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Two Novel Triterpenoids from *Dysoxylum hainanense*

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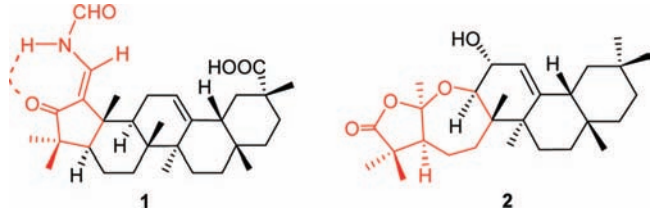
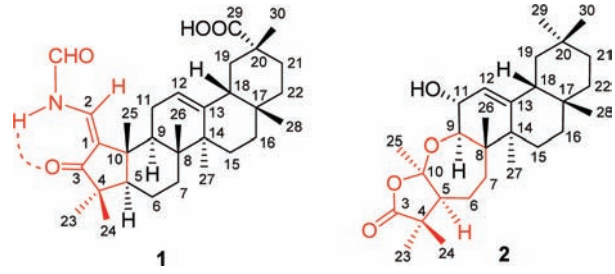
ABSTRACT



Two novel rearranged oleanane-type triterpenes, dysoxyhainanin A (1) possessing a unique 1,3-*cyclo*-2,3-*seco* A ring with a formamido-containing appendage and dysoxyhainanin B (2) featuring an unprecedented 1,2-dinor-3,10:9,10-*bisseco* skeleton, were isolated from *Dysoxylum hainanense*. Dysoxyhainanin A (1) exhibited significant antibacterial activity against Gram-positive bacteria.

The genus *Dysoxylum* (Meliaceae), comprising about 200 species, is mainly distributed in India and Southeast Asia with 14 species being native to China. Many plants in this genus have applications in folk medicine.[1,2] *Dysoxylum hainanense* Merr., a tall tree, mainly grows in the southern Provinces of China.[3] Previous investigation on this species collected from the Yunnan Province of China by Luo's group has led to the isolation of a number of antifeedant tetranortriterpenoids,[4,5] triterpenoids,[6,7] diterpenoids,[8] steroids,[9] and flavonoids.[9] In our work, two novel rearranged oleanane-type triterpenoids, dysoxyhainanins A (**1**) and B (**2**), were isolated from the twigs and leaves of *D. hainanense*, which were collected from its indigenous place of the Hainan Province of China. Dysoxyhainanin A (**1**) possessed a unique 1,3-*cyclo*-2,3-*seco* A ring with a formamido-containing appendage, and dysoxyhainanin B (**2**) featured an unprecedented 1,2-dinor-3,10:9,10-*bisseco*-oleanane skeleton. Their structures were elucidated by extensive spectroscopic methods, and that of **2** was confirmed by a single-crystal X-ray diffraction. Dysoxyhainanin A (**1**) exhibited significant antibacterial activity against Gram-positive bacteria.



The air-dried powder of the plant material (2.0 kg) was percolated with 95% EtOH three times (each 5 L) at rt to give 105 g of crude extract, which was then suspended in water (1 L) and partitioned successively with petroleum ether and EtOAc. The EtOAc soluble fraction (35 g) was subjected to a column of MCI gel (MeOH/H_2O, 0:10 to 10:0) to give five fractions 1−5. Fraction 4 (6 g) was

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extensively separated over columns of silica gel, RP-18 silica gel, and Sephadex LH-20 to obtain **1** (10 mg) and **2** (6 mg).

Dysoxyhainanin A (**1**),[10] a colorless solid, presented a molecular formula of $C_{31}H_{45}NO_4$ as determined by the HREIMS at *m/z* 495.3340 $[M]^+$ (calcd 495.3349) requiring 10 double-bond equivalents. The IR absorptions revealed the presence of amide (3423 and 1612 cm^{-1}) and unsaturated carbonyl groups (1678 cm^{-1}). Its 100 MHz ^{13}C NMR spectrum in $CDCl_3$ with the assistance of DEPT experiments resolved 31 carbon resonances that came from seven methyls, eight methylenes, six methines (two olefinic and one formyl), and ten quaternary carbons (two olefinic, one carboxyl, and one keto-carbonyl). Analysis of the 400 MHz ^1H NMR spectrum in $CDCl_3$ indicated that all seven methyls were tertiary ones. Two olefinic−proton resonances at δ_H 5.35 (brd, $J = 3.3$ Hz; δ_C 122.1) and δ_H 7.48 (d, $J = 10.8$ Hz; δ_C 125.2), and the proton resonance of the formyl group at δ_H 8.27 (s) (δ_C 159.4) were distinguished by analysis of the HSQC spectrum. One proton resonance at δ_H 10.97 (d, $J = 10.8$ Hz) that did not show any cross peak with the carbon signals in the HSQC spectrum implied the presence of a chelated-exchangeable amine proton. To confirm this, a D_2O exchange experiment was thus performed, in which the doublet resonated at δ_H 10.97 disappeared and its coupled proton resonated at δ_H 7.48 became a singlet after the addition of one drop of D_2O in the NMR tube. Careful analysis of 1D and 2D NMR data revealed that compound **1** shared the same B/C/D/E rings with 3-oxo-olean-12en-29-oic acid,[11] and the only differences occurred in ring A. A detailed account of the assignments of ring A and its appendages is given below.

To establish the structure of the unusual ring A and its appendage, HMBC and $^1H-^1H$ COSY spectra were applied. In the HMBC (Figure 1), the correlations from H_3-23 (δ_H 1.07) and H_3-24 (δ_H 1.05) to C-3 (δ_C 216.2) rationalized the existence of a ketone group at C-3. The evidence that the correlations of H-2/C-1 and C-3, H_3-25/C-1, and H-9/C-1 were observed in the HMBC spectrum enabled us to incorporate the sp^2 quaternary carbon at δ_C 130.7 and the sp^2 tertiary carbon at δ_C 125.2 into C-1 and C-2, respectively, to form an exocyclic Δ^1 double bond. The HMBC correlations from H-2 to C-1 and C-3 also allowed the connection of C-1 and C-3 to construct a novel 1,3-*cyclo*-2,3-*seco* A ring for compound **1**. Finally, the HMBC correlation of H-2 to the formyl carbon at δ_C 159.4 and that of the formyl proton at δ_H 8.27 to C-2 allowed the attachment of the formamido group at C-2, which was further confirmed by the correlation between N*H* and H-2 observed in the $^1H-^1H$ COSY spectrum.

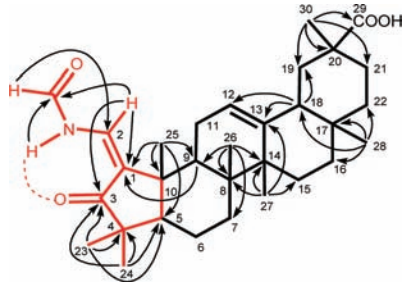

Figure 1. Key HMBC correlations (H→C) of **1**.

As widely reported in the literature,[12−14] there is a peak-doubling phenomenon in the compounds containing the formamido group due to rotational isomerism of the formamido group (the ratio of major and minor rotamers normally ranges from 2:1 to 4:1). However, this phenomenon was notably weakened in **1** (the ratio of the major and minor rotamers was approximately 15:1). It was speculated that the rotational feature of the formamido group was largely fixed by a H-bond formed between the amine proton and the oxygen atom of the C-3 ketone.

The relative stereochemistry of **1** was established by the ROESY spectrum (Figure 2). The ROESY correlations of

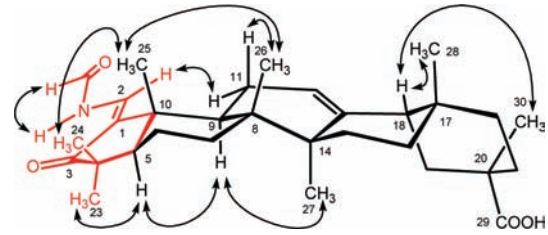

Figure 2. Selected ROESY correlations (H↔H) of **1**.

H-5/H-9, H-5/H_3-23, and H-9/H_3-27 revealed that H-5, H-9, Me-23, and Me-27 were cofacial and were randomly placed in an α-orientation. The ROESY correlations of H_3-24/H_3-25, H_3-25/H_3-26, H-18/H_3-28, and H-18/H_3-30 indicated that H_3-24, H-18, Me-25, Me-26, Me-28, and Me-30 were

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(10) Dysoxyhainanin A (**1**): Colorless solid; $[\alpha]^{20}_D = +66.0°$ (*c* 0.160, MeOH); UV (MeOH) λ_{max} (log ε) = 294 nm (3.91); IR (KBr) ν_{max} 3423, 2925, 1687, 1612, 1458, 1383, 1325, 1164, 1056 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS 70eV *m/z* (relative intensity) 495 $[M]^+$ (7), 249 (16), 248 (100), 233 (6), 204 (9), 192 (13), 164 (22), 147 (5); HREIMS *m/z* 495.3340 $[M]^+$ (calcd for $C_{31}H_{45}NO_4$, 495.3349).

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Table 1. ^{1}H and ^{13}C NMR Data of **1** and **2**[a]

	1		**2**	
no.	δ_H (mult, J, Hz)	δ_C	δ_H (mult, J, Hz)	δ_C
1		130.7		
2	7.48 (d, 10.8) [6.92 (d, 11.0)][b]	125.2		
3		216.2		180.0
4		45.8		44.1
5	1.46 (m)	58.3	2.15 (dd, 11.4, 4.8)	57.0
6a	1.55 (2H, m)	17.2	1.55 (m)	21.3
6b			1.76 (m)	
7a	1.47 (m)	33.1	1.59 (2H, m)	32.0
7b	1.62 (m)			
8		41.4		45.0
9	2.24 (m)	44.2	3.87 (d, 7.8)	80.9
10		43.2		109.3
11a	2.21 (m)	26.3	4.08 (dd, 7.8, 2.8)	70.0
11b	2.33 (m)			
12	5.35 (brd, 3.3)	122.1	5.34 (d, 2.8)	123.5
13		144.7		148.3
14		41.8		43.0
15a	1.01 (m)	26.3	0.85 (m)	26.4
15b	1.80 (dt, 3.6, 13.9)		1.77 (m)	
16a	0.91 (m)	26.9	1.15 (m)	26.3
16b	1.97 (m)		1.99 (dt, 4.4, 13.7)	
17		32.0		32.7
18	2.01 (dd, 3.4, 13.3)	48.1	2.07 (dd, 3.7, 13.4)	47.7
19a	1.32 (m)	31.1	1.18 (m)	45.8
19b	1.95 (m)		1.67 (m)	
20		44.0		31.1
21a	1.62 (m)	42.3	1.13 (m)	34.6
21b	1.88 (m)		1.31 (m)	
22a	1.37 (2H, m)	38.2	1.25 (m)	36.7
22b			1.46 (m)	
23	1.07 (3H, s)	26.9	1.29 (3H, s)	27.9
24	1.05 (3H, s)	20.7	1.23 (3H, s)	21.9
25	1.22 (3H, s)	20.0	1.77 (3H, s)	28.4
26	1.08 (3H, s)	17.9	1.09 (3H, s)	16.0
27	1.19 (3H, s)	26.1	1.23 (3H, s)	24.5
28	0.83 (3H, s)	28.1	0.85 (3H, s)	28.4
29		182.3	0.88 (3H, s)	33.2
30	1.22 (3H, s)	28.6	0.87 (3H, s)	23.4
HCONH	8.27 (s) [8.39 (d, 11.3)]	159.4		
HCO***NH***	10.97 (d, 10.8) [10.72 (dd, 11.0,11.3)]			

[a] Data were recorded in $CDCl_3$ at 400 and 100 MHz for ^{1}H and ^{13}C, respectively. [b] Chemical shifts for the minor rotamer are bracketed.

β-directed. The *Z*-geometry of the Δ^1 double bond was determined by the ROESY correlation of H-2/H_α-11. The ROESY spectrum only gave a strong cross-peak between the formyl proton and the amine proton, and no correlation between the formyl proton and H-2 was observed, indicating that the formyl proton and the amine proton were close in space. The structure of **1** was thus determined as depicted.

To the best of our knowledge, this is the first report of an oleanane-type triterpenoid possessing an unprecedented 1,3-*cyclo*-2,3-*seco* A ring with the appendage of a formamido group.

Dysoxyhainanin B (**2**),[15] which crystallized as colorless crystals from MeOH, showed a molecular formula $C_{28}H_{44}O_4$ as determined by the HREIMS at *m*/*z* 444.3248 $[M]^+$ (calcd 444.3240). The IR absorptions at 3450 and 1743 cm^{-1} indicated the presence of hydroxyl and ester groups, respectively. The 400 MHz ^{1}H NMR spectrum of **2** in $CDCl_3$ exhibited resonances for eight tertiary methyls, one coupling system comprising two oxymethines (at δ_H 3.87, d, J = 7.8 Hz; 4.08, dd, J = 7.8, 2.8 Hz), and one olefinic proton (at δ_H 5.34, d, J = 2.8 Hz) (Table 1). The 100 MHz ^{13}C NMR spectrum in $CDCl_3$ with the aid of the DEPT technique resolved 28 carbon signals including 8 methyls, 7 methylenes, 5 methines (two oxygenated and one olefinic), and 8 quaternary carbons (one carbonyl, one olefinic, and one ketal). The aforementioned data implied that **2** was likely an oleanane-type dinortriterpenoid.

Extensive analysis of the 1D and 2D NMR spectra, particularly HMBC, revealed that **2** had a unique 1,2-dinor-3,10:9,10-*bisseco*-oleanane triterpenoid skeleton, in which the C−E rings remained intact, while the A and B rings were degraded and/or

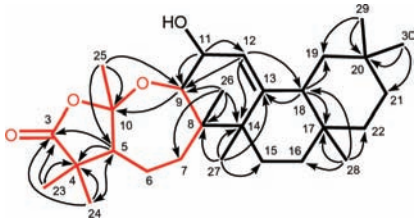

Figure 3. Key HMBC correlations (H→C) of **2**.

rearranged. In the HMBC spectrum in $CDCl_3$ (Figure 3), the only carbonyl group was placed at C-3 by the correlations of H_3-23 (δ_H 1.29) and H_3-24 (δ_H 1.23) with C-3 (δ_C 180.0). The chemical shifts of C-9 (δ_C 80.9) and C-10 (δ_C 109.3) as well as the HMBC correlations from H_3-25 to C-5 and C-10 suggested that a ketal group was located at C-10 and that the C-9−C-10 bond was probably cleaved; instead, an oxygen bridge formed between them. The HMBC correlations of H-9/C-10 and H_3-25/C-9 (^4J) confirmed this speculation. The above analysis also suggested that the carbonyl C-3 and the C-10 were linked through an oxygen atom in a five-membered γ-lactone. Assignment of the remaining oxygenated methine at C-11 bearing a hydroxyl and the location of a Δ^{12} double bond were achieved by the mutual HMBC correlations of H-11/C-9 and C-12, and H-12/C-9, C-14, and C-18. This was supported by the observed AMX spin system of H-9/H-11/H-12 as readily deduced from the coupling constants of these protons (Table 1). Thus, the planar structure of **2** was established.

The relative configuration of **2** was fixed by the 400 MHz ROESY spectrum in $CDCl_3$. The ROESY correlations of H-5/H_3-23, H_3-23/H_3-25, H-9/H_3-25, and H-9/H_3-27 showed that they were cofacial and were arbitrarily assigned as α-oriented. In consequence, the ROESY correlations of H-11/H_3-26, H-18/H_3-28, and H-18/H_3-30 indicated that they were on the same side and β-directed (Figure 4).

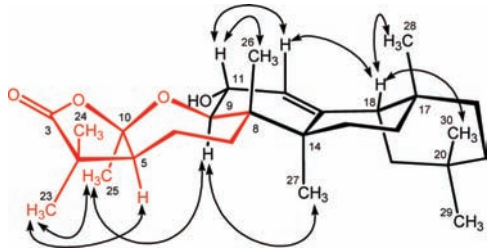

Figure 4. Selected ROESY correlations (H↔H) of **2**.

The structure and conformation of **2** were finally confirmed by a single-crystal X-ray diffraction study (Figure 5, Supporting Information).[16] Dysoxyhainanin B (**2**) was therefore assigned as a novel compound with an unprecedented 1,2-dinor-3,10:9,10-*bisseco*-oleanane skeleton.

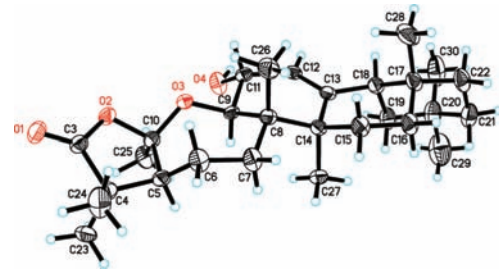

Figure 5. Single-crystal X-ray structure of **2**.

Dysoxyhainanins A (**1**) and B (**2**) were evaluated for antimicrobial activity against Gram-positive and negative bacteria and fungi by microdilution assay (for details see Supporting Information).[17] Dysoxyhainanin A (**1**) showed significant activities against four Gram-positive bacteria, *Staphylococcus aureus* ATCC 25923 (MIC 12.5 μg/mL), *Staphylococcus epidermidis* ATCC 12228 (MIC 6.25 μg/mL), *Micrococcus luteus* ATCC 9341 (MIC 12.5 μg/mL), and *Bacillus subtilis* CMCC 63501 (MIC 6.25 μg/mL). Dysoxyhainanin B (**2**) was inactive for all the tested microbes.

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Supporting Information Available: Experimental procedures; 1D and 2D NMR, EIMS, IR spectra of dysoxyhainanins A (**1**) and B (**2**); and CIF data for the crystal structure of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) Dysoxyhainanin B (**2**): Colorless crystal (MeOH), mp 268 °C; $[\alpha]^{20}_D = -5.0°$ (*c* 0.065, MeOH); IR (KBr) ν_{max} 3450, 2945, 1772, 1743, 1385, 1248, 1057, 1035, 943 cm^{-1}; ^1H NMR and ^{13}C NMR, see Table 1; EIMS 70eV *m/z* (relative intensity) 444 $[M]^+$ (53), 287 (51), 274 (48), 259 (59), 191 (55), 139 (100), 113 (62), 69 (75); HREIMS *m/z* 444.3248 $[M]^+$ (calcd for $C_{28}H_{44}O_4$, 444.3240).

(16) Crystallographic data for dysoxyhainanin B (**2**) have been deposited at the Cambridge Crystallographic Data Centre (deposition no. CCDC-656630). Copies of these data can be obtained free of charge viawww.ccdc.cam.ac.uk/conts/retrieving.htm.

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