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ARTICLE TYPE

Denticulatains A and B: unique stilbene–diterpene heterodimers from *Macaranga denticulata*†

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Two novel heterodimers, denticulatains A (**1**) and B (**2**), were isolated from the fronds of *Macaranga denticulata*. They possess an unprecedented stilbene–diterpene–type skeleton, which represent a unique class of prenylated stilbene. Their structures were elucidated by comprehensive analyses of extensive NMR and MS spectroscopic data. Compounds **1** and **2** exhibited inhibitory activity against acetylcholinesterase with the inhibition ratios of 22.1% and 27.5% at concentration of 50 μM, respectively.

Stilbenes are a class of plant polyphenols with promising bioactivities and potential in therapeutic or preventive applications, such as antitumor, antioxidant, antidiabetic, antifungal and acetylcholinesterase inhibitory effect.[1] Many structurally fascinating stilbene derivatives, especially these oligomers, have gained greater attention in natural product chemistry, and their intricate molecular architectures have brought ambitious targets for organic synthesis endeavors.[1] Prenylated stilbenes, a group of miscellaneous stilbenes, have been a hot research topic due to their complex structures and sufficient biological activities, and it is interesting to study their structures, bioactivities and synthesis.[1–7]

The genus *Macaranga* is one of the largest genera of the Euphorbiaceae, previously studies showed that prenylated stilbenes[3,4,8,9] and flavones[10–14] are their typical secondary metabolites, and a number of diterpenes[15–18] were also isolated. *Macaranga denticulata* (Blume) Müll. Arg (Euphorbiaceae), whose stem water decoction has been used traditionally for washing wounds and drunk as tonic by woman after child labor[19] and its roots have been used for the treatment of icteric hepatitis,[20] has been found to contain prenylated flavones.[11] The discovery of denticulaflavonol (Fig. 1),[11] suggesting that flavone can be substituted by complex terpenoids through combinatorial chemical synthesis in Nature. Considering the presence of meta-dihydroxyl groups increases the reactivity of the ortho position of aromatic rings in the isoprenylation process and plants in the family Euphorbiaceae are a rich source of terpenoid constituents,[21] the report of denticulaflavonol are legitimate. While the isolation of macapruinosins A and B[3] (Fig. 1) from *M. pruinosa* has

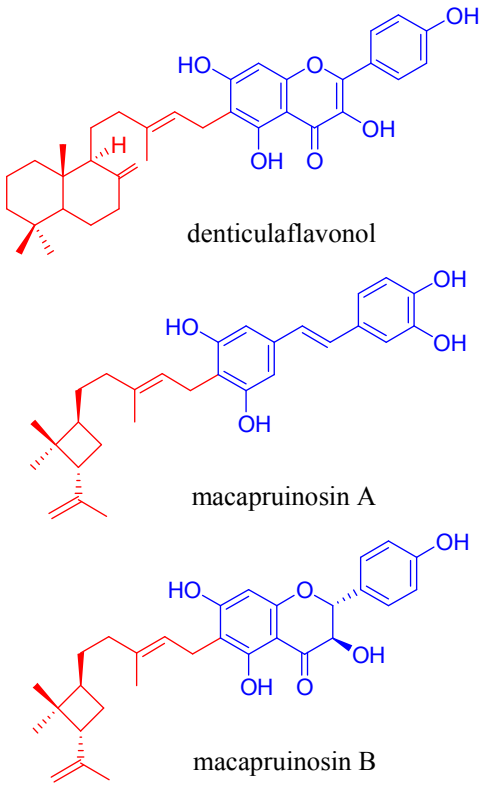

Fig. 1 The structures of denticulaflavonol, macapruinosins A and B.

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†Electronic supplementary information (ESI) available: Detailed experimental procedures, 1D and 2D NMR, MS, IR, UV and ORD spectra of compounds **1** and **2**. See DOI: 10.1039/c4ra00000x.

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suggested that the stilbene could also be substituted by the terpenoids in the similar patterns like flavone. Therefore, the exploration of stilbene-diterpene heterodimers from *M. denticulata* also appears prospective.

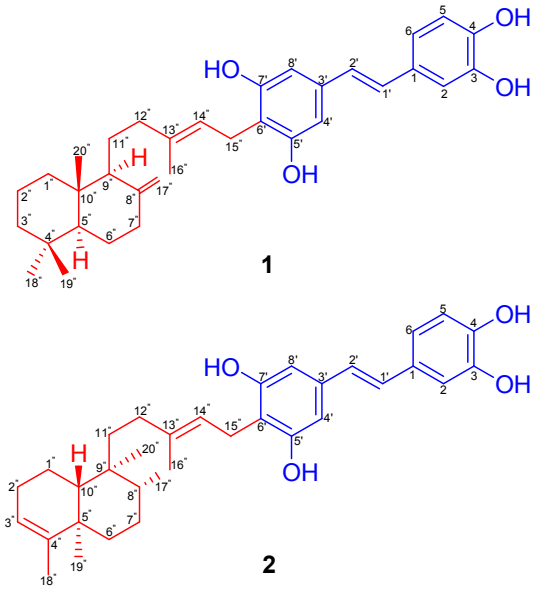


Fig. 2 The structures of denticulatains A (**1**) and B (**2**).

Despite the rapid development of separation techniques and great advances of phytochemical research on genus *Macaranga* in the past decade, the stilbene-diterpene heterodimers remain undiscovered. In our current experiments, two intriguing stilbene-diterpene heterodimers, denticulatains A (**1**) and B (**2**), were isolated and identified from the fronds of *M. denticulata*. Although numbers of oligomeric stilbenes have been reported, their variety of skeletons are produced by coupling between homogeneous or heterogeneous monomeric stilbenes, and the linkage points of oligomers were all located at the vinyl group of monomeric stilbene units.[1] Thus, denticulatains A and B (**1** and **2**) represent a rare class of stilbene-diterpene heterodimers. Their biosynthesis might provide an example of Nature's strategy for combinatorial chemical synthesis and diversity. In addition, compounds **1** and **2** were tested for their antiangiogenic activities using a zebrafish model and inhibitory activity against acetylcholinesterase. Described herein are the isolation, structure elucidation, plausible biogenetic pathway, and biological activities of the two compounds.

The air-dried and powdered fronds of *M. denticulata* (11 kg) were extracted with 90% aqueous ethanol. After removal of the ethanol in vacuo, the residue was partitioned between H_2O and EtOAc. The EtOAc portion was decolorized on MCI gel (eluting with 95% EtOH), the residue (185 g) was chromatographed on silica gel column with a gradient elution of $CHCl_3$/acetone (10:0 to 3:7) to furnish five fractions A–E. Fraction C was purified over a Sephadex LH–20 eluted with $CHCl_3$/MeOH (1:1) and then fractionated by RP–18 with a gradient elution of MeOH/H_2O (2:8 to 10:0) to yield subfractions C1–C6. Subsequently subfraction C2 was purified by a silica gel column ($CHCl_3$/acetone 1:0 to 1:1) to give three parts (P1–P3). P2 was purified over a Sephadex LH-20 eluted with $CHCl_3$/MeOH (1:1) and then separated further by semipreparative HPLC (MeOH/H_2O 85:15) to yield **1** (40 mg, t_R = 22 min) and **2** (25 mg, t_R = 28 min).

Table 1. NMR data of **1** and **2** in acetone-d_6 (δ in ppm)

No.	**1**		**2**	
	δ_C[a]	δ_H[b] (mult, *J* in Hz)	δ_C[a]	δ_H[b] (mult, *J* in Hz)
1	137.0 s		137.2 s	
2	113.6 d	6.98 (d, 1.8)	113.6 d	7.02 (d, 1.8)
3	146.0 s		146.0 s	
4	145.8 s		145.8 s	
5	116.2 d	6.74 (d, 8.2)	116.2 d	6.78 (d, 8.3)
6	119.7 d	6.79 (dd, 8.2, 1.8)	119.7 d	6.84 (dd, 8.3, 1.8)
1′	128.2 d	6.79 (d, 16.3)	128.3 d	6.83 (d, 16.3)
2′	126.9 d	6.71 (d, 16.3)	126.9 d	6.75 (d, 16.3)
3′	130.7 s		130.7 s	
4′	105.6 d	6.54 (s)	105.6 d	6.56 (s)
5′	156.9 s		156.9 s	
6′	115.2 s		115.1 s	
7′	156.9 s		156.9 s	
8′	105.6 d	6.54 (s)	105.6 d	6.56 (s)
1"	39.4 t	1.65 (m)	18.9 t	1.60 (m)
		0.92 (td, 13.0, 3.7)		1.39 (m)
2"	22.2 t	1.53 (m)	27.4 t	1.98 (m)
		1.35 (m)		
3"	42.6 t	1.28 (td, 13.0, 3.7)	121.2 d	5.13 (m)
		1.15 (m)		
4"	34.0 s		144.7 s	
5"	55.7 d	1.00 (dd, 12.6, 2.6)	38.8 s	
6"	25.1 t	1.61 (m)	37.9 t	1.44 (m)
		1.19 (td, 12.9, 4.2)		1.35 (m)
7"	38.8 t	2.23 (ddd, 12.6, 3.9, 2.4)	28.2 t	1.43 (dd, 13.0, 3.2)
		1.78 (m)		1.37 (m)
8"	149.5 s		36.9 d	1.50 (m)
9"	55.8 d	1.57 (m)	39.2 s	
10"	40.0 s		47.1 d	1.37 (m)
11"	20.0 t	1.50 (m)	37.5 t	1.68 (m)
		1.39 (m)		1.14 (m)
12"	38.8 t	2.00 (m)	33.7 t	1.84 (m)
				1.77 (m)
13"	134.7 s		135.4 s	
14"	124.3 d	5.23 (t, 7.0)	123.6 d	5.30 (t, 6.6)
15"	22.9 t	3.36 (dd, 13.7, 7.8)	23.1 t	3.34 (d, 7.0)
		3.26 (dd, 13.7, 6.8)		
16"	16.2 q	1.73 (s)	16.5 q	1.78 (s)
17"	106.4 t	4.72 (s)	16.3 q	0.78 (d, 6.5)
		4.45 (s)		
18"	33.8 q	0.80 (s)	18.2 q	1.54 (s)
19"	22.0 q	0.73 (s)	20.2 q	0.98 (s)
20"	15.0 q	0.61 (s)	18.8 q	0.70 (s)

[a] Recorded in 100 MHz. [b] Recorded in 500 MHz.

Denticulatain A (**1**),[22] obtained as optically yellow oil ($[\alpha]_D^{13}$ +22.5), was assigned the molecular formula $C_{34}H_{44}O_4$ by HRESIMS *m/z* 539.3137 $[M + Na]^+$ (calcd 539.3137), indicating 13 degrees of unsaturation. The UV maximum at 330 nm was typical for a stilbene chromphore such as macapruinosin A,[3] and the IR spectra of **1** shared many features with this unit (3078, 1612, 1517, 958, 825, 807 cm^{-1}).[3] Its ^1H NMR spectrum indicated the presence of an *trans*-vinyl group (δ_H 6.79 and 6.71, *J* = 16.3 Hz); a 1,3,4-trisubstituted benzene ring (δ_H 6.74, d, *J* = 8.2 Hz; 6.79, dd, *J* = 8.2, 1.8 Hz; 6.98, d, *J* = 1.8 Hz) and an AA' benzene ring (δ_H 6.54, s, 2H). Further analysis of the ^{13}C- (Table 1) and 2D- (Fig. 3) NMR revealed that compound **1** has the unit of a C-6' substituted piceatannol.[3] The remaining moiety possessed 20 carbons, including 4 methyls, 9 methylenes (one sp^2 at δ_C 106.4, terminal double bonds), 3 methines (one sp^2 at δ_C 124.3, trisubstituted double bonds), 4 quaternary carbons (two sp^2 at δ_C

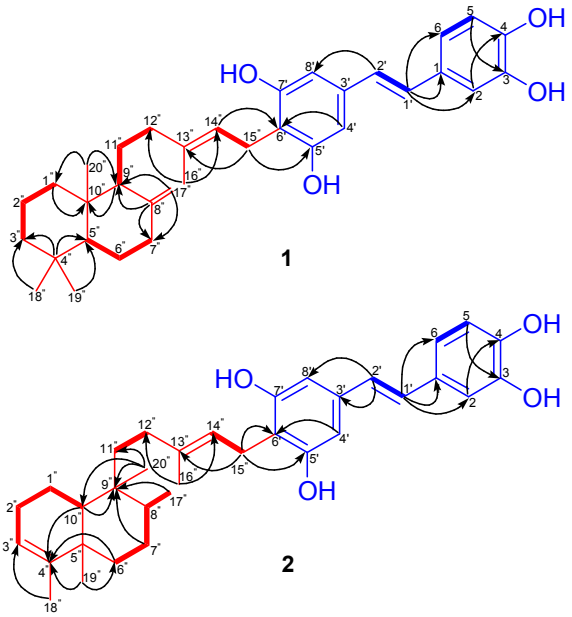


Fig. 3 Selected ^{1}H–^{1}H COSY (bold bond) and HMBC (arrows) correlations of **1** and **2**.

149.5 and 134.7) and 4 degrees of unsaturation (bicyclic C_{20}-unit), was identified as a labdane type diterpene conjugate (Fig. 2) by comparison of the NMR spectrum with that of denticulaflavonol.[11] This assumption was confirmed by comprehensive analyses of ^{1}H-^{1}H COSY (correlations of H-1"/H-2"/H-3", H-5"/H-6"/H-7", H-9"/H-11"/H-12", H-14"/H-15") and HMBC (correlations of Me-18", Me-19"/C-3", C-5"; H-17"/C-7", C-9"; Me-20"/C-1", C-9"; Me-16"/C-12", C-14") spectrum (Fig. 3). Connection of the labdanyl unit to C-6' was established by the HMBC cross-peaks of H-14" with C-6' and H-15" with C-5'.

The relative stereochemistry of **1** was deduced from the analysis of ROESY spectrum and compare to the reported natural labdane diterpenes[23] whose relative configuration had been confirmed by single-crystal X-ray analysis.[24] The ROESY correlation (Fig. 4) of Me-18"/H-5", H-5"/H-9" showed that Me-18", H-5" and H-9" were on the same face of the molecular and assigned as *α*-oriented, the same as reported.[23,24] ROESY cross-peak of Me-19"/Me-20" indicated that Me-19" and Me-20" were on another side. Accordingly, the structure and relative configuration of **1** was established as shown.

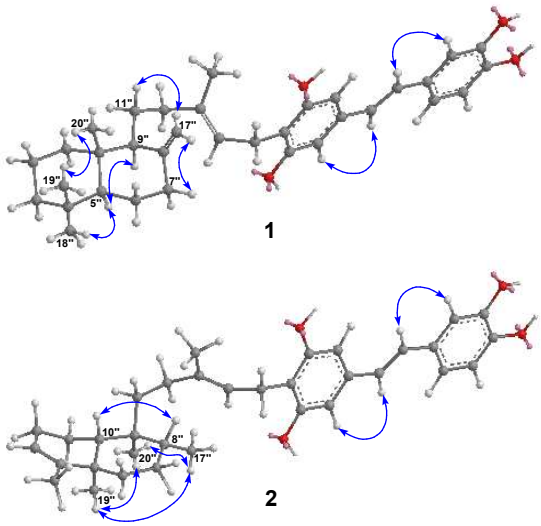
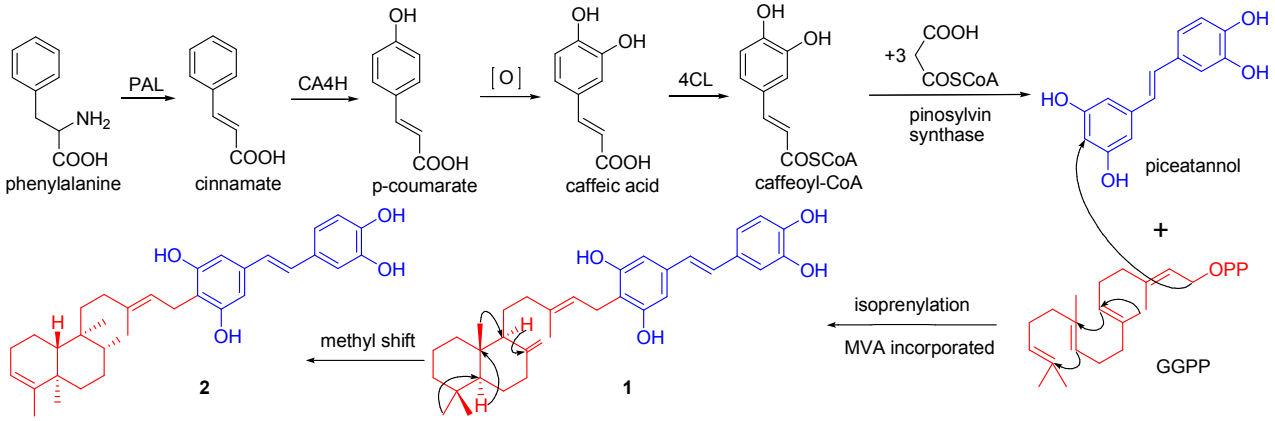


Fig. 4 Key ROESY correlations of **1** and **2**.

Denticulatain B (**2**)[25] was isolated as yellow oil and has the molecular formula of $C_{34}H_{44}O_4$ as determined by HRESIMS (found 539.3130, calcd 539.3137), the same as that of **1**. Detailed comparison of ^{1}H and ^{13}C NMR spectral data of two compounds and analysis of HMBC correlations of **2** indicated that **2** was another stilbene-diterpene heterodimer. Their major difference was the C_{20} unit, including 5 methyls, 7 methylenes, 4 methines (two sp^2 at δ_C 121.2 and 123.6, trisubstituted double bonds), 4 quaternary carbons (two sp^2 at δ_C 144.7 and 135.4) and 4 degrees of unsaturation (bicyclic skeleton), which was determined to be a clerodane type diterpene conjugate (Fig. 2) by comparison of the NMR spectrum with that of kolavenic acid.[15] This deduction was supported by the observed ^{1}H-^{1}H COSY cross-peaks of H-10"/H-1"/H-2"/H-3"; H-6"/H-7"/H-8"/Me-17"; H-11"/H-12" and H-14"/H-15", together with HMBC correlations of Me-18"/C-3"; Me-19"/C-4", C-6"; Me-17"/C-9"; Me-20"/C-10", C-11"; Me-16"/C-12", C-14" and H-15"/C-5', C-6' (Fig. 3). The *trans* relationship between H-8", H-10" and Me-17", Me-19", Me-20" was determined from the ROESY spectrum (Fig. 4),[15] which were consistent with those reported analogs confirmed by single-crystal X-ray analysis.[26] Therefore, the structure of **2** was assigned as shown.



Scheme 1 Proposed biogenetic pathway for compounds **1** and **2**.

Denticulatains A and B (**1** and **2**) were the first two stilbene-diterpene heterodimers and represent a unique carbon skeleton. A plausible biogenetic pathway for **1** and **2** was presented in Scheme 1. The sequence is initiated from a phenylalanine, then a conjugate addition of geranylgeranyl-PP onto the intermediate piceatannol, which undergo an intricate cyclizaton cascade lead to the formation of **1**. Then, compound **2** was produced by a methyl shifting reaction. Previously phytochemical research on genus *Macaranga* lead the isolation of prenylated piceatannol,[3] labdane-kaempferol[11] and clerodane diterpenoids[15] indicated that the related enzyme may exist in the plants of this genus. Additionally, the discovery of compounds **1** and **2** gives insight into how Nature has combined utilization of terpenoid cyclases and polyphenol synthetase to produce heteromers.

Since some prenylated stilbenes isolated from *Macaranga* genus are reported to have modest or strong anticancer[1,2,4] and acetylcholinesterase inhibitory[1,27] activities, the new compounds **1** and **2** were evaluated for their antiangiogenic activities using a zebrafish model by the same method as previously described, and PTK787 was used as the positive control (IC_{50} 0.28 μM).[28] Unfortunately, none of the compounds exhibited significant activities with IC_{50} values greater than 40 μM. In addition, the inhibitory activity against acetylcholinesterase of compounds **1** and **2** were tested using the method previously described, with tacrine used as a positive control (IC_{50} 0.19 μM).[29] Both compounds exhibited weak inhibitory activity against acetylcholinesterase. The inhibition ratios were 22.1% (**1**) and 27.5% (**2**) at a concentration of 50 μM, respectively.

Acknowledgement

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22. Denticulatain A (**1**): yellow oil, $[\alpha]_D^{13}$ = +22.5 (*c* 0.75, MeOH); UV (MeOH) λ_{max} nm (log ε): 201 (4.32) nm, 223 (4.26) nm, 330 (4.29) nm; IR (KBr) ν_{max} 3417, 3078, 2926, 2842, 1694, 1612, 1517, 1441, 1364, 1271, 1191, 1157, 1109, 1035, 958, 886, 855, 825, 807, 641 cm^{-1}; NMR data see Table 1; positive ESIMS *m/z* 539 $[M + Na]^+$; HRESIMS *m/z* 539.3137 $[M + Na]^+$ (calcd for $C_{34}H_{44}O_4Na$, 539.3137).
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25. Denticulatain B (**2**): yellow oil, $[\alpha]_D^{13}$ = –29.1 (*c* 0.45, MeOH); UV (MeOH) λ_{max} nm (log ε): 201 (4.28) nm, 222 (4.27) nm, 330 (4.29) nm; IR (KBr) ν_{max} 3423, 2924, 2871, 1691, 1617, 1581, 1517, 1439, 1340, 1270, 1160, 1107, 1029, 957, 825 cm^{-1}; NMR data see Table 1; positive ESIMS: *m/z* 539 $[M + Na]^+$; HRESIMS *m/z* 539.3130 $[M + Na]^+$ (calcd. for $C_{34}H_{44}O_4Na$, 539.3137).
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Graphic Abstract

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Da-Song Yang, Zi-Lei Li, Xue Wang, Hui Yan, Yong-Ping Yang, Huai-Rong Luo, Ke-Chun Liu, Wei-Lie Xiao and Xiao-Li Li

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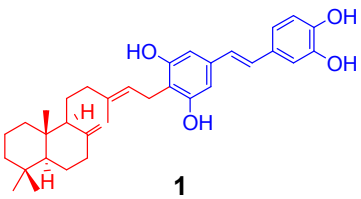

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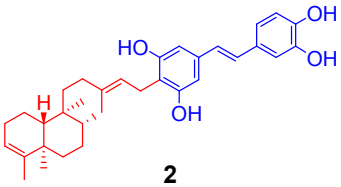

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