Prenylated Benzoic Acid Derivatives from *Ferula kuhistanica*

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Four new prenylated benzoic acid derivatives, kuhistanols A−D (**1**−**4**), along with one known compound, 3-farnesyl-*p*-hydroxybenzoic acid (**5**), have been isolated from the roots of the Uzbekistan medicinal plant, *Ferula kuhistanica*. Their structures were established on the basis of chemical and spectral evidence. Compound **4** showed a significant inhibitory effect on cytokine production in lipopolysaccharide-stimulated human peripheral mononuclear cells.

The exclusively Old World genus *Ferula* belongs to the family Umbelliferae, with some 130 species distributed throughout the Mediterranean area and Central Asia. Several species of this genus have been used in folk medicine.[1] This genus is well documented as a good source of biologically active compounds such as coumarins, terpene alcohols, and sesquiterpene derivatives.[2] As a part of an ongoing study on Uzbekistan folk medicinal plants, we have investigated the constituents of *Ferula kuhistanica* Korouin, which is used traditionally to treat skin diseases and wounds. We describe herein the isolation and characterization of four new prenylated benzoic acid derivatives, kuhistanols A−D (**1**−**4**) as well as the known compound, 3-farnesyl-*p*-hydroxybenzoic acid (**5**),[3−5] which were isolated from the methanolic extract of the air-dried roots of *F. kuhistanica*. This is the first report of prenylated benzoic acid derivatives from a plant in the genus *Ferula*. Compounds **1**−**4** were evaluated for their effects on cytokine production in lipopolysaccharide-stimulated human peripheral mononuclear cells.

Results and Discussion

Kuhistanol A (**1**) showed absorption bands for hydroxyl (3422 cm^{-1}) and carboxyl groups (1685 cm^{-1}) in the IR spectrum. The UV spectrum indicated the presence of an aromatic ring (259 and 214 nm). The ^{13}C NMR spectrum of **1** showed 22 carbon signals, including benzene-ring carbons, two double bonds, one methine, one quaternary carbon attached to an oxygen function, five methylenes, and four methyl carbons (Table 1). The HRFABMS of **1** showed the $[M - H]^-$ ion peak at *m/z* 375.2180, which indicated the molecular formula for **1** to be $C_{22}H_{32}O_5$. The splitting pattern in the ^1H NMR spectrum of the three aromatic protons [δ_H 7.66 (d, J = 2.0 Hz), 7.61 (dd, J = 2.0, 8.4 Hz), and 6.69 (d, J = 8.4 Hz)] established that the aromatic ring was a 1,3,4-trisubstituted benzene ring. In the HMBC spectrum of **1** (Table 2), the proton signal at δ_H 7.66 (H-2) showed cross-peaks to a carbonyl carbon at δ_C 170.4 and a hydroxy-bearing quaternary carbon at δ_C 161.1 (C-4), as well as with a protonated carbon at δ_C 130.4 (C-6) and a methylene carbon at δ_C 28.9 (C-1′), which was considered to be located on the side-chain. This evidence indicated that compound **1** is a 3-substituted 4-hydroxybenzoic acid derivative. The ^{13}C NMR spectral data of **1** were very similar to those of 3-farnesyl-*p*-hydroxybenzoic acid (**5**), except for the resonances of a methine carbon (δ_C 79.1, C-10′) and a quaternary carbon (δ_C 73.8, C-11′) in **1**, replacing two olefinic carbons (δ_C 125.3 and 131.9). In the HMBC spectrum of **1** (Table 2), the oxygen-bearing proton at δ_H 3.14 (H-10′) showed long-range correlations with the carbon signals at δ_C 73.8 (C-11′), 25.5 (C-12′), 25.0 (C-15′), and 37.8 (C-8′), and the olefinic proton at δ_H 5.11 (H-6′) showed long-range correlations with the carbon signals at δ_C 37.8 (C-8′), 16.2 (C-14′), 27.6 (C-5′), and 40.9 (C-4′). Thus, **1** was confirmed to be a prenylated benzoic acid with hydroxyl groups at C-10′ and C-11′. In addition, due to the correlations of H-2′ and H-4′, H-6′ and H-8′, H_3-13′ and H-1′, and H_3-14′ and H-5′, observed in the NOESY spectrum, the stereochemistry of the double bonds could be deduced as 2′*E* and 6′*E*. Methylation of **1** with trimethylsilyldiazomethane gave the dimethylate **1a**, which was acetylated to give **1b**. In the ^1H NMR spectrum of **1b**, the proton signal at δ_H 3.14 (H-10′) in **1** was shifted downfield

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**5** (100 MHz, CD_3OD, δ values)

carbon	1	2	3	4	5
1	122.5	122.6	122.9	121.2	122.5
2	132.5	132.6	132.6	133.3	132.6
3	129.2	129.3	129.2	117.9	129.2
4	161.1	161.0	161.0	158.9	161.0
5	115.3	115.3	115.2	117.9	115.3
6	130.4	130.4	130.4	130.5	130.4
1′	28.9	28.9	29.0	31.9	28.9
2′	123.3	123.5	123.6	68.2	123.4
3′	137.4	137.6	137.3	80.9	137.2
4′	40.9	38.0	37.6	38.9	40.7
5′	27.6	30.4	31.3	22.5	27.5
6′	125.5	78.1	77.4	125.4	125.5
7′	136.1	75.5	87.3	136.3	135.9
8′	37.8	39.5	33.4	40.8	40.8
9′	30.8	23.0	27.6	27.7	27.7
10′	79.1	126.0	85.9	125.4	125.3
11′	73.8	131.9	72.7	132.1	131.9
12′	25.5	25.9	27.0	25.8	25.9
13′	16.2	16.3	16.2	19.0	16.3
14′	16.2	21.9	23.1	16.0	16.1
15′	25.0	17.7	25.8	17.7	17.7
COOH	170.4	170.5	171.2	170.0	170.4

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Table 2. ^{1}H NMR Data and HMBC Correlations in Compounds **1** and **3** (400 MHz, CD_3OD, δ values)

	1		**3**	
position	^{1}H	HMBC	^{1}H	HMBC
2	7.66, d (2.0)	1′, 4, 6, COOH	7.66, d (1.7)	1′, 4, 6, COOH
5	6.69, d (8.4)	1, 3, 4	6.68, d (8.5)	1, 3, 4
6	7.61, dd (2.0, 8.4)	2, 4, COOH	7.60, dd (1.7, 8.5)	2, 4, COOH
1′	3.21, d (7.0)	2, 3, 4, 2′, 3′	3.24, d (7.0)	2, 3, 4, 2′, 3′
2′	5.24, br t (7.0)	3, 1′, 4′, 13′	5.30, br t (7.0)	3, 1′, 4′, 13′
4′	1.99b, 2.05, m	2′, 3′, 5′, 13′	2.02, 2.20, m	2′, 3′, 5′, 13′
5′	1.98b, 2.03, m	4′, 6′, 7′	1.30, 1.61, m	4′
6′	5.11, br t (6.5)	4′, 5′, 8′, 14′	3.37, br d (9.1)	4′, 5′, 7′, 8′, 14′
8′	1.90, 2.12, m	5′, 6′, 7′, 9′, 10′, 14′	1.46, 1.95, m	6′, 7′, 10′, 14′
9′	1.23, 1.57, m	8′, 10′	1.79, 1.79, m	8′, 10′, 11′
10′	3.14, dd (1.4, 10.4)	8′, 10′, 12′, 15′	3.69, br t (7.4)	15′
12′	1.02, s	10′, 11′, 15′	1.09, s	10′, 11′, 15′
13′	1.62, s	2′, 3′, 4′	1.63, s	1′, 2′, 3′, 4′
14′	1.51, s	6′, 7′, 8′	1.04, s	6′, 7′, 8′
15′	1.05, s	10′, 11′, 12′	1.00, s	10′, 11′, 12′

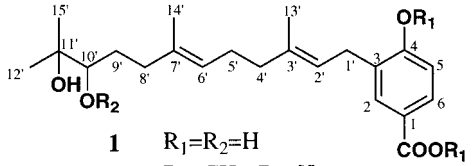
to δ_H 4.76. This additional evidence clearly supported the structure of **1**. Hence, the structure of **1** was determined to be 3-(10′,11′-dihydro-10′,11′-dihydroxyfarnesyl)-*p*-hydroxybenzoic acid. To elucidate the absolute configuration of a secondary alcohol at C-10′, the high-field FT NMR application of Mosher's method was used.[6] Compound **1** was determined to be a racemic mixture at C-10′ because the same ^{1}H NMR signals appeared for both the (*R*)-MTPA ester and the (*S*)-MTPA ester.

Kuhistanol B (**2**) showed the same molecular formula $C_{22}H_{32}O_5$ as that of compound **1**. The ^{1}H and ^{13}C NMR spectral data of **2** were very similar to those of **1**, except for one upfield-shifted methyl in **2** instead of two upfield methyl groups in **1**. It was apparent that only one methyl linked to an oxygen-bearing quaternary carbon (δ_C 75.5, C-7′) was present in **2**. In the HMBC spectrum, an oxygen-bearing methine proton signal at δ_H 3.21 showed long-range correlations with the carbon signals at δ_C 21.9 (C-14′), 30.4 (C-5′), and 38.0 (C-4′), so the hydroxyl groups could be located at C-6′ and C-7′. Furthermore, the stereochemistry of the C-2′−C-3′ double bond was assigned as 2′*E* due to the correlations of H-2′ and H-4′ and of H_3-13′ and H-1′, as seen in the NOESY spectrum. The structure of **2** was determined to be 3-(6′,7′-dihydro-6′,7′-dihydroxyfarnesyl)-*p*-hydroxybenzoic acid.

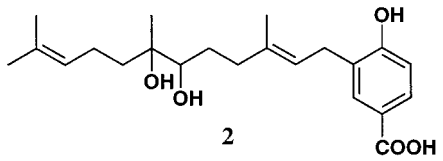
Kuhistanol C (**3**), $C_{22}H_{32}O_6$, contained a 1,3,4-trisubstituted aromatic ring, four tertiary methyls [δ_H 1.00, 1.04, 1.09, and 1.63 (each 3H, s)], five methylenes, one olefinic proton [δ_H 5.30 (1H, br t, J = 7.2 Hz, H-2′)], and two methines [δ_H 3.69 (1H, br t, J = 7.4 Hz, H-10′) and 3.37 (1H, br d, J = 9.1 Hz, H-6′)] attached to an oxygen function, as indicated by the ^{1}H NMR spectrum. The ^{13}C NMR spectral data (Table 1) of **2** and **3** were similar except for the side chain signals of C-7′ through C-15′. In the HMBC spectrum (Table 2), the correlations of C-1 to C-6 and C-1′ to C-16′ were confirmed. There were two oxygen-bearing quaternary carbons (δ_C 72.7 and 87.3) and two oxygen-bearing methines (δ_C 85.9 and 77.4), as shown from the ^{13}C NMR spectrum. Acetylation of **3** gave diacetate **3a**, with the proton signal at δ_H 3.37 (H-6′) in **3** being shifted downfield to δ_H 4.89 in **3a**. The proton signal of H-6′ showed cross-peaks with carbon signals of C-4′, -5′, -7′, -8′, and -14′ in the HMBC spectrum (Table 2). These facts clearly indicated that the position of one of the hydroxyl groups was at C-6′. The HMBC data shown in Table 2 and the correlation between H_3-14′ and H-10′, as observed in the NOESY spectrum, confirmed the presence of a dihydrofuran ring between C-7′ and C-10′ in **3**. Moreover, the stereochemistry of the double bond was assigned as 2′*E*, and the configuration of the furan ring was cis, according to the NOESY spectrum. Hence, the structure of **3** was determined as 3-(6′,7′,10′,11′-tetrahydro-6′,11′-dihydroxy-7′,10′epoxyfarnesyl)-*p*-hydroxybenzoic acid.

Kuhistanol D (**4**) was obtained as an amorphous powder. The molecular formula $C_{22}H_{30}O_4$ was determined on the basis of HRMS. The ^{1}H and ^{13}C NMR spectral data revealed the presence of a 1,3,4-trisubstituted benzene ring with a carboxylic acid substituent. The ^{13}C NMR chemical shifts (Table 1) of C-6′ to C-12′, C-14′, and C-15′ in **4** were almost the same as those of **5**, so the existence of a prenylated unit in **4** could be deduced. Furthermore, the ^{13}C NMR data showed one methine (δ_C 68.2); one quaternary carbon (δ_C 80.9), which was bonded to the oxygen function; and two double bonds. Methylation of **4** with trimethylsilyldiazomethane yielded a monomethyl derivative **4a**. Acetylation of **4a** gave monoacetate **4b**, which revealed an acetoxy group at δ_H 2.06 in the ^{1}H NMR spectrum. Meanwhile, the proton at δ_H 3.95 (H-2′) in **4a** was shifted downfield to δ_H 5.12 in **4b**, which suggested that the hydroxyl group was connected to this methine instead of being connected to the benzene ring as in the other compounds (**1**−**3**). Accordingly, the phenolic hydroxyl group in **4** was connected to the side chain through an ether bond. In the HMBC spectrum, the proton signal at δ_H 3.87 (H-2′) showed long-range correlations with the carbon signals at δ_C 31.9 (C-1′), 38.9 (C-4′), and 19.0 (C-13′); the proton signals of the methylene at δ_H 2.78 and 3.04 (H-1′) showed cross-peaks with the carbon signals at δ_C 133.3 (C-2), 117.9 (C-3), 158.9 (C-4), 68.2 (C-2′), and 80.9 (C-3′); and the proton signals of the methyl at δ_H 1.26 (H_3-13′) showed cross-peaks with the carbon signals at δ_C 68.2, 80.9, and 38.9. From these facts, the existence of a chroman ring was postulated between C-4 and C-3′. In addition, from the other long-range correlations, the structure of **4** could be fully deduced. A series of NOE difference spectroscopy experiments was carried out to confirm the structure and relative stereochemistry of the hydroxyl group and the side chain. In particular, irradiation of the H_3-13′ methyl signal strongly enhanced the H-2′ signal and, to some extent, the H-5′ protons as well as one of the H-1′ protons. These NOE effects clearly indicated a trans relationship between the C-3′ methyl group and the C-2′ hydroxyl group. Also, the $\Delta^{6',7'}$ double bond was assigned as 6′*E* from the NOESY spectrum. To confirm the absolute configuration, we prepared the (*R*)- and (*S*)-MTPA esters of **4**, but both gave the same ^{1}H NMR signals. Thus, **4** was determined to be a racemic mixture at C-2′. The structure of **4** was determined

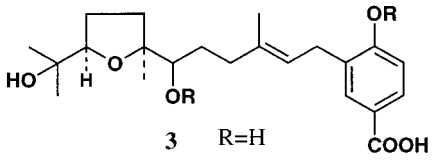
as 3-(2′,3′-dihydro-2′-hydroxy-3′,4-epoxyfarnesyl)-*p*-hydroxybenzoic acid.



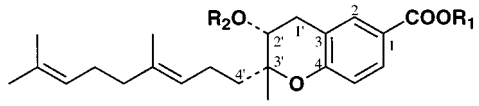
1 R_1=R_2=H
1a R_1=CH_3, R_2=H
1b R_1=CH_3, R_2=$COCH_3$
1c R_1=CH_3, R_2=(+)-MTPA
1d R_1=CH_3, R_2=(-)-MTPA



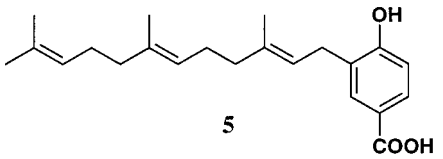
2



3 R=H
3a R=Ac



4 R_1=R_2=H
4a R_1=CH_3, R_2=H
4b R_1=CH_3, R_2=Ac
4c R_1=CH_3, R_2=(+)-MTPA
4d R_1=CH_3, R_2=(-)-MTPA



5

In a screen for immunosuppresive activity, isolated compounds **1**−**4** were examined for inhibitory effects on cytokine production.[7,8] Compound **4** (3 μg/mL) showed a significant inhibitory effect (%) on cytokine production [IL-4; 70.3%, IL-2: 77.3%, IFN-γ: 61.8%] in lipopolysaccharide-stimulated human peripheral mononuclear cells, when compared with the reference compound, prednisolone (0.3 μg/mL) [IL-4; 55.3%, IL-2: 65.6%, IFN-γ: 60.2%]. Compounds **1**−**3** (10 μg/mL) showed no significant inhibitory effects on cytokine production.

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 spectrometer (Perkin-Elmer) and UV spectra on a UV2100 UV−vis recording spectrometer (Shimadzu). NMR (400 MHz for ^{1}H NMR, 100 MHz for ^{13}C NMR, both in TMS) spectra were measured on Bruker ARX400 spectrometer and MS spectra on a JEOL JMSD-300 instrument. Column chromatographic supports: Si gel 60 (Merck), Sephadex LH-20 (Pharmacia), Toyo Pearl HW-40 (Tosoh); HPLC supports: gel permeation chromatography (GPC) (Asahipak GS-310 2G, MeOH; Shodex H-2001, 2002, $CHCl_3$), Si gel (Si 60, Hibar TR250-25), ODS (RP_{18}, Hibar RT250−25).

Plant Material. The roots of *Ferula kuhistanica* were collected in July 1997, in Qashiqaderyo, Uzbekistan. Herbarium specimens (98C008) are deposited in the herbarium of the Institute of Botany, Academy of Sciences, Uzbekistan, and the Faculty of Pharmaceutical Sciences, Kyoto University.

Extraction and Isolation. The roots of *F. kuhistanica* (2.25 kg) were crushed and extracted with MeOH at 60 °C three times. The MeOH extracts were concentrated in vacuo to give a residue, which was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (247 g), which was chromatographed over a Si gel column and eluted with *n*-hexanes−EtOAc (4:1 to 1:3), pure EtOAc, and EtOAc−MeOH (9:1 to 7:3) to give 21 fractions. Fraction 4 (75 g) was chromatographed on a Si gel column using $CHCl_3$−MeOH (99:1 to 9:1) to give six subfractions (4.1−4.6). Fraction 4.3 (48 g) was chromatographed on a Si gel column using $CHCl_3$−MeOH (24:1) to give **5** (253.3 mg), and fraction 11 (7.6 g) was chromatographed on a Si gel column using $CHCl_3$−MeOH (49:1 to 9:1) to give 17 subfractions (11.1−11.17). Fraction 11.10 (782.9 mg) was chromatographed on Sephadex LH-20 with MeOH to give seven subfractions (11.10.1−11.10.7). Fraction 11.10.3 (585 mg) was purified by HPLC [ODS, MeOH−H_2O (8:2)] to give **4** (113.5 mg). Fraction 15 (9 g) was chromatographed on a Si gel column using $CHCl_3$−MeOH (97:3 to 9:2) to give 15 subfractions (15.1−15.15), and fraction 15.10 (920 mg) was chromatographed on Sephadex LH-20 with MeOH to give seven subfractions (15.10.0−15.10.7). Fraction 15.10.5 (580 mg) was purified by HPLC [ODS, MeOH−H_2O (8:2)] to give six subfractions (15.10.5.1−15.10.5.6), with fraction 15.10.5.4 (19.4 mg) purified by HPLC [Si gel, hexanes−EtOAc (1:5)] to give **3** (10.0 mg). Fraction 15.10.5.6 (108.3 mg) was purified by HPLC [Si gel, hexanes−EtOAc (1:5)] to give **1** (91.3 mg) and **2** (4.6 mg).

Kuhistanol A (1): amorphous powder; $[\alpha]^{25}_D$ +1.6° (c 1.0, MeOH); IR (KBr) ν_{max} 3422, 2930, 1685, 1278, 1076 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 259 (4.1), 214 (4.3) nm; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.02 (3H, s, H-12′), 1.05 (3H, s, H-15′), 1.23 (1H, m, Ha-9′), 1.51 (3H, s, H-14′), 1.57 (1H, m, Hb-9′), 1.62 (3H, s, H-13′), 1.90 (1H, m, Ha-8′), 2.12 (1H, m, Hb-8′), 3.14 (1H, dd, J = 1.4, 10.4 Hz, Ha-10′), 3.21 (2H, d, J = 7.0 Hz, H-1′), 5.11 (1H, br t, J = 6.6 Hz, H-6′), 5.24 (1H, br t, J = 7.2 Hz, H-2′), 6.69 (1H, d, J = 8.4 Hz, H-5), 7.61 (1H, dd, J = 2.0, 8.4 Hz, H-6), 7.66 (1H, d, J = 2.0 Hz, H-2); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS *m*/*z* 358 (14), 340 (22), 299 (45), 203 (23), 189 (55), 153 (47), 151 (100), 143 (51), 107 (32), 91 (32), 71 (83), 59 (86), 43 (88); HRFABMS *m*/*z* 375.2180 [M − H]$^-$ (calcd for $C_{22}H_{31}O_5$, 375.2171).

Methylation of Kuhistanol A (1). A solution of **1** (18.2 mg) was treated with $(CH_3)_3SiCHN_2$, for 4 h at room temperature to give **1a** (17.5 mg). **1a**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.11, 1.14, 1.61, 1.71 (each 3H, s, H-12′, H-15′, H-14′, and H-13′), 3.23 (1H, dd, J = 1.2, 10.7, H-10′), 3.85 (3H, s, OMe), 3.89 (3H, s, OMe), 5.18 (1H, br t, J = 6.5 Hz, H-6′), 5.28 (1H, br t, J = 7.1 Hz, H-2′), 6.97 (1H, d, J = 8.6 Hz, H-5), 7.77 (1H, d, J = 1.8 Hz, H-2), 7.86 (1H, dd, J = 8.6, 1.8 Hz, H-6).

Acetylation of 1a. Compound **1a** (5.0 mg) was subjected to acetylation with Ac_2O−pyridine for 2.5 h at room temperature to give **1b** (3.5 mg). **1b**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.13 (6H, s, H-12′ and H-15′), 1.59, 1.72 (each 3H, s, H-14′ and H-13′), 2.06 (3H, s, OAc), 3.86, 3.90 (each 3H, s, OMe), 4.76 (1H, br d, J = 7.6 Hz, H-10′), 5.15 (1H, br t, J = 6.0 Hz, H-6′), 5.30 (1H, br t, J = 7.4 Hz, H-2′), 6.98 (1H, d, J = 8.5 Hz, H-5), 7.78 (1H, d, J = 1.9 Hz, H-2), 7.87 (1H, dd, J = 8.5, 1.9 Hz, H-6).

Determination of the Absolute Configuration of Kuhistanol A (1). A solution of **1a** (2.0 mg, 5.3 mmol), dehydrated pyridine (0.1 mL), and 2,4,6-trinitrochlorobenzene (25 mg, 0.1 mmol) were treated with (+)-MTPA (25 mg, 0.1 mmol) under N_2 and stirred for 18 h. Then, 5% $NaHCO_3$ (2 mL) and diethyl ether (2 mL) were added, and, after stirring until the

sediments were dissolved and the solvents evaporated, the residue was washed with H_2O, saturated $CuSO_4$, and brine and dried with Na_2SO_4. The residue was applied to preparative TLC ($CHCl_3$−CH_3COCH_3, 8:1) to give (*R*)-MTPA ester **1c** (1.2 mg). Using (−)-MTPA and the same experimental method gave (*S*)-MTPA ester **1d** (1.2 mg). **1c**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.06, 1.09, 1.14, 1.17, 1.50, 1.58, 1.70, 1.71 (each 3H, s), 1.78−2.15 (12H, m), 3.85, 3.89 (each 6H, s, OMe), 5.03, 5.11 (each 1H, br t, J = 6.0 Hz), 5.28 (2H, m), 6.98 (2H, d, J = 8.8 Hz), 7.86, 7.88 (each 1H, dd, J = 8.8, 1.9 Hz), 8.00 (2H, s). The ^{1}H NMR spectrum of **1d** was same as that of **1c**.

Kuhistanol B (2): amorphous powder; $[\alpha]^{25}_D$ 0° (*c* 0.4, MeOH); IR (KBr) ν_{max} 3435, 2924, 1686, 1605, 1278, 1099 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 258 (4.0), 208 (4.0) nm; ^{1}H NMR (CD_3-OD, 400 MHz) δ 1.00 (3H, s, H-14′), 1.33 (1H, m, Ha-5′), 1.39 (2H, m, H-8′), 1.51 (3H, s, H-15′), 1.57 (3H, s, H-12′), 1.64 (3H, s, H-13′), 1.71 (1H, m, Hb-5′), 1.96 (2H, m, H-9′), 2.03 (1H, m, Ha-4′), 2.22 (1H, m, Hb-4′), 3.21 (1H, m, H-6′), 5.01 (1H, br t, J = 7.2 Hz, H-10′), 5.31 (1H, br t, J = 7.2 Hz, H-2′), 6.68 (1H, d, J = 8.4 Hz, H-5), 7.60 (1H, dd, J = 8.4, 2.0 Hz, H-6), 7.66 (1H, d, J = 2.0 Hz, H-2); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS *m*/*z* 358 (7), 340 (5), 231 (92), 207 (51), 189 (25), 151 (100), 109 (72), 69 (85), 55 (19), 43 (61); HRFABMS *m*/*z* 375.2150 [M − H]$^-$ (calcd for $C_{22}H_{31}O_5$ 375.2171).

Kuhistanol C (3): amorphous powder; $[\alpha]^{25}_D$ +2.6° (*c* 0.8, MeOH); IR (KBr) ν_{max} 3379, 2926, 1683, 1606, 1278, 1083 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 258 (4.0), 207 (4.2) nm; ^{1}H NMR (CD_3-OD, 400 MHz) δ 1.00 (3H, s, H-15′), 1.04 (3H, s, H-14′), 1.09 (3H, s, H-12′), 1.63 (1H, br s, H-13′), 3.24 (2H, d, H-1′), 3.37 (1H, br d, J = 9.1 Hz, H-6′), 3.69 (1H, br t, J = 7.4 Hz, H-10′), 5.30 (1H, br t, J = 7.0 Hz, H-2′), 6.68 (1H, d, J = 8.5 Hz, H-5), 7.60 (1H, dd, J = 8.5, 1.7 Hz, H-6), 7.66 (1H, d, J = 1.7 Hz, H-2); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS *m*/*z* 205 (5), 189 (21), 151 (48), 143 (46), 107 (18), 85 (22), 71 (36), 59 (79), 43 (100); HRFABMS *m*/*z* 391.2101 [M − H]$^-$ (calcd for $C_{22}H_{31}O_6$ 391.2121).

Acetylation of 3. Compound **3** (1.0 mg) was subjected to acetylation with Ac_2O−pyridine for 12 h at room temperature to give **3a** (1.0 mg). **3a**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.12 (6H, s, H-14′,15′), 1.18 (3H, s, H-12′), 1.71 (1H, br s, H-13′), 2.03, 2.29 (each 3H, s, OAc), 3.77 (1H, dd, J = 3.6, 8.3 Hz, H-10′), 4.89 (1H, dd, J = 1.9, 8.5 Hz, H-6′), 5.23 (1H, br t, J = 6.8 Hz, H-2′), 7.02 (1H, d, J = 8.2 Hz, H-5), 7.82 (1H, br d, J = 8.2 Hz, H-6), 7.88 (1H, br s, H-2); HRFABMS *m*/*z* 499.2320 [M + Na]$^+$ (calcd for $C_{26}H_{36}O_8Na$ 499.2295).

Kuhistanol D (4): amorphous powder, $[\alpha]^{25}_D$ +2.4° (*c* 1.0, MeOH); IR (KBr) ν_{max} 3425, 3329, 2918, 1681, 1263, 1111 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 288 (3.6), 260 (4.2), 212 (4.3) nm; ^{1}H NMR (CD_3OD, 400 MHz) δ 1.26 (3H, s, H-13′), 1.57 (3H, s, H-14′), 1.59 (3H, s, H-15′), 1.64 (3H, s, H-12′), 2.78 (1H, dd, J = 16.7, 5.37 Hz, Ha-1′a), 3.04 (1H, dd, J = 16.7, 7.4 Hz, Hb-1′), 3.87 (1H, dd, J = 7.4, 5.3 Hz, H-2′), 5.06 (1H, t, J = 7.0 Hz, H-10′), 5.13 (1H, t, J = 7.2 Hz, H-6′), 6.80 (1H, d, J = 8.4 Hz, H-5), 7.73 (1H, dd, J = 8.4, 1.9 Hz, H-6), 7.76 (1H, d, J = 1.9 Hz, H-2); ($CDCl_3$, 400 MHz) δ 1.37 (3H, s, H-13′), 1.59 (6H, s, H-14′, H-15′), 1.68 (3H, s, H-12′), 2.85 (1H, dd, J = 16.8, 5.9 Hz, Ha-1′), 3.11 (1H, dd, J = 16.8, 4.4, Hz, Hb-1′), 3.95 (1H, br t, H-2′), 5.09 (2H, m, H-6′, H-10′), 6.88 (1H, d, J = 9.0 Hz, H-5), 7.87 (2H, br s, H-2, H-6); ^{13}C NMR (CD_3OD, 100 MHz), see Table 1; EIMS *m*/*z* 358 (10), 315 (28), 297 (32), 207 (39), 151 (61), 136 (15), 109 (47), 69 (100); HREIMS *m*/*z* 358.2151 [M]$^+$ (calcd for $C_{22}H_{30}O_4$, 358.2144).

Methylation of Kuhistanol D (4). A solution of **4** (5.5 mg) was treated with $(CH_3)_3SiCHN_2$ and left overnight at room temperature to give **4a** (5.0 mg). **4a**: ^{1}H NMR($CDCl_3$, 400 MHz) δ 1.37 (3H, s, H-13′), 1.59 (6H, s, 14′, H-15′), 1.68 (3H, s, H-12′), 2.81 (1H, dd, J = 16.8, 5.8 Hz, Ha-1′), 3.10 (1H, dd, J = 16.8, 4.4 Hz, Hb-1′), 3.88 (3H, s, OMe), 3.95 (1H, dd, 2′-H), 5.08 (2H, m, H-6′, H-10′), 6.85 (1H, d, J = 9.2 Hz, H-5), 7.80 (2H, br s, H-2, H-6).

Acetylation of 4a. Compound **4a** (5.0 mg) was subjected to acetylation with Ac_2O−pyridine and left overnight at room temperature to give **4b** (3.0 mg). **4b**: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.33 (3H, s, H-13′), 1.56 (3H, s, H-14′), 1.57 (3H, s, H-15′), 1.66 (3H, s, H-12′), 2.06 (3H, s, OAc), 2.81 (1H, dd, J = 17.2, 5.0 Hz, Ha-1′), 3.16 (1H, dd, J = 17.2, 4.8 Hz, Hb-1′), 3.87 (3H, s, OMe), 5.06 (2H, br t, J = 6.6 Hz, H-6′, H-10′), 5.12 (1H, br t, J = 4.9 Hz, H-2′), 6.85 (1H, d, J = 8.5 Hz, H-5), 7.77 (1H, s, H-2), 7.81 (1H, d, J = 8.5 Hz, H-6).

Determination of the Absolute Configurations of Kuhistanol D (4). A solution of **4a** (2.55 mg, 6.7 mmol), dehydrated pyridine (0.2 mL), and 2,4,6-trinitrochlorobenzene (40 mg, 0.17 mmol) was treated with (+)-MTPA (40 mg, 0.16 mmol) under N_2 and stirred for 18 h. Then, 5% $NaHCO_3$ (2 mL) and diethyl ether (2 mL) were added and stirred until the sediments were dissolved. After evaporation of the solvent, the residue was washed with H_2O, saturated $CuSO_4$, and brine, and dried with Na_2SO_4 in turn, to yield on removal of solvent, (*R*)-MTPA ester **4c** (2.0 mg). Using (−)-MTPA and the same experimental method gave (*S*)-MTPA ester **4d** (2.0 mg). **4c**: ^{1}H NMR (CD_3OD, 400 MHz) δ 1.28, 1.32 (each 3H, s), 1.54 (12H, s), 1.63 (6H, s), 3.86, 3.87 (each 3H, s, OMe), 5.01 (4H, m), 5.33 (2H, m), 6.79 (1H, d, J = 8.3 Hz), 6.86 (1H, d, J = 9.3 Hz), 7.72, 7.81 (each 1H, s), 7.73, 7.80 (each 1H, br d). The ^{1}H NMR spectrum of **4d** was the same as that of **4c**.

Bioassay. Whole blood from healthy volunteers was used in a screen for immunosuppresive activity of isolated compounds **1**−**4**. The procedure was the same as a previously published method.[8]

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