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Aphagrandinoids A–D, cycloartane triterpenoids with antibacterial activities from *Aphanamixis grandifolia*

Xiao-Ying Wang [a,b], Gui-Hua Tang [b], Chun-Mao Yuan [b], Yu Zhang [b], Tao Zou [a], Chun Yu [a], Qing Zhao [a,*], Xiao-Jiang Hao [b,*], Hong-Ping He [a,b,**]

[a] Faculty of Pharmacy, Yunnan University of TCM, Kunming 650500, Yunnan, People's Republic of China
[b] State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China

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ABSTRACT

Three new cycloartane triterpenoids, aphagrandinoids A–C (**1**–**3**), and a new natural product, aphagrandinoid D (**4**), together with a known compound, were isolated from the leaves and twigs of *Aphanamixis grandifolia*. Their structures were elucidated by extensive NMR and MS techniques. Aphagrandinoid A (**1**), a 29-nor-cycloart triterpenoid, features with a sprio ring system at the side chain, while aphagrandinoid C (**3**) is a pentnortriterpenoid. Antibacterial activities of these five compounds were also evaluated. Compounds **1** and **5** showed weak antibacterial activities (MIC values: 1.57–3.13 μg/mL) against *Staphylococcus aureus*, MRSA 92#, and MRSA 98#.

1. Introduction

The genus *Aphanamixis* (Meliaceae), comprising about 25 species, is native to the tropical regions of Asia such as Southern China, India, Malaysia, and Indonesia [1]. In recent years, the investigations of this genus have been becoming a hot topic because of abundant secondary metabolites with highly complicated structures [2] and significant bioactivities including antifeedant, insecticidal, antitumor, and antioxidant activities [3,4]. The *Aphanamixis grandifolia* Blume is an evergreen and timber tree, whose roots and leaves have been applied in primitive medicine to cure cold and rheumatic joint pain due to arthritis as well as numbness of limbs owing to wind–cold–dampness. Up to date, phytochemical investigations on the genus *Aphanamixis* have led to the occurrence of a series of chemical constituents such as triterpenes [5–8], limonoids [9–11], lignans [12], and alkaloids [13].

During our seeking for potentially biological active components from the plants of Meliaceae family [14–16], three new 29-nor-cycloart triterpenoids, aphagrandinoids A–C (**1**–**3**), and a new natural product, aphagrandinoid D (**4**), along with a structurally related known one (20*R*)-3β-hydroxy-24,25,26,27-tetranor-5α-cycloartan-23,21-olide (**5**), were isolated from the leaves and twigs of *A. grandifolia* [17]. Antibacterial activities of these five compounds were evaluated, and compounds **1** and **5** showed weak antibacterial activities (MIC values: 1.57–3.13 μg/mL) against MRSA 92#, MRSA 98#, and *Staphylococcus aureus*. Herein, we report the isolation, structural elucidation, and antibacterial activities of these five compounds.

2. Experimental

2.1. General experimental procedure

Optical rotations were determined on a JASCO P-1020 digital polarimeter. UV spectra were detected on a Shimadzu

* Corresponding author. Tel.: +86 871 65223263; fax: +86 871 65223070.
** Correspondence to: H.-P. He, State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, Yunnan, People's Republic of China. Tel.: +86 871 65223263; fax: +86 871 65223070.
E-mail addresses: tempzhao@126.com (Q. Zhao), haoxj@mail.kib.ac.cn (X.-J. Hao), hehongping@mail.kib.ac.cn (H.-P. He).

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UV-2401 PC spectrophotometer. IR spectra were scanning with a Bruker Tensor-27 infrared spectrometer and a KBr disk. A Brucker HCT/E squire and a Waters Autospec Premier P776 spectrum were used to measure ESIMS and HREIMS spectra, respectively. 1D and 2D NMR spectra were recorded on a Bruker AM-400, DRX-500, and Avance III 600 spectrometers with TMS as internal standard. Column chromatography was performed on silica gel (200–300 and 300–400 mesh, Qingdao Marine Chemical Inc.), MCI gel CHP 20P (75–150 μm, Mitsubishi Chemical Corporation, Tokyo), Sephadex LH-20 (40–70 μm, Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex RP-C_{18} gel (20–45 μm, Merck, Darmstadt, Germany).

2.2. Plant material

The leaves and twigs of *A. grandifolia* were collected in Xishuangbanna from Yunnan Province, China, in August 2011. The plant was authenticated by Mr. Yu Chen, Kunming Institute of Botany, Chinese Academy of Sciences (CAS). A voucher specimen (No. H20110802) has been deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, CAS.

2.3. Extraction and isolation

The leaves and twigs of *A. grandifolia* (9.0 kg) were extracted by refluxing with 95% EtOH three times. In a vacuum, the EtOH solvent was concentrated to obtain a residue (428.5 g), which was partitioned with PE (petroleum ether), EtOAc, and *n*-BuOH, successively. The PE fraction (133.6 g) was submitted to column chromatography (CC) over silica gel (200–300 mesh) using PE/Acetone (100:1 → 1:1) to give five fractions 1 − 5. Fr. 1 (21.1 g) was subjected to an MCI gel (MeOH/H_2O from 4:6 to 10:0), RP-C_{18} (MeOH/H_2O from 6:4 to 10:0), Sephadex LH-20 (MeOH and Acetone), and further purified on a series of silica gel columns to yield compounds **2** (13.4 mg) and **4** (8.2 mg). Fr. 2 (12.4 g) was repeatedly chromatographed over a RP-C_{18} column (MeOH/H_2O from 6:4 to 10:0), Sephadex LH-20 (MeOH and Acetone), and silica gel columns to obtain compounds **1** (3.7 mg), **3** (6.8 mg), and **5** (13.4 mg).

Aphagrandinoid A (**1**): colorless oil; $[\alpha]_D^{26}$ −1.67 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ε) 204 (3.38) nm; IR (KBr) ν_{max} 3440, 2955, 2923, 2869, 1751, 1462, 1377, 997 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 477 $[M+Na]^+$, 931 $[2\ M+Na]^+$; HREIMS *m/z* 454.3086 (calcd for $C_{30}H_{46}O_2$ $[M]^+$, 454.3083).

Aphagrandinoid B (**2**): white, amorphous powder; $[\alpha]_D^{28}$ + 20.7 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.35) nm; IR (KBr) ν_{max} 3470, 2930, 2871, 2830, 1377, 1036 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 449 $[M+Na]^+$, 875 $[2\ M+Na]^+$; HREIMS *m/z* 426.3487 (calcd for $C_{29}H_{46}O_2$ $[M]^+$, 426.3498).

Aphagrandinoid C (**3**): colorless oil; $[\alpha]_D^{28}$ +28.7 (*c* 0.15, MeOH); UV (MeOH) λ_{max} (log ε) 204 (2.30) nm; IR (KBr) ν_{max} 3456, 2921, 1766, 1196 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 409 $[M+Na]^+$, 795 $[2\ M+Na]^+$; HREIMS *m/z* 386.2827 (calcd for $C_{25}H_{38}O_3$ $[M]^+$, 386.2821).

Aphagrandinoid D (**4**): white, amorphous powder; $[\alpha]_D^{26}$ + 18.6 (*c* 0.19, MeOH); UV (MeOH) λ_{max} (log ε) 203 (2.53) nm; IR (KBr) ν_{max} 2983, 2931, 2867, 1779, 1705, 1177 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; ESIMS *m/z* 421 $[M+Na]^+$, 819 $[2\ M+Na]^+$; HREIMS *m/z* 398.2815 (calcd for $C_{26}H_{38}O_3$ $[M]^+$, 398.2821).

Table 1
^1H NMR data for **1**–**4** in $CDCl_3$ (δ in ppm).

No.	1[a]	2[b]	3[a]	4[a]
1α	1.25, overlapped	1.27, m	1.25, m	1.51, m
1β	1.52, m	1.54, m	1.51, m	1.84, m
2α	1.37, m	1.41, m	1.53, m	2.29, m
2β	1.95, m	1.97, m	1.97, m	2.69, td (13.8, 6.5)
3α	3.18, m	3.20, m	3.20, m	
4	1.12, m	1.17, m	1.13, m	
5α	1.16, m	1.19, m	1.18, m	1.70, dd (12.4, 4.3)
6α	0.56, m	0.58, m	0.57, qd (16.0, 3.0)	0.94, m
6β	1.64, m	1.67, m	1.66, m	1.55, m
7α	1.03, m	1.06, m	1.04, dd (11.6, 3.3)	1.13, dd (12.3, 2.5)
7β	1.29, m	1.31, m	1.30, m	1.37, overlapped
8β	1.58, m	1.61, m	1.57, m	1.58, overlapped
9				
10				
11α	1.21, m	1.23, m	1.23, m	1.21, m
11β	1.92, m	1.93, m	1.95, m	2.04, m
12α	1.25, overlapped	1.33, m	1.31, overlapped	1.37, overlapped
12β	1.46, m	1.49, m	1.54, m	1.58, overlapped
13				
14				
15	1.36, overlapped	1.35, m	1.37, m	1.39, m
16α	1.36, overlapped	1.36, m	1.31, overlapped	1.33, m
16β	1.83, m	1.87, m	1.86, m	1.88, m
17α	1.74, m	1.74, m	1.85, m	1.86, m
18	0.97, s	0.98, s	0.99, s	1.01, s
19a	0.10, d (4.0)	0.12, d (3.7)	0.13, d (4.3)	0.57, d (4.8)
19b	0.39, d (4.0)	0.40, d (3.7)	0.39, d (4.3)	0.79, d (4.8)
20	2.67, m	2.21, m	2.54, m	2.54, m
21α	3.58, dd (9.7, 8.5)	3.19, overlapped	3.86, t (9.3)	3.87, t (9.1)
21β	4.27, t (8.5)	4.03, t (7.4)	4.41, t (8.4)	4.41, t (8.2)
22α	1.78, t (12.9)	1.72, overlapped	2.18, m	2.20, m
22β	2.16, dd (12.9, 6.2)		2.53, overlapped	2.51, m
23		4.59, m		
24	6.67, q (1.4)	5.21, d (8.4)		
25				
26	1.90, d (1.4)	1.69, s		
27		1.71, s		
28α	0.95, d (6.0)	0.97, d (5.4)	0.96, d (6.6)	1.02, s
29β				1.08, s
30	0.85, s	0.88, s	0.88,s	0.90, s

[a] Recorded at 600 MHz.
[b] Recorded at 400 MHz.

2.4. Antibacterial bioassays [18]

We have tested the antibacterial activities of all the compounds by a 2-fold dilution method against *S. aureus*, *Pseudomonas aeruginosa*, MRSA (methicillin-resistant *S. aureus*) 92#, and MRSA 98#. In the antibacterial tests, all the strains

Table 2
^{13}C NMR data for **1**–**4** in $CDCl_3$ (δ in ppm).

No.	1[a]	2[b]	3[a]	4[a]
	δ_C, type	δ_C, type	δ_C, type	δ_C, type
1	30.9, CH_2	30.6, CH_2	30.7, CH_2	33.5, CH_2
2	34.9, CH_2	34.7, CH_2	34.7, CH_2	37.6, CH_2
3	76.6, CH	76.4, CH	76.4, CH	216.7, C
4	44.7, CH	44.5, CH	44.6, CH	50.4, C
5	43.3, CH	43.1, CH	43.1, CH	48.4, CH
6	24.7, CH_2	24.5, CH_2	24.5, CH_2	21.5, CH_2
7	25.2, CH_2	25.0, CH_2	25.1, CH_2	26.0, CH_2
8	46.7, CH	46.6, CH	46.6, CH	47.8, CH
9	23.5, C	23.4, C	23.3, C	21.0, C
10	29.9, C	29.7, C	29.7, C	26.4, C
11	26.8, CH_2	26.7, CH_2	26.6, CH_2	26.5, CH_2
12	31.4, CH_2	31.2, CH_2	31.5, CH_2	31.6, CH_2
13	45.8, C	45.4, C	45.5, C	45.7, C
14	48.6, C	48.4, C	48.5, C	48.5, C
15	35.6, CH_2	35.5, CH_2	35.4, CH_2	35.8, CH_2
16	27.9, CH_2	27.8, CH_2	27.4, CH_2	27.6, CH_2
17	51.2, CH	50.5, CH	50.8, CH	51.0, CH
18	18.9, CH_3	18.5, CH_3	18.7, CH_3	19.2, CH_3
19	27.4, CH_2	27.2, CH_2	27.3, CH_2	29.8, CH_2
20	41.0, CH	42.4, CH	39.3, CH	39.5, CH
21	74.3, CH_2	72.0, CH_2	72.6, CH_2	72.7, CH_2
22	42.7, CH_2	38.9, CH_2	34.8, CH_2	34.9, CH_2
23	112.2, C	74.4, CH	177.1, C	177.2, C
24	144.8, CH	126.8, CH		
25	133.5, C	135.0, C		
26	10.8, CH_3	18.1, CH_3		
27	171.6, C	25.8, CH_3		
28	14.6, CH_3	14.4, CH_3	14.4, CH_3	22.4, CH_3
29				20.9, CH_3
30	19.2, CH_3	19.0, CH_3	19.0, CH_3	19.4, CH_3

[a] Recorded at 150 MHz.
[b] Recorded at 100 MHz.

were obtained from the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command. Under the same conditions, vancomycin hydrochloride (Eli Lilly Japan K.K., Seishin Laboratories, purity ≥ 98%) was used as a positive control without samples. The compounds were dissolved in DMSO at a concentration of 500 μg/mL and then diluted with the final concentrations in a range of 0.78–50 μg/mL. The MIC value was the lowest concentration without any colony growth after incubating at 37 °C for 18 h. As shown in Table 3, compound **5** displayed weak activities with MIC values of 1.57 μg/mL against MRSA 92# and MRSA 98#, and compound **1** with MIC value of 3.13 μg/mL against *S. aureus*.

3. Results and discussion

Aphagrandinoid A (**1**) was obtained as colorless oil and its molecular formula was determined to be $C_{29}H_{42}O_4$ with 9° of unsaturation due to the HREIMS at *m/z* 454.3086 (calcd for 454.3083). Its IR spectrum exhibited the existence of hydroxyl (3440 cm^{-1}) and carbonyl (1751 cm^{-1}) groups. The ^{13}C NMR and DEPT spectra (Table 2) permitted identification of four methyls, eleven methylenes, seven methines, and seven quaternary carbons including a ketal carbon (δ_C 112.2, C-23), an olefinic carbon (δ_C 133.5, C-25) and a lactone carbonyl (δ_C 171.6, C-27). Its ^{1}H NMR spectrum displayed two singlet

Table 3
Antibacterial activities of compounds **1**–**5**.[a]

Compound	Antibacterial activities (MIC in μg/mL)			
	S. aureus	*P. aeruginosa*	MRSA 92#	MRSA 98#
1	3.13	25	25	25
2	25	25	25	25
3	25	25	50	25
4	50	25	25	25
5	25	25	1.57	1.57
Positive control	0.78[b]	25[b]	0.78[b]	0.78[b]

[a] *S. aureus* (*Staphylococcus aureus*), *P. aeruginosa* (*Pseudomonas aeruginosa*), MRSA (methicillin-resistant *Staphylococcus aureus*).
[b] Vancomycin hydrochloride as positive control.

methyls at δ_H 0.85, 0.97, two doublet methyls at δ_H 0.95 (d, J = 6.0 Hz), 1.90 (d, J = 1.4 Hz), and a characteristic up-field methylene at δ_H 0.10 (d, J = 4.0 Hz) and 0.39 (d, J = 4.0 Hz). The above information together with cross-peaks of H-4/H_3-28, H-3/H-4, and H-4/H-5 in the ^{1}H–^{1}H COSY spectrum suggested that compound **1** might be a typical 29-nor-cycloart triterpene.

Carefully comparing the ^{1}H and ^{13}C NMR (Tables 1 and 2) data with those of uvariastrol [19], the structures of rings A with an OH group at C-3, B, C, D and cyclopropane ring were established the same as uvariastrol. The characteristics of ketal and lactone carbonyl carbons, along with the two remaining degrees of unsaturation, revealed that **1** might possess a spiro-ring system at C-17. The HMBC correlations from H-24 to C-27 (δ_C 171.6), H_3-26 to C-24 (δ_C 144.8), C-25 (δ_C 133.5), and C-27, and H_2-21 to C-17(δ_C 51.2), C-22 (δ_C 42.7), and C-23 (δ_C 112.2) as well as the ^{1}H–^{1}H COSY of H-20/H_2-21 and H-20/H_2-22 further demonstrated that a spiro-ring moiety was linked to C-17. Thus, compound **1** with an unusual spiro-ring system at C-17 was determined as depicted in Fig. 1.

The relative configuration of compound **1** was established by the ROESY correlations. As shown in Fig. 2.1b, the ROESY correlations of H-4/H_2-19, H_2-19/H-8, H-8/H_3-18, H_3-18/H-21β, and H_3-18/H-20 indicated that these groups were co-facial and assigned arbitrarily as β-oriented. Meanwhile, the ROESY correlations of H-3/H_3-28, H-3/H-5, H-16α/H-22α, H-22α/H-24, and H_3-30/H-17 implied their α-orientation. Thus, compound **1** was characterized as 3β-hydroxyl-21/23,23/27-diepoxy-29-nor-5α-cycloart-24-en-27-one.

Aphagrandinoid B (**2**), amorphous white powder, had the molecular formula $C_{29}H_{46}O_2$ with 7° of unsaturation deduced from its HREIMS at *m/z* 426.3487 $[M]^+$ (calcd for 426.3498). Comparing the NMR data (Tables 1 and 2) of **2** with those of **1**, it demonstrated the differences that **2** have an oxidative methine (δ_C 74.4, C-23, δ_H 4.59, 1H, m) and a singlet methyl (δ_C 25.8, C-27, δ_H 1.71, 3H, s) rather than the ketal carbon (δ_C 112.2, C-23) and the lactone carbonyl (δ_C 171.6, C-27) in **1**, respectively. This information suggested that the spiroketal moiety was nonexistent in **2**. An analysis of the HMBC correlations of H_2-21 with C-17 (δ_C 50.5), C-22 (δ_C 38.9), and C-23 (δ_C 74.4), and H-24 (δ_H 5.21, d, J = 8.4 Hz) with C-22, C-26 (δ_C 18.1) and C-27 (δ_C 25.8), together with the ^{1}H–^{1}H COSY cross-peaks of H_2-22/H-23 and H-23/H-24 confirmed that an isobutenyl moiety was connected to C-23 in **2**. Thus, compound **2** was elucidated as depicted. The relative stereochemistry of

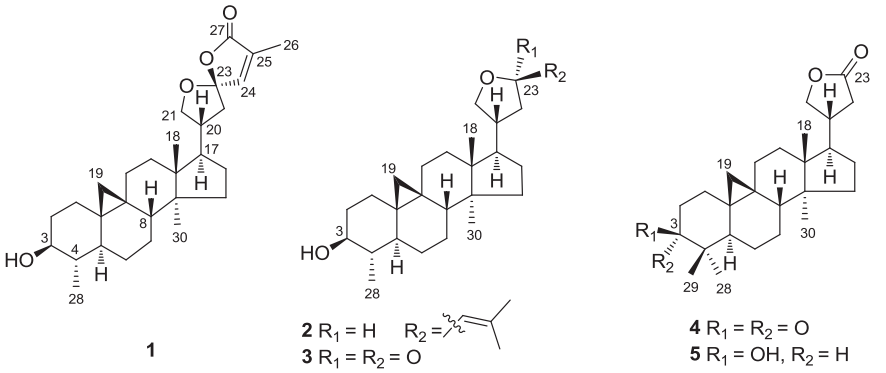

Fig. 1. The chemical structures of compounds **1**–**5**.

compound **2** was almost consistent with that of compound **1** (Fig. 3.2b). In particular, H-23 was assigned as α-oriented deduced by the ROESY correlations of H-21α/H-23 and a direct comparison with 1D NMR data reported in the literature [17]. Therefore, the framework of compound **2** was deduced to be 21,23-epoxy-29-nor-5α-cycloart-24-en-3β-ol.

Aphagrandinoid C (**3**), showed the quasi-molecular ion peak $[M]^+$ at *m/z* 386.2827 (calcd for 386.2821) in the HREIMS, corresponding to the molecular formula $C_{25}H_{38}O_3$, with the same 7° of unsaturation as those of **2**. Based on the comparison of the NMR data (Tables 1 and 2) and MS of **3** with those of **2**, the appearance of a carbonyl signal (δ_C 177.1, C-23) and the missing of five carbon signals (two singlet methyls, a trisubstituted double bond, and an oxygenated methine) in the 1D NMR spectra of **3**, together with the molecular weight of **3** less than that of **2** by 40 suggested that the isobutenyl at C-23 in **2** might be oxidized in **3**. This assignment was further confirmed by the HMBC correlations of H_2-21 to C-17 (δ_C 50.8), C-22 (δ_C 34.8), and C-23 (Supporting information). As shown in the ROESY spectrum (Supporting information), the relative configuration was identical with that of compound **2**. Hence, compound **3** was assigned as 3-oxo-24,25,26,27,29-pentnor-5α-cycloart-23,21-olide.

Aphagrandinoid D (**4**) revealed the molecular formula of $C_{26}H_{38}O_3$ with one unsaturation degree more than **5**, which was inferred from the HREIMS at *m/z* 398.2815 $[M]^+$ (calcd for 398.2821). Extensive interpretation of the 1D and 2D NMR data of **4** and **5** exhibited that they were of the similar structure except for a ketone carbonyl (δ_C 216.7) at C-3 in **4** rather than a hydroxyl at C-3 in **5**. The key HMBC correlations from H_2-1, H-5, H_3-28, and H_3-29 to C-3 placed the ketone carbonyl at C-3. The relative configuration of **4** was similar to that of **5** as shown in the ROESY spectrum (Supporting information). Thus, compound **4** was established as 3-oxo-24,25,26,27-tetranor-5α-cycloartan-23,21-olide, which was identical to the PCC-oxidation of **5** that was reported in the literature [17], and compound **4** was a new natural product isolated from *A. grandifolia*.

Additionally, the known compound **5** was identical to (20*R*)-3β-hydroxy-24,25,26,27-tetranor-5α-cycloartan-23,21-olide [17], which was confirmed by comparing the NMR data with the corresponding literature data.

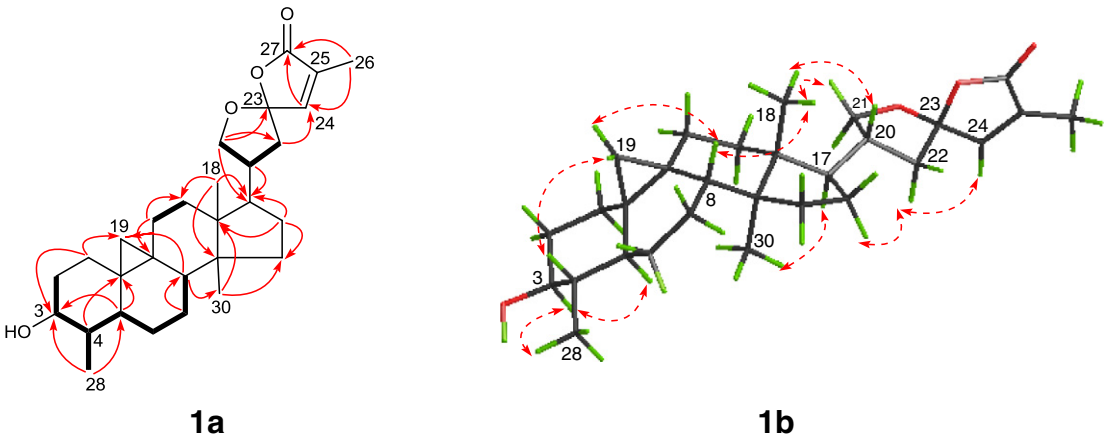

Fig. 2. Selected ^{1}H–^{1}H COSY (—), HMBC (H⟶C) correlations and key ROESY (H⇠⇢H) correlations of compound **1**.

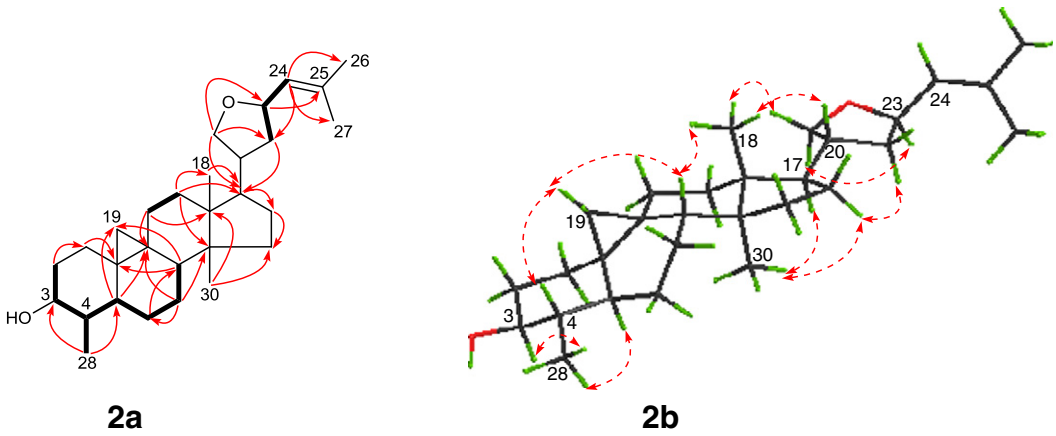

Fig. 3. Selected $^{1}H–^{1}H$ COSY (—), HMBC (H⟶C) correlations and key ROESY (H⇢H) correlations of compound **2**.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fitote.2012.12.030.

References

[1] Chen SK, Li H, Chen PY. Flora of China, vol. 43. Beijing: Science Press; 1997. p. 75–80.
[2] Mohamad K, Hirasawa Y, Lim CS, Awang K, Hadi AHA, Takeya K, et al. Ceramicine A and walsogyne A, novel limonoids from two species of Meliaceae. Tetrahedron Lett 2008;49:4276–8.
[3] Carpinella MC, Defago MT, Valladares G, Palacios SM. Antifeedant and insecticide properties of a limonoid from *Melia azedarach* (Meliaceae) with potential use for pest management. J Agric Food Chem 2003;51:369–74.
[4] Abdelgaleil SAM, Okamura H, Iwagawa T, Sato A, Miyahara I, Doe M, et al. Khayanolides, rearranged phragmalin limonoid antifeedants from *Khaya senegalensis*. Tetrahedron 2001;57:119–26.
[5] Kundu AB, Ray S, Chatterjee A. Aphananin, a Triterpene from *Aphanamixis Polystachya*. Phytochemistry 1985;24:2123–5.
[6] Liu QA, Chen CJ, Shi XA, Zhang L, Chen HJ, Gao K. Chemical constituents from *Aphanamixis grandifolia*. Chem Pharm Bull 2010;58:1431–5.
[7] Wang JS, Zhang Y, Wang XB, Wei DD, Luo J, Luo JG, et al. A pair of tirucallane C_{27}-triterpenoid cyclopentenone epimers from the stem barks of *Aphanamixis grandifolia*. Tetrahedron Lett 2012;53:1705–9.
[8] Zhang Y, Wang JS, Luo J, Kong LY. Novel nortriterpenoids from *Aphanamixis grandifolia*. Chem Pharm Bull 2011;59:282–6.
[9] Cai JY, Zhang Y, Luo SH, Chen DZ, Tang GH, Yuan CM, et al. Aphanamixoid A, a potent defensive limonoid, with a new carbon skeleton from *Aphanamixis polystachya*. Org Lett 2012;14:2524–7.
[10] Tong L, Zhang Y, He HP, Hao XJ. Four new limonoids from *Aphanamixis grandifolia*. Chin J Chem 2012;30:1261–4.
[11] Yuan CM, Zhang Y, Tang GH, Li SL, Di YT, Hou L, et al. Senegalensions A–C, three limonoids from *Khaya senegalensis*. Chem Asian J 2012;7:2024–7.
[12] Sadhu SK, Phattanawasin P, Choudhuri MSK, Ohtsuki T, Ishibashi M. A new lignan from *Aphanamixis polystachya*. J Nat Med 2006;60:258–60.
[13] Harmon AD, Weiss U, Silverton JV. Structure of rohitukine, the main alkaloid of *Amoora rohituka* (syn *Aphanamixis polystachya*) (Meliaceae). Tetrahedron Lett 1979;8:721–4.
[14] Fang X, Di YT, He HP, Liu HY, Zhang Z, Ren YL, et al. Cipadonoid A, a novel limonoid with an unprecedented skeleton, from *Cipadessa cinerasecns*. Org Lett 2008;10:1905–8.
[15] Yin JL, Di YT, Fang X, Liu ED, Liu HY, He HP, et al. Tabulvelutin A, the first 19-nor limonoid with unprecedented ring system from *Chukrasia tabularis* var. *velutina*. Tetrahedron Lett 2011;52:3083–5.
[16] Geng ZL, Fang X, Di YT, Zhang Q, Zeng Y, Shen YM, et al. Trichilin B, a novel limonoid with highly rearranged ring system from *Trichilia connaroides*. Tetrahedron Lett 2009;50:2132–4.
[17] Achenbach H, Frey D. Cycloartanes and other terpenoids and phenylpropanoids from *Monocyclanthus vignei*. Phytochemistry 1992;31:4263–74.
[18] Tang GH, Zhang Y, Gu YC, Li SF, Di YT, Wang YH, et al. Trigoflavidols A–C, degraded diterpenoids with antimicrobial activity, from *Trigonostemon flavidus*. J Nat Prod 2012;75:996–1000.
[19] Waterman PG, Mohammad I. Uvariastrol, a novel cycloartane triterpene from the stem bark of *Uvariastrum zenkeri*. Phytochemistry 1984;23:2077–9.