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Ring A Modified Novel Triterpenoids from *Dysoxylum hainanense*

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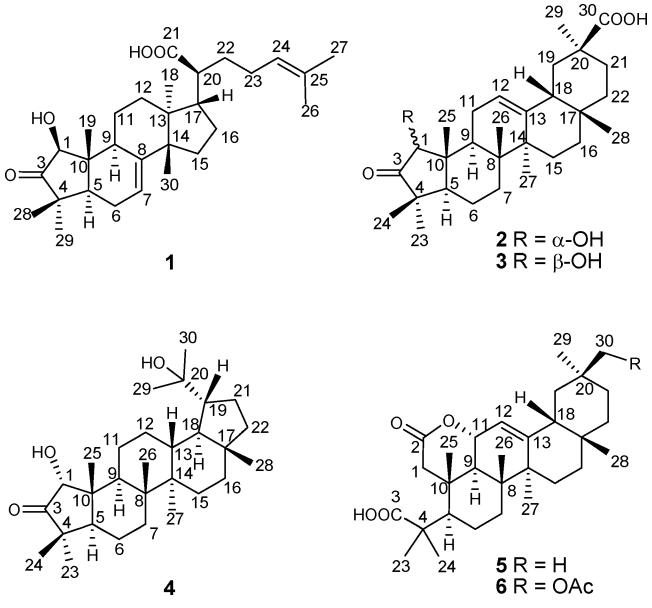
Six ring A modified novel triterpenoids, dysoxyhainic acids A–E (**1**–**3**, **5**, and **6**) and dysoxyhainol (**4**), were isolated from the twigs and leaves of *Dysoxylum hainanense*. Compounds **1**–**4** are a group of triterpenoids featuring a contracted five-membered ring A. Dysoxyhainic acid A (**1**) possessed an unprecedented 2-nor-1,3-cyclotirucallane skeleton, whereas **5** and **6** possessed a rare six-membered δ-lactone formed between C-2 and C-11. The structures of **1**–**6** were established by extensive spectroscopic analysis, and that of **1** was confirmed by single-crystal X-ray diffraction. A hypothetical biosynthetic pathway for compound **1** was also proposed. All the compounds were tested on antimicrobial assays, and compounds **2** and **3** exhibited moderate antibacterial activity against Gram-positive bacteria.

Introduction

Plants of the Meliaceae family are known to produce structurally diverse limonoids and triterpenoids with significant biological activities.[1–4] The genus of *Dysoxylum* of the family Meliaceae are mainly found in India and Southeast Asia, and many of them have been applied in traditional medicine.[5–7] These plants produce an array of biologically active metabolites, including antitumor triterpenoid saponins,[8] anti-RSV and antifeeding tetranortriterpenoids,[9,10] cytotoxic diterpenoids,[11] and cardiac active alkaloids.[12] *Dysoxylum hainanense* is a tall tree distributed in south of China.[5] Recently, we reported the isolation and characterization of two triterpenoids with unusual skeletons from the twigs and leaves of *Dysoxylum hainanense*, which were collected in Hainan Province of P. R. China.[13] In this study, six novel triterpenoids, dysoxyhainic acids A–E (**1**–**3**, **5**, and **6**) and dysoxyhainol (**4**), were further isolated from the same sample of *D. hainanense*. Compounds **1**–**4** featured a contracted five-membered ring A; dysoxyhainic acid A (**1**) possessed an unprecedented 2-nor-1,3-cyclotirucallane skeleton, whereas **5** and **6** possessed a rare six-membered δ-lactone formed between C-2 and C-11. A hypothetical biosynthetic pathway for compound **1** was also proposed. Antimicrobial activity of all the compounds against fungi and bacteria were tested. Compounds **2** and **3** exhibited moderate antibacterial activity against Gram-positive bacteria. Herein, we report the isolation, structural elucidation, and antimicrobial property of these compounds.



Results and Discussion

Structural Elucidation of Dysoxyhainic Acids A–C (1–3) and Dysoxyhainol (4)

Dysoxyhainic acid A (**1**), a colorless crystal (EtOAc/petroleum ether, 1:1), showed the molecular formula of $C_{29}H_{44}O_4$ as determined by HRMS (EI) at m/z = 456.3232 $[M]^+$ (calcd. for $C_{29}H_{44}O_4$ 456.3240). The IR spectrum ex-

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hibited absorption bands for hydroxy (3437 cm^{-1}) and carbonyl (1741 and 1720 cm^{-1}) groups, along with the typical broad band absorption from 3500 to 2500 cm^{-1}, which can be assigned to the carboxyl group. Its ^1H NMR (400 MHz, $CDCl_3$, 25 °C) spectrum displayed two olefinic proton resonances at δ_H = 5.08 (t, J = 7.1 Hz, 1 H) ppm and δ_H = 5.35 (br. s, 1 H) ppm, one oxygenated methine at δ_H = 4.04 (s, 1 H) ppm, and seven methyl groups at δ_H = 0.60, 0.96, 1.00, 1.02, 1.07, 1.58, and 1.67 (s, each 3 H) ppm, the last two of which were assignable to the methyl groups at the terminus of an isopropylidene moiety (Table 1). The ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) and DEPT experiments resolved 29 carbon resonances including 1 oxygenated carbon at δ_C = 83.5 ppm, 2 trisubstituted double bonds at δ_C = 118.2, 147.5, 123.5, and 132.3 ppm, 1 keto carbonyl at δ_C = 222.7 ppm, and 1 carboxyl carbon atom at δ_C = 183.0 ppm (Table 2). The aforementioned data revealed that compound **1** shared the same B/C/D rings and the same side chain with 3-oxotirucalla-7,24-dien-21-oic acid,[14] and the only difference occurred in ring A. To establish the unusual ring A, 2D NMR (HMBC and HSQC) experiments were performed. The keto group was placed at C-3 by HMBC correlations from H_3-28 (δ_H = 1.02, s, 3 H) and H_3-29 (δ_H = 1.07, s, 3 H) to C-3 (δ_C = 222.7 ppm), and the key HMBC correlations from H-1 (δ_H = 4.04, s, 1 H) to C-3, C-9, C-10, and C-19 connected C-3, C-1, and C-10. This enabled the establishment of the 3-oxo five-membered ring A (Figure 1). In comparison to the very common keto carbonyl group of six-membered ring A analogues,[15–17] the chemical shift of C-3 was notably downfield shifted due to the higher tension of the contracted five-membered ring A, which further confirmed the assignment for the planar structure of **1**.

The relative configuration of **1** was mainly deduced by the ROESY spectrum (Figure 2). The ROESY correlations of H_3-29/H-1, H-1/H-5, H-5/H-9, and H-9/H_3-18 showed that they were cofacial and were arbitrarily assigned as α-oriented. In consequence, the ROESY correlations of H_3-28/H_3-19 and H-17/H_3-30 indicated that they were on the same side and β-directed. However, the stereocenter at C-20 remained unknown. Fortunately, a single-crystal X-ray diffraction study allowed not only the assignment of the relative configuration for C-20 but also confirmed the structure of **1** (Figure 3) as 3-oxo-1β-hydroxy-2-nor-1,3-cyclotirucalla-7,24-dien-21-oic acid. To the best of our knowledge, this is the first report of a 2-nor-1,3-cyclotirucallane-type triterpenoid featuring an unprecedented contracted ring A skeleton.

Table 1. ^1H NMR (400 MHz, $CDCl_3$) spectroscopic data of compounds **1**–**6**.

No.	δ_H (mult., J [Hz]) [ppm]					
	1	**2**	**3**	**4**	**5**	**6**
1	4.04 (s)	3.44 (s)	3.93 (s)	3.38 (s)	2.78 (d, 17.6)	2.79 (d, 17.0)
	–	–	–	–	2.61 (d, 17.6)	2.62 (d, 17.0)
2	–	–	–	–	–	–
3	–	–	–	–	–	–
4	–	–	–	–	–	–
5	1.95 (m)	2.04 (m)	1.46 (m)	2.03 (dd, 10.5, 4.7)	1.63 (m)	1.64 (m)
6	2.17 (m), 1.94 (m)	1.55 (m, 2 H)	1.59 (m, 2 H)	1.50 (m, 2 H)	1.54 (m, 2 H)	1.57 (m), 1.51 (m)
7	5.35 (br. s)	1.66 (m), 1.41 (m)	1.65 (m), 1.45 (m)	1.52 (m), 1.44 (m)	1.70 (m), 1.39 (m)	1.71 (m), 1.41 (m)
8	–	–	–	–	–	–
9	2.69 (m)	2.47 (dd, 11.5, 5.9)	2.09 (m)	2.20 (dd, 12.4, 3.8)	1.95 (d, 11.3)	1.96 (d, 11.0)
10	–	–	–	–	–	–
11	1.74 (m, 2 H)	2.04 (m), 1.82 (m)	2.12 (m, 2 H)	1.31 (m, 2 H)	4.85 (dd, 11.3, 2.4)	4.87 (dd, 11.0, 2.0)
12	1.77 (m), 1.53 (m)	5.30 (br. s)	5.33 (br. s)	1.92 (m, 2 H)	5.34 (d, 2.4)	5.33 (d, 2.0)
13	–	–	–	1.72 (m)	–	–
14	–	–	–	–	–	–
15	1.53 (m, 2 H)	1.82 (m)	1.82 (dt, 3.7, 13.6)	1.78 (m)	1.78 (dt, 4.0, 13.3)	1.80 (m)
		1.06 (m)	1.02 (m)	1.05 (m)	1.02 (m)	1.08 (m)
16	1.32 (m, 2 H)	1.98 (m), 0.88 (m)	2.00 (m), 0.90 (m)	1.39 (m), 1.48 (m)	1.98 (m), 0.86 (m)	2.00 (m), 0.90 (m)
17	2.04 (m)	–	–	–	–	–
18	0.96 (s, 3 H)	1.94 (m)	2.01 (m)	1.34 (m)	2.01 (m)	2.00 (m)
19	0.60 (s, 3 H)	1.87 (m), 1.65 (m)	1.89 (m), 1.68 (m)	1.82 (m)	1.67 (m), 1.14 (m)	1.64 (m), 1.41 (m)
20	2.29 (dt, 3.7, 10.6)	–	–	–	–	–
21	–	1.91 (m), 1.29 (m)	1.97 (m), 1.34 (m)	1.92 (m), 1.32 (m)	1.31 (m), 1.13 (m)	1.37 (m, 2 H)
22	1.54 (m, 2 H)	1.35 (m, 2 H)	1.38 (m, 2 H)	1.30 (m), 1.12 (m)	1.43 (m), 1.22 (m)	1.37 (m), 1.29 (m)
23	1.98 (m, 2 H)	0.98 (s, 3 H)	1.08 (s, 3 H)	1.11 (s, 3 H)	1.28 (s, 3 H)	1.30 (s, 3 H)
24	5.08 (t, 7.1)	1.13 (s, 3 H)	1.01 (s, 3 H)	0.95 (s, 3 H)	1.24 (s, 3 H)	1.25 (s, 3 H)
25	–	0.83 (s, 3 H)	0.82 (s, 3 H)	0.72 (s, 3 H)	1.30 (s, 3 H)	1.32 (s, 3 H)
26	1.58 (s, 3 H)	1.03 (s, 3 H)	1.02 (s, 3 H)	1.10 (s, 3 H)	1.06 (s, 3 H)	1.07 (s, 3 H)
27	1.67 (s, 3 H)	1.25 (s, 3 H)	1.22 (s, 3 H)	1.06 (s, 3 H)	1.24 (s, 3 H)	1.26 (s, 3 H)
28	1.02 (s, 3 H)	0.82 (s, 3 H)	0.82 (s, 3 H)	0.82 (s, 3 H)	0.82 (s, 3 H)	0.83 (s, 3 H)
29	1.07 (s, 3 H)	1.18 (s, 3 H)	1.21 (s, 3 H)	1.22 (s, 3 H)	0.86 (s, 3 H)	0.93 (s, 3 H)
30	1.00 (s, 3 H)	–	–	1.11 (s, 3 H)	0.88 (s, 3 H)	3.94 (d, 11.1)
						4.00 (d, 11.1)
OAc						2.09 (s, 3 H)

Table 2. ^{13}C NMR (100 MHz, $CDCl_3$) spectroscopic data of compounds **1**–**6**.

No.	δ_C [ppm]					
	1	**2**	**3**	**4**	**5**	**6**
1	83.5	79.5	87.0	79.8	49.0	49.0
2	–	–	–	–	171.3	171.6
3	222.7	224.6	223.4	224.3	183.0	182.3
4	41.7	44.2	42.3	44.4	45.3	45.2
5	48.9	52.5	53.0	52.4	54.6	54.7
6	23.0	17.6	17.3	17.7	20.4	20.4
7	118.2	32.1	32.4	33.7	33.0	33.0
8	147.5	40.1	40.7	41.7	40.9	41.0
9	47.4	35.6	46.0	38.4	45.4	45.4
10	43.1	42.9	44.1	43.4	38.0	38.0
11	20.0	24.7	25.9	23.5	74.7	74.7
12	29.9	122.2	122.7	28.3	120.2	120.7
13	43.3	144.7	144.2	37.6	149.7	149.0
14	51.1	42.0	41.7	43.9	42.9	42.9
15	33.6	26.2	26.2	27.7	25.5	25.4
16	27.0	26.9	26.9	35.6	26.8	27.0
17	49.8	31.9	32.0	44.6	32.7	32.6
18	22.0	48.3	48.2	48.4	47.4	46.7
19	7.3	42.6	42.5	50.2	45.6	40.7
20	47.3	44.0	44.1	73.6	31.0	34.1
21	183.0	31.0	31.1	28.6	34.6	30.3
22	32.2	38.2	38.3	40.1	36.7	36.1
23	25.9	29.0	27.8	28.8	27.0	27.2
24	123.5	21.9	20.8	21.6	22.9	22.8
25	132.3	15.7	11.5	16.2	17.7	17.7
26	17.6	17.5	17.2	16.7	17.1	17.1
27	25.7	26.1	26.3	15.0	25.3	25.4
28	20.7	28.2	28.1	19.2	28.4	28.2
29	28.2	28.6	28.7	31.6	33.1	27.8
30	27.8	181.9	182.5	24.7	23.5	67.8
OAc						171.6
						21.0

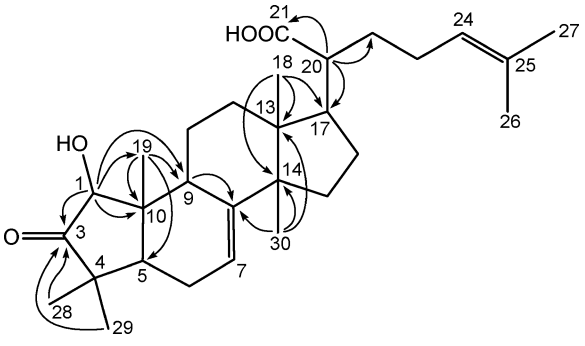


Figure 1. Selected HMBC correlations (H→C) of **1**.

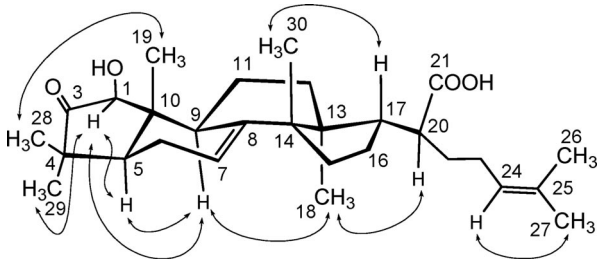


Figure 2. Key ROESY correlations (H↔H) of **1**.

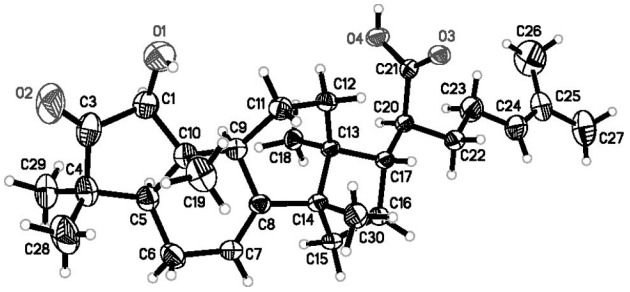
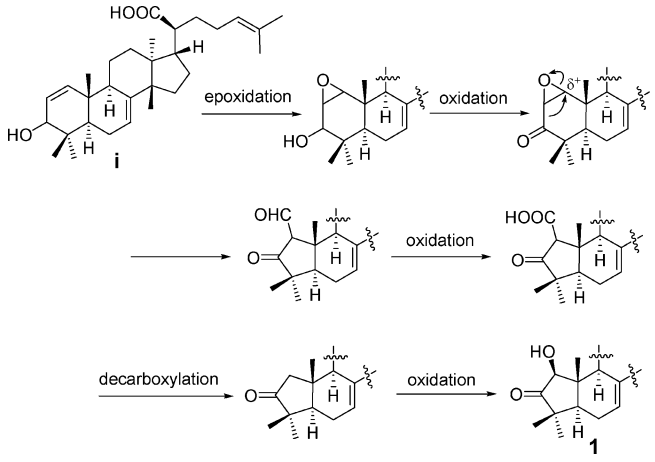


Figure 3. Single-crystal X-ray structure of **1**.

The biogenetic origin of **1** could be traced back to the postulated precursor 3-hydroxytirucalla-1,7,24,trien-21-oic acid (**i**) through a unique biosynthetic pathway involving an α,β-epoxyketone rearrangement reaction[18] as the key step (Scheme 1). This biogenetic pathway fully supports the structure of **1** as assigned.



Scheme 1. Hypothetical biosynthetic pathway for **1**.

Dysoxyhainic acid B (**2**) was obtained as a colorless solid. The HRMS (EI) gave an $[M]^+$ ion at m/z = 456.3230, corresponding to a molecular formula $C_{29}H_{44}O_4$ (calcd. 456.3240). The IR spectrum exhibited the presence of hydroxy (3437 cm^{-1}) and carbonyl (1728 and 1747 cm^{-1}) groups, together with the broad band absorption from 3500 to 2500 cm^{-1} of the typical carboxyl group. The ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) including DEPT experiments showed that three of the eight degrees of unsaturation came from one trisubstituted double bond at δ_C = 122.2 and 144.7 ppm, one keto group at δ_C = 224.6 ppm, similar to the C-3 keto carbonyl in **1**, and one carboxyl group at 181.9 (Table 2). The remaining five degrees of unsaturation were therefore indicative of the pentacyclic skeleton for **2**. In addition, the 1D NMR spectroscopic data along with the HSQC spectrum showed the presence of seven methyl groups at δ_H = 0.82, 0.83, 0.98, 1.03, 1.13, 1.18, and 1.25 (s, each 3 H) ppm, one oxymethine at δ_H = 3.44 (s, 1 H) ppm, and one olefinic proton at δ_H = 5.30 (br. s, 1 H) ppm (Table 1). The above analysis implied that **2** was a noroleanane triterpenoid.[19] The downfield-shifted carbon resonance at δ_C = 224.6 ppm due to the keto carbonyl of C-3

suggested that **2** also possessed a contracted five-membered ring A, which was confirmed by an HMBC experiment. In the HMBC spectrum of **2**, the correlations of H_3-23 (and 24)/C-3, C-4 and C-5, and H-1/C-4, C-5 and C-25 constructed a five-membered 3-oxo-1-hydroxy ring A. Careful analysis of 1D and 2D NMR spectroscopic data revealed that rings B–E of **2** are reminiscent of 3-oxoolean-12-en-30-oic acid.[19] Thus, the planar structure of **2** was completed. The ROESY experiment allowed the establishment of the relative configuration of **2** (Supporting Information, S19). The result showed that H-5, H-9, H_3-23, H_3-27, and H_3-29 were α-oriented, whereas H-1, H-18, H_3-24, H_3-25, H_3-26 were in the β-direction. Compound **2** was therefore determined as 3-oxo-1α-hydroxy-2-nor-1,3-cycloolean-12-en-30-oic acid.

Dysoxyhainic acid C (**3**), obtained as a colorless solid, was analyzed as $C_{29}H_{44}O_4$, which is an isomer of **2**, on the basis of the HRMS (EI) ion at m/z = 456.3242 $[M]^+$ (calcd. 456.3240). Its IR, ^1H NMR and ^{13}C NMR spectroscopic data showed great resemblance to those of **2** except that the oxygenated C-1 was downfield shifted to δ_C = 87.0 ppm in the ^{13}C NMR spectrum (Table 2). HMBC and HSQC analysis suggested that **3** shared the same planar structure with **2**. The ROESY correlations of H_3-23/H-1 and H_3-23/H-5 revealed that the hydroxy group at C-1 was β-oriented. Compound **3** was thus determined as the C-1 epimer of **2**, 3-oxo-1β-hydroxy-2-nor-1,3-cycloolean-12-en-30-oic acid.

Dysoxyhainol (**4**), a white amorphous powder, had a molecular formula of $C_{29}H_{48}O_3$ as determined by HRMS (EI) at m/z = 444.3607 $[M]^+$ (calcd. 444.3603). The IR spectrum exhibited absorption bands due to hydroxy (3562 and 3346 cm^{-1}) and carbonyl (1730 cm^{-1}) groups. The ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) spectrum displayed 29 carbon signals, which were identified by the assistance of a DEPT spectrum as 8 methyl groups, 8 methylene units, 6 methine units (one oxygenated at δ_C = 79.8 ppm), and 7 quaternary carbon atoms (one oxygenated at δ_C = 73.6 ppm and one keto carbonyl at δ_C = 224.3 ppm; Table 2). Its ^1H NMR (400 MHz, $CDCl_3$, 25 °C) spectrum showed proton signals for eight tertiary methyl groups at δ_H = 0.72, 0.82, 0.95, 1.06, 1.10, 1.11, 1.11, and 1.22 (s, each 3 H) ppm, along with an oxygenated methine at δ_H = 3.38 (s, 1 H) ppm (Table 1). The aforementioned data, especially the characteristic keto carbonyl at δ_C = 224.3 (C-3) ppm, indicated that compound **4** was a 3-oxo-20-hydroxy-2-nor-1,3-cyclolupane triterpenoid. Extensive analysis of its 2D NMR spectra further proved this speculation. The HMBC correlations from H_3-23 and H_3-24 to C-3 (δ_C = 224.3 ppm) placed the keto group at C-3; the HMBC correlations from H-1 (δ_H = 3.38, s, 1 H) to C-4, C-5, and C-25 along with the HSQC analysis located one hydroxy to C-1; the HMBC cross peaks from H_3-29 and H_3-30 to the oxygenated carbon at δ_C = 73.6 ppm revealed that the other hydroxy was attached at C-20 (see Supporting Information). To establish the relative configuration of **4**, a ROESY experiment was undertaken. The significant ROESY correlations of H-1/H_3-25, H_3-25/H_3-26, H_3-26/H-13, H-13/H_3-28, and H_3-28/H-19 indicated that they were on the same side of the molecule, and were arbitrarily assigned as β-oriented. As a consequence, the ROESY correlations of H_3-23/H-5, H-5/H-9, H-9/H_3-27, and H_3-27/H-18 revealed that they are α-configured (Supporting Information, S20). Thus, the structure of **4** was determined as 3-oxo-2-nor-1,3-cyclolup-1α,20-diol.

Structural Elucidation of Dysoxyhainic Acids D (5) and E (6)

Dysoxyhainic acid D (**5**), a colorless solid, presented a molecular formula of $C_{30}H_{46}O_4$ as determined by HRMS (EI) at m/z = 470.3390 $[M]^+$ (calcd. 470.3396) with eight double-bond equivalents. The IR absorptions revealed the presence of a carboxyl group (a broad band from 3500 to 2500 and 1699 cm^{-1}) and an ester carbonyl group (1730 cm^{-1}). Its ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C) spectrum with DEPT experiments resolved 30 carbon resonances that came from 8 methyl groups, 8 methylene units, 5 methine groups (one oxygenated and one olefinic), and 9 quaternary carbon atoms (one olefinic and two carbonyls). The ^1H NMR (400 MHz, $CDCl_3$, 25 °C) spectrum further indicated that all the eight methyl groups at δ_H = 0.82, 0.86, 0.88, 1.06, 1.24, 1.24, 1.28, and 1.30 (s, each 3 H) ppm were tertiary ones, and that the only olefinic proton at δ_H = 5.34 (d, J = 2.4 Hz, 1 H) ppm of a trisubstituted double bond coupled with the proton of the oxymethine at δ_H = 4.85 (dd, J = 11.0, 2.4 Hz, 1 H) ppm as deduced from their coupling constants (Table 1). The spectral analysis mentioned above and the HMBC spectrum revealed that compound **5** featured a 2,3-secooleanane backbone. The HMBC correlations from H_3-23 [δ_H = 1.28 (s, 3 H) ppm] and H_3-24 [δ_H = 1.24 (s, 3 H) ppm] to C-3 (δ_C = 183.0 ppm) rationalized the existence of a carboxyl group at C-3. The ester carbonyl carbon was then placed at C-2 on the basis of the HMBC correlations from H_2-1 [an AB spin system at δ_H = 2.61 and 2.78 (d, J = 17.6 Hz, each 1 H) ppm] to C-2 (δ_C = 171.3 ppm) and C-10. A Δ^{12} double bond was determined by the HMBC correlations of H-12/C-9, C-14, and C-18 (Figure 4a). The oxygenated methine (δ_C = 74.7 ppm) was then assigned to C-11 by the HMBC correlations of H-11/C-9 and C-12, as well as the observation of an AMX spin system of H-9 [δ_H = 1.95 (d, J = 11.3 Hz, 1 H) ppm], H-11 [δ_H = 4.85 (dd, J = 11.3, 2.4 Hz, 1 H) ppm], and H-12 [δ_H = 5.34 (d, J = 2.4 Hz, 1 H) ppm] in the ^1H NMR spectrum. The aforementioned functionalities accounted for seven out of eight degrees of unsaturation, indicating that one additional ring system was required. Although no HMBC correlation between H-11 and C-2 was observed; the significant downfield shifted H-11, as compared to the corresponding proton in dysoxyhainanin B,[13] clearly indicated the presence of a six-membered δ-lactone in **4**. This was supported by the IR absorption at 1730 cm^{-1}.

The relative stereochemistry of **5** was furnished by the ROESY spectrum (Figure 4b). The ROESY correlations of H-5/H-9 and H-9/H_3-27 implied that H-5, H-9, and H_3-27 were cofacial and were randomly placed in α-orientation. The ROESY correlations of H-11/H_3-25, H-11/H_3-26, H_3-

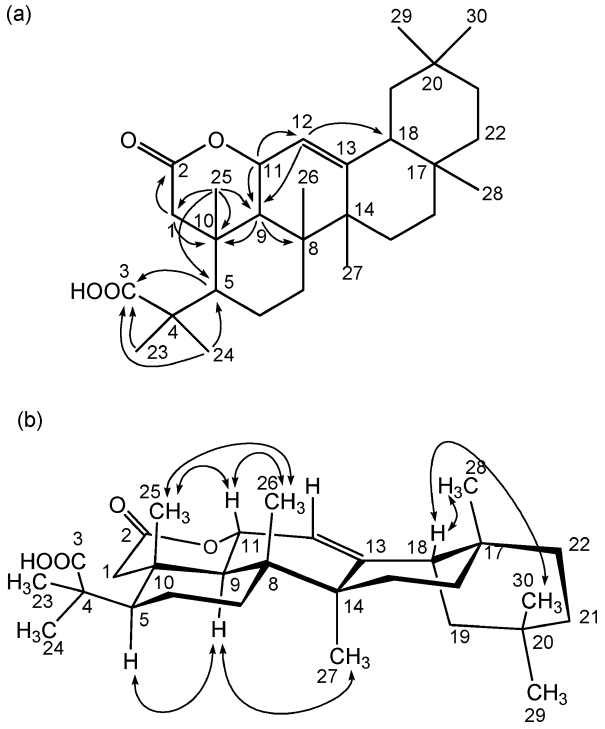


Figure 4. (a) Selected HMBC correlations (H→C) of **5**; (b) key ROESY correlations (H↔H) of **5**.

25/H_3-26, H-18/H_3-28, and H-18/H_3-30 then indicated that H-11, H-18, H_3-25, H_3-26, H_3-28, and H_3-30 were β-directed. The structure of **5** was thus determined as 2,3-secoolean-12-en-3-oic-2,11-olide.

Dysoxyhainic acid E (**6**) was isolated as a white amorphous solid. It was assigned to have a molecular formula of $C_{32}H_{48}O_6$ by HRMS (EI) at m/z = 528.3449 $[M]^+$ (calcd. 528.3451). The ^1H and ^{13}C NMR spectra of **6** were highly similar to those of **5** except for the absence of Me-30 and the presence of an additional oxygenated methylene and an additional acetyl group, suggesting that an acetoxyl group was attached to C-30. This was supported by its proton resonances of H_2-30 at δ_H = 3.94, 4.00 (d, J = 11.1 Hz, each 1 H) ppm as well as the downfield shifted carbon resonance of C-20 at δ_C = 34.1 ppm as compared with those of compound **5**. The structure of **6** was further confirmed by 2D NMR spectra, especially HMBC, in which the key correlations from H_2-30 to C-19, C-20, C-21, C-29, and the carbonyl carbon of acetoxyl proved that **6** was a 30-oxygenated derivative of **5**, 30-acetoxy-2,3-secoolean-12-en-3-oic-2,11-olide.

Antimicrobial Activity Evaluation

All the triterpenoids were evaluated for antimicrobial activity against bacteria, and fungi by microdilution assay.[20,21] Two well-known natural antimicrobial agents, magnolol[22] and pseudolaric acid B,[21] were used as positive controls in this tests for bacteria and fungi, respectively. The antimicrobial minimum inhibitory concentrations (MICs) of compounds **1**–**6** and positive controls are listed in Table 3. Compounds **2** and **3** showed moderate antibacterial activity.

Table 3. Antimicrobial activities of compounds **1**–**6**.[a–c]

Compounds and controls	MICs [μg mL^{-1}]										
	Sa	Se	Ml	Bs	Ec	Sf	Pa	Ca	Ss	Mg	Tr
1	–	–	–	–	–	–	–	–	–	–	–
2	50	50	25	25	–	–	–	–	–	–	–
3	25	25	50	25	–	–	–	–	–	–	–
4	–	–	–	–	–	–	–	–	–	–	–
5	–	–	–	–	–	–	–	–	–	–	–
6	–	–	–	–	–	–	–	–	–	–	–
Magnolol[d]	25	12.5	12.5	12.5	–	–	–				
Pseudolaric acid B[e]								6.25	12.5	12.5	25

[a] Sa = *S. aureus*, Se = *S. epidermidis*, Ml = *M. luteus*, Bs = *B. subtilis*, Ec = *E. coli*, Sf = *S. flexneri*, Pa = *P. aeruginosa*, Ca = *C. albicans*, Ss = *S. sake*, Mg = *M. gypseum*, Tr = *T. rubrum*. [b] MIC was defined as the lowest concentration that inhibited visible growth. [c] MIC > 50 mg mL^{-1} was defined as inactive, and was represented as "–". [d] For bacteria, magnolol was used as positive control. [e] For fungi, pseudolaric acid B was used as positive control.

Experimental Section

General: Melting points were measured with an SGW X-4 melting point apparatus and are uncorrected. Optical rotations were measured with a Perkin–Elmer 341 polarimeter. UV spectra were obtained with a Shimadzu UV-2550 spectrophotometer. IR spectra were recorded with a Perkin–Elmer 577 spectrometer in KBr discs. NMR spectra were recorded with a Bruker AM-400 spectrometer. MS (EI, 70 eV) was measured with a Finnigan MAT-95 mass spectrometer. Semipreparative HPLC was performed with a Waters 515 pump equipped with a Waters 2487 detector (210 nm) and an YMC-Pack ODS-A column (250 × 10 mm, S-5 μm, 12 nm). All solvents used were of analytical grade (Shanghai Chemical Plant, Shanghai, P. R. China). Silica gel (200–300 mesh), silica gel H60, Sephadex LH-20 (Amersham Biosciences), reversed-phase C_{18} silica gel (150–200 mesh, Merck), and MCI gel (CHP20P, 75–150 μm, Mitsubishi Chemical Industries Ltd.) were used for column chromatography. Precoated silica gel GF_{254} plates (Qingdao Haiyang Chemical Co. Ltd. Qingdao, P. R. China) were used for TLC.

Plant Material: The twigs and leaves of *Dysoxylum hainanense* Merr. were collected in September of 2005 from Hainan Province of P. R. China. The plant was authenticated by Prof. S. M. Huang, Department of Biology, Hainan University of China. A voucher specimen has been deposited in Shanghai Institute of Materia Medica, Chinese Academy of Sciences (access number: DHTS-2005–1Y).

Extraction and Isolation: The air-dried powder of the plant material (2 kg) was percolated with 95% EtOH (3 × 5 L) 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 MCI gel column (MeOH/H_2O, 0 to 100%) to give five fractions 1–5. Fraction 4 (6 g) was separated on a silica gel column (petroleum ether/acetone, 100:1 to 3:1) to afford seven fractions 4a–4g. Fraction 4b (1.6 g) was subjected to reverse-phase column chromatography with C_{18} silica gel (MeOH/H_2O, 50:50 to 100:0) to give five parts (4b1–4b5). Frac-

tion 4b4 (670 mg) was purified by chromatography over a silica gel column (CH_2Cl_2/MeOH, 200:1 to 100:1) to afford four parts (4b4a–4b4d). Fraction 4b4b (130 mg) was purified over silica gel column (petroleum ether/acetone, 10:1) to afford **1** (20 mg, 0.001%) and **4** (12 mg, 0.0006%). Fraction 4b4c (120 mg) was separated by preparative HPLC ($CH_3CN/H_2O/CF_3COOH$, 92:8:0.1; 3 mL min^{-1}) to give **2** (15 mg, 0.0008%, t_R = 6.3 min) and **3** (6 mg, 0.0003%, t_R = 6.7 min). Fraction 4f (520 mg) was purified by the following procedure: (1) silica gel column (petroleum ether/acetone, 100:1 to 3:1); (2) reverse-phase C_{18} silica gel column (MeOH/H_2O, 50:50 to 100:0); (3) silica gel column (CH_2Cl_2/MeOH, 200:1 to 100:1), to give **5** (15 mg, 0.0008%) and **6** (5 mg, 0.0003%).

Dysoxyhainic Acid A (1): Colorless crystal. $[a]_D^{20}$ = +7.0 (*c* = 0.120, MeOH); m.p. 145 °C. IR (KBr): $\tilde{\nu}$ = 3437, 2958, 2891, 1741, 1720, 1450, 1385, 1188, 1036, 3500–2500 (br.) cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) = 456 [M]$^+$ (17), 441 (67), 356 (100), 341 (31), 299 (53), 213 (23), 121 (28), 105 (37), 83 (50). HRMS (EI): calcd. for $C_{29}H_{44}O_4$ [M]$^+$ 456.3240; found 456.3232.

Crystal Structure Analysis of Dysoxyhainic Acid A (1): Crystal data were obtained with a Bruker SMART CCD detector employing graphite monochromated Mo-K_α radiation (λ = 0.71073 Å) at 293 K and operating in the ϕ-ω scan mode. The structure was solved by direct methods[23] and refined with full-matrix least-squares calculations on F^2 using SHELXL-97.[24] CCDC-710876 (for **1**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Dysoxyhainic Acid B (2): Colorless solid. $[a]_D^{20}$ = +170.0 (*c* = 0.115, MeOH). IR (KBr): $\tilde{\nu}$ = 3421, 2966, 1747, 1728, 1454, 1381, 1221, 1175, 1049, 3500–2500 (br.) cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) = 456 [M]$^+$ (4), 423 (2), 356 (2), 248 (100), 233 (8), 187 (8), 173 (6), 147 (8), 121 (6). HRMS (EI): calcd. for $C_{29}H_{44}O_4$ [M]$^+$ 456.3240; found 456.3230.

Dysoxyhainic Acid C (3): Colorless solid. $[a]_D^{20}$ = +183.0 (*c* = 0.105, MeOH). IR (KBr): $\tilde{\nu}$ = 3489, 2945, 1741, 1730, 1456, 1381, 1203, 1138, 1032, 3500–2500 (br.) cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) = 456 [M]$^+$ (6), 441 (4), 248 (100), 233 (8), 187 (9), 173 (6), 147 (10), 121 (7). HRMS (EI): calcd. for $C_{29}H_{44}O_4$ [M]$^+$ 456.3240; found 456.3242.

Dysoxyhainol (4): White amorphous powder. $[a]_D^{20}$ = +87.0 (*c* = 0.09, MeOH). IR (KBr): $\tilde{\nu}$ = 3562, 3346, 2971, 1730, 1456, 1379, 1165, 1055, 941 cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) = 444 [M]$^+$ (6), 426 (100), 411 (31), 234 (30), 205 (20), 189 (30), 149 (35), 121 (34), 107 (34). HRMS (EI): calcd. for $C_{29}H_{48}O_3$ [M]$^+$ 444.3603; found 444.3607.

Dysoxyhainic Acid D (5): Colorless solid. $[a]_D^{20}$ = +37.0 (*c* = 0.090, MeOH). IR (KBr): $\tilde{\nu}$ = 3431, 2951, 1730, 1699, 1275, 1230, 1134, 754, 3500–2500 (br.) cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) 471 (33), 470 [M]$^+$ (100), 455 (31); 265 (20), 234 (31), 191 (68), 107 (26), 95 (10). HRMS (EI): calcd. for $C_{30}H_{46}O_4$ [M]$^+$ 470.3396; found 470.3390.

Dysoxyhainic Acid E (6): White amorphous solid. $[a]_D^{20}$ = +103.0 (*c* = 0.075, MeOH). IR (KBr): $\tilde{\nu}$ = 3433, 2925, 1735, 1464, 1392, 1230, 1135, 1035, 3500–2500 (br.) cm^{-1}. ^{1}H NMR spectroscopic data, see Table 1. ^{13}C NMR spectroscopic data, see Table 2. MS (EI, 70 eV): *m*/*z* (%) = 528 [M]$^+$ (39), 469 (36), 468 (85), 453 (100), 385 (71), 381 (41), 85 (37), 83 (57). HRMS (EI): calcd. for $C_{32}H_{48}O_6$ [M]$^+$ 528.3451; found 528.3449.

Antibacterial Tests: The in vitro antibacterial activities against *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Micrococcus luteus* ATCC 9341, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25922, *Shigella flexneri* ATCC 12022, and *Pseudomonas aeruginosa* ATCC 14502 were conducted by following the manipulations described in the literature.[20] The microbial cells were suspended in Mueller Hinton broth to form a final density of 5×10^{-5} to 10^{-6} CFU mL^{-1} and incubated at 37 °C for 18 h under aerobic conditions with the respective compounds and positive control, which were dissolved in DMSO. The blank controls of microbial culture were incubated with limited DMSO under the same condition. DMSO was determined not to be toxic at a limited amount under the experimental conditions.

The in vitro antifungal activity against *Candida albicans* ATCC 1600, *Saccharomyces sake* ATCC 26421, *Microsporum gypseum* ATCC 14683, and *Trichophyton rubrum* ATCC 10218 was completed according to the protocols described in the literature.[21] The fungi were incubated in Sabouraud dextrose broth at 37 °C for 48 h with the respective compounds and the positive control dissolved in DMSO. The blank controls of fungi cultures were incubated with limited DMSO under the same condition.

Supporting Information (see footnote on the first page of this article): ^{1}H NMR, ^{13}C NMR, and mass spectra of **1**–**6**; selected HMBC correlations; key ROESY correlations of **2** and **4**.

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