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Aphanamixoid A, a Potent Defensive Limonoid, with a New Carbon Skeleton from *Aphanamixis polystachya*

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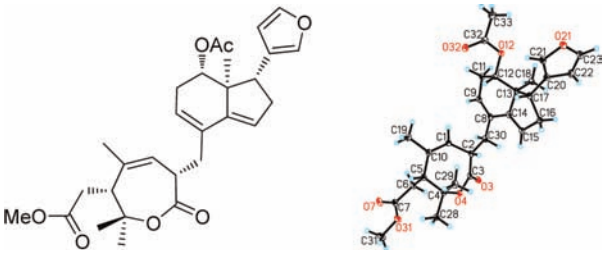
ABSTRACT



Aphanamixoid A (1), a limonoid with a new carbon skeleton, along with its biogenetically related limonoid aphanamixoid B (2), was isolated from the leaves and twigs of *Aphanamixis polystachya*. Their structures with the absolute stereochemistry were determined by spectroscopic analysis, X-ray crystallography and computational methods. The significant antifeedant activity of 1 against the generalist plant-feeding insect *Helicoverpa armigera* (EC_{50} = 0.015 μmol/cm^2) suggested it may be a potent defensive component of *A. polystachya*.

Limonoids, a series of structurally diverse and highly oxygenated tetranortriterpenoids mainly found in the family of Meliaceae, have been attracting continuous attention from biogenetic and synthetic points of view.[1] In recent years, a number of limonoids have still been isolated by several research groups, a few of which exhibited biological activities including cytotoxic,[2] antimalarial,[3] insect antifeedant,[4] insecticidal,[4a,5] and insect growth regulatory[4b] activities.

The plant *Aphanamixis polystachya* (Wall.) R. N. Parker (Meliaceae), a timber tree, is mainly distributed in the tropical areas of Asia, such as India, Malaysia, Indonesia, and southern China.[6] Previous chemical studies on this

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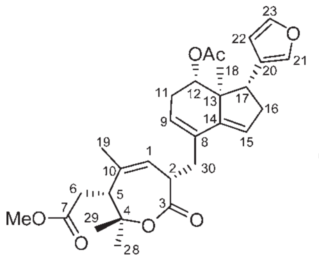
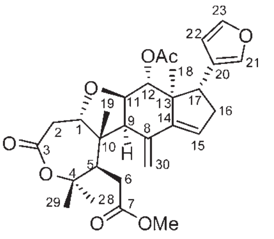
plant have resulted in the isolation of a few new limonoids; however, no significant bioactivity has been found from those compounds.[7] In the current study, a new limonoid with potent antifeedent activity, aphanamixoid A (**1**), was isolated from the leaves and twigs of *A. polystachya* collected in the Yunnan province of China. **1** could be derived from a new biogenetically related limonoid, aphanamixoid B (**2**), via the unique cleavage of a C-9–C-10 bond as well as the formation of a C-2–C-30 bond by means of 3,3-rearrangement. In this paper, we report the isolation, structure elucidation, plausible biogenetic pathway, and the bioactivities of aphanamixoids A (**1**) and B (**2**).



1



2

Aphanamixoid A (**1**)[8] was obtained as colorless crystals (in acetone). Its molecular formula, $C_{29}H_{36}O_7$, was established from the quasi-molecular ion peak at *m/z* 519.2361 $[M + Na]^+$ (calcd 519.2358, $C_{29}H_{36}O_7Na$) in the positive HRESIMS, which indicated 12 degrees of unsaturation. UV absorption at 242 nm (3.44) indicated the presence of conjugated double bonds. IR peaks at 1732 and 1717 cm^{-1} as well as ^{13}C NMR signals at δ 173.4, 172.7, and 170.9 revealed three ester carbonyl groups. Besides a methoxy group (δ_H 3.70; δ_C 52.1) and an acetyl group (δ_H 1.90; δ_C 21.3, 170.9), **1** contained 26 carbons, including a β-furan ring (δ_H 6.32, 7.23, 7.35; δ_C 111.6, 140.0, 142.1) and four tertiary methyl groups (δ_H 0.90, 1.36, 1.61, 1.78). The above evidence suggested that **1** was a tetranortriterpenoid. Furthermore, apart from the five double bonds and three carbonyl groups, the remaining four degrees of unsaturation indicated **1** to be tetracyclic system.

Extensive comparison of ^1H and ^{13}C NMR data with those of a known limonoid, munronoid B, suggested both compounds shared the same A, D, and E ring systems, as further confirmed by 2D NMR studies. Furthermore, the replacement of a C-30 sp^2 methylene signal in munronoid B[5b] with a sp^3 methylene signal (δ_H 2.30, 2.97; δ_C 34.7) in **1**, as well as the striking presence of two double bonds ($\Delta^{8(9)}$ and $\Delta^{1(10)}$) in **1** implied that the methylene group (C-30) might be the core linkage between rings A and C instead of the usual connectivity via C-9 and C-10.

Table 1. ^1H and ^{13}C NMR Data for **1** and **2** in $CDCl_3$

	1		**2**	
	δ_C[a]	δ_H (mult; *J*, Hz)[a]	δ_C[a]	δ_H (mult; *J*, Hz)[b]
1	120.2 (d)	5.18 (s)	81.6 (d)	4.07 (dd, 10.9, 4.8)
2	43.7 (d)	3.42 (br s)	38.2 (t)	2.87 (m, 2H)
3	172.7 (s)		170.3 (s)	
4	81.4 (s)		84.8 (s)	
5	47.8 (d)	2.74 (dd, 6.2, 4.0)	48.6 (d)	2.90 (dd, 6.9, 3.8)
6a	34.4 (t)	2.23 (dd, 17.0, 4.0)	33.4 (t)	2.41 (dd, 17.0, 6.9)
6b		2.82 (dd, 17.0, 6.2)		3.07 (dd, 17.0, 3.8)
7	173.4 (s)		173.7 (s)	
8	131.0 (s)		140.3 (s)	
9	125.0 (d)	5.50 (d, 3.4)	55.6 (d)	3.06 (d, 8.2)
10	137.8 (s)		50.5 (s)	
11α	29.9 (t)	2.22 (m)	79.3 (d)	4.11 (dd, 9.7, 8.2)
11β		2.47 (m)		
12	77.6 (d)	5.07 (dd, 10.0, 6.1)	75.8 (d)	5.35 (d, 9.7)
13	50.2 (s)		51.7 (s)	
14	147.6 (s)		149.4 (s)	
15	121.7 (d)	5.65 (s)	122.9 (d)	5.76 (t, 3.0)
16α	37.7 (t)	2.58 (m, 2H)	37.8 (t)	2.39 (ddd, 16.5, 10.5, 3.0)
16β				2.62 (ddd, 16.5, 8.4, 3.0)
17	46.4 (d)	3.15 (dd, 9.9, 8.7)	47.2 (d)	3.30 (dd, 10.5, 8.4)
18	13.2 (q)	0.90 (s)	15.1 (q)	0.80 (s,CH_3)
19	25.5 (q)	1.78 (s)	20.4 (q)	1.17 (s, CH_3)
20	124.6 (s)		124.3 (s)	
21	140.0 (d)	7.23 (s)	139.9 (d)	7.19 (s)
22	111.6 (d)	6.32 (s)	111.1 (d)	6.24 (s)
23	142.1 (d)	7.35 (br s)	142.4 (d)	7.33 (br s)
28	28.8 (q)	1.36 (s)	30.6 (q)	1.40 (s, CH_3)
29	25.7 (q)	1.61 (s)	27.7 (q)	1.45 (s, CH_3)
30a	34.7 (t)	2.30 (dd, 14.2, 9.1)	118.8 (t)	5.12 (s)
30b		2.97 (dd, 14.2, 3.7)		5.40 (s)
12-OAc	170.9 (s)		170.8 (s)	
	21.3 (q)	1.90 (s)	21.2 (q)	1.95 (s, CH_3)
7-OMe	52.1 (q)	3.70 (s)	52.3 (q)	3.76 (s, CH_3)

[a] ^1H NMR spectra were recorded at 400 MHz and ^{13}C NMR spectrum at 100 MHz. [b] ^1H NMR spectrum was recorded at 500 MHz.

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(8) Aphanamixoid A (**1**): colorless crystals (in acetone); mp 148–150 °C; HRESIMS at *m/z* 519.2361 $[M + Na]^+$ (calcd 519.2358, $C_{29}H_{36}O_7Na$); $[\alpha]_D^{14.0}$ = +39.9° (*c* 0.260, MeOH); UV (MeOH) λ_{max} (log ε) 242 (3.44), 202 (3.39); CD (0.000511 M, MeOH) λ_{max} ($\Delta\varepsilon$) 231 (1.14); IR ν_{max} (KBr) cm^{-1} 1732, 1717, 1265, 1255, and 1245; ^1H and ^{13}C NMR data, see Table 1.

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(10) Aphanamixoid B (**2**): colorless amorphous powder; mp 170–171 °C; HRESIMS at *m/z* 535.2314 $[M + Na]^+$ (calcd 535.2307, $C_{29}H_{36}O_8Na$); $[\alpha]_D^{14.3}$ = +81.8° (*c* 0.140, MeOH); UV (MeOH) λ_{max} (log ε) 210 (3.06), 202 (3.07); CD (0.000342 M, MeOH) λ_{max} ($\Delta\varepsilon$) 236 (1.81), 205 (0.56); IR (KBr) ν_{max} 2957, 2930, 1739, 1373, 1235, and 1030 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1.

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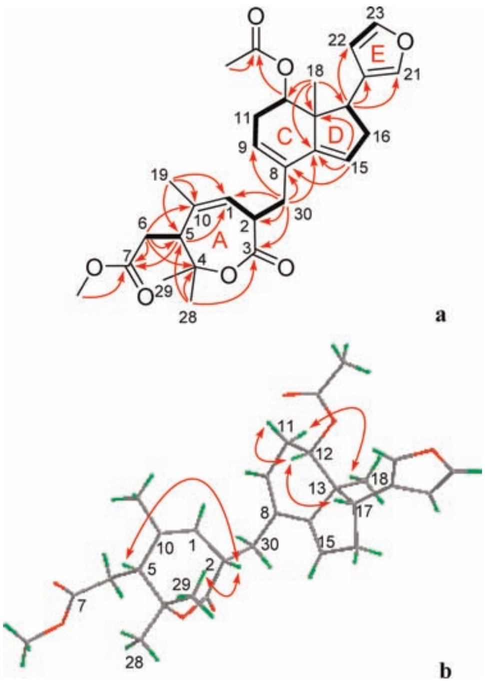

Figure 1. Key $^1H-^1H$ COSY (a: —), HMBC (a: → (red)), and ROESY (b: ↔ (red)) correlations of **1**.

HMBC correlations of H_3-19/C-1 (δ_C 120.2), C-5 (δ_C 47.8), and C-10 (δ_C 137.8), H-5 (δ_H 2.74)/C-5 and C-10 as well as the downfield-shifted hydrogen resonances of H-1 (δ_H 5.18) and $^1H-^1H$ COSY correlations (H-5−H-6) indicated the location of a double bond between C-1 and the quaternary carbon atom C-10, which also suggested the cleavage of C-9 and C-10. The $^1H-^1H$ COSY correlations (H-1−H-2−H_2-30) and the HMBC correlations of H_2-30/C-1, C-2, C-3, C-8 (δ_C 131.0), and C-9 (δ_C 125.0) confirmed that C-2 and C-8 were linked through C-30 as shown in Figure 1. HMBC correlations of H-30/C-14 (δ_C 147.6), H-15 (δ_H 5.65)/C-8, and C-14 indicated the presence of $\Delta^{14(15)}$ double bond, which was conjugated with $\Delta^{9(8)}$ double bond. Additionally, the acetoxy group was located at C-12 by the HMBC cross signal H-12/C-12-OAc, and the HMBC correlations of H_3-28/C-3 together with the downfield-shifted carbon resonances of C-3 and C-4 definitely indicated the linkage of C-3 and C-4 via an oxygen atom to form the unsaturated lactone ring A. Thus, the aforementioned data suggested a unique ring A, B-seco limonoid with a unique C-2−C-30 bond, and the planar structure of **1** was established as shown in Figure 1.

The relative stereochemistry of **1** was determined by ROESY spectrum (Figure 1b). Furthermore, the successful performance of the X-ray diffraction experiment with Cu Kα radiation confirmed the proposed structure and also allowed unambiguous assignment of the absolute configuration of **1** as drawn [Flack parameter: 0.1(2)][9] (Figure 2).

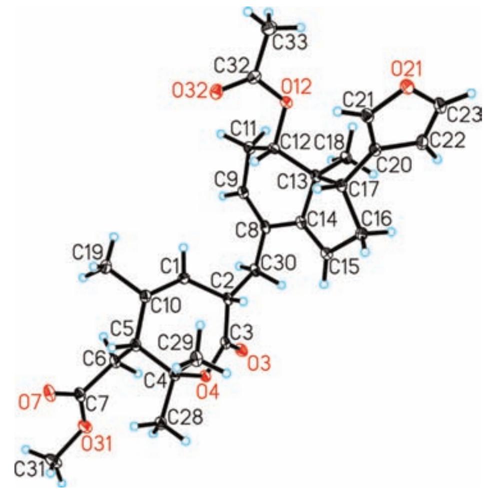

Figure 2. Single crystal X-ray structure of **1**.

Aphanamixoid B (**2**)[10] was obtained as colorless amorphous powder. The molecular formula was determined as $C_{29}H_{36}O_8$ with 12 degrees of unsaturation deduced by HRESIMS at m/z 535.2314 $[M + Na]^+$ (calcd 535.2307, $C_{29}H_{36}O_8Na$). The IR absorption band at 1739 cm^{-1} indicated the presence of ester carbonyl groups. The observation for a β-furan ring (δ_H 6.24, 7.19, 7.33; δ_C 111.1, 139.9, 142.4), a methoxy group (δ_H 3.76; δ_C 52.3), four tertiary methyl groups (δ_H 0.80, 1.17, 1.40, 1.45), and a characteristic exocyclic double bond (δ_H 5.12, 5.40) in the ^1H and ^{13}C NMR spectra of **2** strongly suggested that **2** was a prieurianin-type limonoid.[4d,11] The ^1H and ^{13}C NMR spectral data of **2** showed close similarity to those of the reported compound, munronoid A,[5b] except for the absence of an acetyl group, as well as the presence of an additional oxygenated methine (δ_H 4.11). Compared with munronoid A, the observed significant downfield shifts of C-1 (δ_C 81.6) and C-11 (δ_C 79.3) together with the strong HMBC correlation (Figure 3a) connected via an oxygen atom and formed a tetrahydrofuran ring. The structure of **2** was confirmed by 2D NMR (HSQC, HMBC, $^1H-^1H$ COSY, and ROESY) experiments (Figure 3).

The absolute configuration of aphanamixoid B (**2**) was assigned using the quantum chemical method. The optical rotation (OR) value of **2** was calculated using density functional theory (DFT) methods[12] in the Gaussian 03 program package.[13] The "self-consistent reaction field" method (SCRF) was employed to perform the OR calculation of the most stable conformer of **2** in MeOH solution at the B3LYP/6-31G (d,p) level. The calculated OR value (+91.4°) for **2** is close to its experimental value (+81.8°), which suggested a reliable absolute configuration assignment for **2**. In addition, its electronic circular dichroism (ECD)[14] was also calculated on the Gaussian 03 program using TD-DFT-B3LYP/6-31G(d,p) level,[13] which showed a good agreement with those of experimentally recorded

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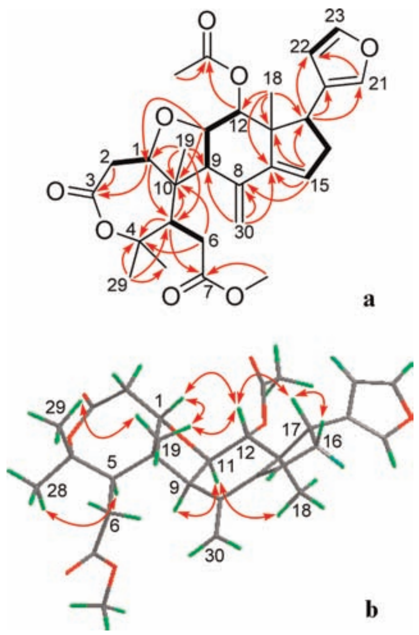


Figure 3. Key $^1H-^1H$ COSY (a: —), HMBC (a: → (red)), and ROESY (b: ↔ (red)) correlations of **2**.

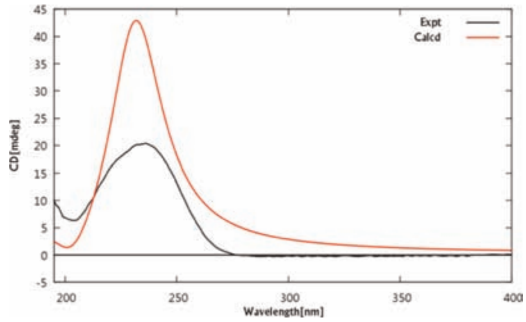
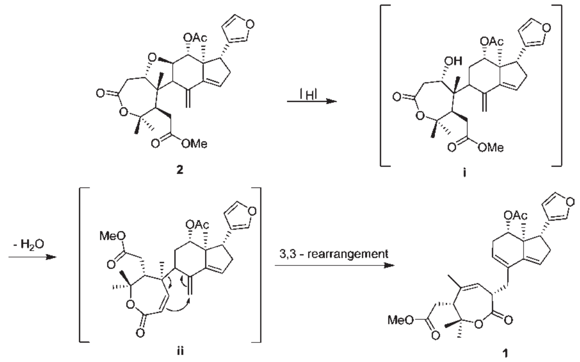


Figure 4. Comparison of the experimental CD and calculated CD spectrum of **2**.

CD spectrum (Figure 4). Thus, the absolute configuration of **2** was unambiguously assigned as depicted.

The biogenetic origin of aphanamixoid A (**1**) might be derived from aphanamixoid B (**2**). The cleavage of ether linkage followed by reduction of **2** formed **i**, and then dehydration to yield the key intermediate **ii**, which produced **1** by means of 3,3-rearrangement, as shown in Scheme 1.

Scheme 1. Plausible Biosynthetic Pathway for **1**



The antifeedant activity of aphanamixoid A (**1**) against the larvae of two generalist insects, beet armyworm (*Spodoptera exigua*) and cotton bollworm (*Helicoverpa armigera*), were evaluated.[15] The compound **1** exhibited a potent antifeedant activity with an EC_{50} value of 0.052 and 0.015 $\mu mol/cm^2$, respectively. The results suggested a potent defensive role of **1** against herbivore enemies, while aphanamixoid B (**2**) showed moderate antifeedant activity against *S. exigua* at 2000 ppm with AFI of 17%.

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Supporting Information Available. 1D and 2D NMR spectra of **1** and **2**, experimental procedures, plant material, bioactivity assay, the X-ray crystallographic data for **1**, and computational calculations for **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.