An Unusual Sesquiterpene Derivative from *Ferula kuhistanica*

Kimiko Tamemoto,[§] Yoshihisa Takaishi,*[,§] Kazuyoshi Kawazoe,[§] Gisho Honda,[†] Michiho Ito,[†] Fumiyuki Kiuchi,[†] Yoshio Takeda,[‡] Olimjon K. Kodzhimatov,[⊥] Ozodbek Ashurmetov,[⊥] Katsuhide Shimizu,[#] Hideko Nagasawa,[#] Yoshihiro Uto,[#] and Hitoshi Hori[#]

Faculty of Pharmaceutical Sciences, University of Tokushima, Shomachi 1-78, Tokushima, 770-8505, Japan, Faculty of Pharmaceutical Sciences, Kyoto University, Yoshida Sakyoku, Kyoto 606-8501, Japan, Faculty of Integrated Arts and Sciences, University of Tokushima, Minamijyosanjima, Tokushima 770-8502, Japan, Academy of Sciences, Uzbekistan Institute of Botany, F. Khodzhaev, St. 32, 700143 Tashkent, Uzbekistan, and Faculty of Engineering, University of Tokushima, Minamijosanjimacho-2, Tokushima 770-8506, Japan

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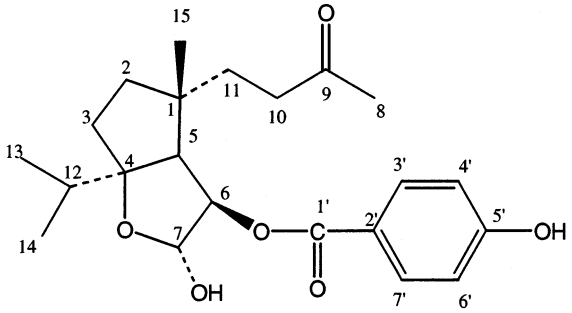
An unusual new sesquiterpene derivative, kuhistaferone (**1**), was isolated from the fruits of the Uzbekistan medicinal plant *Ferula kuhistanica*. The structure of **1** was established on the basis of spectroscopic evidence. Compound **1** showed moderate cytotoxicity against the human colon tumor cell line HCT116.

The exclusively Old World genus *Ferula* belongs to the family Umbelliferae and has some 130 species distributed throughout the Mediterranean area and Central Asia. Plants of this genus have been shown to be a good source of biologically active compounds such as coumarins and sesquiterpene derivatives.[1] *Ferula kuhistanica* Korov. (Umbelliferae) has been used in Uzbekistan folk medicine to treat skin disease and wounds. As part of our studies on Uzbekistan folk medicinal plants,[2−4] we investigated the constituents of *F. kuhistanica* and describe here the isolation and characterization of an unusual sesquiterpene derivative, for which we propose the name kuhistaferone (**1**).



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Ethyl acetate extracts of the air-dried fruit of *F. kuhistanica* were separated by repeated column chromatography to give kuhistaferone (**1**). Compound **1** showed absorption bands for hydroxy (3588 cm^{-1}), ester (1710 cm^{-1}), and aromatic (1609 cm^{-1}) groups in its IR spectrum. The UV spectrum of **1** indicated the presence of an aromatic ring (259 nm). The ^{13}C NMR spectrum of **1** showed 22 carbon signals, including a ketone group (δ_C 210.1), an ester carbonyl (δ_C 166.4), a benzene ring (δ_C 115.6 × 2, 121.3, 133.2 × 2, 161.0), two oxygenated methines (δ_C 82.3, 101.3), a quaternary carbon (δ_C 100.4), four methyls, four methylenes, two methines, and a quaternary carbon. The HRFABMS of **1** showed a $[M + Na]^+$ ion peak at *m/z* 413.1924, which indicated that the molecular formula of **1** was $C_{22}H_{30}O_6$. The ^1H NMR spectrum of **1** showed the presence of an isopropyl group [δ_H 0.97, 1.04 (each 3H, d, J = 6.7 Hz), 1.93 (1H, sep, J = 6.7 Hz)] and a *p*-hydroxybenzoyl group [δ_H 6.87 (2H, d, J = 8.5 Hz), 7.94 (2H, d, J = 8.5 Hz)].

On the basis of these spectral data, compound **1** appeared to be a daucane ester, many of which have been isolated from *F. kuhisutanica*.[3] However additional spectral data of compound **1** were very different from those characteristic of daucane esters. The $^1H-^1H$ COSY spectrum of **1** showed proton correlations of δ_H 1.34 (2-Hα) with δ_H 1.60 (2-Hβ) and δ_H 1.86 (3-H_2), δ_H 5.40 (6-H) with δ_H 2.56 (5-H) and δ_H 5.59 (7-H), and δ_H 2.39 (10-H_2) with δ_H 1.46 (11-Hα) and δ_H 1.72 (11-Hβ), indicating the presence of partial structures (I: $-CH_2-CH_2-$; II $-O-CH-CH-CH-$; III: $-CH_2-CH_2-$). In the HMBC spectrum of **1**, correlations of δ_H 1.25 (15-H_3) with δ_C 44.7 (C-1), δ_C 40.4 (C-2), δ_C 54.7 (C-5), and δ_C 36.0 (C-11); δ_H 2.56 (5-H) with δ_C 44.7 (C-1) and δ_C 36.0 (C-12); δ_H 2.06 (8-H_3) with δ_C 210.1 (C-9); δ_H 2.39 (10-H) with δ_C 210.1 (C-9); and δ_H 0.97 (13-H_3) and δ_H 1.04 (14-H_3) with δ_C 100.4 (C-4) were observed (Figure 1). These results clearly show that the partial structures (I−III) are connected and indicate the presence of a five-membered ring. The remaining problems in determining the structure of **1** were whether C-4 and C-7 were connected via oxygen and the position of *p*-hydroxybenzoic acid.

The carbon chemical shifts (δ_C 100.4 and δ_C 101.3) of C-4 and C-7 suggested the presence of a hemiacetal structure at C-7.[5,6] In the HMBC spectrum (400 MHz, J = 10 Hz), correlations of H-7 with C-4, H-6 with C-1′, and H-5 with C-7 were not apparent. Therefore, we measured the HMBC spectrum under different conditions (500 MHz, J = 8.0 Hz). In this HMBC experiment, correlations of δ_H 5.59 (7-H) with δ_C 100.4 (C-4), δ_H 5.40 (6-H) with δ_C 44.7 (C-1) and 166.4 (C-1′), and δ_H 2.56 (5-H) with δ_C 101.3 (C-7) were observed. These results clearly indicated that C-4 and C-7 are connected via oxygen and that the *p*-hydroxybenzoate group is located at C-6. In addition, the correlation of 15-H_3 with 7-H in the NOESY spectrum shows that 7-OH has an α configuration. Furthermore, the correlation of 5-H with 6-H, 12-H, and 13-H_3 shows that the configuration of the isopropyl group is α, while that of the ester moiety is β. Hence, the structure of **1** is as shown.

Compound **1** has a very unique cyclopentane ring fused and tetrahydrofuran ring structure. Compounds similar to **1** have been isolated from *Catalpae fructus*[5] and tunicate.[6]

* Corresponding author. Tel: 0081-88-6337275. Fax: 0081-88-6339501. E-mail: takaishi@ph.tokushima-u.ac.jp.
[§] Faculty of Pharmaceutical Sciences, University of Tokushima.
[†] Faculty of Pharmaceutical Sciences, Kyoto University.
[‡] Faculty of Integrated Arts and Sciences, University of Tokushima.
[⊥] Academy of Sciences, Uzbekistan Institute of Botany.
[#] Faculty of Engineering, University of Tokushima.

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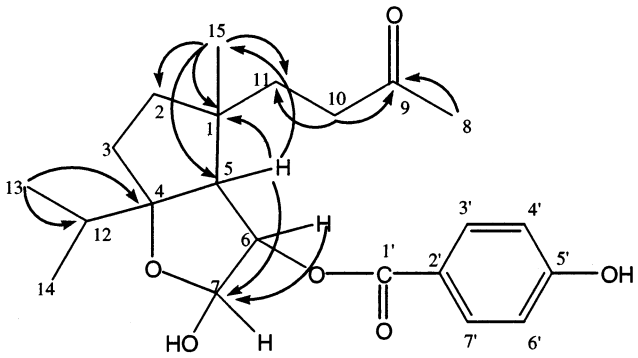

Figure 1. Long-range correration of kuhistaferone (**1**).

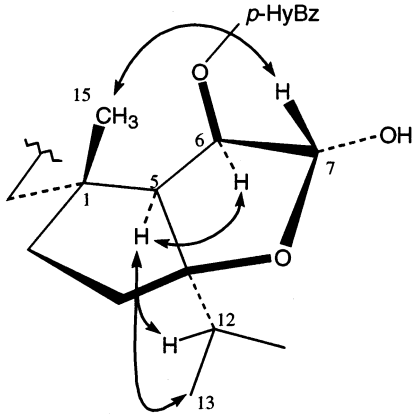

Figure 2. NOESY for kuhistaferone (**1**).

Compound **1** did not have antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-sensitive *S. aureus* (MSSA). We evaluated the cytotoxicity of kuhistaferone against human colon tumor cell line HCT116. MTT assay showed that **1** has moderate antitumor activity, with an IC_{50} of 181 μM against HCT116 cells.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. IR spectra were recorded on a 1720 infrared Fourier transform spectrophotometer (Perkin-Elmer) and UV spectra using a UV2100 UV−vis recording spectrophotometer (Shimadzu). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both in $CDCl_3$ referenced to TMS) spectra were measured on a Bruker ARX400 spectrometer, and MS spectra were measured on a JEOL JMSD-300 instrument. Column chromatographic supports: silica gel 60 (Merck). HPLC supports: silica gel (Si 60, Hibar TR250-25).

Plant Material. The fruits of *Ferula kuhistanica* were collected in July 1997 in Uzbekistan. Herbarium specimens were deposited in the herbarium of the Institute of Botany, Academy of Sciences, Uzbekistan.

Extraction and Isolation. The dried fruits of *F. kuhistanica* (600 g) were crushed and extracted with hexane. The residue was extracted with AcOEt. The AcOEt extracts were concentrated in vacuo to give a residue (60 g), which was subjected to column chromatography on a silica gel column eluted with solvents of increasing polarity (hexane−AcOEt) to give 16 fractions. Fraction 3 (3.3 g) was chromatographed on a silica gel column using hexane−AcOEt (2:1) to give three subfractions (3.1−3.3). Fraction 3.1 (230.3 mg) was fractionated by HPLC [silica gel, hexane−AcOEt (1:1)] to give **1** (9.6 mg).

Kuhistaferone (1): yellowish oil; $[\alpha]^{25}_D$ +10.1° (*c* 0.77, MeOH); IR (NaCl) ν_{max} 3588, 1710, 1609, 1514, 1273, 1165 cm^{-1}; UV (MeOH) λ_{max}(log ϵ) 259 (4.1) nm; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.94 (2H, d, J = 8.5 Hz, H-3′, H-7′), 6.87 (2H, d, J = 8.5 Hz, H-4′, H-6′) 5.59 (1H, d, J = 5.0 Hz, H-7), 5.40 (1H, dd, J = 8.1, 5.0 Hz, H-6), 2.56 (1H, J = 8.1 Hz, H-5), 2.39 (2H, m, H_2-10), 2.06 (3H, s, H-8), 1.93 (1H, sep, J = 6.7 Hz, H-12), 1.86 (2H, m, H-3), 1.72 (1H, m, H-11), 1.60 (1H, m, H-2), 1.46 (1H, m, H-11), 1.34 (1H, m, H-2), 1.25 (3H, s, H_3-15), 1.04 (3H, d, J = 6.7 Hz, H_3-14), 0.97 (3H, d, J = 6.7 Hz, H_3-13); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 210.1 (s, C-9), 166.4 (s, C-1′), 161.0 (s, C-5′), 133.2 (d, C-3′, C-7′), 121.3 (s, C-2′), 115.6 (d, C-4′, C-6′), 101.3 (d, C-7), 100.4 (s, C-4), 82.3 (d, C-6), 54.7 (d, C-5), 44.7 (s, C-1), 40.4 (t, C-2), 39.5 (t, C-10), 36.0 (t, C-11, d, C-12), 32.5 (t, C-3), 29.9 (q, C-8), 21.9 (q, C-15), 18.4 (q, C-14), 17.6 (q, C-13); HRFABMS(matrix: *m*-nitrobenzyl alcohol) *m*/*z* [M + Na]$^+$ 413.1924 (calcd for $C_{22}H_{30}O_6Na$, 413.1940).

Tumor Cells. Human colon carcinoma HCT116 cells were grown at 37 °C in the presence of 5% CO_2 in McCoy's 5A medium supplemented with 10% (50 mL/500 mL) fetal bovine serum (GIBCO BRL, inactivated) and 2.2 g/mL $NaHCO_3$.

Measurement of Cytotoxicity Using MTT. HCT116 cells were inoculated at a cell density of 5.0×10^3 cells/well in 100 μL of the cell culture medium using 96-well plates. One day later, the monolayer culture was incubated in the cell culture medium with or without the compound to be tested at 37 °C for 2 days. Thereafter, the MTT assay was carried out according to the method reported by Mosman.[7] MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diftenyl-2*H*-tetrazolium bromide] was purchased from Dojindo Laboratories (Kumamoto, Japan). Ten microliters of MTT reagent (5 mg/mL phosphate-buffered saline without potassium and magnesium) was added to each well. The cells were incubated at 37 °C for 4 h. Formazan was extracted with 100 μL of 0.04 N HCl in 2-propanol. The optical density of each well using 96-well ELISA plates was measured spectrophotometrically with a microplate reader at wavelength 570 nm (BIO-RAD Model 450).[7]

References and Notes

(1) Gonzalez, A. G.; Barrera, J. B. *Prog. Chem. Org. Nat. Prod.* **1995**, *64*, 1−92.
(2) Chen, B.; Kawazoe, K.; Takaishi, Y.; Honda, G.; Itoh, M.; Takeda, Y.; Kodozhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod.* **2000**, *63*, 362−365.
(3) Chen, B.; Teranishi, R.; Kawazoe, K.; Takaishi, Y.; Honda, G.; Itoh, M.; Takeda, Y.; Kodozhimatov, O. K. *Phytochemistry* **2000**, *54*, 717−722.
(4) Chen, B.; Takaishi, Y.; Kawazoe, K.; Tamemoto, K.; Honda, G.; Itoh, M.; Takeda, Y.; Kodozhimatov, O. K.; Ashurmetov, O. *Chem. Pharm. Bull.* **2001**, *49*, 707−710.
(5) Machida, K.; Ogawa, M.; Kikuchi, M. *Chem. Pharm. Bull.* **1998**, *46*, 1056−1057.
(6) Niels, L.; William, F.; David, F. S.; Chris, M. I.; Gregory, D. V. D.; Craig, J. F.; Jon, C. *J. Am. Chem. Soc.* **1988**, *110*, 1308−1309.
(7) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55−63.

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