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Macropodumines A–C: Novel Pentacyclic Alkaloids with an Unusual Skeleton or Zwitterion Moiety from *Daphniphyllum macropodum* Miq.

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Abstract: Three novel alkaloids, macropodumines A–C (**1**–**3**), were isolated from the stem of *Daphniphyllum macropodum* Miq. Interestingly, the structure of macropodumine A (**1**) was characterized as having a fused pentacyclic system including an unusual eleven-membered macrolactone ring, whereas macropodumine B (**2**) contains a rare cyclopentadienyl carbanion, which is stabilized as a zwitterion by an internal iminium cation. The structures of these new metabolites were established on the basis of their detailed spectroscopic analysis. In particular, the unique structure of zwitterion **2** was further confirmed by using single-crystal X-ray diffraction analysis.

Keywords: alkaloids · macropodumines · natural products · structure elucidation · zwitterions

Introduction

The *Daphniphyllum* alkaloids are a structurally intriguing group of polycyclic, fused heterocyclic natural products produced by plants of the genus *Daphniphyllum* (Daphniphyllaceae).[1] These ring systems have attracted great interest as challenging targets for total synthesis[2] as well as biosynthetic studies.[3] Knowledge of this fascinating group of metabolites was recently widened by Kobayashi and other researchers by the discovery of a new series of *Daphniphyllum* alkaloids, which result from ring cleavage or rearrangement, from a different species of the genus *Daphniphyllum*.[4] *Daphniphyllum* species such as *D. macropodum*, *D. calycinum*, and *D. oldhami* are used in Chinese traditional medicine. For example, the extract from the leaves and fruits of *D. macropodum* Miq. is used in the treatment of inflammations.[5] In 1966, the first *Daphniphyllum* alkaloid, daphnimacin, was discovered from *D. macropodum*,[6s] marking the beginning of the chemical investigation on the genus *Daphniphyllum*. A series of *Daphniphyllum* alkaloids, mainly the derivatives of daphniphylline and yuzurimine, were isolated from this species in the ten years that followed.[6] However, strangely enough, to the best of our knowledge, there have been no chemical reports on this species in the long gap from the end of 1970 until now.

In the course of our search for bioactive metabolites from Chinese medicinal plants,[7] we have reinvestigated the extract of the stems of *D. macropodum*, resulting in the discovery of three novel *Daphniphyllum* alkaloids named macropodumines A–C (**1**–**3**). The pentacyclic, fused framework of macropodumine A (**1**) contains a macrolactone structural element that is the first of which to be found within the *Daphniphyllum* alkaloid family. In addition, the remarkable feature of macropodumine B (**2**) is a rare cyclopentadienyl anion, which is stabilized as a zwitterion by an internal iminium counterion (**2**). This paper describes the isolation and structural elucidation of these new compounds **1**–**3**.

Results and Discussion

The air-dried, powdered stem of *D. macropodum* Miq. was extracted with 95% EtOH, and this extract was partitioned between EtOAc and an acidic aqueous liquor (pH 4–5). The aqueous layer, adjusted to pH 9–10 by addition of Na_2CO_3, was then extracted with $CHCl_3$. The $CHCl_3$-soluble material was subjected to repeated column chromatography on silica gel to afford three pure compounds (**1**–**3**).

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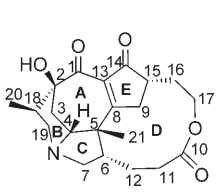

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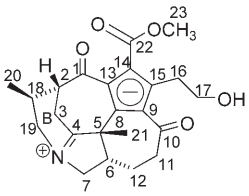

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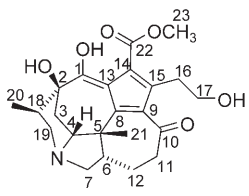

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Macropodumine A (**1**) was isolated as an optically active ($[\alpha]_D^{20} = +30.0$) light yellow oil. The molecular formula $C_{21}H_{27}NO_5$ was established by using high-resolution (HR) electrospray ionization mass spectroscopy (ESIMS) from the pseudo-molecular ions at *m/z* 396.1790 $[M+Na]^+$, 374.1965 $[M+H]^+$, and 372.1808 $[M-H]^-$, which indicated nine degrees of unsaturation. The IR absorption spectrum showed the presence of a hydroxy group ($\tilde{\nu} = 3444\ cm^{-1}$) and conjugated carbonyl functionalities ($\tilde{\nu} = 1720,\ 1707\ cm^{-1}$). This was in agreement with the presence of a tertiary oxygenated carbon atom ($\delta = 77.7$ ppm), and signals for one ester carbonyl atom ($\delta = 172.4$ ppm) and two ketone carbonyl atoms ($\delta = 189.1,\ 213.1$ ppm) as deduced from the ^{13}C NMR spectrum (Table 1), while taking into account the three degrees of unsaturation. The remaining six degrees of unsaturation were due to one double bond and five rings in the molecule.

Four fragments, **a** (C20/C18/C19), **b** (C3/C4), **c** (C7/C6/C12/C11), and **d** (C9/C15/C16/C17), shown in Figure 1, were identified from the analysis of the 2D NMR spectra (HSQC, $^1H,^1H$ COSY, and HMBC). The

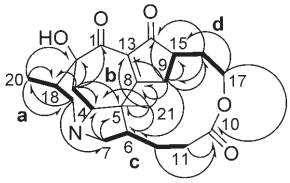


Figure 1. $^1H,^1H$ COSY (—) and selected HMBC (⌒) correlations of **1**.

HMBC correlations were applied to confirm the assignments for the structural fragments **a**–**d** in those cases in which the overlapping proton signals were not sufficient to prove the assignments of these structural fragments by HSQC and $^1H,^1H$ COSY analysis alone. Finally, the linkage of the fragments **a**–**d** was possible by means of analysis of the HMBC correlations. Meanwhile, the "loose ends" resulting from the insertion of the oxygen and nitrogen atoms, and the tertiary and quaternary carbon atoms of C1, C2, C5, C8, C10, C13, and C14 into the fragments, could be fully connected by using an HMBC experiment. The HMBC correlations of H_2-3 with C1, C2, C4, C5, and C18, as well as H_2-7 with C4, and H_2-19 with C2, C4, and C7 indicated the connection of the partial structures **a**, **b**, and **c** by the nitrogen atom N1, and the linkage of both fragments **a** and **b** to C1 and C2. Partial fragments **c** and **d** were found to be linked to each other from the presence of the long-range correlation between H_2-17 and the ester carbonyl carbon at C10, which resulted in the formation of the lactone ring in the structure. Significant HMBC correlations of H_3-21 with C4, C5, C6, and C8, and of H_2-9 with C6, C8, C13, C14, C15, and C16 led to the connection of partial structures **b** and **c** with the quaternary carbon C5, in addition to the linkage from both parts **b** and **c** to **d** by a quaternary carbon bridge of

Table 1. NMR[a] data for macropodumine A (**1**).

Atom no.	In $CDCl_3$[b] δ_H [ppm] (mult., *J* [Hz])	In $CDCl_3$[b] δ_C[d] [ppm] (mult.)	In C_5D_5N[c] δ_H [ppm] (mult., *J* [Hz])	In C_5D_5N[c] δ_C[d] [ppm] (mult.)
1		–		189.1 (s)
2		79.5 (s)		77.7 (s)
3α	2.12 (ov)	25.8 (t)	2.16 (ov)	27.4 (t)
3β	1.78 (ddd, 13.6, 2.8, 1.9)		2.16 (ov)	
4	2.88, (dd, 10.7, 2.8)	70.7 (d)	2.51 (ov)	69.8 (d)
5		50.6 (s)		51.2 (s)
6	2.42 (m)	47.8 (d)	2.18 (m)	48.2 (d)
7α	2.72 (dd, 10.8, 10.3)	49.4 (t)	2.37 (t, 10.0)	51.1 (t)
7β	2.79 (t, 10.8)		2.71 (dd, 10.0, 8.8)	
8		139.0 (s)		141.1 (s)
9α	2.92 (dd, 21.8, 4.2)	30.7 (t)	3.17 (dd, 19.2, 1.8)	32.3 (t)
9β	2.63 (ov)		2.66 (dd, 19.2, 7.3)	
10		172.1 (s)		172.4 (s)
11α	2.38 (ov)	32.4 (t)	2.32 (ddd, 16.5, 7.4, 3.2)	32.7 (t)
11β	2.12 (ov)		2.22 (ov)	
12α	1.72 (m)	18.1 (t)	1.67 (m)	19.9 (t)
12β	2.26 (m)		2.48 (m)	
13		169.0 (s)		167.3 (s)
14		212.4 (s)		213.1 (s)
15	2.63 (m)	42.5 (d)	2.51 (m)	42.6 (d)
16α	2.59 (m)	29.1 (t)	2.67 (m)	29.5 (t)
16β	1.91 (ddd, 15.1, 4.3, 2.2)		1.69 (m)	
17α	3.91 (ddd, 11.7, 3.2, 1.4)	60.3 (t)	3.87 (dd, 11.6, 4.0)	60.2 (t)
17β	4.42 (dt, 11.7, 2.4)		4.53 (dt, 11.6, 2.8)	
18	3.12 (m)	35.3 (d)	3.54 (m)	35.6 (d)
19α	3.30 (t, 11.0)	55.3 (t)	3.00 (t, 11.0)	53.2 (t)
19β	2.36 (ov)		2.02 (dd, 11.0,5.2)	
20	1.09 (d, 7.2)	13.4 (q)	1.15 (d, 7.1)	14.0 (q)
21	1.38 (s)	16.1 (q)	1.53 (s)	19.1 (q)

[a] See the Experimental Section for full details. [b] Chemical shifts referred to $CHCl_3$ and to $CDCl_3$. [c] Chemical shifts referred to C_5H_5N and to C_5D_5N. [d] By DEPT sequence.

C5/C8. Finally, the clear HMBC correlation between H_2-16 and C9 and C14 confirmed the assignment of C14 as a ketone carbonyl atom and consequently established the planar skeleton of **1** by the linkage from C13 to both keto groups.

The relative stereochemistry of **1** was subsequently elucidated by using NOESY experiments (Figure 2). The NOE cross peaks between the C21 methyl group and H3β, H4, H6, and H9β, indicated the β configuration of these protons. The NOE correlations between H9α and H16β and H17β suggested an α configuration for the C16 methylene group and, consequently, the β configuration of H15 was thus established. The β orientation of the C20 methyl group was deduced from the NOE effect between H_3-20 and H3α, while the upfield shift of the C20 atom ($\delta =$ 14.0 ppm) indicated the presence of a β-OH group at C2 due to the γ-gauche effect.[8]

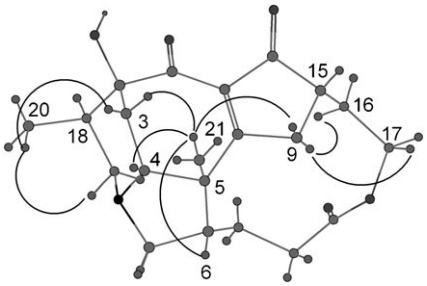


Figure 2. Key NOESY (⌒) correlations of **1**.

All of these data confirm structure **1** for macropodumine A with an unprecedented skeleton including the eleven-membered lactone ring.

Macropodumine B (**2**) was isolated as an optically active ($[\alpha]_D^{20} = -235.0$) light yellow crystalline compound ($CHCl_3$/CH_3OH 1:1). The HR-ESIMS analysis of **2** established the molecular formula $C_{23}H_{27}NO_5$ from the presence of the pseudo-molecular ion $[M+Na]^+$ at m/z 420.1786, which indicated eleven degrees of unsaturation. The IR absorption spectrum showed the presence of a hydroxy ($\tilde{\nu} = 3438\ cm^{-1}$), a conjugated carbonyl, and an iminium ($\tilde{\nu} = 1672$, 1643, 1619, 1599 cm^{-1}) functionality. The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) NMR spectra revealed nine tetrasubstituted sp^2 carbon atoms at lower field and fourteen sp^3 carbon atoms ($1\times C$, $3\times CH$, $7\times CH_2$, $2\times CH_3$, and $1\times OCH_3$) at higher field, which were assigned to their corresponding proton signals by using HSQC experiments (Table 2). Clearly, there is only one free hydroxyl group present in the structure based on the established molecular formula and the relevant NMR data.

Analysis of the $^1H,^1H$ COSY spectrum of **2** readily allowed the recognition of the proton connectivities of three partial structural units **a**–**c**, as shown in Figure 3. Detailed analyses of the HMBC spectrum led to the linking of the above-mentioned substructures through a nitrogen atom (N1) and the quaternary carbon atoms of C1, C4, C5, C8, C9, C10, C13, C14, and C15. The planar structure of **2** was thus elucidated, and showed the same framework as that of daphnicyclidin H (**5**), found in *D. humile* (see below, Scheme 1).[9] Moreover, careful comparison of the NMR data of

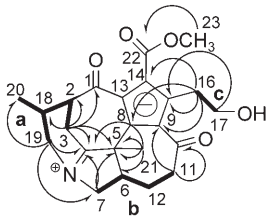


Figure 3. $^1H,^1H$ COSY (▬) and selected HMBC (⌒) correlations of **2**.

Table 2. NMR data[a] for macropodumines B (**2**)[b] and C (**3**)[c].

	Macropodumine B (**2**)		Macropodumine C (**3**)	
Atom no.	δ_H [ppm] (mult., J [Hz])	δ_C[d] [ppm] (mult.)	δ_H [ppm] (mult., J [Hz])	δ_C[d] [ppm] (mult.)
1		195.8 (s)		192.1 (s)
2	2.98 (m)	53.8 (d)		71.3 (s)
3α	2.68 (ov)	27.6 (t)	2.23 (dd, 15.6, 7.1)	24.7 (t)
3β	3.60 (dd, 13.6, 2.6)		1.91 (d, 15.6)	
4		199.5 (s)	3.52 (d, 7.1)	69.2 (d)
5		60.5 (s)		48.9 (s)
6	2.87 (m)	46.0 (d)	2.34 (m)	48.0 (d)
7α	3.50 (ov)	64.9 (t)	2.41 (ov)	59.1 (t)
7β	4.36 (dd, 13.4, 8.6)		3.60 (ov)	
8		127.7 (s)		131.6 (s)
9		123.8 (s)		126.5 (s)
10		204.2 (s)		202.8 (s)
11α	2.55 (dd, 18.0, 5.9)	40.6 (t)	2.44 (ov)	38.6 (t)
11β	2.73 (ov)		2.44 (ov)	
12α	1.55 (ddd, 23.1, 12.8, 1.1)	27.1 (t)	1.66 (ddd, 21.0, 10.8, 3.3)	28.0 (t)
12β	2.12 (m)		1.87 (m)	
13		122.2 (s)		119.1 (s)
14		122.4 (s)		121.4 (s)
15		132.0 (s)		132.5 (s)
16a	2.68 (m)	30.4 (t)	2.52 (m)	29.4 (t)
16b	3.09 (dt, 13.2, 4.6)		2.66 (dt, 13.2, 4.6)	
17a	3.54 (m)	65.3 (t)	3.70 (m)	64.2 (t)
17b	3.66 (m)		3.75 (m)	
18	2.36 (m)	36.3 (d)	2.36 (m)	34.0 (d)
19α	4.03 (dd, 14.1, 6.6)	54.0 (t)	3.77 (ov)	56.3 (t)
19β	3.30 (dd, 14.1, 11.0)		3.00 (dd, 13.0, 2.6)	
20	1.30 (d, 7.0)	19.5 (q)	1.30 (d, 7.0)	11.7 (q)
21	1.77 (s)	29.2 (q)	1.77 (s)	34.2 (q)
22		173.4 (s)		171.6 (s)
23	3.69 (s)	52.6 (q)	3.69 (s)	51.4 (q)

[a] See the Experimental Section for full details. [b] In $CDCl_3/CD_3OD$ (1:1), chemical shifts referred to $CHCl_3$ and to $CDCl_3$. [c] In CD_3OD, chemical shifts referred to CH_3OH and to CD_3OD. [d] By DEPT sequence.

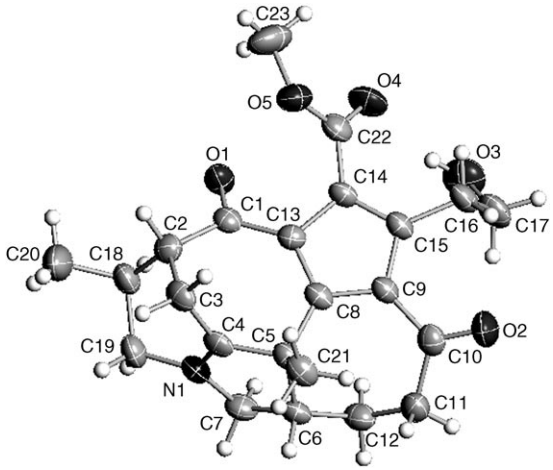
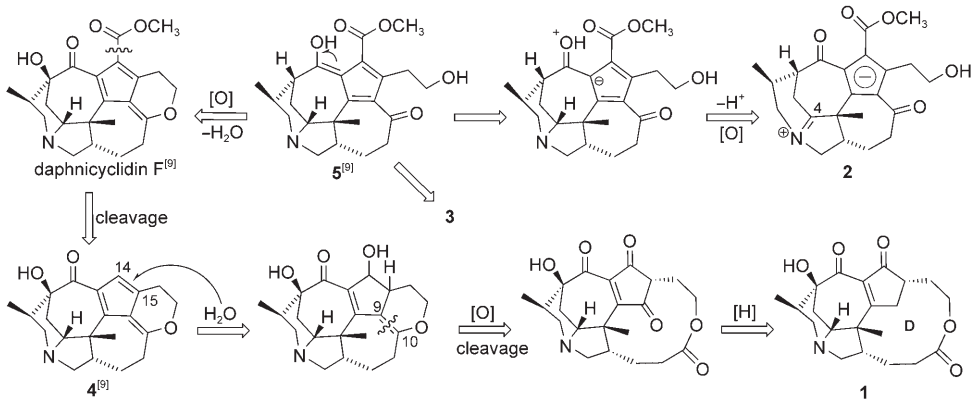


Figure 4. Single-crystal X-ray structure of **2** (ORTEP drawing).

represents the second reported example with such an amazing structural feature after the report of juglorubin from cultures of *Streptomyces* spp.[11]

Macropodumine C (**3**) was isolated as an optically active ($[\alpha]_D^{20} = -156.0$) light yellow oil. Its molecular formula $C_{23}H_{29}NO_6$ was deduced from the protonated molecular ion $[M+H]^+$ at m/z 416.2068 in the HR-ESIMS spectrum. Ten degrees of unsaturation were attributed to two carbonyl groups, three double bonds, and five rings in the molecule. The strong absorption bands at $\tilde{\nu} = 3406$, 1666, and 1597 cm^{-1} indicated the presence of one OH function and two conjugated carbonyl groups in the structure. The ^{13}C NMR and DEPT spectra revealed 23 carbon signals due to six tetrasubstituted sp^2 carbon atoms (including one enolic carbon atom), two carbonyl groups, and 15 sp^3 carbon atoms ($2\times C$, $3\times CH$, $7\times CH_2$, $2\times CH_3$, and $1\times OCH_3$), which were completely assigned to their corresponding proton signals by means of HSQC experiments (Table 2).

Analysis of the ^1H and ^{13}C NMR data of **3** revealed a great similarity to those of daphnicyclidin H (**5**) (Scheme 1).[9] The replacement of the methine carbon atom at C2 ($\delta = 48.1$ ppm) in **5** by a tertiary oxygenated carbon



Scheme 1. Proposed biogenetic connection of macropodumines A–C (**1**–**3**) with daphnicyclidins G (**4**) and H (**5**).

2 and **5** revealed that the striking differences between **2** and **5** were the absence of the proton at C4, as well as the disappearance of the enol moiety at C1/13 in **5**. The β configurations of H2, H6, H_3-20, and H_3-21 were deduced from NOESY correlations of H_3-20/H2, H3α, H19β and H_3-21/H3β, H6, and H11β.

Because compound **2** is crystalline, we were able to undertake an X-ray diffraction analysis to confirm the assigned structure and the relative configurations. The X-ray structure of **2** is shown in Figure 4, and is in agreement with the structure for **2** deduced from the NMR data. Consequently, the relative configurations for the four chiral carbon atoms of **2** were unambiguously determined as 2*R**, 5*S**, 6*R**, and 18*R**.

In particular, the presence of the cyclopentadienylium zwitterion structure in **2** was supported by the results from the X-ray diffraction analysis. The crystallographic study revealed similar O–C bond lengths for O–C1 (1.236 Å) as those of the other two keto carbonyl groups in the structure (O–C10, 1.226 Å; O–C22, 1.221 Å) in contrast with that of an enolic group (1.299 Å).[9] The bond lengths in the cyclopentadienylium anion are all very similar (1.417 ± 0.023 Å). Also, the bond length of the iminium ion (C=N^+, 1.282 Å) corresponds to that of related compounds (1.278 Å)[10] in contrast to the much longer length of an amine bond (1.514 Å).[9]

It is noteworthy that cyclopentadienyl carbanions, stabilized as a zwitterion by an internal iminium cation in **2**, are unique structural elements in the class of alkaloids and within natural products in general. In addition, natural products containing the cyclopentadienyl anion are also very rare. To the best of our knowledge, macropodumine B (**2**) atom resonating at $\delta = 71.3$ ppm in the ^{13}C NMR spectrum of **3** was supported by the increase of the molecular weight of **3** by 16 mass units. The assignment of the tertiary carbon atom as C2 was deduced from the HMBC correlations of H_2-3 with C1, C2, C4, C5, and C18, of H4 with C2, C3, C8, and C19, and of H_3-20 with C2, C18, and C19. The marked upfield shift of C20 from $\delta = 17.1$ ppm in **5** to $\delta = 11.7$ ppm in **3** unambiguously indicated a β-OH group at C2, due to the γ-gauche effect.[8] The β orientation of H4, H6, Me20, and Me21 in **3**, deduced from the NOESY spectrum, was the same as that in **5**. The structure of **3** was thus assigned as 2β-hydroxy daphnicyclidin H.

A biosynthetic relationship between the new macropodumines A–C (**1**–**3**) and those of the previously isolated daphnicyclidins G (**4**) and H (**5**), found in *D. humile*,[9] is obviously that they differ only in their oxidation state, as shown in Scheme 1. The macrocyclic lactone ring of **1** probably origi-

nated from **4** by addition of water to the C14,15 double bond, followed by oxidation at C14 and oxidative cleavage of the C9,10 double bond to form the macrocyclic lactone ring (ring **D**, Scheme 1).

Evidently, the zwitterion **2** is an oxidation product of daphnicyclidins H (**5**); the two hydrogen atoms at C1-OH and at C4 are removed. The unusual fulvene structural element presented in **5** is certainly stabilized by the hydrogen bond of the C1 enol hydroxy group with the C22 ester carbonyl group. However, the cyclopentadienyl anion present in **2** is not only stabilized by the formation of an inner salt with the iminium cation, but also by the electron-withdrawing effect of the three adjacent carbonyl groups. Thus, the observation of a stable cyclopentadienyl anion in the natural product is due to a favorable combination of several structural features within the molecule of macropodumine B (**2**). Finally, the hydroxylation of daphnicyclidin H (**5**) at C2 may lead to macropodumine C (**3**).

The cytotoxic activities of **1–3** against the growth of tumor cell lines (P-388 (mouse lymphocytic leukemia), A549 (human lung adenocarcinoma), and HT-29 (human colon adenocarcinoma)) were evaluated. Unfortunately, the results indicated that all the tested compounds were inactive against the above cancer cells (50% effective dose of clonal inhibition (ED_{50}) $>10\,\mu g\,mL^{-1}$). In addition, **1–3** also showed no inhibitory effects toward COX-2, an enzyme involved in the inflammation pathway.

The discovery of macropodumines A–C (**1–3**) has added to a diverse and complex array of *Daphniphyllum* alkaloids that are rapidly expanding. Further studies should be conducted to prove the biosynthetic origin of these compounds and to understand their real ecological roles played in the life cycle of the plant, as well as to confirm their unusual structures by total synthesis.

Experimental Section

General: Commercially available silica gel (Qing Dao Hai Yang Chemical Group Co., 200–300 and 400–600 mesh) was used for the column chromatography. Precoated silica gel plates (Yan Tai Zi Fu Chemical Group Co., G60 F-254) were used for the analytical thin-layer chromatography (TLC). TLC R_f values are reported. The NMR spectra were recorded at 293 K on a Bruker DRX-400 spectrometer at 400 MHz (for ^1H) and 100 MHz (for ^{13}C). Chemical shifts (δ) are reported with the residual $CHCl_3$ ($\delta_H=7.26$ ppm), CH_3OH ($\delta=3.31$ ppm), or C_5H_5N ($\delta_H=7.20, 7.57, 8.73$ ppm) as the internal standards for the ^1H NMR spectroscopy, and $CDCl_3$ ($\delta_C=77.0$ ppm), CD_3OD ($\delta=49.5$ ppm), or C_5D_5N ($\delta_C=123.6, 135.8, 150.0$ ppm) for the ^{13}C NMR spectroscopy. ^1H and ^{13}C NMR assignments were supported by $^1H,^1H$ COSY, heteronuclear single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC), and nuclear Overhauser enhancement spectroscopy (NOESY) NMR experiments. The following abbreviations are used to describe spin multiplicity: s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplet, ddd = doublet of doublets of doublets, m = multiplicity, ov = overlapped signal. Optical rotations were measured on a Perkin–Elmer polarimeter 341 at the sodium D-line. Infrared spectra were recorded on a Nicolet Magna FTIR 750 spectrophotometer. Melting points were measured on an X-4 digital micro-melting point apparatus and are uncorrected. UV spectra were recorded on a 756 CRT spectrophotometer. The mass spectra and HRMS were performed on a Q-TOF Micro LC–MS–MS mass spectrometer, with resolution of 5000 fwhm (full width at half-maximum). An solution of sodium iodide ($2\,mg\,mL^{-1}$) in isopropyl alcohol was used as the reference compound. The X-ray diffraction study was carried out on a Bruker SMART APEX CCD diffractometer with $Mo_{K\alpha}$ radiation ($\lambda=0.71073$ Å).

Plant material: The *Daphniphyllum macropodum* Miq. plant was collected in the Guangxi Province of the P.R. China, in July 2000, and identified by Associate Professor B.-H. Chen of the South China Institute of Botany, Chinese Academy of Sciences. A voucher specimen (reg. no. GX-18) is available for inspection at the Herbarium of the Institute of Materia Medica, Shanghai Institute for Biological Sciences, Chinese Academy of Sciences.

Extraction and isolation: The air-dried, powdered plant stem (2.1 kg) of *D. macropodum* Miq. was immersed in 95% EtOH at RT for 21 d. Evaporation of the solvent (under vacuum, 40 °C) gave a residue, which was suspended in water (1 L) and adjusted to pH 4–5 with 2 N H_2SO_4. The acidic mixture was defatted with EtOAc (3 × 1 L), and the aqueous layer was basified to pH 9–10 with saturated Na_2CO_3, and then extracted with $CHCl_3$ (3 × 1 L) to yield the crude alkaloids (1.1 g). The crude alkaloids were subjected to silica gel column chromatography eluted with a $CHCl_3$/CH_3OH/Et_2NH (50:1:0.1 to 3:1:0.1) gradient to give two major fractions, F1 and F2, of crude alkaloids. Further silica gel column chromatography on fraction F1 ($CHCl_3$/CH_3OH/Et_2NH 30:1:0.1) afforded daphnimacrolactone A (**1**) (8.9 mg). Purification of fraction F2 over a silica gel column ($CHCl_3$/CH_3OH/Et_2NH 4:1:0.1) yielded daphnicyclidin L (**2**) (76.7 mg) and M (**3**) (7.3 mg).

Macropodumine A (1): Light yellow oil; $R_f=0.38$ ($CHCl_3$/MeOH 4:1); $[\alpha]_D^{20}=+30.0$ ($c=0.25$ in MeOH); ^1H and ^{13}C NMR: see Table 1; IR ($CHCl_3$): $\tilde{\nu}=3444, 3363, 2926, 2854, 1720, 1707, 1456, 1259\,cm^{-1}$; UV/Vis (MeOH): λ_{max} (ε) = 239 (6245), 210 nm ($3262\,mol^{-1}m^3cm^{-1}$); MS (ESI): m/z: 374.3 $[M+H]^+$; HR-ESIMS: m/z: calcd for $C_{21}H_{27}NO_5Na$ $[M+Na]^+$: 396.1787; found: 396.1790; calcd for $C_{21}H_{28}NO_5$ $[M+H]^+$: 374.1967; found: 374.1965; calcd for $C_{21}H_{26}NO_5$ $[M-H]^-$: 372.1811; found: 372.1808.

Macropodumine B (2): Light yellow block crystals ($CHCl_3$/CH_3OH 1:1); $R_f=0.52$ ($CHCl_3$/MeOH 7:3); m.p. 245–247 °C; $[\alpha]_D^{20}=-235.0$ ($c=0.25$ in MeOH); ^1H and ^{13}C NMR: see Table 2; IR (KBr): $\tilde{\nu}=3439, 2924, 2852, 1672, 1643, 1620, 1599, 1385\,cm^{-1}$; UV/Vis (MeOH): λ_{max} (ε) = 362 (13300), 323 (7146), 295 (14888), 245 (2978), 210 nm ($7940\,mol^{-1}m^3cm^{-1}$); ESIMS: m/z: 420.15 $[M+Na]^+$, 396.4 $[M-H]^-$; HR-ESIMS: m/z: calcd for $C_{23}H_{27}NO_5Na$ $[M+Na]^+$: 420.1787; found: 420.1786.

Macropodumine C (3): Light yellow oil; ($CHCl_3$/CH_3OH 1:1); $R_f=0.49$ ($CHCl_3$/MeOH 7:3); $[\alpha]_D^{20}=-156.0$ ($c=0.175$ in MeOH); ^1H and ^{13}C NMR: see Table 2; FTIR (MeOH): $\tilde{\nu}=3406, 2924, 2852, 1666, 1597, 1423, 1119\,cm^{-1}$; UV/Vis (MeOH): λ_{max} (ε) = 367 (14110), 325 (7055), 301 (15148), 250 (2698), 209 nm ($7262\,mol^{-1}m^3cm^{-1}$); ESIMS: m/z: 416.2 $[M+H]^+$, 414.4 $[M-H]^-$; HR-ESIMS: m/z: calcd for $C_{23}H_{30}NO_6$ $[M+H]^+$: 416.2073; found: 416.2068.

X-ray crystallographic studies of macropodumine B (2): Light yellow block crystals of **2** were obtained by recrystallization in $CHCl_3$/CH_3OH (1:1). The crystal ($0.507\times0.488\times0.365$ mm) belongs to the orthorhombic system, with formula $C_{23}H_{27}NO_5$ ($M_r=433.49$), space group $P2_12_12_1$ with $a=9.8625$ (16), $b=20.953(3)$, $c=21.035(3)$ Å; $\alpha=\beta=\gamma=90.0°$; $V=4346.9(12)$ Å^3; $Z=8$; and $\rho_{calcd}=1.325\,mg\,m^{-3}$. A total of 26240 reflections were collected to a maximum 2θ value of 55.00° by using the ϕ/ω scan technique at 293(2) K. The structure was solved by using direct methods and was refined by means of the full-matrix least-squares procedure. The collection data were reduced by using the Saint program[12] and the empirical absorption correction was performed by using the Sadabs program.[13] All non-hydrogen atoms were given anisotropic thermal parameters. The hydrogen atom positions were geometrically idealized and allowed to ride on their parent atoms. The refinement converged to the final $R=0.0472$, $wR=0.0802$ for 9845 observed reflections ($I>2\sigma(I)$, $2\theta=51.12°$) and 615 variable parameters.

CCDC 287274 (structure **2**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the

Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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