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Aphanamolide A, a New Limonoid from *Aphanamixis polystachya*

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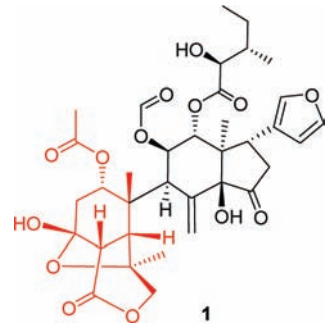
ABSTRACT



Two new limonoids, namely aphanamolides A (1) and B (2), were isolated from the seeds of *Aphanamixis polystachya*. Their structures were established by spectroscopic methods. Aphanamolide A (1) featured an unprecedented carbon skeleton via the formation of a C-3−C-6 bond. Compounds 1 and 2 showed cytotoxic activity against two tumor cell lines.

The plant of *Aphanamixis polystachya* (Wall) J. N. Barker (Meliaceae) is a timber tree mainly growing in the tropical areas of Asia, such as China, India, Malaysia, and Indonesia.[1] Previous chemical investigations on this plant species growing in some other parts of the world led to the isolation of a series of compounds including alkaloids,[2] flavonoids,[3] sesquiterpes,[4] diterpenes,[5] triterpenes,[6] and limonoids.[3a,7] In the current study, two novel limonoids aphanamolides A (**1**) and B (**2**), along with a structurally related known limonoid, Tr-B,[7c] were isolated from the EtOH extract of the seeds of *A. polystachya*, which was collected from the Hainan Province of China. Aphanamolide A (**1**) featured an unprecedented carbon skeleton via the formation of a C-3−C-6 bond. We present herein the isolation and structural elucidation of limonoids **1** and **2**.

Aphanamolide A (**1**)[8] was isolated as white amorphous powders. The molecular formula was determined to be $C_{35}H_{44}O_{14}$ by HREIMS requiring 14 degrees of unsaturation.

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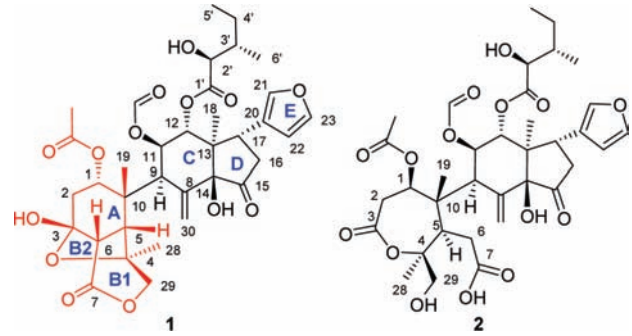
Table 1. NMR Data of Aphanamolides A and B (**1** and **2**) and Tr-B

	1			**2**		Tr-B
	δ_C[a]	δ_C[b]	δ_H[a]	δ_C[c]	δ_H[c]	δ_C[b]
1	76.2	74.0	4.93, d (5.0)	74.1	5.58, d (10.3)	75.3
2α	42.0	39.9	2.00, d (15.5)	35.6	2.97, d (15.6)	37.3
2β			2.43, dd (15.5, 5.0)		2.58, dd (15.6,10.3)	
3	104.4	102.3		171.3		169.3
4	82.8	80.3		90.2		79.9
5	51.6	51.5	2.91, s	41.6	3.10, brs	42.5
6	53.8	48.2	3.25, s	33.5	2.70, m	32.0
7	172.5	169.7		174.2		169.6
8	141.2	139.0		138.4		139.4
9	50.7	48.7	4.01, d (8.1)	52.7	3.24, d (9.1)	50.6
10	46.3	48.2		47.8		45.7
11	73.6	74.0	5.49, dd (11.0, 8.1)	70.5	5.27, dd (9.7, 9.1)	71.2
12	75.6	73.0	6.21, d (11.0)	73.7	5.90, d (9.7)	72.1
13	51.3	49.1		49.1		49.1
14	82.3	79.9		79.9		79.4
15	208.6	206.9		207.2		206.6
16α	42.9	41.0	2.84, dd (19.2, 9.0)	41.2	2.84, dd (19.2, 9.2)	41.0
16β			2.37, dd (19.2, 9.0)		2.41, dd (19.2, 9.2)	
17	37.1	34.9	3.92, t (9.0)	35.0	3.81, t (9.2)	34.7
18	14.1	12.6	0.97, s	12.3	0.88, s	12.3
19	23.6	21.4	1.71, s	19.0	1.34, s	19.8
20	125.3	123.4		122.7		123.3
21	142.7	140.6	7.32, s	140.0	7.37, s	140.6
22	112.5	111.4	6.39, s	110.8	6.41, s	111.3
23	144.6	143.0	7.43, s	142.3	7.48, s	142.9
28	22.8	22.0	1.76, s	19.0	1.49, s	21.0
29	80.7	78.5	4.05, d (11.1)	65.3	3.59,d (13.0)	76.9
			3.95, d (11.1)		3.63,d (13.0)	
30a	125.3	123.4	5.91, brs	120.9	5.71, brs	123.6
30b			5.69, brs		5.47, brs	
1′	175.6	173.3		172.6		173.2
2′	76.5	74.0	3.16, d (3.0)	74.0	3.24, m	74.0
3′	39.9	37.6	1.44, m	37.1	1.43, m	37.5
4′a	24.8	23.1	1.05, m	22.7	1.13, m	23.0
4′b			1.15, m			
5′	12.5	11.6	0.74, t (7.6)	10.7	0.75, t (7.5)	11.5
6′	16.3	15.5	0.81, d (7.2)	14.8	0.75, d (7.7)	15.4
OAc	22.2, 172.4	22.0, 168.9	2.03, s	20.2, 169.2	1.97, s	23.0, 169.3
HCOO	162.7	161.2	7.90, s	161.6	8.14, s	161.1

[a] Data were recorded in CD_3OD at 298 K. [b] Data were recorded in DMSO-d_6 at 298 K. [c] Data were recorded in DMSO-d_6 at 333 K.

The IR absorptions indicated the presence of hydroxyl (3600−3000 cm^{-1}) and carbonyl (1728 cm^{-1}) groups. All the 35 carbons in the molecuar formula were well resolved in the ^{13}C NMR spectrum, and were classified by chemical shifts and HSQC spectrum as six methyls, five methylenes (one olefinic and one oxygenated), 13 methines (four oxygenated, and three olefinic, and one formyloxy carbon), and 11 quaternary carbons (one ketone, three esters, two olefinic, one hemiketal, and two oxygenated carbons). In addition, the presence of one acetoxyl (δ_H 2.03, 3H, s), one formyloxy group (δ_H 7.90), three tertiary methyls (δ_H 0.97, 1.71, and 1.76, each 3H, s), an exocyclic double bond (δ_H 5.91, 5.69, each 1H, br s), and a β-substituted furan ring (δ_H 6.39, 7.32, and 7.43) were distinguished by the analysis of its ^1H and ^{13}C NMR data (Table 1). The aforementioned data suggested that **1** was a ring B-*seco* limonoid bearing a typical $\Delta^{8(30)}$ double bond.[7d]



The planar structure of **1** was constructed by the detailed analysis of 1D and 2D NMR data, especially the HMBC spectrum. The rings C−E were readily established by comparison with those of several known limonoids, such as Tr-B (Supporting Information, S1, S21 to S23),[7c] and the linkages of the substituents to the rings C and D were comfirmed by the HMBC spectrum (Figure 1a), in which

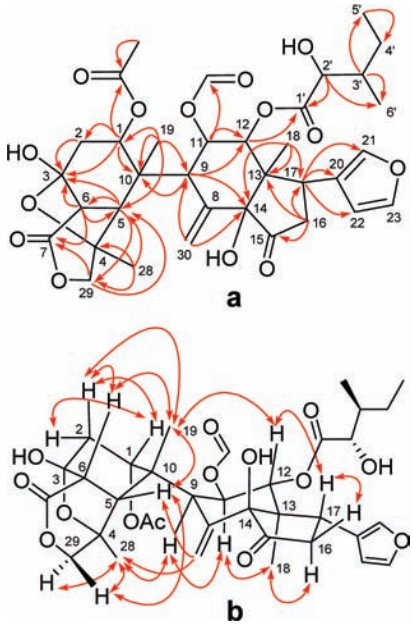


Figure 1. (a) Selected HMBC (H→C) correlations of **1**; (b) key ROESY (H↔H) correlations of **1**.

the formyloxy group was attached to C-11 by the correlation between H-11 and the carbonyl of the formyl group at δ_C 162.7; a 2-hydroxy-3-methylpentanoyloxy moiety was identified by the ^{1}H and ^{13}C NMR data (Table 1) along with the multiple HMBC correlations within this group, and it was placed at C-12 by the key HMBC correlation between H-12 and C-1′ at δ_C 175.6; a hydroxyl and a keto group were assigned to C-14 and C-15 by their chemical shifts and HMBC correlations of CH_3-18 and H-30/C-14, and H_2-16/C-15, respectively. The most unique scaffold of rings A, B1, and B2 were then established mainly by comprehensive analysis of the HMBC spectrum (Figure 1a), in which the correlation from H-1 to the carbonyl signal at δ_C 172.4 of an acetyl located the only acetoxyl group at C-1; the multiple HMBC correlations from H-1 to C-2 and C-3 at δ_C 104.4, and from H_2-2 to C-3 indicated the presence of a hemiketal motif at C-3; the linkages between C-3 and C-6, and between C-6 and C-5 were demonstrated by the key HMBC correlations of H-6/C-3 and H-6/C-5, respectively; the HMBC correlations of H-1/C-10, H-9/10, Me-19/C-10, and Me-19/C-5 attached C-1, C-9, Me-19, and C-5 to the quaternary C-10; the strong HMBC correlations from Me-28 to C-5, C-4 (oxygenated quaternary carbon, δ_C 82.8) and C-29 (an oxygenated methylene, δ_C 80.7), suggested the linkages of C-5, Me-28, and C-29 to C-4, and this was supported by the mutual ^3J HMBC correlations of H-5/C-29 and H_2-29/C-5; the HMBC correlation between H_2-29 and C-7 (δ_C 172.5) clearly indicated the linkage of C-29 and C-7 via an oxygen atom to form a six-membered lactone; the connectivity of C-6 and C-7 was assigned by the HMBC correlation between H-6 and C-7. The above assigned functional groups and rings system acounted for 13 (a formyl, a ketone, three esters, a β-furyl, a double bond, and rings A, B1, C, and D) out of the 14 degrees of unsaturation, the remaining one degree of unsaturation required the presence of an additional ring in **1**. Although there are no HMBC correlations available to furnish the two "loose ends" of C-3 and C-4, the downfield-shifted carbon resonance of C-4 at δ_C 82.8 and C-3 at δ_C 104.4 definitely indicated the linkage of C-3 to C-4 via an oxygen atom to form the hemiketal group. The planar structure of **1** was thus established as an unprecedented carbon skeleton formed by the key linkage between C-3 and C-6.

The relative stereochemistry of **1** was fixed by the performance of a ROESY experiment (Figure 1b). The ROESY cross-peaks of Me-19/H-2β, H-2β/H-6, and H-6/Me-19 indicated that Me-19 and H-6 took the axial position of ring A that adopted a chair conformation, and were arbitraryly assigned β-configured. In consequence, the ROESY correlations of H-1/H-2α and H-1/H-2β, and the small coupling constant of H-1 (d, J = 5.0 Hz), revealed that they were in a *gauche* relationship, and H-1 was in the equatorial bond and β-directed. The ROESY cross-peaks of Me-19/H-5 and H-5/H-29a showed that the H-5 was in a β-orientation, and the ring B1 took a half-chair conformation. The ROESY correlations of Me-28/H_2-29 and H-9 revealed that Me-28 was α-oriented. The formation of the five-membered ring B2 of an envelope conformation occupied two 1,3-axial bonds at the α-face of rings A, indicating that HO-3 was definitely in a β-configuration. In rings C and D, the ROESY cross-peaks of H-12/H-17 and H-17/H-16β indicated that they were cofacial, and β-oriented. The stereochemistry of 2-hydroxy-3-methylpentanoyloxy moiety at C-12 of **1** was assigned to be identical with that of rubrins A−F,[7b] based on the very similar NMR patterns within this structural moiety. Subsequently, the ROESY correlations of H-9/H-11, H-11/Me-18, and Me-18/H-16α indicated that they were α-directed. The HO-14 of **1** was assigned in a β-orientation on the basis of chemical shift of C-14 at δ_C 79.9, which was very close to that (at δ_C 79.4) of Tr-B, a coexisting known compound with the identical D and E rings to **1** (Table 1, both were mesured in DMSO-d_6).[7c] The key ROESY correlating network of Me-19/H-12 and Me-28/H-9 indicated that the free rotation around the C-9−C-10 bond was fixed thanks to the stereohindrance of two bulky fragments in the molecule of **1**.

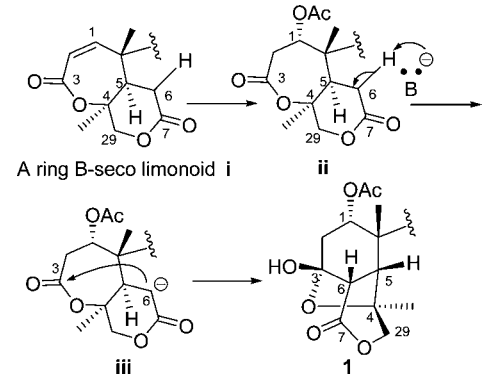
(8) Aphanamolide A (**1**): white amorphous powder; $[\alpha]^{21}_D$ −47.0 (*c* 0.0380, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) = 207 (−0.71), 222 (+3.63), 311 (−2.28) nm; IR (KBr) ν_{max} 3440, 2966, 1776, 2937, 1728, 1383, 1296, 1246, 1188, 1030, 874, 604 cm^{-1}; ^{1}H NMR and ^{13}C NMR see Table 1, respectively; positive mode ESIMS *m*/*z* 711.1 [M + Na]$^+$; EIMS *m*/*z* 688 (4), 582 (66), 549 (37), 450 (43), 311(88), 177 (92), 121 (100), 76 (82), 60 (76); HREIMS *m*/*z* 688.2702 (calcd for $C_{35}H_{44}O_{14}$ 688.2731).

The molecular formula of aphanamolide B (**2**)[9] was determined to be $C_{35}H_{46}O_{15}$ by HRESIMS. Its IR absorptions at 3600−3200 and 1743 cm^{-1} revealed the existence of hydroxyl and carbonyl groups. All 35 carbon atoms in the molecule were fully resolved as 35 signals in the ^{13}C NMR spectrum (Table 1). Analysis of ^{1}H NMR spectrum showed the presence of one acetyl group (δ_H 1.97, 3H, s), one formyl group (δ_H 8.14), three tertiary methyls (δ_H 0.88, 1.34, and 1.49, each 3H, s), a typical $\Delta^{8(30)}$ double bond (δ_H 5.71, and 5.41, each 1H, br s),[7d] and a β-substituted furan ring (δ_H 6.41, 7.37, and 7.48) (Table 1), suggesting that **2** was also a ring B-*seco* limonoid.

The chemical shifts and ^{13}C NMR pattern of **2** showed many similarities to those of a coexisting known compound Tr-B[7c] (Table 1, both in DMSO-d_6), except for the obvious changes at C-4, C-7, and C-29, indicating that they were structural analogues. As compared with Tr-B, the C-4 and C-7 of **2** were downfield shifted at δ_C 90.2 ($\Delta\delta$ +10.3) and δ_C 174.2 ($\Delta\delta$ +4.6), and C-29 of **2** was upfield shifted at δ_C 65.3 ($\Delta\delta$ −11.6), suggesting that **2** was most likely the hydrolysate of 7,29-lactone of this class of ring B-*seco* limonoids, such as Tr-B. The severe changes of chemical shifts of C-4, C-7, and C-29 could be demonstrated by the formation of multiple intramolecular H-bonds.[10] The H-11 of **2** was assigned in an α-configuration by the key ROESY correlation of H-11/Me-18 (Supporting Information, S2). The stereochemistry of the 2-hydroxy-3-methylpentanoyloxy group was identical with that of **1** based on NMR analysis. The structural assignment of **2** was finally confirmed by 2D NMR (Supporting Information, S2).

The biogenetic origin of aphanamolide A (**1**) could be traced back to a common ring B-*seco* limonoid (**i**) (Scheme 1). Limonoid **i** would be transformed into **ii** by a cascade of oxidation and ethylation procedures. Intermediate **ii**, which underwent an Aldol reaction,[11] would finally produce **1** via the key anion intermediate **iii** formed by a typical base-catalyzed chemical step.

Scheme 1. The Plausible Biogenetic Origin of **1**



Aphanamolides A and B were tested for cytotoxicity against two tumor cell lines A-549 (human lung adenocarcinoma) and HL-60 (human premyelocytic leukemia) by using the SRB method[12] and the MTT method,[13] respectively. Both compounds **1** and **2** showed cytotoxic activity against A-549 (IC_{50}: 88.1 and 60.4 μM) and HL-60 (IC_{50}: 191.0 and 20.6 μM) tumor cell lines, respectively.

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Supporting Information Available: Experimental section, selected HMBC and key ROESY correlations of **2**, and ^{1}H and ^{13}C NMR, EIMS, IR, 2D NMR, and CD spectra of aphanamolides A (**1**) and B (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Aphanamolide B (**2**): white amorphous powder; $[\alpha]^{21}_D$ −50.7 (*c* 0.3255, MeOH); CD (MeOH) λ ($\Delta\varepsilon$) = 206 (+3.78), 220 (+4.80), 311 (−1.67) nm; IR (KBr) ν_{max} 3448, 2968, 1743, 1246, 1203, 1167, 1034, 1026, 960, 876 cm^{-1}; ^{1}H NMR and ^{13}C NMR see Table 1, respectively; positive mode ESIMS *m/z* 729.1 $[M + Na]^+$; EIMS *m/z* 688 (14), 660 (21), 603 (16), 468 (12), 241 (30), 121 (51), 94 (56), 76 (100), 57 (35); HRESIMS *m/z* 729.2735 (calcd for $C_{35}H_{46}O_{15}Na$ 729.2734).

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