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Two New Highly Oxygenated and Rearranged Limonoids from *Phyllanthus cochinchinensis*

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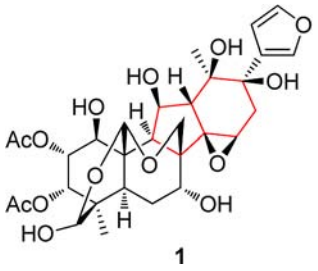
ABSTRACT



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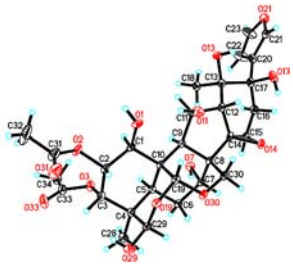

A new 6/6/5/6-fused limonoid, phyllanthoid A (1), possessing both 19/30 and 19/29 oxygen bridges was isolated from *Phyllanthus cochinchinensis* (Euphorbiaceae), together with a new related limonoid, phyllanthoid B (2). Their structures were determined by spectroscopic analysis and single-crystal X-ray 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] insecticidal[5] 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**).

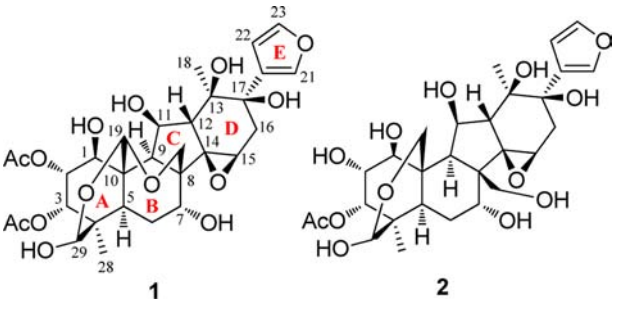

Figure 1. Structures of **1** and **2**.

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 × 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, H_3-28; 1.03, s, H_3-18), 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−H_2-6−H-7, and the HMBC correlations of H_3-28 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-6α 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)−CH_2(16) fragments of **1** (bold lines in Figure 2), and the HMBC correlations (Figure 2) of H_3-18 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 A[10] 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 H_3-28/H-5, H_3-18/H-9, H-19/H-30β, H-12/H-30β 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 H_3-18. 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 H_2-30. 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 Kα 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, $C_{30}H_{38}O_{14}Na$); $[\alpha]_D^{19.1} = -10.0°$ (*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. ^{1}H and ^{13}C NMR Data for **1** and **2** in CD_3OD

	1[a]		**2**[b]	
position	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]
6α	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)
16α	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)
30α	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 (^{1}H) and 125 MHz (^{13}C). [b] Data were recorded at 600 MHz (^{1}H) and 150 MHz (^{13}C). [c] Overlapped with each other.

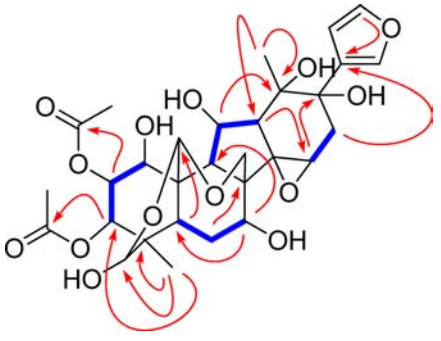

Figure 2. Key $^{1}H-^{1}H$ COSY (bold —) and HMBC (→) correlations of **1**.

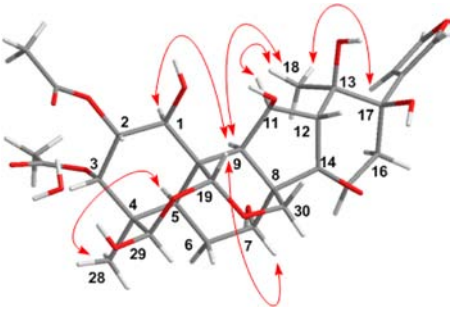

Figure 3. Key ROESY (↔) correlations of **1**.

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*,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

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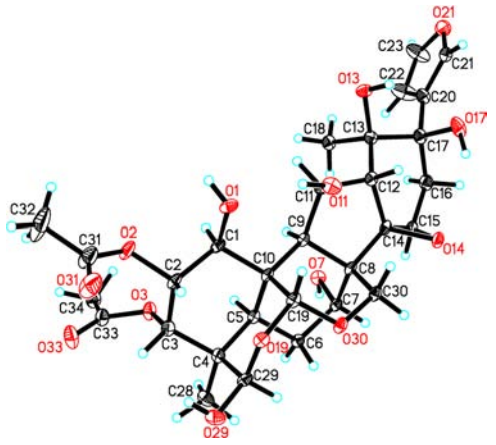

Figure 4. Single crystal X-ray structure of **1**.

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_C141.7, 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_2-6−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-29 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 μM, while **2** showed no cytotoxicity against any of the five human cancer cell lines at a concentration of 40 μM.

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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° (*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.