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Medicinal Flowers. XXIII.[1] New Taraxastane-Type Triterpene, Punicanolic Acid, with Tumor Necrosis Factor-α Inhibitory Activity from the Flowers of *Punica granatum*

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The methanolic extract from the flowers of *Punica granatum* L. (Punicaceae) was found to show inhibitory effect on tumor necrosis factor-α (TNF-α, 1 ng/ml)-induced cytotoxicity in L929 cells. By bioassay-guided separation, a new taraxastane-type triterpene, punicanolic acid (1), was isolated from the active fraction (ethyl acetate-soluble fraction) together with four triterpenes (2—5), two galloyl glucoses (6, 7), two flavones (8, 9), and β-sitosterol. Among the constituents, 1, oleanolic acid (2), maslinic acid (4), 1,2,6-tri-*O*-galloyl β-D-glucopyranoside (6), 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl β-D-glucopyranoside (7), and luteolin (8) significantly inhibited TNF-α-induced cytotoxicity in L929 cells at 30 μM.

Key words *Punica granatum*; punicanolic acid; triterpene; tumor necrosis factor-α inhibitory activity; pomegranate

The Punicaceae plant, *Punica granatum* Linn. (pomegranate in English), is widely distributed in Middle East, extending throughout the Mediterranean, eastward to China and India, and on the Southwest American countries, *etc.* The bark and roots of *P. granatum* are believed to have anthelmintic and vermifuge properties in Ayurvedic medicinal system. From India, Tunisia, and Guatemala, the dried peels are decocted in water and employed both internally and externally for numerous problems demanding astringent and/or germicides, especially for aphthae, diarrhea, and ulcers. The mixtures of pomegrantae seed, juice, and peel products paradoxically have been reported not only to prevent abortion but also conception. In Unani medicinal system, the flower parts serve as a remedy for diabetes mellitus.[2] The chemical constituents from this herbal medicine, several fatty acids, sterols, triterpenes, anthocyanins, flavonoids, and tannins were identified and isolated from the juice, pericarps, leaves, and seeds, and flower parts.[2,3] Pharmacological studies of the flowers of this natural medicine have reported that some constituents exhibited antioxidant activity[3] and the aqueous ethanolic extract showed antidiabetic activity.[4] During the course of our characterization studies on medicinal flowers,[1,5—25] we found that the methanolic extract from the flowers of *P. granatum* was found to inhibit on tumor necrosis factor-α (TNF-α)-induced cytotoxicity in L929 cells. TNF-α mediates a number of forms of organ injury through its induction of cellular apoptosis. In the case of liver, the biological effects of TNF-α have been implicated in hepatic injury induced by hepatic toxins, ischemia/reperfusion, vital hepatitis, and alcohol.[26—28] Therefore, TNF-α is considered to be an important target in research to discover anti-inflammatory and hepatoprotective agents. On the basis of above-mentioned concept, we investigated protective constituents from naturally occurring products on TNF-α-induced cell death in L929 cells, a TNF-α-sensitive cell line.[29] Previously, we have reported the isolation and structure elucidation of several constituents from *Piper chaba*[30] and *Boesenbergia rotunda*[31] with inhibitory effect on TNF-α/actinomycin D-induced cytotoxicity in L929 cells. By bioassay-guided separation, a new taraxastane-type triterpene, punicanolic acid (**1**), was isolated from the active fraction (ethyl acetate-soluble fraction) together with four triterpenes (**2**—**5**), two galloyl glucoses (**6**, **7**), two flavones (**8**, **9**), and β-sitosterol. This paper deals with the isolation and structure elucidation of a new triterpene (**1**) and the effects of the constituents from this herbal medicine on TNF-α inhibitory activity.

The flowers of *P. granatum* (purchased in Urumqi, Xinjiang province, China) were extracted with methanol to give a methanolic extract (59.8% from the dried flowers). As shown in Table 1, the methanolic extract was found to inhibit TNF-α-induced cytotoxicity in L929 cells (inhibition: 44.4±1.1% at 100 μg/ml). The methanolic extract was parti-

Table 1. Effects of the Methanolic Extract from the Flowers of *P. granatum* and Its Fractions on TNF-α-Induced Cytotoxicity in L929 Cells

	Inhibition (%)					
TNF-α (1 ng/ml) Conc. (μg/ml)	− 0	+ 0	+ 3	+ 10	+ 30	+ 100
MeOH ext.	100.0±7.9**	0.0±0.7	−0.1±1.2	2.8±0.8	6.7±2.6*	44.4±1.1**
EtOAc-soluble fraction	100.0±2.4**	0.0±1.0	3.1±1.7	9.8±3.6*	45.3±2.6**	−7.1±2.6
MeOH-eluted fraction	100.0±3.0**	0.0±0.8	3.0±1.1	5.1±1.0**	13.5±0.6**	−3.4±1.3
H_2O-eluted fraction	100.0±2.5**	0.0±1.7	−0.8±0.5	−2.1±0.6	−2.8±1.4	−5.3±0.7*

Each value represents the mean±S.E.M. ($n=4$). Significantly different from the control, $*p<0.05$, $**p<0.01$.

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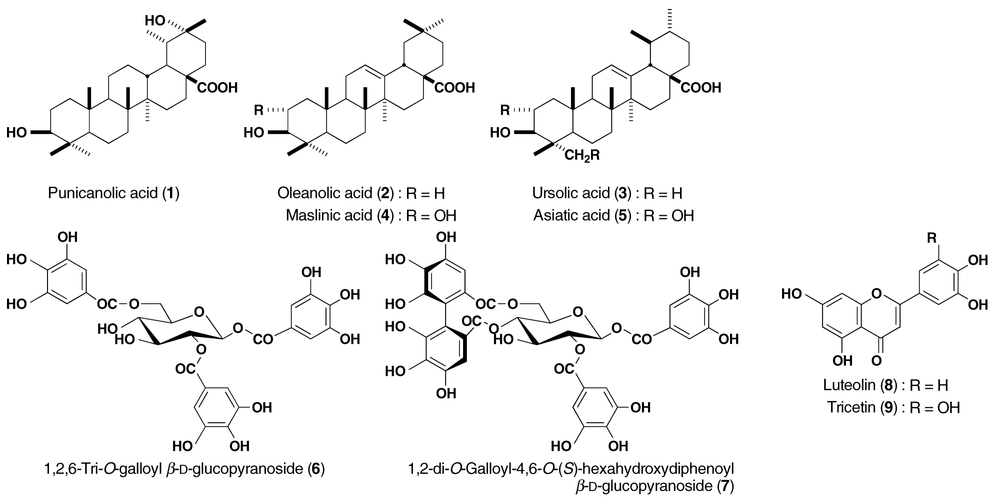

Chart 1

tioned into an EtOAc–H_2O (1 : 1, v/v) mixture to furnish an EtOAc-soluble fraction (9.9%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (H_2O→MeOH→acetone) to give H_2O-, MeOH-, and acetone-eluted fractions (32.2, 13.7, 1.48%), respectively. Among them, the EtOAc-soluble fraction was obtained as an active fraction (45.3±2.6% at 30 μg/ml).

The active fraction (EtOAc-soluble fraction) was subjected to normal- and reversed-phase column chromatographies, and finally HPLC to give punicanolic acid (**1**, 0.005% from the dried flowers) together with oleanolic acid[32] (**2**, 0.30%), ursolic acid[32,33] (**3**, 0.36%), maslinic acid[34] (**4**, 0.0006%), asiatic acid[35,36] (**5**, 0.0019%), 1,2,6-tri-*O*-galloyl β-D-glucopyranoside[37] (**6**, 0.016%), 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl β-D-glucopyraoside[38] (**7**, 0.028%), luteolin[39] (**8**, 0.0095%), tricetin[40] (**9**, 0.0047%), and β-sitosterol[41] (0.076%).

Structure of Punicanolic Acid (1) Punicanolic acid (**1**) was obtained as colorless needles from MeOH (mp 280—281 °C) with negative optical rotation ($[\alpha]_D^{28}$ −7.4° in MeOH). The IR spectrum of **1** showed absorption bands at 3451 and 1719 cm^{-1} ascribable to hydroxy and carbonyl groups. In the EI-MS of **1**, molecular ion peak was observed at *m/z* 474 (M^+), and high-resolution EI-MS analysis revealed the molecular formula of **1** to be $C_{30}H_{50}O_4$. The ^1H- and ^{13}C-NMR (Table 2) spectra (pyridine-d_5) of **1**, which were assigned by various NMR experiments,[42] showed signals assignable to seven methyls [δ 0.86, 1.01, 1.02, 1.09, 1.23, 1.43 (3H each all s, 25, 24, 27, 26, 23, 30-H_3), 1.41 (3H, d, J=6.2 Hz, 29-H_3)], a methine [δ 3.46 (1H, dd, J=5.8, 10.3 Hz, 3-H)] and quaternary carbon (δ_C 72.5, 20-C) bearing an oxygen function, and a carboxyl group (δ_C 179.3, 28-C) together with 10 methylenes, five methines, and five quaternary carbons. As shown in Fig. 1, the ^1H–^1H correlation spectroscopy (^1H–^1H COSY) experiment on **1** indicated the presence of partial structures written in bold lines. In the heteronuclear multiple-bond correlations (HMBC) experiment on **1**, long-range correlations were observed between the following protons and carbons (1-H_2 and 10-C; 3-H, 5-H and 4-C; 9-H and 8-C, 10-C; 13-H, 15-H_2 and 14-C; 16-H_2 and 28-C; 18-H and 17-C, 20-C, 28-C; 21-H_2 and 20-C; 22-H_2 and 28-C; 23-H_3 and 3—5-C, 24-C; 24-H_3 and 3—5-C, 23-C; 25-H_3 and 1-C, 5-C, 9-C, 10-C; 26-H_3 and 7—9-C, 14-C; 27-H_3 and 14-C, 15-C; 29-H_3 and 18-C, 20-C; 30-H_3 and 19—21-C) as shown in Fig. 1. Thus the connectivities of quaternary

Table 2 ^1H- and ^{13}C-NMR Data (600 MHz, Pyridine-d_5) of Punicanolic Acid (**1**)

Position	**1**	
	δ_H	δ_C
1	α 0.98 (m)	39.5
	β 1.70 (ddd, 3.2, 3.6, 14.0)	
2	1.86 (2H, m)	28.3
3	3.46 (dd, 5.8, 10.3)	78.2
4		39.3
5	0.81 (dd, 1.9, 11.6)	55.9
6	1.41 (m)	18.8
	1.55 (m)	
7	1.40 (2H, m)	35.2
8		41.7
9	1.30 (m)	50.5
10		37.4
11	α 1.53 (m)	22.0
	β 1.28 (m)	
12	α 1.37 (m)	30.0
	β 2.03 (m)	
13	2.77 (ddd, 4.0, 10.4, 14.0)	41.0
14		43.1
15	1.22 (m)	29.8
	1.27 (m)	
16	α 1.60 (ddd, 4.0, 13.5, 14.2)	36.1
	β 2.38 (ddd, 2.9, 4.1, 14.2)	
17		51.4
18	1.79 (dd, 10.4, 10.5)	47.9
19	2.50 (dq, 10.5, 6.2)	40.0
20		72.5
21	α 1.86 (m)	37.5
	β 2.10 (ddd, 3.2, 12.9, 16.9)	
22	α 2.32 (ddd, 2.9, 12.9, 14.9)	33.9
	β 2.03 (m)	
23	1.23 (3H, s)	28.7
24	1.01 (3H, s)	16.4
25	0.86 (3H, s)	16.5
26	1.09 (3H, s)	16.8
27	1.02 (3H, s)	15.3
28		179.3
29	1.41 (3H, d, 6.2)	19.0
30	1.43 (3H, s)	30.9

carbons (4, 8, 10, 14, 17, 20, 28-C) in **1** were clarified and the planar structure of **1** was elucidated. Next, the taraxastane-type triterpene skeleton of **1** was characterized by the coupling constants in ^{1}H-NMR experiment and by nuclear Overhauser enhancement spectroscopy (NOESY) experiment. Thus, the NOE correlations in **1** were observed between the 13β axial proton [δ 2.77 (ddd, J=4.0, 10.4, 14.0 Hz)] and the 11β axial proton [δ 1.28 (m)], the 19β axial proton [δ 2.50 (dq, J=10.5, 6.2 Hz)], and the 26 axial methyl proton, between the 18α axial proton [δ 1.79 (dd, J=10.4, 10.5 Hz)] and the 22α axial proton [δ 2.32 (ddd, J=2.9, 12.9, 14.9 Hz)], the 27α axial methyl proton, and the 29α equatorial methyl proton, and between the following proton pairs (19-H and 21β-H, 30-H$_3$; 23-H$_3$ and 3-H, 5-H; 25-H$_3$ and 1β-H, 24-H$_3$, 26-H$_3$; 27-H$_3$ and 9-H, 16α-H, 18-H) as shown in Fig. 1. Consequently, the stereostructure of **1** was determined to be 3β,20α-dihydroxytaraxastane-28-oic acid.

Effects of the Constituents on TNF-α-Induced Cytotoxicity in L929 Cells To clarify the active constituents, we examined the effects of the constituents from *P. granatum* on TNF-α-induced cytotoxicity in L929 cells. As shown in Table 3, punicanolic acid (**1**, inhibition: 13.9±1.0% at 30 μM), oleanolic acid (**2**, 21.1±2.2%), maslinic acid (**4**, 20.0±0.8%), 1,2,6-tri-*O*-galloyl β-D-glucopyranoside (**6**, 30.7±1.4%), 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl β-D-glucopyranoside (**7**, 32.0±2.0%), and luteolin (**8**, 30.7±0.4%), were found to show inhibitory activity, which were stronger than those of piperine (10.6±0.9% at 30 μM).[30,43] This evidence indicated that those constituents were found to decrease in the sensitivity of L929 cells to TNF-α. Many compounds, which inhibit cell death by production on TNF-α have been reported,[9,44,45] but there are few reports about compounds which selectively reduce the sensitivity of L929 cells to TNF-α.

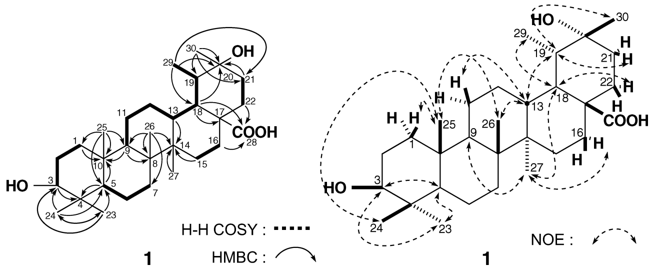

Fig. 1

Experimental

The following instruments were used to obtain physical data: melting points, Yanaco micro-melting point apparatus MP-500D (values are uncorrected); specific rotations, Horiba SEPA-300 digital polarimeter (l=5 cm); IR spectra, Shimadzu FTIR-8100 spectrometer; EI-MS and high-resolution MS, JEOL JMS-GCMATE mass spectrometer; ^{1}H-NMR spectra, JEOL JNM-ECA600 (600 MHz) and JNM-ECS400 (400 MHz) spectrometer; ^{13}C-NMR spectra, JEOL JNM-ECA600 (150 MHz) and JNM-ECS400 (100 MHz) spectrometer with tetramethylsilane as an internal standard; and HPLC detector, Shimadzu RID-6A refractive index and SPD-10A*vp* UV–VIS detectors.

The following experimental conditions were used for chromatography: ordinary-phase silica gel column chromatography, Silica Gel 60N (Kanto Chemical, Co., Inc., spherical, neutral, 63—210 μM); reverse-phase silica gel column chromatography, Diaion HP-20 (Nippon Rensui) and Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100—200 mesh); TLC, precoated TLC plates with Silica gel 60F$_{254}$ (Merck, 0.25 mm) (ordinary phase) and Silica gel RP-18 F$_{254S}$ (Merck, 0.25 mm) (reverse phase); reverse-phase HPTLC, precoated TLC plates with Silica gel RP-18 WF$_{254S}$ (Merck, 0.25 mm); and detection was achieved by spraying with 1% $Ce(SO_4)_2$–10% aqueous H_2SO_4 followed by heating.

Plant Material The flowers of *P. granatum* were purchased in Urumqi, Xinjiang province, China in August 2006. The plant material was identified by Dr. Xiao-guang Jia (director of Xinjiang Institute of Chinese Materia Medica and Ethnodrug, China). A voucher specimen (2006.08. Xinjiang-02) of this plant is on file in our laboratory.

Extraction and Isolation The dried flowers of *P. granatum* (510 g) were extracted three times with MeOH under reflux for 3 h. Evaporation of the solvent under reduced pressure provided a methanolic extract (305.0 g, 59.8%). The methanolic extract (255.0 g) was partitioned between an EtOAc–H_2O (1 : 1, v/v) mixture, and removal of the solvents *in vacuo* yielded an EtOAc-soluble fraction (42.0 g, 9.9%) and an aqueous phase. The aqueous phase was subjected to Diaion HP-20 column chromatography (2.0 kg, $H_2O \rightarrow$MeOH$\rightarrow$acetone) to give H_2O-eluted fraction (137.3 g, 32.2%), MeOH-eluted fraction (58.4 g, 13.7%), and acetone-eluted fraction (6.3 g, 1.48%). The EtOAc-soluble fraction (35.0 g) was subjected to normal-phase silica gel column chromatography [1.0 kg, *n*-hexane–EtOAc (10 : 1$\rightarrow$3 : 1$\rightarrow$1 : 1, v/v)$\rightarrow$EtOAc$\rightarrow$MeOH] to give 10 fractions {Fr. 1 (1.59 g), Fr. 2 (0.65 g), Fr. 3 (0.10 g), Fr. 4 [=oleanolic acid (**2**, 0.40 g, 0.11%)], Fr. 5 (2.38 g), Fr. 6 [=ursolic acid (**3**, 0.30 g, 0.084%)], Fr. 7 (0.15 g), Fr. 8 (0.29 g), Fr. 9 (25.30 g), and Fr. 10 (5.28 g)}. Fraction 2 (0.65 g) was subjected to reversed-phase silica gel column chromatography (30 g, MeOH$\rightarrow$acetone) to give β-sitosterol (268.3 mg, 0.076%). Fraction 3 (60.0 mg) was purified by HPLC [Cosmosil 5C$_{18}$-MS-II, CH_3CN–1% aqueous AcOH (55 : 45, v/v)] to furnish asiatic acid (**5**, 4.0 mg, 0.0019%). Fraction 5 (500 mg) was purified by HPLC [Wakopak Navi C30-5, CH_3CN–H_2O

Table 3. Effects of Constituents from the Flowers of *P. granatum* on TNF-α-Induced Cytotoxicity in L929 Cells

	Inhibition (%)					
TNF-α (1 ng/ml)	−	+	+	+	+	+
Conc. (μM)	0	0	3	10	30	100
Punicanolic acid (**1**)	100.0±4.1**	0.0±0.8	0.1±0.4	3.4±1.4	13.9±1.0**	52.3±1.3**
Oleanolic acid (**2**)	100.0±3.8**	0.0±2.3	−0.3±2.4	5.9±2.2	21.1±2.2**	38.1±2.0**
Ursolic acid (**3**)	100.0±1.9**	0.0±1.7	−0.1±1.8	2.2±1.6	−10.5±1.2	−35.4±0.1[a]
Maslinic acid (**4**)	100.0±5.3**	0.0±0.8	0.4±0.8	2.7±1.6	20.0±0.8**	−23.0±4.8[a]
Asiatic acid (**5**)	100.0±5.4**	0.0±4.0	−0.4±3.9	4.4±1.8	11.2±2.2*	−41.8±1.4[a]
1,2,6-Tri-*O*-galloyl β-D-glucopyranoside (**6**)	100.0±3.7**	0.0±2.3	5.1±1.6	9.8±1.8**	30.7±1.4**	34.8±1.0**
1,2-Di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl β-D-glucopyranoside (**7**)	100.0±3.1**	0.0±1.6	3.5±1.7	7.4±1.3*	32.0±2.0**	17.0±2.6**
Luteolin (**8**)	100.0±8.6**	0.0±0.5	7.3±0.5**	28.5±2.5**	30.7±0.4**	−15.7±0.8[a]
Tricetin (**9**)	100.0±1.8**	0.0±2.0	3.7±4.1	1.2±1.8	−1.2±1.6	15.6±0.6**
Piperine	100.0±2.6**	0.0±1.3	5.5±1.6*	5.3±1.4*	10.6±0.9**	41.8±1.4**
Sylibin	100.0±3.6**	0.0±2.6	5.3±2.8	22.0±3.8**	48.0±4.1**	50.8±3.9**

Each value represents the mean±S.E.M. (n=4). Significantly different from the control, *$p<0.05$, **$p<0.01$. *a*) Cytotoxic effect was observed.

(95 : 5, v/v)] to give **2** (134.4 mg, 0.18%) and **3** (200.3 mg, 0.27%). Fraction 7 (150 mg) was purified by HPLC [Wakopak Navi C30-5, CH_3CN–H_2O (95 : 5, v/v)] to afford eight fractions {Fr. 7-1 [=punicanolic acid (**1**, 17.8 mg, 0.005%)], Fr. 7-2 (14.7 mg), Fr. 7-3 (16.7 mg), Fr. 7-4 (6.5 mg), Fr. 7-5 (8.1 mg), Fr. 7-6 (5.6 mg), Fr. 7-7 [=**2** (9.7 mg, 0.0027%)], and Fr. 7-8 [=**3** (25.4 mg, 0.0071%)]}. Fraction 7-3 (16.7 mg) was further separated by HPLC [Cosmosil 5C_{18}-MS-II, CH_3CN–1% aqueous AcOH (55 : 45, v/v)] to furnish maslinic acid (**4**, 2.0 mg, 0.0006%). Fraction 9 (25.30 g) was subjected by reversed-phase silica gel column chromatography [1.0 kg, MeOH–H_2O (20 : 80→40 : 60→60 : 40, v/v)→MeOH→acetone] to afford 11 fractions [Fr. 9-1 (1158.1 mg), Fr. 9-2 (2726.3 mg), Fr. 9-3 (1666.7 mg), Fr. 9-4 (1153.1 mg), Fr. 9-5 (9364.2 mg), Fr. 9-6 (3033.8 mg), Fr. 9-7 (1063.5 mg), Fr. 9-8 (1285.6 mg), Fr. 9-9 (93.8 mg), Fr. 9-10 (117.8 mg), and Fr. 9-11 (951.4 mg)]. Fraction 9-3 (500 mg) was separated by HPLC [Wakopak Navi C30-5, MeOH–1% aqueous AcOH (20 : 80, v/v)] to give 1,2-di-*O*-galloyl-4,6-*O*-(*S*)-hexahydroxydiphenoyl β-D-glucopyranoside (**7**, 29.7 mg, 0.028%). Fraction 9-4 (500 mg) was separated by HPLC [Wakopak Navi C30-5, MeOH–1% aqueous AcOH (25 : 75, v/v)] to give 1,2,6-tri-*O*-galloyl β-D-glucopyranoside (**6**, 25.2 mg, 0.016%). Fraction 9-8 (500 mg) was purified by HPLC [Wakopak Navi C30-5, MeOH–1% aqueous AcOH (55 : 45, v/v)] to give luteolin (**8**, 13.1 mg, 0.0095%). Fraction 9-10 (117.8 mg) was separated by HPLC [Wakopak Navi C30-5, MeOH–1% aqueous AcOH (55 : 45, v/v)] to give tricetin (**9**, 16.7 mg, 0.0047%).

Punicanolic Acid (**1**): Colorless needles from MeOH, mp 280—281 °C, $[\alpha]_D^{28}$ −7.4° (c=0.19, MeOH). High-resolution EI-MS: Calcd for $C_{30}H_{50}O_4$ (M^+): 474.3709. Found: 474.3712. IR (KBr): 3451, 1719 cm^{-1}. ^1H-NMR (600 MHz, pyridine-d_5) δ_H: given in Table 2. ^{13}C-NMR data (150 MHz, pyridine-d_5) δ_C: given in Table 2. EI-MS (%) m/z: 474 (M^+, 4), 456 (M^+-H_2O, 19), 395 (100).

Inhibitory Effect on TNF-α-Induced Cytotoxicity in L929 Cells Inhibitory effect on TNF-α-induced cell death in L929 cells was assayed by the method described in our previous report with slight modification.[30,31] Briefly, a suspension of 1×10^4 cells [obtained from Dainippon Pharmaceutical (Osaka, Japan)] in 100 μl of minimum essential medium Eagle supplemented with 1% non-essential amino acid solution (Invitrogen), fetal calf serum (FCS, 10%), penicillin G (100 units/ml), and streptomycin (100 μg/ml) was incubated in a 96-well microplate. After 20 h of incubation in the medium containing TNF-α (1 ng/ml) with or without a test sample, the viability of the cells was assessed by the MTT colorimetric assay. Piperine was obtained from the fruit of *Piper chaba*, which was described previously.[30,43] Silybin was purchased from Funakoshi Co., Ltd. (Tokyo, Japan). Each test sample was dissolved in DMSO, and the solution was added to the medium (final DMSO concentration was 0.5%).

Statistics Values were expressed as means±S.E.M. One-way analysis of variance (ANOVA) followed by Dunnett's test was used for statistical analysis. Probability (p) values less than 0.05 were considered significant.

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References and Notes

1) Part XXII: Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **56**, 1297—1303 (2008).
2) Lansky E. P., Newman R. A., *J. Ethanopharmacol.*, **109**, 177—206 (2007).
3) Wang R., Wang W., Wang L., Liu R., Ding Y., Du L., *Fitoterapia*, **77**, 534—537 (2006).
4) Jafri M. A., Aslam M., Javed K., Singh S., *J. Ethnopharmacol.*, **70**, 309—314 (2000).
5) Yoshikawa M., Morikawa T., Murakami T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull.*, **47**, 340—345 (1999).
6) Yoshikawa M., Morikawa T., Toguchida I., Harima S., Matsuda H., *Chem. Pharm. Bull.*, **48**, 651—656 (2000).
7) Yoshikawa M., Murakami T., Kishi A., Kageura T., Matsuda H., *Chem. Pharm. Bull.*, **49**, 863—870 (2001).
8) Murakami T., Kishi A., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 974—978 (2001).
9) Matsuda H., Ninomiya K., Shimoda H., Yoshikawa M., *Bioorg. Med. Chem.*, **10**, 707—712 (2002).
10) Matsuda H., Morikawa T., Toguchida I., Harima S., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 972—975 (2002).
11) Yoshikawa M., Murakami T., Ishiwada T., Morikawa T., Kagawa M., Higashi Y., Matsuda H., *J. Nat. Prod.*, **65**, 1151—1155 (2002).
12) Matsuda H., Morikawa T., Ishiwada T., Managi H., Kagawa M., Higashi Y., Yoshikawa M., *Chem. Pharm. Bull.*, **51**, 440—443 (2003).
13) Yoshikawa M., Morikawa T., Kashima Y., Ninomiya K., Matsuda H., *J. Nat. Prod.*, **66**, 922—927 (2003).
14) Yoshikawa M., Morikawa T., Yamamoto K., Kato Y., Nagatomo A., Matsuda H., *J. Nat. Prod.*, **68**, 1360—1365 (2005).
15) Yoshikawa M., Sugimoto S., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 571—576 (2007).
16) Yoshikawa M., Xu F., Morikawa T., Pongpiriyadacha Y., Nakamura S., Asao Y., Matsuda H., *Chem. Pharm. Bull.*, **55**, 308—316 (2007).
17) Nakamura S., Sugimoto S., Matsuda H., Yoshikawa M., *Heterocycles*, **71**, 577—588 (2007).
18) Yoshikawa M., Nakamura S., Kato Y., Matsuhira K., Matsuda H., *Chem. Pharm. Bull.*, **55**, 598—605 (2007).
19) Yoshikawa M., Morikawa T., Asao Y., Fujiwara E., Nakamura S., Matsuda H., *Chem. Pharm. Bull.*, **55**, 606—612 (2007).
20) Yoshikawa M., Sugimoto S., Nakmaura S., Sakumae H., Matsuda H., *Chem. Pharm. Bull.*, **55**, 1034—1038 (2007).
21) Nakamura S., Sugimoto S., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **55**, 1342—1348 (2007).
22) Yoshikawa M., Wang T., Sugimoto S., Nakamura S., Nagatomo A., Matsuda H., Harima S., *Yakugaku Zasshi*, **128**, 141—151 (2008).
23) Yoshikawa M., Sugimoto S., Kato Y., Nakamura S., Wang T., Yamashita C., Matsuda H., *Chem. Biodiversity*, in press.
24) Morikawa T., Li X., Nishida E., Ito Y., Matsuda H., Nakamura S., Muraoka O., Yoshikawa M., *J. Nat. Prod.*, **71**, 828—835 (2008).
25) Yoshikawa M., Li X., Nishida E., Nakamura S., Matsuda H., Muraoka O., Morikawa T., *Chem. Pharm. Bull.*, **56**, 559—568 (2008).
26) Wang Y., Singh R., Lefkowitch J. H., Rigoli R. M., Czaja M. J., *J. Biol. Chem.*, **281**, 15258—15267 (2006).
27) Tilg H., Day C. P., *Nat. Clin. Pract. Gastroenterol. Hepatol.*, **4**, 24—34 (2007).
28) Seronello S., Sheikh M. Y., Choi J., *Free Radic. Biol. Med.*, **43**, 869—882 (2007).
29) Kouroku Y., Fujita E., Jimbo A., Mukasa T., Tsuru T., Momoi M. Y., Momoi T., *Biochem. Biophys. Res. Commun.*, **270**, 972—977 (2000).
30) Matsuda H., Ninomiya K., Morikawa T., Yasuda D., Yamaguchi I., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **18**, 2038—2042 (2008).
31) Morikawa T., Funakoshi K., Ninomiya K., Yasuda D., Miyagawa K., Matsuda H., Yoshikawa M., *Chem. Pharm. Bull.*, **56**, 956—962 (2008).
32) Seebacher W., Simic N., Weis R., Saf R., Kunert O., *Magn. Res. Chem.*, **41**, 636—638 (2003).
33) Alves J. S., de Castro J. C. M., Freire M. O., da-Cunha E. V. L., Barbosa-Filho J. M., de Silva M. S., *Magn. Res. Chem.*, **38**, 201—206 (2000).
34) Taniguchi S., Imayoshi Y., Kobayashi E., Takamatsu Y., Ito H., Hatano T., Sakagami H., Tokuda H., Nishino H., Sugita D., Shimura S., Yoshida T., *Phytochemistry*, **59**, 315—323 (2002).
35) Matsuda H., Morikawa T., Ueda H., Yoshikawa M., *Heterocycles*, **55**, 1499—1504 (2001).
36) Matsuda H., Morikawa T., Ueda H., Yoshikawa M., *Chem. Pharm. Bull.*, **49**, 1368—1371 (2001).
37) Haddock E. A., Gupta R. K., Al-Shafi S. M. K., Haslam E., Magnolato D., *J. Chem. Soc. Perkin Trans. 1*, **1982**, 2515—2524 (1982).
38) Fukuda T., Ito H., Yoshida T., *Phytochemsitry*, **63**, 795—801 (2003).
39) Matsuda H., Morikawa T., Toguchida I., Yoshikawa M., *Chem. Pharm. Bull.*, **50**, 788—795 (2002).
40) Markham K. R., Ternai B., Stanley R., Geiger H., Mabry T. J., *Tetrahedron*, **34**, 1389—1397 (1978).
41) Kongduang D., Wungsintaweekul J., De-Eknamkul W., *Tetrahedron Lett.*, **49**, 4067—4072 (2008).
42) The ^1H- and ^{13}C-NMR spectra of **1** was assigned with the aid of distortionless enhancement by polarization transfer (DEPT), double quantum filter correlation spectroscopy (DQF COSY), heteronuclear multiple quantum coherence (HMQC), heteronuclear multiple bond connectivity (HMBC), and phase sensitive nuclear Overhauser enhancement spectroscopy (NOESY) experiments.
43) Morikawa T., Matsuda H., Yamaguchi I., Pongpiriyadacha Y., Yoshikawa M., *Planta Med.*, **70**, 152—159 (2004).
44) Matsuda H., Ninomiya K., Morikawa T., Yoshikawa M., *Bioorg. Med. Chem. Lett.*, **8**, 339—344 (1998).
45) Morikawa T., Matsuda H., Ninomiya K., Yoshikawa M., *Biol. Pharm. Bull.*, **25**, 627—631 (2002).