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Amomaxins A and B, Two Unprecedented Rearranged Labdane Norditerpenoids with a Nine-Membered Ring from *Amomum maximum*

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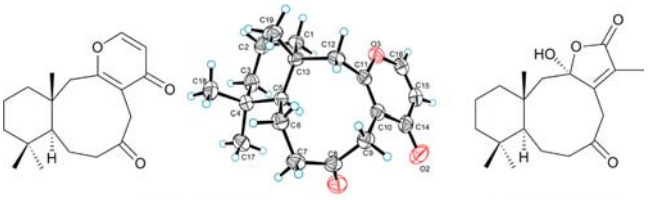
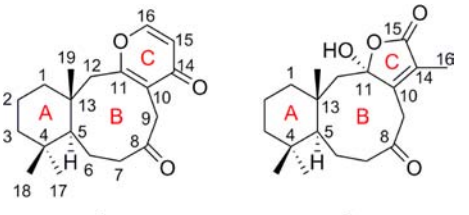
ABSTRACT



Amomaxins A (1) and B (2), featuring an unprecedented rearranged labdane norditerpene skeleton with a nine-membered ring, along with their biosynthetic related known compound isocoronarin D (3) were isolated from the roots of *Amomum maximum*. Their structures with absolute configurations were determined by spectroscopic data, CD experimentation, and single-crystal X-ray diffraction. Compound 2 showed an inhibitory effect on nitric oxide (NO) production in lipopolysaccharide-induced RAW264.7 macrophages.

Amomum maximum Roxb. (Zingiberaceae) is a tropical plant and widely distributed in South China and Southeast Asia. Its fruits and roots are commonly used as folklore medicine to treat stomach diseases and digestive disorders.[1] Our previous investigation on the medicinal plants of the Zingiberaceae family showed some unusual constituents with diverse bioactivities.[2−4] In our continuing endeavor to discover unique structures, two unprecedented rearranged labdane norditerpenes with a nine-membered ring, amomaxins A (**1**) and B (**2**), along with a known labdane diterpene isocoronarin D (**3**) were isolated from the roots of *A. maximum*. We herein present their isolation and structure elucidation, as well as inhibitory activities on nitric oxide (NO) production in lipopolysaccharide-induced RAW264.7 macrophages.

The EtOH extract from the roots of *A. maximum* was suspended in H_2O and then partitioned successively with CH_2Cl_2 and EtOAc. The CH_2Cl_2 fraction was subjected to column chromatography over silica gel, Sephadex LH-20, and ODS and further purified by preparative HPLC, to afford **1** (0.0001%), **2** (0.00007%), and **3** (0.0002%). The known compound **3** was identified as isocoronarin D by comparison of its spectroscopic data with those in the literature.[5]



Amomaxin A (**1**)[6] was obtained as colorless needle crystals (MeOH). The molecular formula was deduced as

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$C_{19}H_{26}O_3$ by the pseudomolecular ion at m/z 303.1961 $[M + H]^+$ (calcd for $C_{19}H_{27}O_3$, 303.1955) in the HRESIMS, corresponding to 7 degrees of unsaturation. The UV absorption at 220, 259 nm and IR bands at 1643, 1620 cm^{-1} indicated the presence of a γ-pyrone moiety,[7] and the IR absorption at 1711 cm^{-1} suggested the other keto group. The ^1H NMR spectrum exhibited three high-field methyl singlets at δ_H 0.84, 0.98, and 1.08 and a pair of coupled olefinic protons at δ_H 6.40 (1H, d, J = 6.0 Hz) and 8.05 (1H, d, J = 6.0 Hz). On the basis of ^{13}C NMR and HSQC spectra, compound **1** showed 19 carbons. Among them, two ketone carbons (δ_C 180.4, 212.3), two olefinic quaternary carbons (δ_C 125.0, 167.4), and a pair of olefinic methine carbons (δ_C 116.1, 157.6) occupied 4 degrees of unsaturation. The above data suggested that compound **1** is a norditerpenoid possessing a tricyclic ring system.

The gross structure of **1** was established by analysis of 2D NMR spectra (HSQC, HMBC, $^1H-^1H$ COSY, and ROESY). In the HMBC spectrum, two singlets at δ_H 0.84 and 0.98 (each 3H, s) ascribable to Me-17 and Me-18, respectively, showed significant correlations with an sp^3 methylene at δ_C 43.3 (C-3) and an sp^3 quaternary carbon at δ_C 36.6 (C-4), another singlet at δ_H 1.08 (3H, s, Me-19) with an sp^3 quaternary carbon at δ_C 41.9 (C-13), and H-5 (δ_H 0.73, dd, J = 7.0, 2.0 Hz) exhibited correlations with C-4 (δ_C 36.6), C-13 (δ_C 41.9), and C-19 (δ_C 23.2). Considering the $^1H-^1H$ COSY spin-coupling systems H-1/H-2/H-3, a six-membered ring A was established (Figure 1).

Furthermore, HMBC correlations from H-12 (δ_H 3.27, 2.21) to an olefinic quaternary carbon at δ_C 125.0 (C-10) and from H-9 (δ_H 3.73, 3.65) to C-10 and the other olefinic quaternary carbon at δ_C 167.4 (C-11) indicated that one olefinic bond was placed at C-10/C-11. The combined spin-coupling systems H-5/H-6/H-7 in the $^1H-^1H$ COSY spectrum with HMBC correlations from H-6 (δ_H 2.01, 1.78), H-7 (δ_H 2.76, 2.32), and H-9 (δ_H 3.73, 3.65) to C-8 (δ_C 212.3) demonstrated that the keto group was placed at C-8. Additionally, key HMBC correlations from H-6β (δ_H 2.01) to C-13 (δ_C 41.9), H-12α (δ_H 2.21) to C-5 (δ_C 53.1), and H-7α (δ_H 2.32) to C-9 (δ_C 35.8) were also observed. Based on the above information, a nine-membered ring B was thus constructed to be fused with ring A at C-5/C-13 (Figure 1).

Similarly, an olefinic proton at δ_H 8.05 (1H, d, J = 6.0 Hz, H-16) showed HMBC correlations with C-11 (δ_C 167.4) and the carbonyl carbon at δ_C 180.4 (C-14), and the other olefinic signal at δ_H 6.40 (1H, d, J = 6.0 Hz, H-15), with C-10 (δ_C 125.0). Again, taking the degrees of unsaturation into consideration, a γ-pyrone unit (ring C) comprising C-10, C-11, C-14, C-15, and C-16 was fused with ring B at C-10/C-11 (Figure 1).

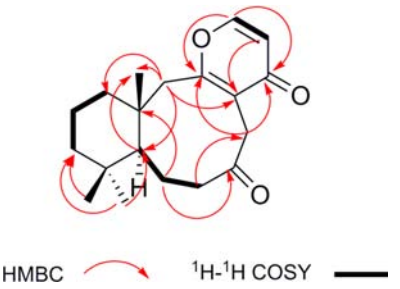

Figure 1. Key HMBC and $^1H-^1H$ COSY correlations of amomaxin (**1**).

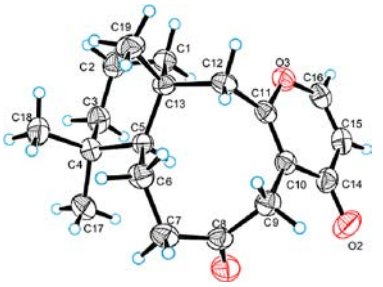

Figure 2. X-ray structure of amomaxin (**1**).

On the basis of ROESY data, correlations between H-5 (δ_H 0.73) and Me-17 (δ_H 0.84), Me-19 (δ_H 1.08) and Me-18 (δ_H 0.98) as well as an absent correlation between H-5 (δ_H 0.73) and Me-19 (δ_H 1.08) indicated the relationship between H-5 and Me-19 to be *trans*. Fortunately, single crystals of **1** were obtained and subjected to an X-ray diffraction experiment using mirror Cu Kα radiation. As shown in Figure 2, the structure of **1** was confirmed as deduced above, and the absolute configuration was finally determined to be 5*S*,13*R* by Flack absolute structure parameter 0.0(2). Accordingly, compound **1** was established and named as amomaxin A.

Amomaxin B (**2**)[8] was isolated as a light yellow oil. HRESIMS data (m/z 343.1878 $[M + Na]^+$, calcd for $C_{19}H_{28}O_4Na$, 343.1880) gave the molecular formula $C_{19}H_{28}O_4$, indicating 6 degrees of unsaturation. The IR spectrum showed the presence of a hydroxyl group (3422 cm^{-1}), a keto group (1709 cm^{-1}), and an α,β-unsaturated-γ-lactone ring (1746, 1643 cm^{-1}).[9] The ^1H NMR spectrum ($CDCl_3$) of **2** exhibited signals for four tertiary methyls at δ_H 0.78, 0.87, 0.87, 1.94. The ^{13}C NMR, DEPT, and HSQC spectra ($CDCl_3$) revealed 19 carbons for four

(6) Amomaxin A (**1**): colorless needle crystals (MeOH); mp 134–136 °C; $[\alpha]^{25}_D$ −37.4 (*c* 0.10, MeOH); UV (MeOH) λ_{max} (log ε) 220 (3.78), 259 (3.74) nm; IR (KBr) ν_{max} 2961, 2925, 1711, 1643, 1620, 1400, 1385, 1129 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 303.2 $[M + H]^+$; HRESIMS m/z 303.1961 $[M + H]^+$ (calcd for $C_{19}H_{27}O_3$, 303.1955).

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(8) Amomaxin B (**2**): light yellow oil; $[\alpha]^{25}_D$ +16.7 (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 218 (3.70) nm; CD (MeOH, *c* 1.67 × 10^{-4}) λ_{max} ($\Delta\varepsilon$) 230 (−3.26), 253 (+2.91) nm; IR (KBr) ν_{max} 3422, 2961, 2925, 1746, 1709, 1643, 1462, 1394, 1379, 1066 cm^{-1}; for ^1H and ^{13}C NMR data, see Table 1; ESIMS m/z 319.1 $[M - H]^-$; HRESIMS m/z 343.1878 $[M + Na]^+$ (calcd for $C_{19}H_{28}O_4Na$, 343.1880).

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Data of Compounds **1** and **2**

	1		**2**			
no.	δ_H[a] (multi, *J* in Hz)	δ_C[a]	δ_H[b] (multi, *J* in Hz)	δ_C[b]	δ_H[c] (multi, *J* in Hz)	δ_C[c]
1α	1.55 (m)	40.1	1.33 (m)[d]	38.6	1.17 (br d, 14.0)	37.8
1*β*	1.66 (m)		1.72 (m)[d]		1.70 (m)	
2α	1.47 (m)	20.1	1.39 (m)	18.9	1.28 (m)[d]	18.3
2*β*	1.71 (m)		1.50 (m)		1.46 (br d, 14.0)	
3α	1.04 (dd, 13.5, 3.5)	43.3	0.97 (m)	42.7	0.80 (m)	42.2
3*β*	1.39 (br d, 13.0)		1.34 (m)[d]		1.27 (m)[d]	
4		36.6		35.6		34.8
5	0.73 (dd, 7.0, 2.0)	53.1	0.98 (m)	50.3	0.90 (dd, 6.5, 2.5)	49.5
6α	1.78 (m)	21.7	1.65 (m)[d]	21.6	1.60 (m)	20.3
6*β*	2.01 (m)					
7α	2.32 (m)	48.3	2.43 (m)	45.6	2.25 (m)[d]	44.7
7*β*	2.76 (dt, 13.5, 5.5)		2.67 (m)		2.60 (ddd, 14.5, 8.0, 4.0)	
8		212.3		207.2		207.3
9α	3.73 (d, 14.5)	35.8	3.55 (d, 14.0)	40.4	3.57 (d, 14.0)	40.1
9*β*	3.65 (d, 14.5)		3.41 (d, 14.0)		3.40 (dd, 14.0, 1.0)	
10		125.0		153.5		154.5
11		167.4		106.0		106.4
12α	2.21 (d, 14.5)	46.0	1.84 (d, 15.5)	48.8	1.72 (d, 16.0)	47.5
12*β*	3.27 (d, 14.5)		2.34 (d, 15.5)		2.24 (d, 16.0)[d]	
13		41.9		38.3		37.3
14		180.4		129.6		127.2
15	6.40 (d, 6.0)	116.1		170.9		170.8
16	8.05 (d, 6.0)	157.6	1.94 (s)	9.4	1.79 (s)	8.6
17	0.84 (s)	34.6	0.78 (s)	34.5	0.71 (s)	33.8
18	0.98 (s)	22.7	0.87 (s)[d]	22.4	0.82 (s)	21.8
19	1.08 (s)	23.2	0.87 (s)[d]	24.0	0.76 (s)	23.8
11-OH					7.15 (s)	

[a] Measured in CD_3OD. [b] Measured in $CDCl_3$. [c] Measured in DMSO-d_6. [d] Signal pattern unclear due to overlapping.

methyls, seven methylenes, one methine, and seven quaternary carbons (including a ketol carbon at δ_C 106.0, two olefinic carbons at δ_C 129.6 and 153.5, a lactone carbonyl at δ_C 170.9, and a ketone carbonyl at δ_C 207.2). All of the proton and carbon signals were assigned unambiguously by comprehensive analysis of the $^{1}H-^{1}H$ COSY, HSQC, and HMBC spectra ($CDCl_3$). Comparison of NMR data with those of **1** (Table 1) indicated structural similarities, except for signals assignable to ring C. In the HMBC spectrum of **2**, correlations from Me-16 (δ_H 1.94) to C-14 (δ_C 129.6), C-10 (δ_C 153.5), and C-15 (δ_C 170.9); from H-9 (δ_H 3.55, 3.41) to C-11 (δ_C 106.0), C-14 (δ_C 129.6), and C-10 (δ_C 153.5); from H-12 (δ_H 2.34, 1.84) to C-11 (δ_C 106.0); and from H-12α (δ_H 1.84) to C-10 (δ_C 153.5) (Figure S1 in the Supporting Information (SI)) suggested that an α,*β*-unsaturated-*γ*-lactone unit was fused with the nine-membered ring B at C-10/C-11. To determine the position of the hydroxyl group, 1D and 2D NMR spectra of **2** were recorded in DMSO-d_6. The hydroxyl proton was observed at δ_H 7.15 (1H, s) and showed HMBC correlations with C-10 (δ_C 154.5), C-11 (δ_C 106.4), and C-12 (δ_C 47.5), which suggested that it was connected to C-11 (Figure S1 in SI).

The relative configuration of **2** was determined by the ROESY experiment in DMSO-d_6. Correlations of H-5 with H-7α, Me-17 with H-7α, H-9α with H-7α and 11-OH, and Me-19 with H-12*β* indicated H-5, 11-OH, Me-17 on the same face of the molecule, while Me-19 was on the other face (Figure S1 in SI). The absolute configuration at C-11 of **2** was established by a CD spectrum (a $\pi-\pi^*$ transition in the α,*β*-unsaturated-*γ*-lactone moiety). The observed negative Cotton effect at 230 nm revealed the 11*R* configuration (Figure S25 in SI).[9−11] Combined with ROESY correlations, the absolute configuration of **2** was designated as 5*S*,11*R*,13*R*. Therefore, the structure of **2** was depicted as shown.

A review concerning the types of skeletons occurring in plants of the Zingiberaceae family suggested that labdane-type diterpenes were common secondary metabolites.[12−16] Consequently, we could tentatively propose that compounds **1** and **2** are originated from isocoronarin D (**3**) with a plausible biogenetic pathway, outlined in Scheme 1.

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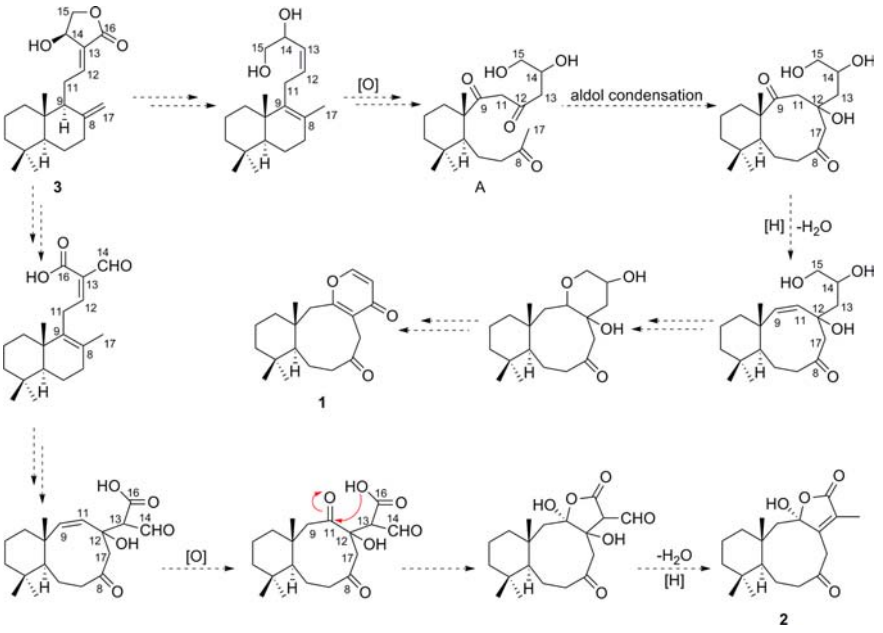
Scheme 1. Plausible Biogenetic Correlations of Compounds **1**–**3**



The early steps of the biosynthetic process of **1** were proposed to involve decarboxylation at C-16 after esterlysis of **3**, oxidation of $\Delta^{12(13),17}$ the double bond migration from $\Delta^{8(17)}$ to $\Delta^{8(9)}$, and further oxidative cleavage of $\Delta^{8(9),18}$ to yield the intermediate A. C-12 and C-17 of A might undergo an aldol condensation[19] to reconstruct an unusual nine-membered ring. After reduction of the keto group at C-9 and further dehydration producing $\Delta^{9(11),18}$ the γ-pyrone unit was finally formed via the addition reaction of $\Delta^{9(11)}$ and further cyclization, followed by dehydration and oxidation. Correspondingly, the formation of **2** from **3** involved oxidative degradation of 14,15-diols after esterlysis of **3**, reconstruction of a nine-membered ring through the same procedures with **1**, oxidation of $\Delta^{9(11),17}$ and nucleophilic addition,[20] dehydration, and reduction of aldehyde. Therefore, from a biogenetic point of view, amomaxins A (**1**) and B (**2**) represent the first example of unprecedented rearranged labdane norditerpenes with a nine-membered ring.

Compounds **1**–**3** were tested for their inhibitory effects on NO production induced by LPS in macrophage cell line RAW264.7. Cell viability was first determined by the MTT method to find whether inhibition of NO production was due to the cytotoxicity of the tested compounds. As a result, no obvious cytotoxic effects (over 90% cell survival) of RAW264.7 cells treated with compounds **1** and **2** at concentrations up to 100 μM were observed, whereas compound **3** showed significant cytotoxicity. Compound **2** showed NO inhibition on LPS-activated RAW264.7 cells with an IC_{50} value of 31.33 μM, comparable to that of the control compound *N*-monomethyl-L-arginine at 40.45 μM, whereas compound **1** was considered inactive (IC_{50} > 100 μM). For compounds **1**–**3**, only **2** showed a NO inhibitory effect, which indicated that the presence of a tertiary alcohol group in **2** played an important role in NO inhibitory activity.[21,22]

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Supporting Information Available. Experimental section; UV, IR, ESIMS, HRESIMS, CD, 1D and 2D NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

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