Aphagranols D – H: Five New Limonoids from the Fruits of *Aphanamixis grandifolia*

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Five new limonoids, aphagranols D – H (**1** – **5**, resp.), were isolated from the EtOH extract of the fruits of *Aphanamixis grandifolia* by chromatographic methods. Their structures were established on the basis of extensive spectroscopic analyses. The absolute configuration of **1** was determined by comparison the experimental and calculated electronic circular dichroism (ECD) spectra. All of the isolates were evaluated for insecticidal activities.

Introduction. – Limonoids that have more than 20 classes of C-skeletons with diverse biological activities, isolated from Meliaceae family, are of interest to both phytochemists and biochemists [1]. Over the past five years, our group has conducted phytochemical investigations on more than seven species of meliaceous plants from the mainland of China, which led to the isolation and characterization of a series of highly oxdized, polycyclic limonoids or triterpenoids endowed with various skeletons, and some of them showed promising bioactivities [2 – 9]. *Aphanamixis grandifolia* BLUME, a wild timber tree, grows in tropical and subtropical areas of Asia, such as Indonesia, Malaysia, and southern mainland China. The roots and leaves of this plant are utilized to relieve rheumatoid joint pain and numbness of limbs in some regions of China. In the course of our search for structurally novel and bioactive limonoids from the Meliaceae family, we reported several rearranged limonoids with a complex ring *A* system obtained from this species previously [8][9]. A subsequent study led to the isolation of five new limonoids, named aphagranols D – H (**1** – **5**, resp.; *Fig. 1*), from the EtOH extract of the fruits of *A. grandifolia* by chromatographic methods. Their structures were established on the basis of extensive spectroscopic analyses. The absolute configuration of **1** was determined by comparison the experimental and the calculated ECD spectra. All of the compounds were evaluated for insecticidal activities. Herein, we describe their isolation and structure elucidation.

Results and Discussion. – Aphagranol D (**1**) was obtained as white, optically active amorphous powder ($[\alpha]_D^{27} = -86.3$). The HR-ESI-MS provided the molecular formula $C_{40}H_{48}O_{15}$ (*m/z* 791.2890 ($[M+Na]^+$; calc. 791.2885)), requiring 17 degrees of unsaturation. The IR absorptions of **1** indicated the presence of a γ-lactone group (1765 cm^{-1}), C=C bonds (1635 cm^{-1}), and a furan ring (873 cm^{-1}) [10]. The ^{13}C-NMR and DEPT spectra of **1** (*Table 1*) displayed signals of nine Me, three sp^3 CH_2 (one O-

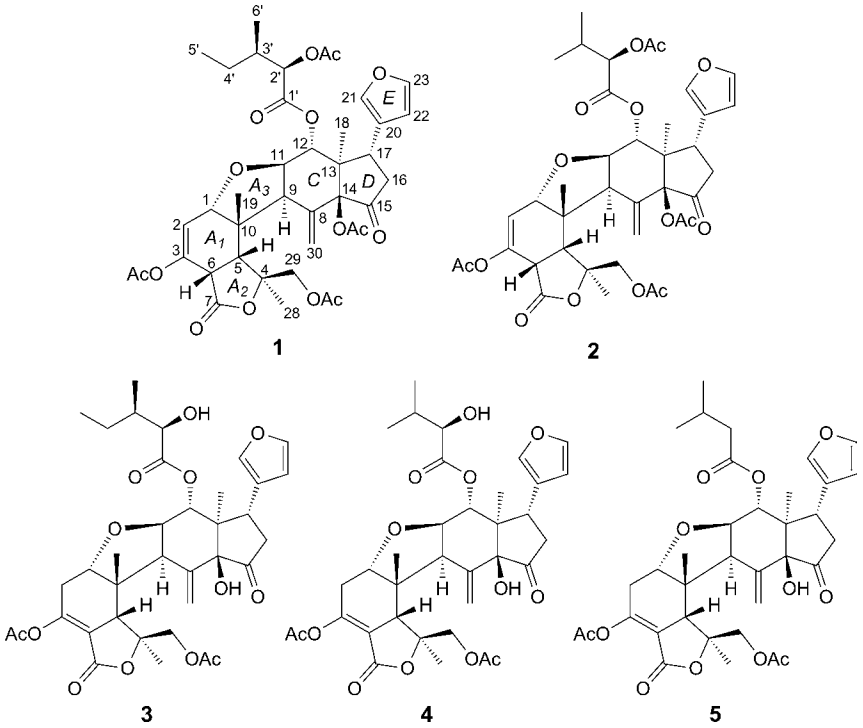

Fig. 1. *Structures of compounds* **1**–**5**

bearing, δ(C) 68.9), and nine sp^3 CH (groups four O-bearing ones; δ(C) 79.8, 78.4, 76.0, and 72.3); four sp^3 quaternary C-atoms (two O-bearing ones; δ(C) 86.8 and 86.0); one ketone (δ(C) 202.5) and six ester C=O bonds (δ(C) 170.6, 170.0, 169.8, 169.5, 169.1, and 168.5), and four C=C bonds (δ(C) 144.8, 143.0, 141.3, 133.4, 127.8, 123.3, 113.8, and 110.7). Accordingly, 48 H-atoms were all bound to C-atoms, and no exchangeable H-atom was present. Furthermore, a combined analyses of its ^{1}H- and ^{13}C-NMR data (*Table 1*) revealed the presence of four AcO groups (δ(H) 2.19, 2.18, 2.16, and 2.13), an exocyclic C=C bond (δ(H) 6.72 (*s*, H_a–C(30)) and 5.39 (*s*, H_b–C(30))), a β-furyl ring (δ(H) 7.46 (*s*, H–C(21)), 7.37 (*s*, H–C(23)), and 6.30 (*s*, H–C(22))), and a 2-hydroxy-3-methylpentanoyloxy moiety (δ(H) 4.72 (*d*, J = 3.0, H–C(2′)), 1.77–1.83 (*m*, H–C(3′)), 1.40–1.45 (*m*, H_a–C(4′)), 1.19–1.26 (*m*, H_b–C(4′)), 0.78 (*t*, J = 7.5, Me(5′)), and 0.93 (*d*, J = 7.0, Me(6′))). Accordingly, a hexacyclic structure was required for **1** to fulfill the degrees of unsaturation. The aforementioned 1D-NMR data implied that **1** was a ring *A*-rearranged and ring *B-seco* limonoid with a typical C(8)=C(30) bond [11].

Comparison of the 1D-NMR data of **1** with those of aphagranol C [8] indicated that they shared the similar rings system. Extensive analyses of 2D-NMR spectra (HSQC and HMBC) allowed the assignment of most functional groups to the limonoid core and verified the framework for **1** (*Fig. 2*). Accordingly, the key points for the structure determination were elucidated to construct the ring A_1. An *AX* spin system at δ(H) 4.18 (br. *t*, J = 3.0, H–C(1)) and 5.68 (*dd*, J = 4.0, 2.0, H–C(2)) were directly assigned to

Table 1. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[a]). Atom numbering as indicated in *Fig 1*.

Position	**1**		**2**	
	δ(H)	δ(C)	δ(H)	δ(C)
1	4.18 (br. *t*, *J* = 3.0)	78.4 (*d*)	4.20 (br. *t*, *J* = 3.0)	78.5 (*d*)
2	5.68 (*dd*, *J* = 4.0, 2.0)	113.8 (*d*)	5.69 (*dd*, *J* = 4.5, 3.0)	113.9 (*d*)
3		144.8 (*s*)		144.8 (*s*)
4		86.8 (*s*)		86.8 (*s*)
5	3.01 (*d*, *J* = 9.0)	48.0 (*d*)	3.01 (*d*, *J* = 8.5)	48.0 (*d*)
6	3.73 (*dt*, *J* = 9.0, 2.0)	42.1 (*d*)	3.73 (*dt*, *J* = 8.5, 2.5)	42.1 (*d*)
7		169.8 (*s*)		169.8 (*d*)
8		133.4 (*s*)		132.4 (*d*)
9	3.43 (*d*, *J* = 10.0)	49.8 (*d*)	3.43 (*d*, *J* = 9.5)	49.9 (*d*)
10		44.3 (*s*)		44.3 (*d*)
11	4.15 (*t*, *J* = 10.0)	79.8 (*d*)	4.15 (*t*, *J* = 10.0)	79.7 (*d*)
12	5.94 (*d*, *J* = 10.0)	72.3 (*d*)	5.94 (*d*, *J* = 9.5)	72.2 (*t*)
13		47.1 (*s*)		47.2 (*s*)
14		86.0 (*s*)		86.0 (*s*)
15		202.5 (*s*)		202.5 (*s*)
16	2.25 (*dd*, *J* = 19.5, 8.0), 2.95 (*dd*, *J* = 19.5, 11.0)	41.1 (*t*)	2.25 (*dd*, *J* = 19.5, 8.0), 2.96 (*dd*, *J* = 19.5, 11.0)	41.2 (*t*)
17	3.77 (*dd*, *J* = 11.0, 8.0)	34.9 (*d*)	3.78 (*dd*, *J* = 11.0, 8.0)	35.0 (*d*)
18	0.84 (*s*)	13.5 (*q*)	0.84 (*s*)	12.7 (*q*)
19	1.06 (*s*)	25.5 (*q*)	1.07 (*s*)	25.6 (*q*)
20		123.3 (*s*)		122.5 (*s*)
21	7.46 (*s*)	141.3 (*d*)	7.46 (*s*)	141.3 (*d*)
22	6.30 (*s*)	110.7 (*d*)	6.31 (*s*)	110.7 (*d*)
23	7.37 (s)	143.0 (*d*)	7.38 (*s*)	143.0 (d)
28	1.47 (*s*)	21.1 (*q*)	1.49 (*s*)	21.2 (*q*)
29	4.33 (*d*, *J* = 12.0), 4.08 (*d*, *J* = 12.0)	68.9 (*t*)	4.44 (*d*, *J* = 12.5), 4.07 (*d*, *J* = 12.5)	68.9 (*t*)
30	6.72 (*s*), 5.39 (*s*)	127.8 (*t*)	6.72 (*s*), 5.40 (*s*)	127.9 (*t*)
1′		169.5 (*s*)		169.5 (*s*)
2′	4.72 (*d*, *J* = 3.0)	76.0 (*d*)	4.66 (*d*, *J* = 3.0)	76.2 (*d*)
3′	1.77–1.83 (*m*)	37.0 (*d*)	2.09–2.13 (*m*)	30.1 (*d*)
4′	1.40–1.45 (*m*), 1.19–1.26 (*m*)	24.2 (*t*)	0.93 (*d*, *J* = 7.0)	18.8 (*q*)
5′	0.78 (*t*, *J* = 7.5)	11.3 (*q*)	0.90 (*d*, *J* = 7.0)	16.3 (*q*)
6′	0.93 (*d*, *J* = 7.0)	14.1 (*q*)		
Me*C*OO–C(3)		169.1 (*s*)		169.2 (*s*)
*Me*COO–C(3)	2.19 (*s*)	20.6 (*q*)	2.20 (*s*)	20.6 (*q*)
Me*C*OO–C(14)		170.0 (*s*)		170.0 (*s*)
*Me*COO–C(14)	2.13 (*s*)	20.7 (*q*)	2.16 (*s*)	20.7 (*q*)
Me*C*OO–C(29)		168.5 (*s*)		169.5 (*s*)
*Me*COO–C(29)	2.16 (*s*)	21.5 (*q*)	2.13 (*s*)	21.6 (*q*)
Me*C*OO–C(2′)		170.6 (*s*)		170.6 (*s*)
*Me*COO–C(2′)	2.18 (*s*)	20.6 (*q*)	2.20 (*s*)	20.6 (*q*)

[a]) Recorded in $CDCl_3$ at 500 (^{1}H) and 125 MHz (^{13}C); chemical shifts, δ, in ppm rel. to Me_4Si; *J* in Hz.

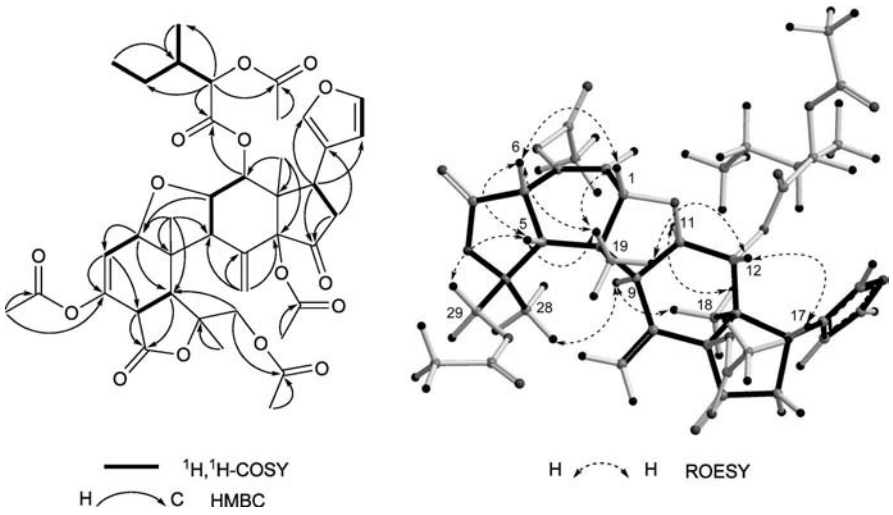

Fig. 2. *[1]H,[1]H-COSY, Key HMB, and ROESY correlations of* **1**

an oxidized C-atom (δ(C) 78.4, C(1)) and olefinic C-atom (δ(C) 113.8, C(2)) in the HSQC spectrum, respectively, and both H-atoms had the correlations with C(3) (δ(C) 144.8), C(6) (δ(C) 42.1), and C(10) (δ(C) 44.3) in the HMBC spectrum. The other *AX* spin system at δ(H) 3.01 (*d*, $J = 9.0$, H–C(5)) and 3.73 (*dt*, $J = 9.0$, 2.0, H–C(6)) correlated with C(3), indicating a trisubstituted C(2)=C(3) bond in ring A_1. Moreover, the *geminal* and oxidized H-atoms CH_2(29) (δ(H) 4.33 (*d*, $J = 12.0$) and 4.08 (*d*, $J = 12.0$)) correlated with C(4) (δ(C) 86.8), while the absence cross-peak to an ester carbonyl C-atom C(7) (δ(C) 169.8) in the HMBC spectrum indicated the presence of a γ-lactone in ring A_2 [12]. The HMBCs from H–C(11) (δ(H) 4.15 (*t*, $J = 10.0$)) to C(1) (δ(C) 78.4), and from Me(19) (δ(H) 1.06 (*s*)) to C(1), C(9) (δ(C) 49.8), and C(10) (δ(C) 44.3) established a tetrahydrofuran ring A_3. The cyclohexane ring *C* was fused with cyclopentanone ring *D via* C(13) and C(14) as deduced from the crucial HMBCs from Me(18) (δ(H) 0.84 (*s*)) to C(13) (δ(C) 47.1) and C(14) (δ(C) 86.0), from CH_2(30) to C(14), and from H–C(17) (δ(H) 3.77 (*dd*, $J = 11.0$, 8.0)) to C(12) (δ(C) 72.3) and C(15) (δ(C) 202.5). The terminal furan ring was linked to ring *D* according to the long-range HMBCs from CH_2(16) (δ(H) 2.25 (*dd*, $J = 19.5$, 8.0) and 2.95 (*dd*, $J = 19.5$, 11.0)) to C(20) (δ(C) 123.3), and from H–C(17) to C(21) (δ(C) 141.3) and C(22) (δ(C) 110.7). In addition, the linkages of the substituents to the core of the limonoid were confirmed by the HMBC spectrum, in which a 2-hydroxy-3-methylpentanoyloxy moiety was identified by their multiple HMBCs within this group, and it was placed at C(12) by the key HMBC between H–C(12) (δ(H) 5.94 (*d*, $J = 10.0$)) and C(1′) (δ(C) 169.5), and four AcO groups were placed at C(3), C(14), C(29) (δ(C) 68.9), and C(2′) (δ(C) 76.0) by the small but key ^3J(H,C) or ^4J(H,C) HMBCs, respectively.

The relative configuration of **1** was mainly established by a ROESY experiment (*Fig. 2*), in which the diagnostic NOE cross-peaks CH_2(29)/H–C(5)/H–C(6)/H–C(1)/Me(19)/H–C(12)/H–C(17), indicated that they were co-facial and were arbitrarily assigned a β-orientation. Then, the substituents of C(12) (2-hydroxy-3-methylpenta-

noyloxy moiety) and C(17) (furan ring) were α-orientated. In consequence, the key correlations Me(28)/H–C(9)/Me(18)/H–C(11) indicated α-orientation for the involved H-atoms and groups.

The electronic circular dichroism (ECD) spectroscopy had been widely employed in the determination of the absolute configurations in chiral molecules [13][14]. The experimental ECD spectrum of **1** showed a positive *Cotton* effect at λ_{max} 195 nm, and three negative *Cotton* effects at λ_{max} 217, 258, and 304 nm. However, lack of proper model compounds as reference and the absence of applicable exciton coupling in the ECD spectrum of **1** made the assignment of its absolute configuration difficult by direct analysis of its ECD curve. It recent years, it has been found that the calculated ECD spectrum of the compound of interest provides critical configurational information: the closer the calculated and experimental ECD spectra are, the better the calculated configuration reflect the compounds behavior [15][16]. Therefore, the absolute configurations of **1** were determined by calculation of their ECD spectra, with Gaussian-09 program package using time-dependent density-functional theory (TDDFT) at the B3LYP/6-311 + G (d, p) level, followed by comparison of the experimental data with those calculated. The ECD spectrum calculated for **1** matched fairly well the experimental ECD spectrum of **1** (*Fig. 3*), thus providing the absolute configuration of **1** as depicted.

Aphagranol E (**2**), obtained as amorphous powder, possessed the molecular formula $C_{39}H_{46}O_{15}$, as deduced from the HR-ESI-MS (*m/z* 777.2730 ($[M+H]^+$)), *i.e.*, 14 mass units less than **1**. Comparison of the ^{1}H- and ^{13}C-NMR data of **2** with those of **1**

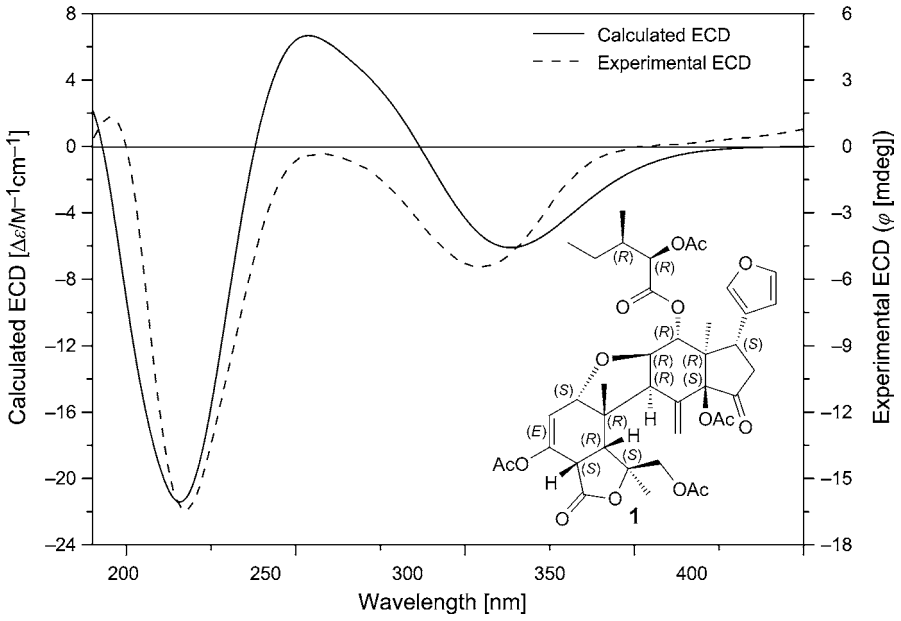

Fig. 3. *Assignment of the absolute configuration in* **1** *by comparison of the experimental with the calculated ECD spectrum for* **1** *by using TDDFT methods*

(*Table 1*) revealed that most signals of **2** were very similar to those of **1**. The only difference between **1** and **2** turned out to be in the ester moiety at C(12). Analyses of ^{1}H,^{1}H-COSY and HSQC spectra allowed the connection of H–C(2′) to H–C(5′), and the correlation of these H-atoms with those directly attached C-atoms (δ(H) 4.66 (*d*, $J = 3.0$, H–C(2′)), 2.09 – 2.13 (*m*, H–C(3′)), 0.93 (*d*, $J = 7.0$, Me(4′)), and 0.90 (*d*, $J = 7.0$, Me(5′)); δ(C) 169.5 (C(1′)), 76.2 (C(2′)), 30.1 (C(3′)), 18.8 (C(4′)), and 16.3 (C(5′))), which, together with the observed HMBCs from H–C(2′) and H–C(3′) to the ester CO C-atom C(1′), revealed the presence of 2-hydroxy-3-methylbutanoyloxy group. The HMBC of H–C(2′) to the ester CO (δ(C) 170.6) indicated that the C(2′) was connected to an AcO unit. The configuration of **2** was identical to that of **1**, as supported by its ^{1}H- and ^{13}C-NMR, HSQC, HMBC, ^{1}H,^{1}H-COSY, and ROESY spectra.

Aphagranol F (**3**), an optically active compound ($[\alpha]_D^{27} = -32.5$), was isolated as white amorphous powder. Its molecular formula was deduced as $C_{36}H_{44}O_{13}$ from HR-ESI-MS (*m*/*z* 707.2680 ($[M + Na]^+$; calc. 707.2674)). Comparison of the NMR data (*Table 2*) of **3** and **1** indicated the presence of a tetrasubstituted C=C bond (δ(C) 151.4 (C(3)) and 113.4 (C(6))) and a CH_2 group (δ(H) 2.75 (*dd*, $J = 19.0, 4.0$), 2.49 (*d*, $J = 19.0$); δ(C) 30.9) in **3**, replacing the trisubstituted C=C bond and a CH group in **1**, respectively. This suggested that the C(2)=C(3) bond in **1** shifted to C(3)=C(6) in **3**, forming a conjugated α,β-unsaturated C=O group between rings A_1 and A_2, verified by the 2D-NMR data, particularly by HMBCs from CH_2(2) to C(3) and C(6), combined with their shifts. In addition, two AcO groups were located at C(3) and C(29) according to the HMBC ^4J(H,C) correlations from one Me of AcO signal at δ(H) 2.25 to C(3), and from the other Me of AcO signal at δ(H) 2.14 to C(29). In the ROESY spectrum, correlations H–C(1)/H–C(5)/Me(19)/H–C(12)/H–C(17) evidenced a β-orientation for H–C(5).

Aphagranol G (**4**) was isolated as white amorphous powder. The HR-ESI-MS data indicated that **4** had the molecular formula $C_{35}H_{42}O_{13}$. Analysis of the ^{1}H- and ^{13}C-NMR, and HSQC data (*Table 2*) revealed that **4** was structurally similar to **3**. Comparison of the spectroscopic data of **4** with those of **3** indicated that they were highly similar, except that the molecular weight of **4** was 14 mass units lower than that of **3**, implying that **4** had a CH_2 group less than **3**. Comparison of the NMR data of **4** and **3** indicated the replacement of a 2-hydroxy-3-methylpentaoyloxy group in **3** by a 2-hydoxy-3-methylbutanoyloxy group in **4**. This group was at C(12) as deduced from the crucial HMBC from H–C(12) to the ester C=O group (δ(C) 175.1).

The HR-ESI-MS of aphagranol H (**5**) provided the molecular formula $C_{35}H_{42}O_{12}$ (*m*/*z* 653.2623 ($[M - H]^-$; calc. 653.2604)), *i.e.*, one OH moiety less than **4**. Comparison of the NMR data of **5** and **4** revealed that the only difference between them was the ester group at C(12). The HMBCs from CH_2(2′) and H–C(3′) to C(1′), and from the Me(4′) and Me(5′) to C(3′) thus established the presence of a 3-methylbutanoyloxy moiety, which was located at C(12) in accordance with the key HMBC between H–C(12) and C(1′).

The discovery of aphagranols F – H (**1** – **5**, resp.) is an important enrichment to the diversity and complexity of limonoids. Aphagranol D and the previously reported aphagranol C are isomers regarding the location of the C=C bond. Aphagranols E and D only differ in the methylation pattern of the ester chain at C(12). Aphagranols F – H (**1** – **5**, resp.) were similar except for the ester chain at C(12), *i.e.*, **1** was 14,2′-

Table 2. *^{1}H- and ^{13}C-NMR Data of* **3**–**5**[a]). Atom numbering as indicated in *Fig. 1*.

Position	**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1	4.00 (*d*, *J* = 4.0)	80.0 (*d*)	4.00 (*d*, *J* = 4.5)	80.0 (*d*)	4.00 (*d*, *J* = 4.0)	79.7 (*d*)
H_α–C(2)	2.49 (*d*, *J* = 19.0)	30.9 (*t*)	2.51 (*d*, *J* = 19.0)	30.9 (*t*)	2.56 (*d*, *J* = 19.0)	30.7 (*t*)
H_β–C(2)	2.75 (*dt*, *J* = 19.0, 4.0)		2.75 (*dt*, *J* = 19.0, 4.5)		2.74 (*dt*, *J* = 19.0, 4.0)	
3		151.4 (*s*)		151.3 (*s*)		151.3 (*s*)
4		85.6 (*s*)		85.6 (*s*)		85.6 (*s*)
5	3.08 (*d*, *J* = 3.5)	52.5 (*d*)	3.08 (*d*, *J* = 4.0)	52.5 (*d*)	3.09 (*d*, *J* = 3.5)	52.5 (*d*)
6		113.4 (*s*)		113.4 (*s*)		113.3 (*s*)
7		164.4 (*s*)		164.3 (*s*)		164.4 (*s*)
8		138.9 (*s*)		138.9 (*s*)		139.3 (*s*)
9	3.42 (*d*, *J* = 10.0)	50.5 (*d*)	3.43 (*d*, *J* = 10.0)	50.4 (*d*)	3.40 (*d*, *J* = 10.0)	50.4 (*d*)
10		45.9 (*s*)		45.7 (*s*)		45.7 (*s*)
11	4.05 (*dd*, *J* = 10.0, 7.0)	80.6 (*d*)	4.05 (*dd*, *J* = 10.0, 7.0)	80.5 (*d*)	4.05 (*dd*, *J* = 10.0, 7.0)	80.9 (*d*)
12	5.87 (*d*, *J* = 7.0)	77.4 (*d*)	5.87 (*d*, *J* = 7.0)	77.4 (*d*)	5.77 (*d*, *J* = 7.0)	75.1 (*d*)
13		48.2 (*s*)		48.1 (*s*)		48.3 (*s*)
14		79.8 (*s*)		79.8 (*s*)		79.8 (*s*)
15		209.2 (*s*)		209.0 (*s*)		209.5 (*s*)
H_α–C(16)	2.86 (*dd*, *J* = 19.5, 8.5)	42.0 (*t*)	2.86 (*dd*, *J* = 19.5, 8.5)	42.0 (*t*)	2.82 (*dd*, *J* = 20.0, 9.0)	42.2 (*t*)
H_β–C(16)	2.40 (*dd*, *J* = 19.5, 10.5)		2.39 (*dd*, *J* = 19.5, 10.5)		2.37 (*dd*, *J* = 20.0, 10.0)	
17	3.83 (*dd*, *J* = 10.5, 8.5)	35.1 (*d*)	3.82 (*dd*, *J* = 10.5, 8.5)	35.1 (*d*)	3.81 (*t*, *J* = 10.0)	35.0 (*d*)
18	0.87 (*s*)	13.2 (*q*)	0.87 (*s*)	13.2 (*q*)	0.87 (*s*)	13.3 (*q*)
19	1.14 (*s*)	25.7 (*q*)	1.14 (*s*)	25.8 (*q*)	1.13 (*s*)	25.8 (*q*)
20		122.6 (*s*)		122.5 (*s*)		122.7 (*s*)
21	7.20 (*s*)	140.4 (*d*)	7.21 (*s*)	140.4 (*d*)	7.21 (*s*)	140.6 (*d*)
22	6.22 (*s*)	110.5 (*d*)	6.21 (*s*)	110.5 (*d*)	6.24 (*s*)	110.5 (*d*)
23	7.37 (*s*)	143.1 (*d*)	7.38 (*s*)	143.0 (*d*)	7.35 (*s*)	142.8 (*d*)
28	1.38 (*s*)	18.5 (*q*)	1.39 (*s*)	18.7 (*q*)	1.40 (*s*)	18.7 (*q*)
29	4.42 (*d*, *J* = 12.5), 4.28 (*d*, *J* = 12.5)	67.4 (*t*)	4.43 (*d*, *J* = 12.5), 4.28 (*d*, *J* = 12.5)	67.4 (*t*)	4.44 (*d*, *J* = 12.5), 4.28 (*d*, *J* = 12.5)	67.3 (*t*)
30	6.24 (*s*), 5.24 (*s*)	121.7 (*t*)	6.25 (*s*), 5.25 (*s*)	121.6 (*t*)	6.22 (*s*), 5.21 (*s*)	121.1 (*t*)

Table 2 (cont.)

Position	**3**		**4**		**5**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1′		175.2 (*s*)		175.1 (*s*)		172.7 (*s*)
2′	3.45 (*d*, *J* = 3.5)	74.5 (*d*)	3.45 (*d*, *J* = 3.5)	74.7 (*d*)	1.89 (*dd*, *J* = 15.0, 7.0), 1.72 (*dd*, *J* = 15.0, 7.0)	43.0 (*t*)
3′	1.60–1.65 (*m*)	39.2 (*d*)	1.87–1.92 (*m*)	32.0 (*d*)	1.87–1.95 (*m*)	25.1 (*d*)
4′	1.25–1.32 (*m*), 1.09–1.15 (*m*)	23.8 (*t*)	0.94 (*d*, *J* = 7.0)	18.5 (*q*)	0.90 (*d*, *J* = 7.0)	22.4 (*q*)
5′	0.79 (*t*, *J* = 7.5)	11.8 (*q*)	0.79 (*d*, *J* = 7.0)	15.7 (*q*)	0.87 (*d*, *J* = 7.0)	22.4 (*q*)
6′	0.91 (*d*, *J* = 7.0)	15.1 (*q*)				
Me*C*OO–C(3)		167.9 (*s*)		167.9 (*s*)		168.0 (*s*)
*Me*COO–C(3)	2.25 (*s*)	20.7 (*q*)	2.26 (*s*)	20.7 (*q*)	2.25 (*s*)	20.6 (*q*)
Me*C*OO–C(29)		170.5 (*s*)		170.4 (*s*)		170.5 (*s*)
*Me*COO–C(29)	2.14 (*s*)	20.6 (*q*)	2.14 (*s*)	20.6 (*q*)	2.14 (*s*)	20.6 (*q*)

[a]) Recorded in $CDCl_3$ at 500 (^1H) and 125 MHz (^{13}C); chemical shifts, δ, are in ppm rel. to Me_4Si; *J* in Hz.

dideaceylated aphagranol C, and **5** was 2′-desoxy aphagranol G. These isolates are genuine natural products, which were confirmed to occur in the EtOH crude extract by the HPLC analysis.

The isolated limonoids, **1**–**5**, were also screened for their initial insecticidal activities against four types of insects, *Sitobion avenae*, *Plutella xylostella*, *Diabrotica balteata*, and *Caenorhabditis elegans*. Unfortunately, no significant activities were observed for these compounds when applied at a relatively high concentration.

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Experimental Part

General. All solvents used were of anal. grade (*Jiangsu Hanbang Science and Technology Co., Ltd.*, Nanjing, P. R. China). Column chromatography (CC): silica gel (SiO_2; 100–200 mesh and 200–300 mesh; *Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China), *Sephadex LH-20* (40–70 μm, *Amersham Pharmacia Biotech AB*, SE-Uppsala), and *YMC-Gel RP-C_{18}* (50 μm, *Milford*, MA, USA). TLC: Silica gel *GF_{254}* plates (*Qingdao Haiyang Chemical Co., Ltd.*, Qingdao, P. R. China); various solvent systems; visualization by heating the silica-gel plates sprayed with H_2SO_4 in EtOH (10 : 90 (*v*/*v*)). Semi-prep. HPLC: *Shimadzu LC-8A* system (*Shimadzu*, Tokyo, Japan) equipped with a *Shim-pack RP-C_{18}* column (200 mm × 20 mm i.d., 10 μm; *Shimadzu*, Tokyo, Japan); flow rate, 10.0 ml/min; column temp., 25°; detection with a binary channel UV detector at 210 and 230 nm. Optical rotations: *JASCO P-1020* polarimeter (*Jasco*, Tokyo, Japan). ECD Spectra: *JASCO P-810* spectrometer (*Jasco*, Tokyo, Japan). Ultraviolet (UV) Spectra: *UV-2450 UV/VIS* spectrophotometer (*Shimadzu*, Tokyo, Japan); λ_{max} (log ε) in nm. IR Spectra: *Bruker Tensor 27* spectrometer (*Bruker*, DE-Karlsruhe); KBr disks; $\tilde{\nu}$ in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker Avance III NMR* instrument; at 500 (^1H) and 125 MHz (^{13}C) (*Bruker*, DE-Karlsruhe); δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Agilent 1100 series LC/MSD* ion trap mass spectrometer (*Agilent Technologies*, Santa Clara, CA, USA). HR-ESI-MS: *Agilent 6520B UHPLC-Q-TOF* instrument (*Agilent Technologies*, Santa Clara, CA, USA).

Plant Material. The fruits of *Aphanamixis gradifolia* BLUME were collected in Xishuangbanna, Yunnan Province, China, in April 2010, and authenticated by Prof. *Jing-Yun Cui* of Xishuangbanna Tropical Garden, Chinese Academy of Sciences. A voucher specimen (No. AG-Fruits-2010-04-ZY) has been deposited with the Department of Natural Medicinal Chemistry, China Pharmaceutical University.

Extraction and Isolation. The air-dried and powdered fruits of *A. grandifolia* (2.8 kg) were extracted with 95% aq. EtOH (3 × 10 l) under reflux. After removal of the solvent under reduced pressure, the crude extract (405.3 g) was suspended in H_2O (1 l) and successively partitioned with petroleum ether (PE; 3 × 1 l) and AcOEt (3 × 1 l). The AcOEt-soluble portion (110.2 g) was subjected to CC (SiO_2 (100–200 mesh; 1.1 kg, *Φ* 10.0 cm × 80.0 cm); CH_2Cl_2/acetone 20 : 1, 10 : 1, 5 : 1, 2 : 1, 1 : 1, 1 : 2, and 0 : 1; each 5000 ml) to give seven fractions, *Frs. A–G. Fr. F* (12.5 g) was seperated by CC (SiO_2 (200–300 mesh; 250.0 g, *Φ* 8.0 cm × 50.0 cm); PE/AcOEt 2 : 1, 1 : 1, 1 : 2, and 0 : 1; each 2000 ml) to afford five subfractions, *Frs. Fa–Fe. Fr. Fa* (3.1 g) was subjected to a CC (*RP-C_{18}* (50 μm, 100.0 g, *Φ* 3.5 cm × 40.0 cm); MeOH/H_2O 50 : 50, 65 : 35, 80 : 20, and 100 : 0; each 1000 ml) to furnish three subfractions, *Frs. Fa1–Fa3*. *Fr. Fa1* (270.5 mg) was purified by semi-prep. HPLC (*Shim-pack RP-C_{18}* (200 mm × 20 mm i.d.); MeOH/H_2O 75 : 25; flow rate, 10 ml/min) to give **5** (t_R = 25.5 min; 7.1 mg) and **2** (t_R = 31.8 min; 6.8 mg). *Fr. Fa2* (731.3 mg) was separated by CC (*Sephadex LH-20* (100.0 g, *Φ* 2.0 cm × 150.0 cm); MeOH 5 ml per bottle; 500 ml) to afford **1** (35.2 mg) and **4** (2.6 mg). *Fr. Fb* (5.0 g) was subjected to a CC (*RP-C_{18}* (50 μm, 150.0 g, *Φ* 4.0 cm × 60.0 cm); MeOH/H_2O 50 : 50, 65 : 35, 80 : 20, and 100 : 0; each 1500 ml) to afford five subfractions, *Frs. Fb1–Fb5*. *Fr. Fb2* (152.2 mg) was purified by semi-

prep. HPLC (*Shim-pack RP-C_{18}* (200 mm × 20 mm i.d.); eluted with MeOH/H_2O 70 : 30; flow rate, 10 ml/min) to give **3** (t_R = 28.5 min; 4.1 mg).

Aphagranol D (= *(1*S*,3a*S*,5a*S*,6a*R*,7*R*,7a*R*,8*S*,10a*S*,11a*R*,11b*R*,11c*R*)-4,10a-Bis(acetyloxy)-1-[(acetyloxy)methyl]-8-(furan-3-yl)-1,3a,5a,6a,7,7a,8,9,10,10a,11,11a,11b,11c-tetradecahydro-1,7a,11b-trimethyl-11-methylidene-3,10-dioxo-3*H*-indeno[5,6-*d*]benzo[1,2-*b*:3,4-*c*′]difuran-7-yl (2*R*,3*R*)-2-(Acetyloxy)-3-methylpentanoate*; **1**). White amorphous powder. $[\alpha]_D^{27} = -86.3$ (c = 0.30, MeOH). UV (MeCN): 193 (4.28), 212 (4.05), 310 (3.66). ECD (MeCN): 195 (+6.42), 217 (−79.65), 258 (−1.63), 304 (−26.38). IR (KBr): 3456, 2971, 1765, 1635, 1462, 1385, 1222, 1156, 1048, 983, 873, 603. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 786.2 ($[M+NH_4]^+$), 767.2 ($[M-H]^-$). HR-ESI-MS: 791.2890 ($[M+Na]^+$, $C_{40}H_{48}NaO_{15}^+$; calc. 791.2885).

Aphagranol E (= *(1*S*,3a*S*,5a*S*,6a*R*,7*R*,7a*R*,8*S*,10a*S*,11a*R*,11b*R*,11c*R*)-4,10a-Bis(acetyloxy)-1-[(acetyloxy)methyl]-8-(furan-3-yl)-1,3a,5a,6a,7,7a,8,9,10,10a,11,11a,11b,11c-tetradecahydro-1,7a,11b-trimethyl-11-methylidene-3,10-dioxo-3*H*-indeno[5,6-*d*]benzo[1,2-*b*:3,4-*c*′]difuran-7-yl (2*R*)-2-(Acetyloxy)-3-methylbutanoate*; **2**). White amorphous powder. $[\alpha]_D^{27} = -56.3$ (c = 0.20, MeOH). UV (MeCN): 194 (4.22), 214 (3.94), 307 (3.08). IR (KBr): 3454, 1765, 1635, 1385, 1224, 1028, 873, 603. ^{1}H- and ^{13}C-NMR: see *Table 1*. ESI-MS: 772.3 ($[M+NH_4]^+$), and 789.2 ($[M+Cl]^-$). HR-ESI-MS: 777.2730 ($[M+Na]^+$, $C_{39}H_{46}NaO_{15}^+$; calc. 777.2729).

Aphagranol F (= *(1*S*,5a*S*,6a*R*,7*R*,7a*R*,8*S*,10a*S*,11a*R*,11b*R*,11c*S*)-4-(Acetyloxy)-1-[(acetyloxy)methyl]-8-(furan-3-yl)-1,5,5a,6a,7,7a,8,9,10,10a,11,11a,11b,11c-tetradecahydro-10a-hydroxy-1,7a,11b-trimethyl-11-methylidene-3,10-dioxo-3*H*-indeno[5,6-*d*]benzo[1,2-*b*:3,4-*c*′]difuran-7-yl (2*R*,3*R*)-2-Hydroxy-3-methylpentanoate*; **3**). White amorphous powder. $[\alpha]_D^{27} = -32.5$ (c = 0.15, MeOH). UV (MeCN): 193 (4.25), 212 (4.10); IR (KBr): 3456, 1766, 1385, 1225, 1020, 875, 605. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 702.2 ($[M+NH_4]^+$), 683.1 ($[M-H]^-$), 719.2 ($[M+Cl]^-$). HR-ESI-MS: 707.2680 ($[M+Na]^+$, $C_{36}H_{44}NaO_{13}^+$; calc. 707.2674).

Aphagranol G (= *(1*S*,5a*S*,6a*R*,7*R*,7a*R*,8*S*,10a*S*,11a*R*,11b*R*,11c*S*)-4-(Acetyloxy)-1-[(acetyloxy)methyl]-8-(furan-3-yl)-1,5,5a,6a,7,7a,8,9,10,10a,11,11a,11b,11c-tetradecahydro-10a-hydroxy-1,7a,11b-trimethyl-11-methylidene-3,10-dioxo-3*H*-indeno[5,6-*d*]benzo[1,2-*b*:3,4-*c*′]difuran-7-yl (2*R*)-2-Hydroxy-3-methylbutanoate*; **4**). White amorphous powder. $[\alpha]_D^{27} = -26.8$ (c = 0.15, MeOH). UV (MeCN): 195 (4.15), 211 (4.03). IR (KBr): 3454, 1765, 1635, 1385, 1224, 1028, 873, 603. ^{1}H- and ^{13}C-NMR: see *Table 2*. ESI-MS: 688.2 ($[M+NH_4]^+$), 705.2 ($[M+Cl]^-$). HR-ESI-MS: 693.2513 ($[M+Na]^+$, $C_{35}H_{42}NaO_{13}^+$; calc. 653.2604).

Aphagranol H (= *(1*S*,5a*S*,6a*R*,7*R*,7a*R*,8*S*,10a*S*,11a*R*,11b*R*,11c*S*)-4-(Acetyloxy)-1-[(acetyloxy)methyl]-8-(furan-3-yl)-1,5,5a,6a,7,7a,8,9,10,10a,11,11a,11b,11c-tetradecahydro-10a-hydroxy-1,7a,11b-trimethyl-11-methylidene-3,10-dioxo-3*H*-indeno[5,6-*d*]benzo[1,2-*b*:3,4-*c*′]difuran-7-yl 3-Methylbutanoate*; **5**). White amorphous powder. $[\alpha]_D^{27} = -34.5$ (c = 0.15, MeOH). UV (MeCN): 193 (4.23), 211 (4.08). IR (KBr): 3455, 2973, 1755, 1636, 1384, 1224, 1028, 873, 605. ^{1}H- and ^{13}C-NMR: *Table 2*. ESI-MS: 672.2 ($[M+NH_4]^+$), 789.3 ($[M+Cl]^-$). HR-ESI-MS: 653.2623 ($[M-H]^-$, $C_{35}H_{41}O_{12}^-$; calc. 653.2604).

Insecticide Activity Assay. The isolates were tested for insecticidal activity against *Sitobion avenae* (1,000 ppm), *Plutella xylostella* (500 ppm), and *Diabrotica balteata* (500 ppm) in a leaf-disk assay. The isolates were also evaluated against the nematode species *Caenorhabditis elegans* in liquid culture at 50 ppm. Chemicals were applied to feed *S. avenae*, prior to infestation with *P. xylostella* larvae, or diluted into the *C. elegans* culture. Thiamethoxam and indoxacarb were included as positive control. The assay plates were stored in controlled environment cabinets for 5 to 9 d (depending on the species). Mortality was then assessed relative to untreated control wells, with wells showing significant levels of mortality scored as 99, and wells without significant mortality scored as 0.

REFERENCES

[1] Q.-G. Tan, X.-D. Luo, *Chem. Rev.* **2011**, *111*, 7437.
[2] J. Luo, J.-S. Wang, J.-G. Luo, X.-B. Wang, L.-Y. Kong, *Org. Lett.* **2009**, *11*, 2281.
[3] J. Luo, J.-S. Wang, J.-G. Luo, X.-B. Wang, X.-F. Huang, J.-G. Luo, L.-Y. Kong, *Tetrahedron* **2009**, *65*, 3425.

[4] J. Luo, J.-S. Wang, X.-B. Wang, J.-G. Luo, L.-Y. Kong, *J. Nat. Prod*. **2010**, *73*, 835.
[5] Y. Zhang, J.-S. Wang, X.-B. Wang, D.-D. Wei, J.-G. Luo, J. Luo, M.-H. Yang, L.-Y. Kong, *Tetrahedron Lett.* **2011**, *52*, 2590.
[6] M.-H. Yang, J.-S. Wang, J.-G. Luo, X.-B. Wang, L.-Y. Kong, *Bioorg. Med. Chem.* **2011**, *19*, 1409.
[7] J.-S. Wang, Y. Zhang, X.-B. Wang, L.-Y. Kong, *Tetrahedron* **2012**, *68*, 3963.
[8] X.-B. Wang, Y. Zhang, J.-S. Wang, Y.-C. Gu, L.-Y. Kong, *Tetrahedron Lett.* **2013**, *54*, 6023.
[9] Y. Zhang, J.-S. Wang, Y.-C. Gu, L.-Y. Kong, *Phytochem. Lett.* **2013**, *6*, 539.
[10] C.-Y. Zhou, H.-Y. Tang, X. Li, Y.-S. Sang, *Helv. Chim. Acta* **2009**, *92*, 1191.
[11] S.-P. Yang, H.-D. Chen, S.-G. Liao, B.-J. Xie, Z.-H. Miao, J.-M. Yue, *Org. Lett.* **2011**, *13*, 150.
[12] X.-D. Luo, S.-H. Wu, D.-G. Wu, Y.-B. Ma, S.-H. Qi, *Tetrahedron* **2002**, *58*, 7797.
[13] R. J. Stonard, D. A. Trainor, M. Nakatani, K. Nakanishi, *J. Am. Chem. Soc.* **1983**, *105*, 130.
[14] F. Bracher, W. J. Eisenreich, J. Mühlbacher, M. Dreyer, G. Bringmann, *J. Org. Chem*. **2004**, *69*, 8602.
[15] P. L. Polavarapu, D. K. Chakraborty, *J. Am. Chem. Soc.* **1998**, *120*, 6160.
[16] P. Sun, D.-X. Xu, A. Mándi, T. Kurtán, T.-J. Li, B. Schulz, W. Zhang, *J. Org. Chem*. **2013**, *78*, 7030.

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