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NOVEL CYTOTOXIC DITERPENES FROM THE STEM OF *DYSOXYLUM KUSKUSENSE*[1]

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Abstract: Three novel diterpenes, dysokusones A (**1**), B (**2**), and C (**3**), were isolated from the stem of *Dysoxylum kuskusense* as cytotoxic substances. The structures were established by spectroscopic examinations. Compounds **1**, **2**, and **3** were cytotoxic toward HL-60(TB) cells with EC_{50} values of 2.25, 6.35, and 2.37 μM, respectively. Compound **1** also displayed cytotoxicity against K-562 and NCI-H522 cells with EC_{50} values of 5.04 and 4.80 μM, respectively.

Our previous papers reported the isolation and characterization of 17 triterpene glucosides, cumingianosides A-Q, as well as a trisnor- and a tetranor-triterpene glucoside, cumindysosides A and B, respectively, from a cytotoxic fraction of the leaves of *Dysoxylum cumingianum* (Meliaceae).[2] Among them, cumingianosides A and C exhibited potent selective cytotoxicity against MOLT-4 human leukemia cells with EC_{50} values of <0.00625 and <0.0045 μM, respectively. In our continuing investigation of cytotoxic compounds in *Dysoxylum* spp. plants as part of our search for novel plant-derived cytotoxic agents, we investigated the cytotoxic consitituents in a MeOH extract of the stem of *Dysoxylum kuskusense* (Meliaceae). Subsequent fractionation of the hexane-soluble fraction (20.7 g from 5.4 kg dried stem) by using chromatography on silica gel, MCI-gel CHP 20P, and YMC ODS-A, and by semi-preparative scale HPLC on YMC ODS and Wakopack Sil has led to the isolation of three new diterpenes, named dysokusones A (**1**, 15 mg), B (**2**, 17 mg), and C (**3**, 130 mg), as cytotoxic compounds.

Dysokusone A (**1**)[3] was obtained as a yellow syrup and had the molecular formula $C_{20}H_{32}O_2$. The ^1H nmr spectrum showed one tertiary methyl group (δ 0.85, s), three secondary methyl groups [δ 0.89 (3H, d, *J*= 6.5 Hz), 0.92 (3H, d, *J*= 6.5 Hz), and 0.93 (3H, d, *J*= 6.5 Hz)] and a vinylic methyl group (δ 1.90, t, *J*= 1.5 Hz). The ^1H and ^{13}C nmr spectra also showed a trisubstituted double bond [δ 5.87 (br s); δ 126.42 (d) and 162.91 (s)] and two carbonyl groups (δ 199.11 and 210.56). Examination of the ^1H-^1H COSY and ^1H-^{13}C COSY spectra provided two fragment structures shown by bold lines in Figure 1. The long-range couplings in the HMBC spectrum established the connectivity of these fragment units, including methyl groups, carbonyl

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groups, and a double bond, as shown in Figure 1, to furnish a prenyleudesmane-type skeleton.

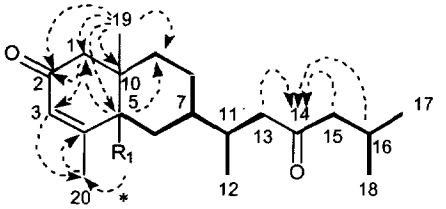


Figure 1. Long-range ^{1}H-^{13}C Correlations (H→C) in **1** and **2** (* Observed in **1**)

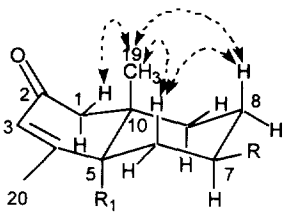
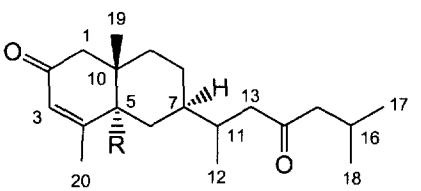
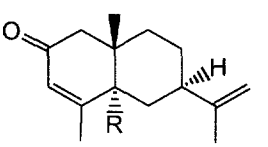


Figure 2. NOE Correlations in **1** (R_1 = H) and **2** (R_1 = OOH)

In the ^{1}H NMR spectrum of **1**, the large coupling constants of H-5, -6, -7, -8 and -9 ($J_{5,6ax} = J_{6ax,7} = J_{7,8ax} = J_{8ax,9ax} = 11.5$ Hz) indicated that the B ring is in a chair conformation. NOE between H-1 (δ 2.25) and H-19, H-6ax and H-8ax, H-6ax and H-19, and H-8ax and H-19 together with long-range ^{1}H-^{1}H coupling between H-1 (δ 2.17) and H-19 (W arrangement), indicated a *trans* ring fusion in **1**.[4] The chemical shift (δ 0.85) for the C-19 methyl group was also consistent with a *trans* ring fusion.[5] In addition, the CD curve of **1** ($[\theta]_{240}$ -8307, $[\theta]_{330}$ +884) was superimposable on that of α-rotunol (**4**).[4] From the observations described above, the structure of dysokusone A was concluded to be represented by formula **1**. However, because the small sample size precluded further examination, the absolute configuration of C-11 is still not clear.



1: R = H
2: R = OOH

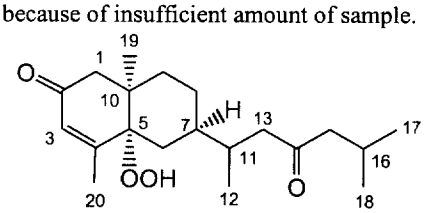


4: R = ···OH
5: R = ◄OH

Dysokusone B (**2**)[6] had the molecular formula $C_{20}H_{32}O_4$, which was 32 units (O_2) more than that of **1**. The ^{1}H and ^{13}C NMR spectra of **2** correlated with those of **1** and showed the presence of the same functional groups seen in **1**, including one tertiary methyl (δ 1.02, s) three secondary [δ 0.89 (3H, d, J = 5.0 Hz), 0.90 (3H, d, J = 5.0 Hz), and 0.93 (3H, d, J = 6.0 Hz)], and one vinylic methyl (δ 2.05, d, J = 1.5 Hz) groups, a trisubstituted double bond [δ 5.80 (br s); δ 129.27 (d) and 162.88 (s)], and two carbonyl groups (δ 198.65 and 210.85). In addition, an oxygenated quaternary carbon resonance at δ 84.23 and a characteristic one-proton singlet at δ 10.29, combined with the loss of H_2O_2 from the molecular ion in the mass spectrum, indicated the presence of a hydroperoxide group in **2**.[7] The location of the hydroperoxide group was determined to be at C-5 by extensive spctroscopic examinations, including ^{1}H-^{1}H COSY and ^{1}H-^{13}C COSY spectra. The *trans* ring fusion of **2** was indicated by the NOE between H-19 and H-1 (δ 1.85) and H-6ax as well as the long-range ^{1}H-^{1}H coupling between H-1 (δ 2.55) and H-19 (W arrangement), along with the large coupling constants of H-6, -7, -8, and -9 ($J_{6ax,7} = J_{7,8ax} = J_{8ax,9ax} = 13.5$ Hz), which were similar to those found in **1**. This conformation (Fig.

2) was in good agreement with the lowfield shift of the protons located on the same side as the hydroperoxide group[7b,8] [H-1α (δ 2.55), H-9α (δ 1.82), and H-7 (δ 1.90)]. Furthermore, the CD curve of **2** ($[\theta]_{239}$ -17560, $[\theta]_{322}$ +2026) was similar to that found in **1**, and thus the structure of dysokusone B is represented by formula **2**. The absolute configuration of C-11 is still not clear, again because of the small sample size.

Dysokusone C (**3**)[9] contained the same molecular formula ($C_{20}H_{32}O_4$) as **2**. The ^1H and ^{13}C NMR spectra revealed the same functional groups in **3** as those found in **2**. Examination of the ^1H-^1H COSY, ^1H-^{13}C COSY, and HMBC spectra indicated that **3** had the same general molecular structure but a different stereostructure from those of **2**. In the ^1H NMR spectra of **3**, the large coupling constants of H-6, -7, -8, and -9 ($J_{6ax,7} = J_{7,8ax} = J_{8ax,9ax}$ = 12 Hz) indicated that the B ring is in a chair conformation. NOE was observed between H-19 and H_2-1, H-20 and H-7, and H-20 and H-6eq in the phase-sensitive NOESY of **3**, indicative of a *cis* ring fusion in **3**. The chemical shift (δ 1.23) for the C-19 methyl group, which is typical of *cis* fused eudesmanes,[5] provided additional support for this assignment. This conformation was also consistent with the lowfield shift (δ 3.04) of the H-1β located on the same side as the hydroperoxide group.[7b,8] Furthermore, the CD curve of **3** showed a positive Cotton effect ($[\theta]_{239}$ -26483, $[\theta]_{328}$ +3641), which was opposite to that reported for the *cis*-fused eudesmane-type sesquiterpene, β–rotunol (**5**),[4] thus indicating that **3** is represented by formula **3**. The configuration of C-11 in **3** was assigned to be *S* based upon the observation of NOE between H-11 and H-8 as well as between H-12 and H-6eq, though the chirality at C-11 has not been fully investigated at this time, because of insufficient amount of sample.



3

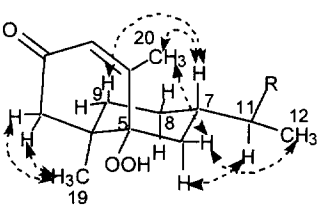


Figure 3. NOE Correlations in **3**

The cytotoxicities of dysokusones A (**1**), B (**2**), and C (**3**) against a panel of about 60 tumor cell lines[10] were evaluated at the National Cancer Institute. Dysokusones B (**2**) and C (**3**) demonstrated selective cytotoxicity against HL-60(TB) leukemia tumor cells with EC_{50} values of 6.35 and 2.37 μM, respectively. Dysokusone A (**1**) also displayed significant cytotoxicity against the HL-60(TB) cell line with an EC_{50} value of 2.25 μM, and showed marginal cytotoxicity against K-562 (leukemia) and NCI-H522 (lung tumor) cells with EC_{50} values of 5.04 and 4.80 μM, respectively.

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TABLE 1. ^{1}H (δ, *J* in Hz) and ^{13}C (δ) NMR Data for Compounds 1-3

	1[a]		2[b]		3[a]	
	H	C	H	C	H	C
1	2.17 (br d, 16.5)	54.48	1.85 (br d, 18)	49.19	2.07 (d, 16)	50.65
	2.25 (br d, 16.5)		2.55 (br d, 18)		3.04 (d, 16)	
2		199.11		198.65		199.59
3	5.87 (br s)	126.42	5.80 (br s)	129.27	6.10 (br s)	132.14
4		162.91		162.88		155.08
5	2.30 (br d, 11.5)	47.87		84.23		85.14
6	1.07 (q, 11.5)	26.00	1.38 (dd, 13.5,14)	27.98	1.33 (t, 12)	29.80
	1.82 (br d, 11.5)		2.39 (dd, 3,14)		1.80 (br d, 12)	
7	1.35 (br t, 11.5)	43.43	1.90 (br t, 13.5)	38.19	1.14 (br t, 12)	39.37
8	1.11 (dq, 3,11.5)	24.38	1.41 (dq, 4.5,13.5)	25.09	1.26 (dq, 4,12)	27.97
	1.51 (br d, 11.5)		1.50 (br d, 13.5)		1.41 (br d, 12)	
9	1.38 (dt, 3,11.5)	40.11	1.27 (dt, 13.5,4.5)	35.54	1.41 (br d, 12)	38.13
	1.55 (dt, 11.5,3)		1.82 (dt, 4.5,13.5)		1.49 (dt, 5.5,12)	
10		37.70		41.27		39.07
11	2.05 (m)	33.76	2.05 (m)	34.67	2.00 (m)	33.20
12	0.89 (d, 6.5)	16.72	0.93 (d, 6)	17.64	0.89 (d, 6.5)	16.79
13	2.25 (dd, 8,16)	47.91	2.31 (dd, 9,16)	48.67	2.23 (dd, 8,12)	47.07
	2.44 (dd, 4,16)		2.58 (dd, 4,16)		2.35 (dd, 4,12)	
14		210.56		210.85		210.23
15	2.28 (m)	52.41	2.34 (m)	53.20	2.26 (m)	52.40
16	2.25 (m)	24.59	2.11 (m)	25.53	2.12 (m)	24.52
17	0.92 (d, 6.5)	22.60	0.90 (d, 5)	23.35	0.91 (d, 6.5)	22.52
18	0.93 (d, 6.5)	22.57	0.89 (d, 5)	23.28	0.91 (d, 6.5)	22.59
19	0.85 (s)	17.03	1.02 (s)	24.23	1.18 (s)	21.02
20	1.90 (t, 1.5)	21.84	2.05 (d, 1.5)	22.91	1.98 (d, 1.5)	19.79
OOH			10.29 (s)		8.78 (s)	

[a]Measured at 400 MHz (^{1}H) and 100 MHz (^{13}C) in $CDCl_3$

[b]Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in acetone-d_6

References and Notes

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6. Mp: 122–123 °C, $[\alpha]_D^{20}$ –52.8° (*c* 0.06, acetone). $C_{20}H_{33}O_4$ [$(M + H)^+$ *m/z* 337.2390, calcd 337.2378].
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