 50% MeCN/H_2O.

*Daphmacromine A (**1**):* colorless crystals; mp 136.0−137.0 °C; $[\alpha]_D^{22}$ −61 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 246 (4.20); ECD (0.000 62 M, MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+2.20), 248 (−12.95), 308 (+4.89) nm; IR (KBr) ν_{max} 3436, 2972, 2942, 2886, 2839, 2785, 2754, 1729, 1694, 1662, 1377, 1047, 890 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1; positive ESIMS *m/z* 432 $[M + H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 432.2759 (calcd for $C_{25}H_{38}NO_5$, 432.2749).

*Daphmacromine B (**2**):* white powder; $[\alpha]_D^{26}$ +24 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.77); ECD (0.000 58 M, MeOH) λ_{max} ($\Delta\varepsilon$) 248 (+14.06), 298 (−2.37) nm; IR (KBr) ν_{max} 3433, 2960, 2934, 2920, 2850, 2786, 2761, 1732, 1703, 1663, 1382, 1039, 892 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1; positive ESIMS *m/z* 455 [M + Na + $H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 432.2760 (calcd for $C_{25}H_{38}NO_5$, 432.2749).

*Daphmacromine C (**3**):* white powder; $[\alpha]_D^{22}$ −24.6 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 246 (4.14); ECD (0.000 29 M, MeOH) λ_{max} ($\Delta\varepsilon$) 204 (+5.34), 247 (−22.64), 306 (+9.07) nm; IR (KBr) ν_{max} 3433, 2970, 2936, 2881, 2785, 1735, 1703, 1666, 1377, 1045, 872 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1; positive ESIMS *m/z* 432 $[M + H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 432.2758 (calcd for $C_{25}H_{38}NO_5$, 432.2749).

*Daphmacromine D (**4**):* white powder; $[\alpha]_D^{26}$ +38.4 (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.99); ECD (0.000 19 M, MeOH) λ_{max} ($\Delta\varepsilon$) 248 (+24.72), 303 (−4.66) nm; IR (KBr) ν_{max} 3432, 2968, 2936, 2882, 2786, 2761, 1737, 1704, 1664, 1375, 1052, 945 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1; positive ESIMS *m/z* 455 [M + Na + $H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 432.2743 (calcd for $C_{25}H_{38}NO_5$, 432.2749).

*Daphmacromine E (**5**):* white powder; $[\alpha]_D^{22}$ −36 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ε) 246 (3.93); ECD (0.000 66 M, MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+2.66), 248 (−12.52), 307 (+5.25) nm; IR (KBr) ν_{max} 3431, 2938, 2883, 2785, 1735, 1703, 1664, 1376, 1044, 891 cm^{-1}; ^1H and ^{13}C NMR data, Tables 2 and 1; positive ESIMS *m/z* 418 $[M + H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 418.2583 (calcd for $C_{24}H_{36}NO_5$, 418.2593).

*Daphmacromine F (**6**):* white powder; $[\alpha]_D^{22}$ +63 (*c* 0.4, MeOH); UV (MeOH) λ_{max} (log ε) 246 (3.93); ECD (0.000 35 M, MeOH) λ_{max} ($\Delta\varepsilon$) 203 (+2.32), 245 (−18.62), 306 (+6.90) nm; IR (KBr) ν_{max} 3437, 2939, 2881, 2786, 1733, 1701, 1665, 1376, 1051, 866 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 1; positive ESIMS *m/z* 418 $[M + H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 418.2603 (calcd for $C_{24}H_{36}NO_5$, 418.2593).

*Daphmacromine G (**7**):* white powder; $[\alpha]_D^{26}$ +28 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 249 (4.04); ECD (0.000 21 M, MeOH) λ_{max} ($\Delta\varepsilon$) 248 (+25.51), 303 (−4.47) nm; IR (KBr) ν_{max} 3440, 2942, 2883, 2786, 2761, 1735, 1703, 1663, 1383, 1040, 892 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 1; positive ESIMS *m/z* 440 $[M + Na]^+$; positive HRESIMS $[M + H]^+$ *m/z* 418.2582 (calcd for $C_{24}H_{36}NO_5$, 418.2593).

*Daphmacromine H (**8**):* white powder; $[\alpha]_D^{25}$ +16.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 249 (3.78); ECD (0.000 34 M, MeOH) λ_{max} ($\Delta\varepsilon$) 248 (+13.68), 298 (−2.62) nm; IR (KBr) ν_{max} 3434, 2960, 2937, 2877, 2787, 1734, 1704, 1660, 1383, 1039, 935 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 1; positive ESIMS *m/z* 418 $[M + H]^+$; HREIMS $[M]^+$ *m/z* 417.2509 (calcd for $C_{24}H_{35}NO_5$, 417.2515).

*Daphmacromine I (**9**):* white powder; $[\alpha]_D^{27}$ −17.5 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 243 (3.82); ECD (0.000 70 M, MeOH) λ_{max} ($\Delta\varepsilon$) 202 (+2.03), 247 (−9.07), 306 (+3.92) nm; IR (KBr) ν_{max} 3432, 2926, 2853, 2787, 1732, 1703, 1659, 1376, 1048, 938 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 1; positive ESIMS *m/z* 386 $[M + H]^+$; positive HRESIMS $[M + H]^+$ *m/z* 386.2329 (calcd for $C_{23}H_{32}NO_4$, 386.2331).

*Daphmacromine J (**10**):* white powder; $[\alpha]_D^{22}$ −29 (*c* 0.26, MeOH); UV (MeOH) λ_{max} (log ε) 245 (3.88); ECD (0.00080 M, MeOH) λ_{max} ($\Delta\varepsilon$) 201 (+2.42), 249 (−10.97), 307 (+5.03) nm; IR (KBr) ν_{max} 3432, 2960, 2932, 2878, 2786, 1735, 1703, 1666, 1376, 1043, 939, 867 cm^{-1}; ^1H and ^{13}C NMR data, Tables 3 and 1; positive ESIMS *m/z* 422 $[M + Na]^+$; positive HRESIMS $[M + H]^+$ *m/z* 400.2489 (calcd for $C_{24}H_{34}NO_4$, 400.2487).

Pesticidal Bioassay. Brine shrimp eggs (*Artemia salina*) obtained locally (Qingdao, China) were hatched in artificial seawater prepared from sea salt (Sigma Chemical Co., U.K.). After 48 h of incubation at 28 °C, nauplii were prepared for the following tests. Daphmacromines A−J and seven known alkaloids were dissolved in DMSO prior to adding artificial seawater to 100, 50, and 10 mg/L. The three dilutions were added to wells of 96-well microplates in triplicate to a total volume of 100 μL. Control wells with 100 μL of DMSO were included in each experiment. A suspension of nauplii containing 15−25 organisms (100 μL) was added to each well, and the covered plate was incubated at 28 °C for 24 h. Plates were examined under a microscope, and the numbers of dead (nonmotile was considered dead) nauplii in each well were counted. No lethal effects were observed for these compounds at 10 and 50 mg/L, while the corrected mortality was calculated for each sample at 100 mg/L.

Five human tumor cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW-480) were used in the cytotoxic activity assay. All cells were cultured in DMEM or RPMI-1640 medium (Hyclone, Logan, UT) supplemented with 10% fetal bovine serum (Hyclone) at 37 °C in a humidified atmosphere with 5% CO_2. Cell viability was assessed by conducting colorimetric measurements of the amount of insoluble formazan formed in living cells based on the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, St. Louis, MO, USA). Briefly, 100 μL of adherent cells was seeded into each well of a 96-well cell culture plate and allowed to adhere for 12 h before drug addition, while suspended cells were seeded just before drug addition, both with an initial density of 5×10^3 to 1×10^4 cells/mL in 100 μL of medium. Each cell line was exposed to the test compound at 40 μM in triplicate for 48 h, with cisplatin and paclitaxel (Sigma) as positive controls. After incubation, 20 μL of MTT (5 g/L) was added to each well, and the incubation continued for 4 h at 37 °C. The cells were next lysed with 200 μL of 10% SDS after removing the medium. The optical density of the lysate was measured at 595 nm in a 96-well microtiter plate reader (Bio-Rad 680). The IC_{50} value of each compound was calculated using the method of Reed and Muench.

X-ray Crystal Structure Analysis. Colorless crystals of **1** were obtained from acetone. Intensity data were collected at room temperature on a Bruker APEX DUO diffractometer equipped with an APEX II CCD using Cu Kα radiation. Cell refinement and data reduction were performed with Bruker SAINT software. The structure was solved by direct methods using SHELXL-97.[15] Refinements were performed with SHELXL-97 using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The H atoms were placed in calculated positions and refined using a riding model. Molecular graphics were computed with PLATON. The absolute configuration was determined by refinement of the Flack parameter based on resonant scattering of the light atoms and

computation of the Hooft parameter. Crystal data: $C_{25}H_{37}NO_5$, M_w = 431.56, tetragonal space group $P4_1$, $a = b$ = 14.3257(4) Å, c = 11.4299(3) Å, $\alpha = \beta = \gamma = 90°$, V = 2345.71(11) Å^3, Z = 4, D_x = 1.222 kg m^3, θ_{max} = 67.6, R = 0.038 for 3647 data and 285 refined parameters. The Flack parameter is 0.12(19), and the Hooft parameter is 0.06(8) for 1431 Bijvoet pairs. Crystallographic data (excluding structure factor tables) for **1** were deposited with the Cambridge Crystallographic Data Center as supplementary publication no. CCDC 835501. Copies of the data can be obtained free of charge by application to CCDC, 12 Union Road, Cambridge CB 1EZ, UK [fax: Int. +44 (0) (1223) 336 033; e-mail: deposit@ccdc.cam.ac.uk].

■ ASSOCIATED CONTENT

Supporting Information

This material (1D and 2D NMR, ESIMS, HRESIMS, IR, and CD spectra of daphmacromines A–J) 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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