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Macropodumines D and E, Two New Alkaloids with Unusual Skeletons from *Daphniphyllum macropodum* Miq

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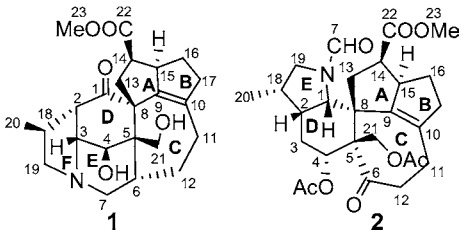
ABSTRACT



1 2

Two new complex polycyclic alkaloids, macropodumines D (1) and E (2), both possessing unprecedented skeletons, along with four known related alkaloids, were isolated from the leaves and barks of *Daphniphyllum macropodum* Miq. The structures including the relative stereochemistry of new compounds 1 and 2 were elucidated on the basis of detailed spectroscopic data analysis.

Daphniphyllum alkaloids, produced by plants of the genus *Daphniphylum*, are a structurally diverse group of natural products with highly complex polycyclic skeletons.[1] These unusual structural features have attracted great attention as challenging targets for total synthesis[2] as well as biosynthetic studies.[3] Recently, a series of new *Daphniphyllum* alkaloids have been discovered and reported,[4] and some of them possessed new carbon skeletons, which have greatly widened the knowledge of this intriguing group of natural products.

Many *Daphniphyllum* species, such as *D. macropodum* and *D. calycinum*, are used in traditional Chinese medicine for the treatment of asthma, cough, rheumatism, inflamma-

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tion, fever, and snakebite. For example, the extracts of the leaves and fruits of *D. macropudum* is used in China for the treatment of inflammation.[5]

D. macropodum Miq. is an evergreen tree which is widely distributed in the southern part of China. Previous chemical investigations on the title plant of Japanese origin carried out by Japanese researchers in the 1960s and 1970s had resulted in the isolation of numerous *Daphniphyllum* alkaloids, which could be classified into six different carbon skeletons.[1]

In the course of our search for bioactive secondary metabolites from Chinese medicinal plants,[6] we made a collection of *D. macropodum* from Guangxi Province, P. R. China, and three novel *Daphniphyllum* alkaloids named macropodumines A−C,[4a] which possess either an unusual skeleton or a rare zwitterion moiety, were isolated from this plant. Very recently, we encountered the same plant from Emei Mountain, Sichuan Province, China. Our continuing studies on the chemical constituents of this new collection led to the isolation of two new alkaloids, macropodumines D (**1**) and E (**2**), both of them possessing unprecedented carbon skeletons, along with four known related compounds, yuzurimine,[7] yuzurimine B,[8,7b] macrodaphniphyllidine,[9] and daphniglaucin D (**3**).[10] We report herein the isolation and structural elucidation of the new compounds **1** and **2**.

The usual workup[4a,11] of the $CHCl_3$-soluble fractions of the 95% EtOH extract of the leaves and barks of *D. macropodum* yielded the new compounds **1** (5.1 mg) and **2** (9.3 mg).

Macropodumine D (**1**)[12] showed the pseudomolecular ion peak at *m/z* 402 $(M + H)^+$ in the ESIMS, and the molecular formula, $C_{23}H_{31}NO_5$, was established by HRESIMS [*m/z* 402.2286, $(M + H)^+$, + 0.6 mmu]. IR absorptions implied the presence of ester carbonyl and ketone (1732 and 1693 cm^{-1}, respectively) functionalities. ^{13}C NMR and DEPT spectra revealed 23 carbon signals due to one tetrasubstituted olefin, one carbonyl, one ester carbonyl, two sp^3 quaternary carbons, seven sp^3 methines, eight sp^3 methylenes, one methyl, and one methoxy. Among them, two methylenes (δ_C 62.2, δ_H 2.43 and 2.95; δ_C 50.2, δ_H 2.18 and 2.51) and one methine (δ_C 63.2, δ_H 3.33) were ascribed to those bearing a nitrogen, while one methine (δ_C 73.0, δ_H 4.42) and one methylene (δ_C70.0, δ_H 3.71 and 4.54) were assigned to those bearing oxygen atoms (Table 1).

Table 1. ^1H NMR (δ_H) and ^{13}C NMR Data (δ_C) for **1** and **2** Measured in $CDCl_3$ at 300 K

	1		**2**	
no.	δ_H (mult, *J*, Hz)[a]	δ_C[b]	δ_H (mult, *J*, Hz)[a]	δ_C[b]
1		215.2, s	3.66 (d, 6.5)	60.9, d
2	2.60 (d, 5.9)	52.3, d	2.54 (m)	37.9, d
3α	3.33 (dd, 5.6, 3.9)	63.2, d	2.28 (dd, 25.3, 12.7)	24.4, t
3*β*			1.79 (m)	
4	4.42 (d, 4.4)	73.0, d	4.99 (dd, 11.7, 5.1)	75.4, d
5		44.3, s		56.5, s
6	1.94 (m)	42.6, d		204.6, s
7a	2.51 (dd, 12.6, 2.7)	50.2, t	8.03 (s)	165.8, d
7b	2.18 (m)			
8		58.7, s		52.0, s
9		145.5, s		141.7, s
10		133.6, s		139.9, s
11a	1.80 (m)	24.4, t	2.18 (m)	24.4, t
11b	1.92 (m)		2.52 (m)	
12a	1.46 (m)	24.4, t	2.66 (m)	42.1, t
12b	1.92 (m)		2.66 (m)	
13α	2.18 (m)	42.1, t	2.12 (m)	39.1, t
13*β*	3.69 (m)		2.50 (m)	
14	2.96 (m)	43.6, d	3.05 (dd, 16.5, 8.4)	41.2, d
15	3.85 (m)	57.9, d	3.52 (m)	51.3, d
16α	1.96 (m)	28.2, t	2.08 (m)	28.5, t
16*β*	1.13 (dd, 11.0, 8.3)		1.41 (m)	
17α	2.72 (m)	43.4, t	2.68 (m)	40.5, t
17*β*	2.32 (dd, 14.8, 7.9)		2.42 (m)	
18	2.98 (m)	33.4, d	2.08 (m)	35.8, d
19a	2.95 (m)	62.2, t	2.70 (m)	49.6, t
19b	2.43 (m)		3.86 (dd, 11.4, 6.8)	
20	1.09 (d, 7.1)	20.6, q	0.93 (d, 7.0)	11.1, q
21a	4.54 (d, 12.1)	70.0, t	4.66 (d, 11.8)	65.6, t
21b	3.71 (d, 12.1)		4.46 (d, 11.8)	
22		178.2, s		173.8, s
23	3.66 (q)	51.8, q	3.67 (q)	51.8, q
24			2.00 (s)	20.9, q
25				169.8, s
26			1.97 (s)	21.2, q
27				171.0, s

[a] Recorded at 600 MHz. [b] Recorded at 100 MHz.

Detailed analysis of the 2D NMR spectra of **1** revealed that compound **1** was composed of two moieties (Figure 1). The right moiety contained three rings (rings A, B, and C) with a double bond between C-9 and C-10, a carbomethoxy group at C-14, and two methylenes at C-5 and C-6, respectively, which was the same as that of daphniglaucin D (**3**).[10] In the left moiety, two partial structures of C-2−C-3−C-4 and C-19−C-18−C-20 were established by analy-

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(11) The leaves and bark of *D. macropodum* were extracted with 95% EtOH, and the extract was partitioned between EtOAc and acidic water (pH 4−5). The aqueous layer was basified to pH 9−10 with saturated $NaCO_3$ and then extracted with $CHCl_3$. The $CHCl_3$-soluble materials were subjected to silica gel column chromatography ($CHCl_3/CH_3OH/Et_2NH$, 50:1:0.1 to 1:1:0.1). Repeated column chromatography on amino silica gel afforded macropodumines D (**1**, 0.0004% yield) and E (**2**, 0.0007%).

(12) Macrodumine D (**1**): colorless oil; $[\alpha]^{20}_D$ −10.6 (*c* 0.24, $CHCl_3$); IR (KBr) ν_{max} 3350, 2924, 1732, 1693, 1437, 1168, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; ESIMS *m/z* 402 $(M + H)^+$; HRESIMS *m/z* 402.2286 ($[M + H]^+$, calcd for $C_{23}H_{32}NO_5$ 402.2280).

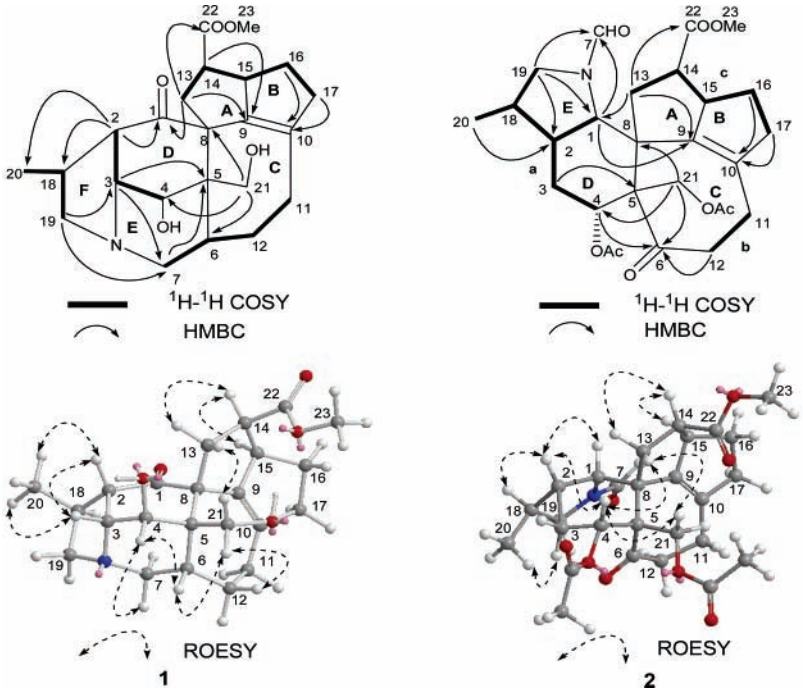
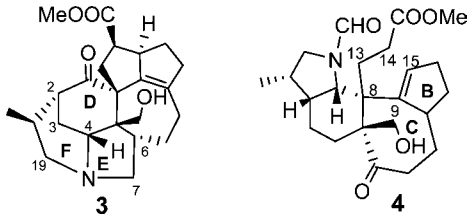


Figure 1. Selected two-dimensional NMR correlations for macropodumines D (**1**) and E (**2**).

sis of ^{1}H−^{1}H COSY spectrum. Further, the connectivity of C-2 (δ_C 52.3) to C-18 (δ_C 33.4) was revealed by the cross-peak of H-2/C-18 and C-20 in the HMBC spectrum. Moreover, several significant HMBC correlations were observed for H-19a to C-7 (δ_C 50.2) and C-3 (δ_C 63.2), H-7b to C-19 (δ_C 62.2) and H-3 to C-7, suggesting that C-3, C-7 and C-19 were connected to each other through a nitrogen atom. The presence of a ketone at C-1 (δ_C 215.2) was suggested by the HMBC correlations for H-2 and H-3 to C-1. Furthermore, the cross-peaks of H-13α to C-1 and H-3 to C-5 suggested that the right and left components are connected by bonds C-1−C-8 and C-4−C-5. Thus, the gross structure of **1**, possessing an unprecedented hexacyclic-fused ring system, which formally would be derivative of daphniglaucin D (**3**), was established as shown in Figure 1.

The relative stereochemistry of **1** was deduced from analyzing its ROESY spectrum. The ROESY correlations (Figure 1) of H-2/H-3, H-2/H$_3$-20 and H-3/H$_3$-20 indicated the β orientation of these protons. While the β oriented C-4 hydroxyl group was suggested by the ROESY cross-peaks between H-4 and H-6; H-4 and H-7b, respectively. In addition, ROESY correlations of H-21a/H-6, H-21b/H-13β, H-13α/H-14, and H-14/H-15 indicated that the relative configuration at C-5, C-6, C-8, C-14 and C-15 is the same as that of **3** leading to structure **1** for macropodumine D.

It is worthy to note that the ^{13}C NMR chemical shifts of carbons at C-9, C-10, C-11 and C-12 of **1** are somewhat different from those of **3** though they share the similar right moiety. This anomaly was probably caused by the difference in the left moiety of the structures between compounds **1** and **3**.

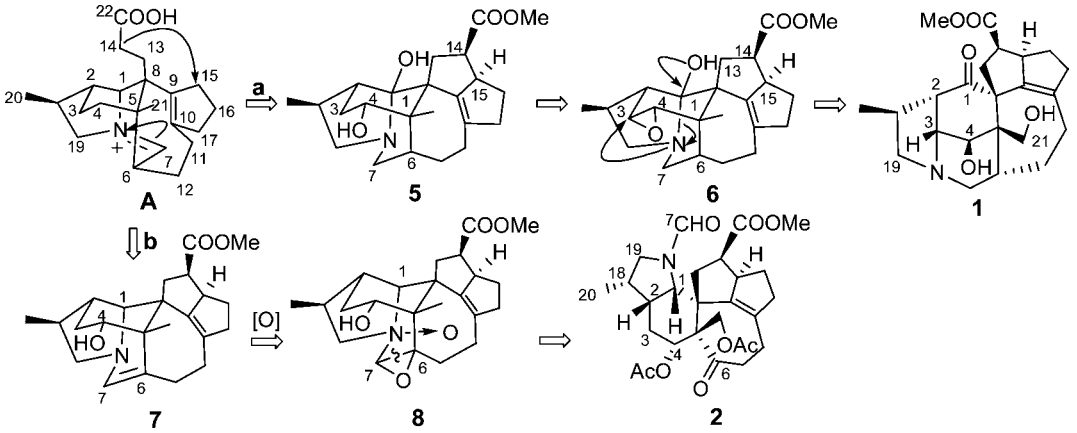


Macropodumine E (**2**)[13] was obtained as a colorless oil. Its molecular formula was deduced to be $C_{27}H_{35}NO_8$ by an HRESIMS pseudomolecular peak *m*/*z* at 524.2234 [(M + Na)$^+$, Δ −2.6mmu], indicating eleven degrees of unsaturation. Detailed analysis of ^{13}C NMR and DEPT data revealed one carbonyl, three ester carbonyls, one formyl carbonyl, and one tetrasubstituted double bond, accounting for six degrees of unsaturation. Thus the remaining degrees of unsaturation were ascribed to five rings in the molecule.

Three partial structures **a** (C-1 to C-4, C-18 to C-19 and C-20, C-2 to C-18), **b** (C-11 to C-12), and **c** (C-13 to C-17) were revealed by analysis of 2D NMR spectra of **2** (HSQC, COSY, HMBC). HMBC correlations suggested that these structural units (**a**−**c**) and the remaining one formyl and one methoxy group had to be connected to each other through six quaternary carbons (C-5, C-6, C-8, C-9, C-10, C-22) and one nitrogen atom as shown in Figure 1 to complete the structure of **2**.

(13) Macropodumine E (**2**): colorless oil; $[\alpha]^{20}_D$ −51.1 (*c* 0.47, $CHCl_3$); IR (KBr) ν_{max} 2922, 1732, 1703, 1660, 1247, 754 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; ESIMS *m*/*z* 524 (M + Na)$^+$; HRESIMS *m*/*z* 524.2234 ([M + Na]$^+$, calcd for $C_{27}H_{35}NO_8$ 524.2260).

Scheme 1. Plausible Biogenetic Pathway for Macropodumines D (**1**) and E (**2**)



The planar structure of **2** was reminiscent of that of daphniglaucin C (**4**).[14] In fact, **2** differs from **4** only by the presence of a five-membered ring A and two acetate functionalities, as well as the isomerization of the $\Delta^{9(15)}$ double bond. COSY correlations of H-14 (δ 3.05)/H-15 (δ 3.52) clearly suggested that C-14 (δ 41.2) and C-15 (δ 51.3) are connected to each other to form the ring A, while the disappearance of the olefinic proton at δ 5.74 in **4** implied that the trisubstituted double bond ($\Delta^{9(15)}$) was replaced by a tetrasubstituted olefin in **2**, which was located at $\Delta^{9(10)}$ as a consequence of cyclization of ring A. Moreover, HMBC cross-peaks between H-4 (δ 4.99), H_2-21 (δ 4.46 and 4.66), and two ester carbonyl carbons (δ 171.0 and 169.8, respectively) revealed that two acetoxyl groups were attached to C-4 and C-21, respectively. Due to the oxygenation at C-4, C-3 was downfield shifted (from δ_C 16.7 in **4** to 24.4 in **2**). Similarly, H_2-21 were also downfield shifted (from δ_H 4.49 and 3.81 in **4** to 4.66 and 4.46 in **2**, respectively) due to the acetylation of the C-21 hydroxy group.

Thus, the gross structure of macropodumine E was assigned as **2** having an unprecedented pentacyclic-fused ring system. Moreover, to the best of our knowledge, macropodumine E represents the second example of *Daphniphyllum* alkaloid with an *N*-formyl group.

The relative configuration of **2** at C-1, C-2, C-5, C-8, and C-18 was the same as that of **4** deduced by analysis of ROESY spectrum as depicted in Figure 1. The α oriented acetoxy group at C-4 was determined by the ROESY cross-peaks of H-2/H-4 and H-4/H-13β. H-14 and H-15 were cis to each other by NOE correlations of H-13α/H-14, H-14/H-15. The ROESY correlations of H-2/H-4, H-4/H-13β suggested that the cyclohexane ring D took a chair form.

By analogy to Kobayashi's hypothesis for biogenetic paths of calyciphylline A,[15] daphniglaucin D (**3**),[10] and daphniglaucin C (**4**),[14] a plausible biogenetic pathway for macropodumines D (**1**) and E (**2**) is proposed (Scheme 1). Both compounds **1** and **2**, like alkaloids **3** and **4**, should have a common precursor, an imine intermediate **A**, which, after the formation of C-14−C-15 bond, will yield hexacyclic intermediate **5**. Successively, epoxidation at C-3 and C-4 of **5** will give **6**. Further cleavage of the C-1−N bond of the epoxide intermediate **6** accompanying the formation of a C-3−N bond will generate the skeleton of macropodumine D (**1**). For compound **2**, the isomerization of the double bond $\Delta^{7(N)}$ in intermediate **A** will afford **7**. Oxidation of N, C-6 and C-7 of **7** and cleavage of C-6 to C-7 bond of intermediate **8** by Polonovski-type reaction[16] will give the skeleton of macropodumine E (**2**), although an alternative path through oxidative cleavage of C-6 to C-7 bond is also possible.

New compounds **1** and **2** were evaluated for their inhibitory activity against hPTP1B (human protein tyrosine phosphatase 1B), a key target for the treatment of type II diabetes and obesity.[17] Unfortunately, the results indicated that both compounds **1** and **2** were inactive. Other bioassay studies for antibacterial and anti-inflammatory activities are currently underway.

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Supporting Information Available: Full experimental procedures and one- and two-dimensional NMR spectra for compounds **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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