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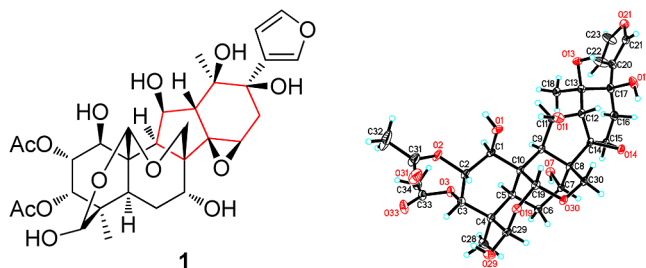
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ABSTRACT



A new 6/6/5/6-fused limonoid, phyllanthoid A (1), possessing both 19/30 and 19/29 oxygen bridges was isolated from *Phyllanthus cochinchinensis* (Euphorbiaceae), together with a new related limonoid, phyllanthoid B (2). Their structures were determined by spectroscopic analysis and single-crystal X-ray diffraction in the case of 1. Compound 1 displayed moderate antifeedant against the generalist plant-feeding insect *Spodoptera exigua* and cytotoxicity against the MCF-7 cell line.

Limonoids are modified triterpenes mainly found in the Meliaceae and Rutaceae families and less frequently in the Euphorbiaceae.¹ A variety of oxidations and skeletal rearrangements occurring in the basic skeleton led to various limonoid skeletons.¹ The diverse structure of limonoids has attracted considerable attention. In recent years, a number of novel limonoids with significant biological activities, e.g. insect antifeedant,² cytotoxic,³ antimalarial,⁴ insecticidal⁵ activities, and blocking activities against the Kv1.2 potassium channel,⁶ have been reported continuously.

Phyllanthus, composed of approximately 600 species, is the largest genus in the family Euphorbiaceae. Previously, a number of structurally diverse and highly oxygenated sesquiterpenoids were isolated from the genus *Phyllanthus*.⁷ *P. cochinchinensis* is a shrub mainly growing in the southern part of P. R. China. So far, no chemical study has been carried out on this species. A subsequent study on bioactive compounds from *Phyllanthus* species^{7a,b} led to the isolation of two new 6/6/5/6-fused limonoids, phyllanthoids A (1) and B (2), from the whole plants of *P. cochinchinensis* (Figure 1).

To date, most of reported limonoids possess a 6/6/6/5 ring system or a δ -lactonyl D ring.¹ Besides, two novel limonoids incorporating a five-membered C ring fused to a six-membered aromatic D ring were reported.⁸ Phyllanthoids

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A (**1**) and B (**2**) feature a rare 6/6/5/6 ring system with a six-membered aliphatic D ring. Furthermore, adding to the structural complexity, compound **1** has an oxide bridge between C-19 and C-30 to furnish an extra tetrahydropyran ring, in addition to the C-19/C-29 lactol bridge. We describe herein the isolation, structural elucidation, and bioactivities of the two new rearranged limonoids, phyllanthoids A (**1**) and B (**2**).

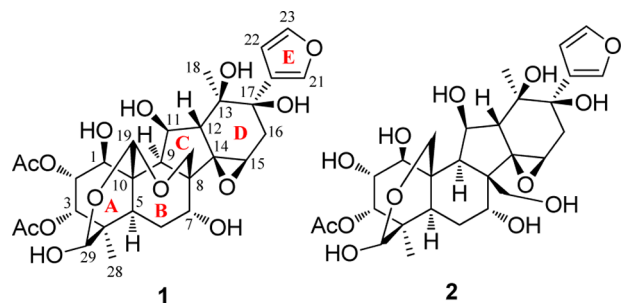


Figure 1. Structures of **1** and **2**.

Phyllanthoid A (**1**)⁹ was obtained as colorless crystals (in MeOH). The molecular formula was deduced to be $C_{30}H_{38}O_{14}$ on the basis of HR(+)ESIMS analysis (m/z 645.2159 [$M + Na$]⁺) and ^{13}C NMR (DEPT), indicating 12 degrees of unsaturation. The IR absorptions at 3439 and 1729 cm^{-1} indicated the existence of hydroxyl and carbonyl groups. The 1H and ^{13}C NMR spectral data (Table 1, Supporting Information (SI)) showed the presence of two acetyls [δ_H 1.97, 2.08 (each 3H, s); δ_C 20.8, 21.0, and 172.3 ($C \times 2$)], a typical β -substituted furan ring [δ_H 6.53, 7.39, 7.45 (each 1H, s); δ_C 112.0, 141.5, 143.2],^{2–4} two tertiary methyls (δ_H 0.85, s, H₃-28; 1.03, s, H₃-18), and other characteristic signals arising from three methylenes including one that is oxygen-bearing (δ_C 67.7, C-30), 11 methines with seven that are oxygen-bearing, and six quaternary carbons with three oxygenated ones (δ_C 69.6, C-14; 75.3, C-17; 75.5, C-13). The aforementioned data indicated that **1** is a limonoid possessing unprecedented high oxidation. Apart from the furan ring and the two carbonyl groups accounting for five degrees of unsaturation, **1** should have seven additional rings in the molecule. The extensive comparison and analysis of 1D NMR data of **1** suggested that it shared the same A, B, E rings and a C-19/C-29 bridged hemiacetal unit [δ_H 5.71 (s) and 5.21 (s); δ_H 97.5 and 93.2] to those of trichilin A, a known limonoid from *Trichilia emetic* (Meliaceae).¹⁰ This was further

confirmed by the 1H – 1H COSY correlations of H-1–H-2–H-3 and H-5–H₂-6–H-7, and the HMBC correlations of H₃-28 to C-3 (δ_C 73.8)/C-4 (δ_C 42.9)/C-5 (δ_C 38.1)/C-29 (δ_C 93.2), H-29 to C-3/C-10 (δ_C 43.3)/C-28 (δ_C 19.1), H-5 to C-19 (97.5)/C-29, H-6 α to C-5/C-7 (δ_C 69.2)/C-8 (δ_C 46.5)/C-10, and H-7 to C-5/C-9 (δ_C 49.7). However, the ^{13}C NMR resonances due to the C and D rings of **1** were different from those of trichilin A. The 1H – 1H COSY spectrum verified the presence of CH(9)–CH(11)–CH(12) and CH(15)–CH₂(16) fragments of **1** (bold lines in Figure 2), and the HMBC correlations (Figure 2) of H₃-18 to C-12 (δ_C 56.6)/C-17 (δ_C 75.3) and H-11 to C-8/C-12/C-13 (δ_C 75.5)/C-14 (δ_C 69.6) were indicative of a five-membered C ring fused with a six-membered aliphatic D ring, in contrast to 6/5-fused C/D rings of a normal limonoid skeleton.¹ In addition, the C-30 methyl and C-19 methylene in trichilin A¹⁰ were replaced respectively with an oxymethylene (δ_H 3.53, 4.41; δ_C 67.7) and a lower-field shifted methine (δ_H 5.71; δ_C 97.5) in **1**, respectively. The HMBC correlation of H-30/C-19 clearly revealed the oxygen bridge between C-30 and C-19, to form an extra tetrahydropyran ring. Moreover, 14,15-epoxy was confirmed by the upfield shifted carbon resonances of C-14 (δ_C 69.6, s) and C-15 (δ_C 59.1, d), which was essentially identical to those of trichilin A.¹⁰ The proposal was further confirmed by the HMBC correlations of H-30/C-14 and H-15/C-17. The β -substituted furan ring was attached to C-17 of the D ring, due to the HMBC correlations of H-15/C-16/C-17, H-16/C-20, H-23/C-20, and H-22/C-20. Two acetyls were located on C-2 and C-3, respectively, by the HMBC correlations from H-2 and H-3 to the corresponding carbonyls at δ_C 172.3. Thus, the planar structure of **1** was established as shown in Figure 1.

The relative configuration of the fused A–B–C–D rings was established as shown in Figure 1, on the basis of the ROESY correlations (Figure 3) of H₃-28/H-5, H₃-18/H-9, H-19/H-30 β , H-12/H-30 β and biosynthetic considerations. The large value for $J_{1,2}$ (9.5 Hz) and small value for $J_{2,3}$ (4.5 Hz) supported the fact that both H-1 and H-2 are located in an axial orientation and H-3 is in an equatorial orientation. Combined with the ROESY correlation (Figure 3) of H-1 with H-5, both C-2-OAc and C-3-OAc were confirmed as α -oriented and 1-OH was β -oriented. The hydroxyls at C-11, C-13, and C-17 were deduced as β -oriented by the ROESY correlations of both H-11 and H-22 to H₃-18. In the same case, H-7 showing as a broad single peak in the 1H NMR spectrum supported an equatorial H-7, corresponding to an α -configuration for 7-OH, which was further confirmed by the ROESY correlation of H-7 with H₂-30. Moreover, the 29-*exo* configuration of **1** was assigned by the chemical shift of H-3 (δ_H 5.50), due to fact that the H-3 resonated between δ_H 5.3 and 5.6 for the 29-*exo* configuration (δ_H 4.9 – 5.1 for 29-*endo* configuration).¹¹ A crystal X-ray diffraction experiment with Cu K α radiation finally confirmed the planar structure of **1** (Deposition No. CCDC-930101) and also

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(9) Phyllanthoid A (**1**): Colorless crystals (in MeOH); HRESIMS at m/z 645.2159 [$M + Na$]⁺ (calcd 645.2153, $C_{30}H_{38}O_{14}Na$); [α_D]^{19.1} = -10.0° (c 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 208 (3.79); IR (KBr) ν_{max} 3439, 2976, 2936, 1729, 1677, 1640, 1375, 1256, and 1065 cm^{-1} ; for 1H and ^{13}C NMR data, see Table 1.

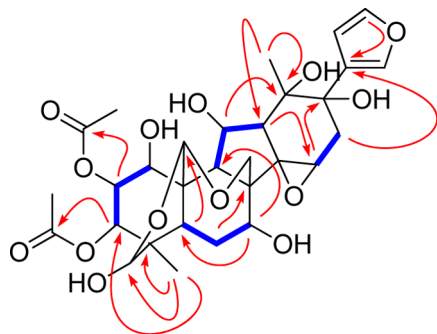
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Table 1. ^1H and ^{13}C NMR Data for **1** and **2** in CD_3OD

position	1^a		2^b	
	δ_{C}	δ_{H} (mult, J , Hz)	δ_{C}	δ_{H} (mult, J , Hz)
1	75.7 (d)	3.80 (d, 9.5)	82.4 (d)	3.53 (d, 9.3)
2	75.1 (d)	5.69 (dd, 9.5, 4.5)	73.3 (d)	4.54 (dd, 9.3, 5.2)
3	73.8 (d)	5.50 (d, 4.5)	76.7 (d)	5.51 (5.2)
4	42.9 (s)		43.2 (s)	
5	38.1 (d)	2.21 (dd, 12.2, 5.5)	37.5 (d)	2.33 ^c
6 α	32.8 (t)	1.65 (dd, 13.3, 5.5)	29.0 (t)	1.65 (dd, 14.0, 3.8)
6 β		2.39 ^c		1.92 (d, 14.0)
7	69.2 (d)	3.67 (brs)	66.6 (d)	3.56 (brs)
8	46.5 (s)		53.3 (s)	
9	49.7 (d)	2.42 ^c	52.5 (d)	2.35 ^c
10	43.3 (s)		45.5 (s)	
11	75.1 (d)	4.64 (d, 5.3)	73.8 (d)	4.77 (d, 4.9)
12	56.6 (d)	2.86 (brs)	58.5 (d)	2.63 (s)
13	75.5 (s)		76.0 (s)	
14	69.6 (s)		69.1 (s)	
15	59.1 (d)	3.38 (s)	61.5 (d)	3.63 (s)
16 α	36.0 (t)	2.44 ^c	36.1 (t)	2.49 (dd, 16.2, 2.2)
16 β		2.35 ^c		2.33 ^c
17	75.3 (s)		75.6 (d)	
18	19.1 (q)	1.03 (s)	19.0 (q)	1.02 (s)
19	97.5 (d)	5.71 (s)	63.7 (t)	4.59 (d, 12.6) 4.12 (d, 11.9)
20	130.2 (s)		129.9 (s)	
21	141.5 (d)	7.45 (s)	141.7 (d)	7.41 (s)
22	112.0 (d)	6.53 (s)	112.1 (d)	6.53 (s)
23	143.2 (d)	7.39 (s)	143.3 (d)	7.47 (s)
28	19.1 (q)	0.85 (s)	19.5 (s)	0.81 (s)
29	93.2 (d)	5.21 (s)	96.9 (d)	4.79 (s)
30 α	67.7 (t)	3.53 (d, 12.1)	64.6 (t)	3.92 (d, 11.5)
30 β		4.41 (d, 12.1)		3.66 (d, 11.5)
2-OAc	20.8 (q) 172.3 (s)	1.97 (s)	21.2 (q) 173.1 (s)	2.10 (s)
3-OAc	21.0 (q) 172.3 (s)	2.08 (s)		

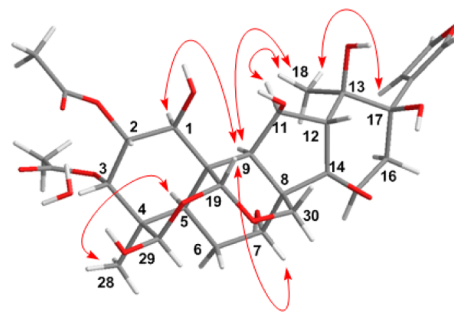
^a Data were recorded at 500 MHz (^1H) and 125 MHz (^{13}C). ^b Data were recorded at 600 MHz (^1H) and 150 MHz (^{13}C). ^c Overlapped with each other.

**Figure 2.** Key ^1H – ^1H COSY (bold —) and HMBC (→) correlations of **1**.

allowing the unambiguous assignment of the absolute configuration of **1** as 1*S*,2*R*,3*S*, 4*R*,5*R*,7*R*,8*R*,9*S*,10*R*,–

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(13) Hooft, R. W. W.; Straver, L. H.; Spek, A. L. *Appl. Crystallogr.* **2008**, 41, 96–103.

**Figure 3.** Key ROESY (↔) correlations of **1**.

11*R*,12*R*,13*R*,14*R*,15*R*,19*R*,29*R* [the Flack parameter is 0.1(2) and the Hooft parameter is 0.10(4) for 2102 Bijvoet pairs]^{12,13} (Figure 4).

To the best of our knowledge, compound **1**, possessing a 6/6/5/6 ring system^{8,14} with both a 19/30-oxygen bridge

(14) Zhou, Z. W.; Yin, S.; Zhang, H. Y.; Fu, Y.; Yang, S. P.; Wang, X. N.; Wu, Y.; Tang, X. C.; Yue, J. M. *Org. Lett.* **2008**, 10, 465–468.

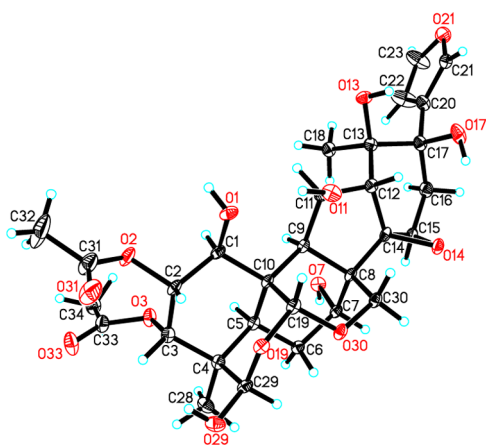


Figure 4. Single crystal X-ray structure of **1**.

and a C-19/29 lactol bridge in the limonoid core, is the first example ever found in the Euphorbiaceae family.

Phyllanthoid **B** (**2**),¹⁵ obtained as a white amorphous powder, possessed the molecular formula $C_{28}H_{38}O_{13}$, as deduced from HR(+)-ESIMS analysis (605.2204 $[M + Na]^+$), with 11 degrees of unsaturation. The IR spectrum showed the presence of hydroxyl (3423 cm^{-1}) and carbonyl (1679 cm^{-1}) groups. A typical β -substituted furan ring (δ_H 7.41, 6.53, 7.47; δ_C 141.7, 112.1, 143.3) and two methyl singlets (δ_H 0.81, δ_C 19.5, H_3 -28; δ_H 1.02, δ_C 19.0, H_3 -18) were observed in the 1H and ^{13}C NMR spectra (SI, Table 1). The aforementioned data indicated that compound **2** is also a limonoid. An extensive comparison of the 1H and ^{13}C NMR data with those of **1** indicated that both compounds shared the same A, B, C, D, and E ring systems, as further confirmed by 2D NMR experiments (SI). The main differences between **2** and **1** were the observation of only one acetyl group in **2** and the replacement of the lower-field C-19 oxymethine in **1** with an oxygen-bearing methylene (δ_H 4.59, 4.12; δ_C 63.7) in **2**. These data implied that the oxide bridge between C-19 and C-30 in **1** was opened in **2**. This was confirmed by the 1H – 1H COSY correlations (H_1 – H_2 – H_3 and H_5 – H_2 – H_6 – H_7) and the HMBC correlations of the H_3 -28/C-3 (δ_C 76.7)/C-4 (δ_C 43.2)/C-5 (δ_C 37.5)/C-29 (δ_C 96.9), the H_2 -9 to C-3/C-4/C-28 (δ_C 19.5), the H_2 -19 to C-1 (δ_C 82.4)/C-5/C-10/C-29, the H_2 -30 to C-9 (δ_C 52.5)/C-7 (δ_C 66.6), and the H_3 -18/C-12 (58.5)/C-17 (75.6). The acetyl group was

located on C-3 by the HMBC correlation from the H_3 to the corresponding carbonyl carbon at δ_C 173.1. Thus, the planar structure of **2** was established as shown in Figure 1.

The relative stereochemistry of **2** was determined by the coupling constants, the ROESY correlation (SI), and biosynthetic considerations. Assignments of α -configurations for 2-OH and C-3-OAc and β -oriented 1-OH in **2** were achieved by consideration of $J_{1,2}$ (9.3 Hz) and $J_{2,3}$ (5.2 Hz) and supported by the ROESY correlation of H_5 with H_1 . Furthermore, H_7 appeared as a broad singlet signal, suggesting an α -configuration for 7-OH, which was confirmed by the ROESY correlation of H_7 with H_30 . The assignments of the β -orientation for all the hydroxyls at C-11, C-13, and C-17 were revealed by the ROESY correlations of both H_11 and H_22 with H_3 -18. It is unfortunate that the absolute configuration of **2** could not be achieved by single-crystal X-ray diffraction due to the small amount available.

From a structural point of view, the rare 5/6 (C/D) ring system of phyllanthoids **A** (**1**) and **B** (**2**) should be originated from a Wagner–Meerwein rearrangement on a trichilin A-type limonoid, via a key carbonium ion intermediate.

The antifeedant activity of phyllanthoid **A** (**1**) against the larvae of a generalist insect (*Spodoptera exigua*) was tested (SI). Compound **1** showed moderate antifeedant activity against *S. exigua* at 2000 ppm with AFI of 17.46%. Moreover, the cytotoxicities of **1** and **2** were evaluated against five human cancer cell lines (breast cancer MCF-7, hepatocellular carcinoma SMMC-7721, human myeloid leukemia HL-60, colon cancer SW480, and lung cancer A-549) using the MTT method (SI). Phyllanthoid **A** (**1**) displayed moderate cytotoxicity against breast cancer MCF-7 with an IC_{50} value of $15.52\text{ }\mu\text{M}$, while **2** showed no cytotoxicity against any of the five human cancer cell lines at a concentration of $40\text{ }\mu\text{M}$.

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Supporting Information Available. NMR, IR, MS, and $[\alpha]_D$ spectra of compounds **1** and **2**, experimental procedures, plant material, bioactivity assay, and the X-ray crystallographic data for **1**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

The authors declare no competing financial interest.

(15) Phyllanthoid **B** (**2**): White amorphous powder; HRESIMS at m/z 605.2203 $[M + Na]^+$ (calcd 605.2204, $C_{28}H_{38}O_{13}Na$); $[\alpha]_D^{20.2} = -3.38^\circ$ (c 0.15, MeOH); UV (MeOH) λ_{max} (log ϵ) 205 (3.91), 272 (2.51); IR (KBr) ν_{max} 3423, 2970, 2929, 1679, 1622, 1444, 1276, 1132, and 1060 cm^{-1} ; for 1H and ^{13}C NMR data, see Table 1.