Cytotoxic Prenyleudesmane Diterpenes from the Fruits of *Dysoxylum kuskusense*

Chang-Yih Duh,*,† Shang-Kwei Wang,‡ and Ih-Sheng Chen§

Department of Marine Resources, National Sun Yat-sen University, Kaohsiung, Taiwan, Department of Microbiology, Kaohsiung Medical University, Kaohsiung, Taiwan, and School of Pharmacy, Kaohsiung Medical University, Kaohsiung, Taiwan

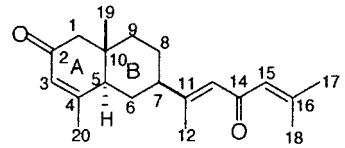
Received May 26, 2000

Two new prenyleudesmane diterpenes, dysokusone D (**1**) and dysokusone E (**2**), were isolated from the fruits of *Dysoxylum kuskusense* and exhibited cytotoxicity against P-388, HT-29, and A549 cell lines. Their structures were determined by 1D and 2D NMR spectral analysis.

The fruits of the Formasan plant *Dysoxylum kuskusense* (Hay) Kanehira & Hatusima (Meliaceae) were studied because methanol extracts showed significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon aenocarcinoma), and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of two new cytotoxic prenyleudesmane diterpenes, dysokusone D (**1**) and dysokusone E (**2**).

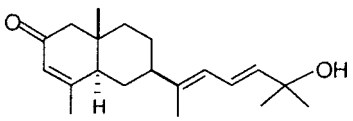
The MeOH-soluble fraction of the fruits of *Dysoxylum kuskusense* was chromatographed over silica gel to give a yellow syrup. HRFABMS and the DEPT spectrum of **1** established a molecular formula of $C_{20}H_{28}O_2$. Thus, 7 degrees of unsaturation were determined for **1**. ^{1}H NMR spectral data suggested that **1** contained four vinylic methyl groups and a tertiary methyl. The ^{1}H and ^{13}C NMR spectra also suggested the presence of three trisubstituted double bonds and two carbonyl groups. These facts in combination with the molecular formula suggested the occurrence of two rings. The ^{1}H and ^{13}C NMR data were quite similar to those of the prenyleudesmane-type diterpene, dysokusone A,[3] except for the lack of three secondary methyl groups, which were replaced by three vinylic methyl groups. The assignment of double bonds at C-11 and C-15 was confirmed by HMBC correlations. The signal at δ 2.17 (H-17) coupled to C-15 and C-16, the signal at δ 1.90 (H-18) to C-15 and C-16, the signal at δ 6.10 (H-15) to C-14 and C-18, the signal at δ 6.09 (H-13) to C-7, C-14, and C-11, and the signal at δ 2.18 (H-12) to C-7, C-11, and C-13. In the ^{1}H NMR spectrum of **1**, the large coupling constants of H-5, -6, -7, -8, and -9 ($J_{5,6ax} = J_{6,7ax} = J_{7,8ax} = J_{8,9ax} =$ 11.6 Hz) indicated that the B ring was in a chair conformation. NOE between H-1β and H-19; H-19 and H-8β; H-5 and H-7; and H-7 and H-6α, H-5, and H-6α indicated *trans* ring fusion in **1**. The CD spectrum of **1** ($[\theta]_{241}$ −12360, $[\theta]_{334}$ +1037) was similar to that of dysokusone A.[3] Therefore, the structure of dysokusone E was concluded to be represented by formula **1**.

Dysokusone E (**2**) had the molecular formula $C_{20}H_{30}O_2$, which was 2 mass units greater than that of **2**. ^{1}H NMR and ^{13}C NMR spectral data showed it to contain two vinylic methyl groups, a tertiary methyl, a conjugated diene, a carbonyl group, and a dimethyl carbinol. The ^{1}H and ^{13}C NMR data of **2** were quite similar to those of **1** except for



1

the C_8 side chain. A combination of COSY, HMQC, and HMBC data revealed **2** to possess the same C_8 side chain as found in fuscol.[4] In the ^{1}H NMR spectrum of **2**, the large coupling constants of H-5, -6, -7, -8, and -9 ($J_{5,6ax} = J_{6,7ax} = J_{7,8ax} = J_{8,9ax} =$ 12.0 Hz) indicated that the B ring was in a chair conformation. NOE between H-1β and H-19; H-5 and H-7; and H-7 and H-6α, H-5, and H-6α indicated a *trans* ring fusion in **1**. The CD spectrum of dysokusone E ($[\theta]_{238}$ −10380, $[\theta]_{331}$ +1238) was similar to that found in **1**; thus the structure of dysokusone E is represented by formula **2**.



2

Compound **1** exhibited cytotoxicity against A549, HT-29, and P-388 cell lines with ED_{50} values of 2.39, 4.68, and 3.87 μg/mL, respectively. Compound **2** exhibited cytotoxicity against A549, HT-29, and P-388 cell lines with ED_{50} values of 3.76, 4.89, and 2.64 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined using a Yanagimoto micromelting point apparatus and are reported uncorrected. Optical rotations were determined on a JASCO DIP-181 polarimeter. UV spectra were obtained on a Shimadzu UV-160A spectrophotometer, and IR spectra were recorded on a Hitachi 26-30 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer at 400 and 100 MHz, respectively, in $CDCl_3$ using TMS as internal standard. EIMS were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 eV. Silica gel 60 (Merck, 230−400 mesh) and precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for column chromatography and TLC analysis.

Plant Material. Fruits of *D. kuskusense* were collected at Taitong Hsien, Taiwan, in September 1994. A voucher specimen is deposited in the School of Pharmacy, Kaohsiung Medical University, Taiwan.

* To whom correspondence should be addressed. Tel.: 886-7-525-2000 ext. 5036. Fax: 886-7-525-5020.
† National Sun Yat-sen University.
‡ Department of Microbiology, Kaohsiung Medical University.
§ School of Pharmacy, Kaohsiung Medical University.

10.1021/np000264z CCC: $19.00
Published on Web 09/27/2000

Extraction and Isolation. Dried fruits (2.01 kg) of *D. kuskusense* were extracted with MeOH. After removal of solvent in vacuo, the residue (40 g) was partitioned between *n*-hexane and H_2O. The H_2O-soluble fraction was further extracted by $CHCl_3$. The $CHCl_3$-soluble fraction was chromatographed over silica gel 60 using $CHCl_3$ and MeOH−$CHCl_3$ mixtures of increasing polarity. Elution by MeOH−$CHCl_3$ (1:49) afforded a fraction containing **1**. Elution by MeOH−$CHCl_3$ (1:20) afforded a fraction containing **2**. Compound **1** was purified by silica gel chromatography using *n*-hexane−EtOAc (5:1). Compound **2** was purified by silica gel chromatography using CH_2Cl_2−EtOAc (5:1).

Dysokusone D (1): colorless oil (17 mg); CD (*c* 0.08, $CHCl_3$): $[\theta]_{241}$ −12360, $[\theta]_{334}$ +1037; UV (MeOH) λ_{max} (log ϵ) 257 (4.26) nm; IR (KBr) ν_{max} 3650, 1745, 1708 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.91 (3H, s, H_3-19), 1.46 (1H, br t, J = 11.6 Hz, H-9α), 1.53 (1 H, br t, J = 11.6 Hz, H-6β), 1.58 (1H, br t, J = 11.6 Hz, H-8β), 1.62 (1H, m, H-9β), 1.63 (1H, m, H-8α), 1.90 (3H, br s, H_3-18), 1.91 (3H, br s, H_3-20), 1.97 (1H, m, H-6α), 2.11 (1H, br t, J = 11.6 Hz, H-7), 2.17 (3H, br s, H_3-17), 2.18 (3H, br s, H_3-12), 2.20 (1H, br d, J = 16.2 Hz, H-1α), 2.29 (1H, br d, J = 16.2 Hz, H-1β), 2.41 (1H, br d, J = 11.6 Hz, H-5), 5.89 (1H, br s, H-3), 6.09 (1H, br s, H-13), 6.10 (1H, br s, H-15); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 16.9 (q, C-19), 17.6 (q, C-12), 20.7 (q, C-17), 21.9 (q, C-20), 25.6 (t, C-8), 27.8 (q, C-18), 28.1 (t, C-6), 37.5 (s, C-10), 39.9 (t, C-9), 47.7 (d, C-5), 49.6 (d, C-7), 54.4 (t, C-1), 124.8 (d, C-13), 126.3 (d, C-15), 127.1 (d, C-3), 154.8 (s, C-16), 160.4 (s, C-11), 162.5 (s, C-4), 192.0 (s, C-14), 198.9 (s, C-2); EIMS *m/z* 300 $[M]^+$ (1), 285 (2), 245 (1), 205 (1), 175 (1), 135 (38), 123 (100); HREIMS *m/z* 300.2091 (calcd for $C_{20}H_{28}O_2$ 300.2082).

Dysokusone E (2): colorless oil (15 mg); CD (*c* 0.10, $CHCl_3$): $[\theta]_{238}$ −10380, $[\theta]_{331}$ +1238; UV (MeOH) λ_{max} (log ϵ) 247 (4.06) nm; IR (KBr) ν_{max} 1704 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 0.90 (3H, s, H_3-19), 1.37 (6H, s, H_3-17, 18), 1.41 (1 H, br t, J = 12.0 Hz, H-6β), 1.44 (1H, br t, J = 12.0 Hz, H-9α), 1.50 (1H, br t, J = 12.0 Hz, H-8β), 1.57 (1H, m, H-9β), 1.59 (1H, m, H-8α), 1.77 (1H, br t, J = 12.0 Hz, H-7), 1.81 (3H, br s, H_3-12), 1.89 (3H, br s, H_3-20), 1.92 (1H, m, H-6α), 2.22 (1H, br d, J = 16.4 Hz, H-1α), 2.28 (1H, br d, J = 16.4 Hz, H-1β), 2.39 (br d, J = 12.0 Hz, H-5), 5.79 (1H, d, J = 11.7 Hz, H-15), 5.89 (1H, br s, H-3), 5.90 (1H, br d, J = 8.1 Hz, H-13), 6.49 (1H, dd, J = 11.4, 8.1 Hz, H-14); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.0 (q, C-12), 16.8 (q, C-19), 21.9 (q, C-20), 25.8 (t, C-8), 28.3 (t, C-6), 29.9 (q, C-17, 18), 37.5 (s, C-10), 40.0 (t, C-9), 47.8 (d, C-5), 48.3 (d, C-7), 54.4 (t, C-1), 70.9 (s, C-16), 122.8 (d, C-13), 123.1 (d, C-14), 126.8 (d, C-3), 139.8 (d, C-15), 142.2 (s, C-11), 163.0 (s, C-4), 199.2 (s, C-2); EIMS *m/z* 302 $[M]^+$ (3), 284 (5), 260 (6), 231 (3), 176 (14), 135 (39), 107 (100); HREIMS *m/z* 302.2236 (calcd for $C_{20}H_{30}O_2$ 302.2238).

Cytotoxicity Testing. P-388 cell culture was kindly supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxicity asssays were carried out according to the procedure described previously.[5]

Acknowledgment. We thank Prof. J. M. Pezzuto, Program for Collaborative Research in Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, for the provision of P-388 cell lines. This work was supported by grants from the National Science Council of Taiwan awarded to C.-Y.D.

References and Notes

(1) Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep.* **1972**, *3*, 1−91.
(2) Hou, R.-S.; Duh, C.-Y.; Chiang, M. Y.; Lin, C.-N. *J. Nat. Prod.* **1995**, *58*, 1126−1130.
(3) Fujioka, T.; Yamamoto, M.; Kashiwada, Y.; Fujii, H.; Mihashi, K.; Ikeshiro, Y.; Chen, I.-S.; Lee, K. *Bioorg. Chem. Lett.* **1998**, *8*, 3479−3482.
(4) Shin, J.; Fenical, W. *J. Org. Chem.* **1991**, *56*, 3153−3158.
(5) Duh, C.-Y.; Wang, S.-K.; Weng, Y.-L.; Chiang, M. Y.; Dai, C.-F. *J. Nat. Prod.* **1999**, *62*, 1518−1521.

NP000264Z