Sesquiterpene Phenylpropanoids from *Ferula fukanensis* and Their Nitric Oxide Production Inhibitory Effects[1]

Tsunetake Motai and Susumu Kitanaka*

College of Pharmacy, Nihon University, 7-7-1 Narashinodai, Funabashi, Chiba 274-8555, Japan

Received November 30, 2004

Five new sesquiterpene phenylpropanoid derivatives, fukanedone A (**1**), fukanedone B (**2**), fukanedone C (**3**), fukanedone D (**4**), and fukanedone E (**5**), and a novel phenyl-oxo-acetate ester, fukaneketoester A (**6**), were isolated from a 80% aqueous methanol extract of the roots of *Ferula fukanensis*. The structures were elucidated on the basis of spectroscorpic evidence, especially heteronuclear multiple-bond connectivity (HMBC) and high-resolution MS. The sesquiterpene phenylpropanoid derivatives inhibited nitric oxide (NO) production and inducible NO synthase (iNOS) gene expression by a murine macrophage-like cell line (RAW 264.7), which was activated by lipopolysaccharide (LPS) and recombinant mouse interferon-γ (IFN-γ).

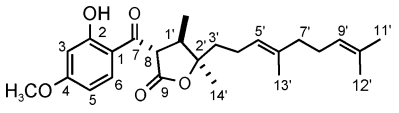
Ferula fukanensis grows on arid land in Central Asia and has been used as a traditional medicine for treatment of rheumatoid arthritis and bronchitis. Previous studies have analyzed the polysulfanes in this plant by GC-MS (CI/EI),[2] and the chemical constituents of plants in the genus *Ferula* (Umbelliferae) have been studied by many groups. In previous papers,[3,4] we reported the isolation of sesquiterpene coumarin derivatives from *F. fukanensis*. Compounds commonly found in this genus are sesquiterpenes[5−11] (especially daucanes, humulanes, and guaianes), sesquiterpene coumarins, and sesquiterpene chromones.[12−17] In our continuing investigations of in vitro anti-inflammatory effects of medicinal herbal extracts, the 80% aqueous methanol extract of the roots of *F. fukanensis* was observed to inhibit nitric oxide (NO) production in a lipopolysaccharide (LPS)-activated murine macrophage-like cell line.

Macrophages play major roles in the immunity and inflammatory responses involved in host defense. Once activated, they initiate the production of cytokines, oxygen and nitrogen species, and eicosanoids. In macrophages, bacterial lipopolysaccharide (LPS), alone or in combination with recombinant mouse interferon-γ (IFN-γ), is the stimulus best known to induce the transcription of gene-encoding pro-inflammatory proteins. Such stimulation results in cytokine release and the synthesis of enzymes such as cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS). The nitric oxide (NO) radical is known to play a central role in inflammatory and immune reactions. However, excessive production of NO may cause tissue damage. In inflammatory diseases such as rheumatoid arthritis, excessive NO production by activated macrophages has been observed.

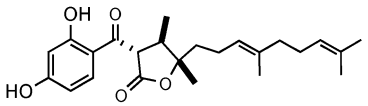
We discovered that 80% aqueous methanol extract of the roots of *F. fukanensis* (FFE) inhibited activated macrophage NO production (IC_{50} = 21.9 μg/mL).[3] Therefore, analysis of FFE was undertaken to identify the active compounds in the extract. Through bioactivity-guided fractionation, active compounds that inhibited NO production were isolated from the $CHCl_3$ fraction. FFE and these compounds also inhibited iNOS mRNA expression in RAW 264.7 cells treated with LPS and IFN-γ.

Results and Discussion

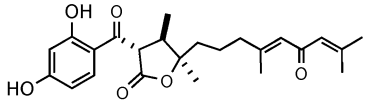
FFE was partitioned by successive extraction with $CHCl_3$, ethyl acetate, and H_2O. The $CHCl_3$-soluble fraction inhibited 60% of NO production at 30 μg/mL. Therefore, the $CHCl_3$-soluble fraction was chromatographed on a silica gel column to yield 11 fractions. Fraction 3 inhibited NO production 100% at 30 μg/mL, and fraction 6 inhibited 96.2% of NO production at 30 μg/mL. Compounds **1**−**6** were isolated from fractions 3 and 6.



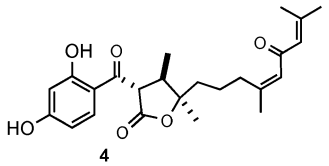
1



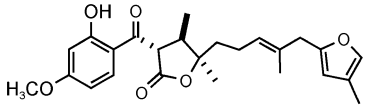
2



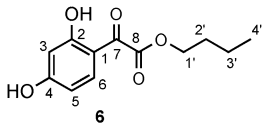
3



4



5



6

Fukanedone A (**1**) was obtained as a colorless oil. HREIMS showed the molecular ion peak at *m/z* 414.2397, indicating a molecular formula of $C_{25}H_{34}O_5$. The ^{1}H NMR

* To whom correspondence should be addressed. Tel: 0081-47-465-5356. Fax: 0081-47-465-5440. E-mail: kitanaka@pha.nihon-u.ac.jp.

10.1021/np040215c CCC: $30.25
Published on Web 03/03/2005

Table 1. ^{1}H NMR Data for Compounds **1** and **5** (300 MHz) and **2**−**4** (500 MHz) ($CDCl_3$, TMS, δ (ppm), J = Hz)[a]

H	1	2	3	4	5
3	6.43 d (2.3)	6.33 brs[b]	6.37 brs[b]	6.40 m[b]	6.43 d (2.3)
5	6.49 dd (2.3, 8.8)	6.33 brdd[b]	6.39 brdd[b]	6.40 m[b]	6.49 dd (2.3, 9.1)
6	7.66 d (8.8)	7.56 d (8.8)	7.59 d (9.4)	7.59 d (9.5)	7.66 d (9.1)
8	4.25 d (12.0)	4.25 d (11.7)	4.23 d (12.0)	4.24 d (11.9)	4.25 d (12.0)
1′	3.09 m	3.15 m	3.10 m	3.06 m	3.09 m
3′	1.57 m	1.82 m	1.56 m	1.65 m[b]	1.62 m
4′	2.18 m	2.18 m	1.68 m	1.65 m[b]	2.23 m
5′	5.09 m[b]	5.09 m[b]	2.18 m	2.60 m	5.20 t (7.0)
				2.78 m	
7′	2.0 m	2.0 m	6.06 brs	6.08 s	3.24 s
8′	2.07 m	2.06 m			
9′	5.09 m[b]	5.09 m[b]	6.09 brs	6.11 s	5.89 s
11′	1.69 s	1.68 s	1.91 s	1.91 s	7.07 brs
12′	1.61 s	1.60 s	2.19 s	2.16 s	1.99 s
13′	1.63 s	1.63 s	2.16 s	1.92 s	1.64 s
14′	1.52 s	1.36 s	1.51 s	1.49 s	1.51 s
15′	1.09 d (7.0)	1.07 d (6.7)	1.06 d (6.7)	1.04 d (7.0)	1.08 d (6.7)
OMe	3.85 s				3.85 s
3OH	12.45 s	12.35 s	12.31 s	12.36 s	12.43 s

[a] Assignments confirmed by decoupling,^{1}H−^{1}H COSY, HMQC, HMBC, NOESY, and difference NOE spectra. [b] Overlapped signal.

data of **1** (Table 1) showed signals of a 1,2,4-trisubstituted benzene ring at δ_H 7.66 (1H, d, J = 8.8 Hz, H-6), 6.49 (1H, dd, J = 8.8, 2.3 Hz, H-5), and 6.43 (1H, d, J = 2.3 Hz, H-3), a methoxy group at δ_H 3.85, a chelated phenolic hydroxyl group at δ_H 12.45, and other signals characteristic of a sesquiterpene unit, as determined on the basis of correlations of ^{1}H−^{1}H COSY, HMQC, and HMBC spectra. The correlations of δ_H 3.85 (OMe) with δ_H 6.49 (H-5) and 6.43 (H-3) in the NOE experiments suggested that the methoxy group was connected to C-4.

In the HMBC spectrum of **1**, the correlations of δ_H 7.66 (H-6) with C-2, C-4, and C-7 and δ_H 12.45 (Ar-OH) with C-1, C-2, and C-3 indicated that the carbonyl group at δ_C 195.9 (C-7) was conjugated with the aromatic ring. In the ^{1}H−^{1}H COSY spectrum of **1**, H-1′ (δ_H 3.09) was correlated with H-15′ (δ_H 1.09) and H-8 (δ_H 4.25) and when considered in combination with the observed HMBC correlations of H-8 with C-7, C-9, C-1′, and C-2′; H-1′ with C-7, C-8, C-9, and C-2′; and H-15′ with C-8, C-1′, and C-2′, verified that C-1′ was connected to C-8. On the basis of the above analysis and the chemical shifts of C-9 and C-2′, the ester group of C-9 must be connected to C-2′. The characteristic carbonyl absorption (ν 1767 cm^{-1}) in the IR spectrum of **1** supported the presence of a γ-lactone in this compound.

The relative configuration of **1** at C-8, C-1′, and C-2′ and the configuration of the double bond of the sesquiterpene unit were determined on the basis of the NOE experiments. Compound **1** showed significant NOE correlations between H-8 and H-3′, H-15′, between H-1′ and H-14′, and between H-5′ and H-7′. Thus, **1** is 3*S**-(2-hydroxy-4-methoxybenzoyl)-4*R**,5*R**-dimethyl-5-[4,8-dimethyl-3(*E*)-7-nonadien-1-yl]tetrahydro-2-furanone.

Fukanedone B (**2**) was obtained as a colorless oil. The HREIMS showed the molecular ion peak at *m/z* 400.2249, which suggested a molecular formula of $C_{24}H_{32}O_5$. The ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1**, except for the absence of a methoxy group (δ_H 3.85 for **1**). However, the NMR spectra of **2** differed slightly from those of **1**, especially at C-3′ (δ_C 40.2 for **2**, δ_C 35.9 for **1**) and C-14′ (δ_C 21.0 for **2**, δ_C 24.4 for **1**). In the NOESY spectrum of **2**, H-8 correlated with H-14′ and H-15′ and H-1′ correlated with H-3′. Thus, **2** is 3*S**-(2,4-dihydroxybenzoyl)-4*R**,5*S**-dimethyl-5-[4,8-dimethyl-3(*E*)-7-nonadien-1-yl]tetrahydro-2-furanone.

Fukanedone C (**3**) was obtained as a colorless oil. The HREIMS showed the molecular ion peak at *m/z* 414.2043, which suggested a molecular formula of $C_{24}H_{30}O_6$. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **2**. The double bond and the carbonyl group of the sesquiterpene unit were conjugated according to their NMR spectral data (δ_H 6.06, brs, H-7′; δ_C 191.5, C-8′; δ_C 156.2, C-6′; δ_C 125.7, C-7′). This was confirmed by the correlations of H-7′ with C-8′, C-9′, and C-6′ and of H-9′ with C-8′, C-7′, C-10′, and C-11′ in the HMBC spectrum of **3**. The double bond at C-6′ has an *E* configuration based on the chemical shifts of C-13′ (δ_C 19.1).[17,18] The relative configuration of **3** at C-8, C-1′, and C-2′ was determined on the basis of the NOE experiments and comparison of the ^{13}C NMR data with those of **1**. Thus, **3** is 3*S**-(2,4-dihydroxybenzoyl)-4*R**,5*R**-dimethyl-5-[4,8-dimethyl-4(*E*)-7-nonadien-6-onyl]tetrahydro-2-furanone.

Fukanedone D (**4**) was obtained as a colorless oil. The HREIMS showed the molecular ion peak at *m/z* 414.2047, which suggested a molecular formula of $C_{24}H_{30}O_6$. The ^{1}H and ^{13}C NMR spectra of **4** were similar to those of **3**. However, the NMR spectra of **4** differed slightly from those of **3**, especially the C-13′ methyl signal (δ_C 25.4 for **4**, δ_C 19.1 for **3**) and the C-5′ methylene signal (δ_C 33.6 for **4**, δ_C 41.0 for **3**). The configuration of the double bond of the sesquiterpene unit was determined on the basis of the differences in NOE experiments. Compound **4** showed significant NOE correlations between H-7′ and H-9′ and H-13′, indicating that the double bond of the sesquiterpene unit of **4** has the *Z* configuration. Thus, **4** is 3*S**-(2,4-dihydroxybenzoyl)-4*R**,5*R**-dimethyl-5-[4,8-dimethyl-4(*Z*)-7-nonadien-6-onyl]tetrahydro-2-furanone.

Fukanedone E (**5**) was obtained as a colorless oil. The HREIMS showed the molecular ion peak at *m/z* 426.20440, which suggested a molecular formula of $C_{25}H_{30}O_5$. The ^{1}H and ^{13}C NMR spectra of **5** were similar to those of **1**, except for the presence of the 4-methyl-2-furyl system (δ_H 1.99, 5.89, 7.07; δ_C 9.8, 108.6, 120.1, 137.3, 153.3) at C-7′ in place of the trisubstitued olefin present in **5**. Thus, **5** is 3*S**-(2-hydroxy-4-methoxybenzoyl)-4*R**,5*R**-dimethyl-5-[4-methyl-5-(4-methyl-2-furyl)-3(*E*)-penten-1-yl]tetrahydro-2-furanone.

Fukaneketoester A (**6**) was obtained as a light yellow oil, with a molecular weight of 238 based on fast atom bombardment mass spectrometry (FABMS) data that showed a protonated molecular ion peak at *m/z* 239 $(M + H)^+$ and a deprotonated molecular ion at *m/z* 237 $(M - H)^-$ in the negative mode. These data, together with the ^{1}H and

Table 2. ^{13}C NMR Data for Compounds **1** and **5** (75 MHz) and **2**−**4** (125 MHz) ($CDCl_3$, TMS, δ (ppm))[a]

C	1	2	3	4	5
1	114.1	114.1	113.5	114.0	113.5
2	166.3	164.4	163.7	164.4	165.6
3	101.1	103.7	103.1	103.5	100.5
4	166.9	166.0	165.5	166.1	166.3
5	108.6	109.0	108.1	108.7	107.9
6	133.1	133.4	133.1	133.8	132.4
7	195.9	195.5	194.8	195.9	195.2
8	54.9	54.9	54.1	54.4	54.2
9	171.2	172.4	171.0	171.7	170.4
1′	44.5	41.8	43.8	44.1	43.9
2′	87.9	89.0	87.4	88.0	87.1
3′	35.9	40.2	34.4	35.0	35.1
4′	22.5	22.7	21.1	22.0	22.0
5′	123.2	123.0	41.0	33.6	124.8
6′	136.5	136.4	156.2	158.3	132.7
7′	40.2	40.1	125.7	126.7	38.2
8′	27.2	27.1	191.5	191.9	153.3
9′	124.3	124.3	125.7	126.4	108.6
10′	131.7	131.6	155.2	155.5	120.1
11′	26.3	26.2	27.8	27.8	137.3
12′	18.3	18.2	20.7	20.8	9.8
13′	16.7	16.6	19.1	25.4	16.0
14′	24.4	21.0	23.8	23.8	23.7
15′	13.3	13.9	12.7	12.6	12.7
OMe	56.2				55.5

[a] Assignments confirmed by decoupling, $^1H-^1H$ COSY, HMQC, HMBC, NOESY, and difference NOE spectra.

Table 3. NMR Data for Compound **6** (300 MHz for ^1H NMR, 75 MHz for ^{13}C NMR, $CDCl_3$, TMS, δ (ppm), J = Hz)[a]

position	δ_C	δ_H
1	109.1	
2	165.1	
3	102.3	6.41 brs
4	163.1	
5	107.7	6.42 dd
6	133.1	7.60 d (9.1)
7	186.7	
8	161.3	
1′	65.1	4.37 t (6.7,13.5)
2′	29.2	1.74 m
3′	17.9	1.44 m
4′	12.5	0.96 t (7.3,14.7)
OH		11.62 s

[a] Assignments confirmed by decoupling, $^1H-^1H$ COSY, HMQC, and HMBC spectra.

^{13}C NMR data (Table 3), suggested a molecular formula of $C_{12}H_{14}O_5$, which was supported by HRFABMS in the negative mode ($C_{12}H_{13}O_5$, *m/z* 237.0760). The ^1H and ^{13}C NMR spectra of **6** showed the existence of a 1,2,4-trisubstituted benzene ring at δ_H 7.60 (1H, d, J = 9.1 Hz, H-6), δ_H 6.41 (2H, m, H-3, 5), a chelated phenolic hydroxyl group at δ_H 11.62 (1H, s), a ketone at δ_C 186.7 (C-7), and an ester at δ_C 161.3 (C-8).

In the $^1H-^1H$ COSY spectrum of **6**, the correlations of δ_H 1.74 (H-2′) with the oxymethylene at δ_H 4.37 (H-1′) and the methylene at δ_H 1.44 (H-3′) with the methyl group at δ_H 0.96 (H-4′) and H-2′ suggested that **6** has a butoxy group. Furthermore, in the HMBC spectrum of **6**, the correlations of H-1′ with C-8 suggested that the butoxy group was conjugated with the carbonyl group at C-8. Thus, **6** is (2,4-dihydroxyphenyl)-oxo-acetic acid butyl ester. This compound is isolated for the first time from natural sources.

When the RAW 264.7 was incubated with LPS and IFN-γ, NO production increased sharply. Compounds **3**−**6** showed inhibitory activity (**3**: IC_{50} = 6.7 μM; **4**: IC_{50} = 27.8 μM; **5**: IC_{50} = 76.2 μM; **6**: IC_{50} = 112.6 μM). The cytotoxic effects of these compounds were measured using the MTT assay.[21] Compounds **3** and **4** (0.3−10 μg/mL) and **5** and **6** (1−30 μg/mL) did not demonstrate any significant cytotoxicity upon LPS/IFN-γ treatment for 24 h. Compounds **1** and **2** did not inhibit NO production at 30 μg/mL. Compounds **1** and **2**, neither of which contain a ketone in the sesquiterpene unit, did not inhibit NO production. Compound **5** showed some NO inhibitory activity. However, **3** and **4**, which have an α,β-unsaturated ketone, inhibited NO production the most. This suggests that the α,β-unsaturated ketone moiety of the sesquiterpene unit may be important for the inhibitory activity of sesquiterpene phenylpropanoid derivatives.

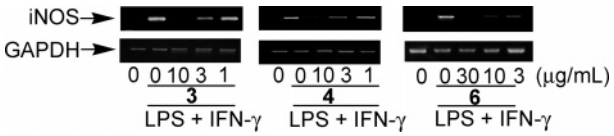


Figure 1. Inhibitory effect of compounds **3**, **4**, and **6** on gene expression stimulated by LPS and IFN-γ.

Experimental Section

General Experimental Procedures. UV spectra were obtained in MeOH on a Shimadzu UV-160 spectrophotometer, and IR spectra were recorded on a JASCO IR A-2 spectrophotometer. The NMR spectra were taken on Mercury-300BB Varian and JNM-GSK 500 FT NMR spectrometers, with TMS as an internal standard. The mass spectra (MS) were obtained on a JEOL GCmate spectrometer. Column chromatography was carried out on silica gel (Wako gel C-300, WAKO Pure Chemical Industry Ltd.). TLC was performed on Merck TLC plates (0.25 mm thickness), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in EtOH and then heating on a hot plate. HPLC was performed on a JASCO PU-2089 apparatus equipped with a JASCO UV-2075 detector. YMC-Pak SIL-06 (10 × 150 mm i.d.) columns and YMC-PAK Pro-C_{18} (10 × 150 mm i.d.) were used for preparative purposes.

Plant Material. The dried roots of *F. fukanensis* were collected in the Urumqi, Xinjiang, People's Republic of China, in October 2002. Voucher specimens (NK03044) have been deposited at College of Pharmacy, Nihon University.

Extraction and Isolation. The dried roots of *F. fukanensis* (5.9 kg) were chopped and extracted twice with 80% MeOH (18 L). The solvent was evaporated under reduced pressure from the combined extract (448 g). The extract was suspended in H_2O (3.0 L) and partitioned with $CHCl_3$ (3 × 3 L) and EtOAc (3 × 3 L), successively. Evaporation of the solvent yielded a $CHCl_3$ fraction [272 g, inhibitory effect 60% (30 μg/mL)], an EtOAc fraction (142 g, cytotoxic effect), and an aqueous fraction (96 g, 33%). The $CHCl_3$ fraction was subjected to silica gel column chromatography (12 × 17 cm, eluted with hexane and EtOAc in increasing polarity). The fractions (200 mL each) were combined according to TLC monitoring into 11 portions. Fraction 3, eluted with hexane−EtOAc (90:10), was isolated and further purified by column chromatography and reversed-phase HPLC (CH_3CN-H_2O, 75:25) to give **1** (13.6 mg) and by normal-phase HPLC (hexane−EtOAc, 85:15) to give **5** (13.3 mg). Fraction 6, eluted with hexane−EtOAc (80:20), was isolated and further purified by column chromatography and reversed-phase HPLC (CH_3CN-H_2O, 72:28) to give **2** (20.6 mg), by normal-phase HPLC (hexane−EtOAc, 68:32) to give **3** (4.0 mg) and **4** (2.6 mg), and by reversed-phase HPLC (CH_3CN-H_2O, 71:29) to give **6** (89.2 mg).

Fukanedone A (1): colorless oil; $[\alpha]^{23}_D$ +20.3° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 322 (3.89), 283 (4.21), 231 (3.93), 211 (4.22) nm; IR (LF) ν_{max} 2970, 2929, 2853, 1767, 1631, 1576, 1509, 1444, 1375, 1332, 1281, 1210, 1158, 1127, 1067, 1027, 962, 903, 838, 800, 735 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 414 $[M]^+$; HREIMS *m/z* 414.2397 $[M]^+$ (calcd for $C_{25}H_{34}O_5$ 414.2406).

Fukanedone B (2): colorless oil; $[\alpha]^{23}_D$ −9.6° (*c* 1.59, MeOH); UV (MeOH) λ_{max} (log ϵ) 329 (3.98), 285 (4.10), 233

(3.84), 214 (4.14) nm; IR (KBr) ν_{max} 3370, 2974, 2912, 2854, 1742, 1637, 1510, 1446, 1388, 1375, 1325, 1289, 1242, 1204, 1175, 1154, 1110, 1059, 1024, 967, 906, 854, 779 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 400 $[M]^+$; HREIMS *m/z* 400.2249 $[M]^+$ (calcd for $C_{24}H_{32}O_5$ 400.2250).

Fukanedone C (3): colorless oil; $[\alpha]^{23}_D$ +72.0° (*c* 0.25, MeOH); UV (MeOH) λ_{max} (log ϵ) 334 (3.96) 276 (4.47), 242 (4.12), 213 (4.21) nm; IR (KBr) ν_{max} 3276, 2963, 2930, 1764, 1629, 1509, 1444, 1375, 1329, 1287, 1261, 1230, 1125, 1073, 1027, 971, 905, 853, 802 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 414 $[M]^+$; HREIMS *m/z* 414.2043 $[M]^+$ (calcd for $C_{24}H_{30}O_6$ 414.2042).

Fukanedone D (4): colorless oil; $[\alpha]^{23}_D$ +35.0° (*c* 0.16, MeOH); UV (MeOH) λ_{max} (log ϵ) 325 (3.64), 277 (4.14), 242 (3.79), 214 (3.87) nm; IR (KBr) ν_{max} 3294, 2959, 2576, 1717, 1681, 1386, 1308, 1287, 1240, 1211, 1170, 1146, 1098, 1006, 945, 913, 863, 810 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 414 $[M]^+$; HREIMS *m/z* 414.2047 $[M]^+$ (calcd for $C_{24}H_{30}O_6$ 414.2042).

Fukanedone E (5): colorless oil; $[\alpha]^{23}_D$ +18.1° (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 321 (4.75), 283 (5.07), 231 (4.88), 213 (5.12) nm; IR (KBr) ν_{max} 2973, 2937, 1765, 1629, 1576, 1629, 1576, 1444, 1373, 1332, 1279, 1210, 1158, 1127, 1069, 1026, 962, 904, 866, 839, 802, 756 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 1 and 2; EIMS *m/z* 426 $[M]^+$; HREIMS *m/z* 426.2044 $[M]^+$ (calcd for $C_{25}H_{30}O_6$ 426.2042).

Fukaneketoester A (6): light yellow oil; UV (MeOH) λ_{max} (log ϵ) 320 (3.53), 289 (3.61), 235 (3.32), 212 (3.58) nm; IR (KBr) ν_{max} 3346. 3181, 2963, 2936, 2875, 1744, 1630, 1503, 1438, 1392, 1348, 1325, 1211, 1129, 1058, 989, 969, 929, 857, 812, 759 cm^{-1}; ^1H and ^{13}C NMR data, see Tables 3; FABMS *m/z* 237 $[M - H]^-$; HRFABMS *m/z* 237.0756 $[M - H]^-$ (calcd for $C_{12}H_{13}O_5$ 237.0763).

Nitrite Assay.[22,23] The cells were seeded at 1.2×10^6 cells/mL onto a 96-well flat bottom plate (Sumitomo Bakelite, #8096R, Tokyo) and then incubated at 37 °C for 2 h. Then the test compound was added to the culture simultaneously with both *Escherichia coli* LPS (100 ng/mL) and recombinant mouse IFN-γ (0.33 ng/mL), and the cells were incubated at 37 °C, usually for 16 h. After incubation, the cells were chilled on ice. One 100 μL aliquot of the culture supernatant was placed in a well in duplicate 96-well flat-bottomed plates. A standard solution of $NaNO_2$ was also placed in other wells on the same plate. To quantify nitrite, Griess reagent 50 μL, 1% sulfanilamide in 5% H_3PO_4, and 0.1% N-1-naphthyletylenediamide dihydrochloride were added to each well. After 10 min, the reaction products were colorimetrically quantified at 550 nm with subtraction of the background absorbance at 630 nm, using a Model 3550 microplate reader (BIO-RAD).

Reverse Transcription-Polymerase Chain Reaction Analysis of iNOS mRNA. The cells were cultured at 1.2×10^6 cells/mL onto a 96-well flat bottom plate at 37 °C for 2 h, then the test compound was added to the culture simultaneously with both LPS (100 ng/mL) and IFN-γ (0.33 ng/mL). The cells were incubated at 37 °C for approximately 8 h. Total RNA was isolated from the cell pellet using an RNA isolation kit (QIAGEN, Hilden, Germany). Total RNA (250 ng) was reverse-transcribed into cDNA by oligo $(dT)_{12-18}$ primer. The PCR sample contained 30 μL of the reaction mixture, comprised of 50 mM KCl, 5 mM $MgCl_2$, 0.2 mM dNTP, 0.6 units of Ampli Taq GOLD (Applied Biosystems, CA), and 0.4 μmol of sense and antisense primers. The sense primer for iNOS was 5′-ACCTACTTCCTGGACATTACGACCC-3′, and the antisense primer was 5′-AAGGGAGCAATGCCCGTACCAGGCC-3′. The sense primer for glyceraldehydes-3-phosphatedehydrogenase (GAPDH) was 5′-ACCACAGTCCATGCCATCAC-3′, and the antisense primer was 5′-TCCACCACCCTGTTGCTGTA-3′. The PCR reaction was performed under the following conditions: 25 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1.5 min, using a thermal cycler (GeneAmp PCR Systems 9770; PE Applied Biosystems). The PCR products were run on a 2% agarose gel and visualized by ethidium bromide staining. The bands in the gel were then photographed.

Acknowledgment. This work was supported by a Special Research Grant-in-Aid for the Promotion and Mutual Aid Corporation for Private Schools of Japan to Nihon University and an "Academic Frontier" Project for Private Universities: matching fund subsidy from MEXT (Ministry of Education, Culture, Sports, Science and Technology) 2002−2006 of Japan.

References and Notes

(1) Part 11 in the series "Antiallergic Agents from Natural Sources". Part 10: Wang, L.; Unehara, T.; Kitanaka, S. *Chem. Pharm Bull*. **2005**, *53*, 137−139.
(2) Min, Z-da.; Mai, Qi-fi.; Mizuno, M.; Tanaka, T.; Iinuma M. *Planta Med*. **1987**, 300−302.
(3) Motai, T.; Daikonya, A.; Kitanaka, S. *J. Nat. Prod*. **2004**, *67*, 432−436.
(4) Motai, T.; Kitanaka S. *Chem. Pharm. Bull*. **2004**, *52*, 1215−1218.
(5) Khasanov, T. K.; Saidkhodzhaev, A. I.; Nikonov, G. K. *Khim. Prir. Soedin*. **1972**, *6*, 807−808.
(6) Potapov, V. M.; Nikonov, G. K. *Khim. Prir. Soedin*. **1976**, *6*, 819−820.
(7) Kushmuradov, T. K.; Makhmudov, M. K.; Saidkhodzhaev, A. I.; Tashkhodzhaev, B.; Malikov, V. M.; Yagudaev, M. *Khim. Prir. Soedin*. **1990**, *1*, 42−46.
(8) Kushmuradov, A. Y.; Saidkhodzhaev, A. I.; Kadyrov, A. Sh. *Khim. Prir. Soedin*. **1981**, *3*, 400.
(9) Kushmuradov, A. Y.; Saidkhodzhaev, A. I.; Kadyrov, A. S. *Khim. Prir. Soedin*. **1986**, *4*, 523−524.
(10) Kushmuradov, A. Y.; Saidkhodzhaev, A. I.; Kadyrov, A. S. *Khim. Prir. Soedin*. **1986**, *1*, 53−56.
(11) Kojima, K.; Isaka, K.; Ondognii, P.; Jargalsaikhan, G.; Suran, D.; Mizukami H.; Ogihara, Y. *Chem. Pharm. Bull*. **1998**, *46*, 1781−1784.
(12) Kojima, K.; Isaka, K.; Ondognii, P.; Jargalsaikhan, G.; Suran, D.; Mizukami, H.; Ogihara, Y. *Chem. Pharm. Bull*. **1999**, *47*, 690−691.
(13) Kojima, K.; Isaka, K.; Ondognii, P.; Zevgeegiin, O.; Davgiin, K.; Mizukami, H.; Ogihara, Y. *Chem. Pharm. Bull*. **1999**, *47*, 1145−1147.
(14) Kojima, K.; Isaka, K.; Ondognii, P.; Zevgeengiin, O.; Gombosurengyin, P.; Davgiin, K.; Mizukami, H.; Ogihara, Y. *Chem. Pharm. Bull*. **2000**, *48*, 353−356.
(15) Isaka, K.; Nagatsu, I.; Ondognii, P.; Zevgeegiin, O.; Gombosurengyin, P.; Davgiin, K.; Kojima, K.; Ogihara, Y. *Chem. Pharm. Bull*. **2001**, *49*, 1072−1076.
(16) Nagatsu, A.; Isaka, K.; Kojima, K.; Ondognii, P.; Zevgeegiin, O.; Gombosurengyin, P.; Davgiin, K.; Irfan, B.; Iqubal, C. M.; Ogihara, Y. *Chem. Pharm. Bull*. **2002**, *50*, 657−677.
(17) Bao-Ning, S.; Takaishi, Y.; Gisho, H.; Itoh, M.; Takeda, Y.; Kodzhimatov, O. K.; Ashurmetov, O. *J. Nat. Prod*. **2000**, *63*, 436−440.
(18) Dorman, D. E.; Jautelat, M.; Roberts, J. D. *J. Org. Chem*. **1971**, *36*, 2757−2766.
(19) Rao, M., K.; Ghosh, B. *Int. Immunopharmacol*. **1999**, *21*, 435−443.
(20) Kim, H, K.; Cheon, B, S.; Kim, Y, H.; Kim, S. Y.; Kim, H. P. *Biochem. Pharmacol*. **1999**, *58*, 759−765.
(21) Zhao, F.; Nozawa, H.; Daikonya, A.; Kondo, K.; Kitanaka, S. *Biol. Pharm. Bull*. **2003**, *26*, 61−65.
(22) Ishii, R.; Saito, K.; Horie, M.; Shibano, T.; Kitanaka, S.; Amano, F. *Biol. Pharm. Bull*. **1999**, *22*, 647−653.
(23) Daikonya, A.; Kastuki, S.; Wu, Jin-Bin; Kitanaka, S. *Chem. Pharm. Bull*. **2002**, *50*, 657−677.

NP040215C