Pancreatic Lipase-Inhibiting Triterpenoid Saponins from Fruits of *Acanthopanax senticosus*

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Sixteen triterpenoid saponins were isolated from the fruits of *Acanthopanax senticosus*, including a new compound, acanthopanaxoside E (1), which was established as 3-*O*-*β*-D-glucuronopyranosyl echinocystic acid 28-*O*-*β*-D-glucopyranoside on the basis of various spectroscopic analyses and chemical degradation. By using a pancreatic lipase-inhibiting assay system, the crude saponin fraction showed inhibitory activity on pancreatic lipase, which is a key enzyme in lipid digestion. Among the isolated compounds, silphioside F (2), copteroside B (3), hederagenin 3-*O*-*β*-D-glucuronopyranoside 6′-*O*-methyl ester (4) and gypsogenin 3-*O*-*β*-D-glucuronopyranoside (5) showed inhibitory activity toward pancreatic lipase with IC_{50} values of 0.22, 0.25, 0.26 and 0.29 mM, respectively, and the free carboxylic acid groups in position 28 within their chemical structures were required for enhancement of pancreatic lipase inhibition.

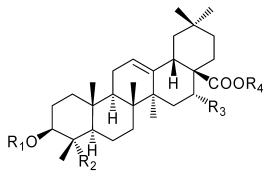
Key words *Acanthopanax senticosus*; Araliaceae; triterpenoid saponin; pancreatic lipase

A increasing number of people are suffering from obesity, which is often associated with the development of type II diabetes, coronary heart diseases, hyperlipidemia, hypertension, and metabolic syndrome.[1,2] It is well known that pancreatic lipase plays an important role in lipid digestion. The inhibition of pancreatic lipase reduces the efficiency of dietary fat absorption in the small intestine and thereby promotes modest long-term reduction in body weight.[3]

Acanthopanax senticosus (RUPR. *et* MAXIM.) HARMS. is a shrub, belonging to the family Araliaceae, which is commonly distributed in the northeast of Asia. Different parts of the plant have been widely used as traditional Chinese medicines for the treatment of a variety of human diseases, such as ischemic heart diseases, neurasthenia, hypertension, arthritis, and tumors.[4] Recently, we have reported fifteen triterpenoid saponins from the leaves of *A. senticosus*, among which ciwujianoside C_1, tauroside H_1, 3-*O*-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl mesembryanthemoidigenic acid, acanthopanaxoside C, sessiloside, and chiisanoside exhibited pancreatic lipase inhibitory activity *in vitro*.[5] The occurrence of triterpenoid saponins has also been demonstrated in a previous chemical investigation of the fruits of *A. senticosus*.[6] As our continuous investigation on bioactive triterpenoid saponins[5,7—9] and our current interest in the natural lipase inhibitors,[5,9,10] we initiated a chemical investigation of the fruits of *A. senticosus*. The total saponin fraction exhibited inhibitory activity on pancreatic lipase with the IC_{50} value of 3.63 mg/ml. Further isolation of this fraction resulted in the isolation of sixteen triterpenoid saponins (**1**—**16**), including a new compound, acanthopanaxoside E (**1**). The biological activities of **1**—**16** against pancreatic lipase were examined. Silphioside F (**2**),[11] copteroside B (**3**),[11] hederagenin 3-*O*-*β*-D-glucuronopyranoside 6′-*O*-methyl ester (**4**)[12] and gypsogenin 3-*O*-*β*-D-glucuronide (**5**)[13] showed inhibitory activity, with IC_{50} value of 0.22, 0.25, 0.26 and 0.29 mM, respectively.

Results and Discussion

The air-dried fruits of *A. senticosus* were extracted with 70% EtOH. The 70% EtOH extract was suspended in H_2O and then partitioned successively with EtOAc and *n*-BuOH. The *n*-BuOH soluble fraction was subjected to a Diaion HP-20 column, followed by washing with H_2O and 50% MeOH to remove phenolic compounds, and then with MeOH to result in a crude saponin fraction, which inhibited pancreatic lipase (IC_{50} value: 3.63 mg/ml). Further isolation of this fraction by combination of silica gel column chromatography (CC), ODS CC and preparative HPLC afforded sixteen triterpenoid saponins, including a new compound, acanthopanaxoside E (**1**). The known saponins were identified as silphioside F (**2**),[11] copteroside B (**3**),[11] hederagenin 3-*O*-*β*-D-glucuronopyranoside 6′-*O*-methyl ester (**4**),[12] gypsogenin 3-*O*-*β*-D-glucuronide (**5**),[13] silphioside G (**6**),[14] ilexoside XLVIII (**7**),[15] hederagenin 3-*O*-*β*-D-glucuronopyranosyl methyl ester-28-*O*-*β*-D-glucopyranoside (**8**),[16] 3-*O*-*β*-D-glucuronopyranosyl gypsogenin 28-*O*-*β*-D-glucopyranoside (**9**),[17] hederagenin 28-*O*-*β*-D-glucopyranoside (**10**),[18] hemsgiganoside B (**11**),[19] anhuienside C (**12**),[20] ciwujianoside C_3 (**13**),[21] hederasaponin B (**14**),[22] eleutheroside L (**15**)[23] and ciwujianoside A_1 (**16**)[24] by detailed NMR analyses and comparison of the



	R_1	R_2	R_3	R_4
1	GlcA	CH_3	OH	Glc
2	GlcA	CH_3	H	H
3	GlcA	CH_2OH	H	H
4	6-*O*-methyl-GlcA	CH_2OH	H	H
5	GlcA	CHO	H	H
6	GlcA	CH_3	H	Glc
7	GlcA	CH_2OH	H	Glc
8	6-*O*-methyl-GlcA	CH_2OH	H	Glc
9	GlcA	CHO	H	Glc
10	H	CH_2OH	H	Glc
11	GlcA	CH_3	H	Glc-(1→6)-Glc
12	Rha-(1→2)-Ara	CH_3	H	Glc-(1→6)-Glc
13	Ara	CH_3	H	Rha-(1→4)-Glc-(1→6)-Glc
14	Rha-(1→2)-Ara	CH_3	H	Rha-(1→4)-Glc-(1→6)-Glc
15	Rha-(1→4)-Ara	CH_3	H	Rha-(1→4)-Glc-(1→6)-Glc
16	Glc-(1→2)-Ara	CH_3	H	Rha-(1→4)-Glc-(1→6)-Glc

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Table 1. ^{1}H- and ^{13}C-NMR Spectroscopic Data (δ) of **1** in pyridine-d_5[a)]

Position	δ_H	δ_C	Position	δ_H	δ_C
1	ax 0.92 (td, 11.7, 2.5)	38.8	22	ax 2.11 (td, 12.8, 3.8)	33.5
	eq 1.45 (m)			eq 2.38 (dt, 12.8, 2.5)	
2	ax 1.89[a)]	26.7	23	1.29 (s)	28.2
	eq 2.26 (br dd, 11.7, 3.2)		24	0.99 (s)	17.0
3	ax 3.42 (dd, 11.7, 3.9)	89.1	25	0.87 (s)	15.7
4		39.6	26	1.12 (s)	17.6
5	ax 0.83 (br d, 11.6)	55.9	27	1.86 (s)	27.3
6	1.29[a)]	18.6	28		176.0
7	ax 1.61 (td, 11.6, 3.9)	32.2	29	1.01 (s)	33.2
	eq 1.47 (m)		30	1.04 (s)	24.6
8		40.1			
9	ax 1.79 (m)	47.2	GlcA		
10		37.0	1	5.04 (d, 7.8)	107.2
11	ax 1.97 (br dd, 11.7, 2.8)	23.8	2	4.13 (t, 8.2)	75.5
	eq 1.78 (m)		3	4.33 (t, 9.4)	78.2
12	5.59 (t-like, 3.2)	122.8	4	4.61 (t, 9.4)	73.5
13		144.4	5	4.70 (d, 9.4)	77.8
14		42.1	6		172.9
15	ax 2.56 (dd, 14.9, 2.8)	36.2			
	eq 1.79 (m)		Glc		
16	eq 5.30 (br s)	74.4	1	6.32 (d, 8.0)	95.9
17		49.2	2	4.16 (t, 8.5)	74.2
18	ax 3.51 (dd, 13.8, 3.9)	41.3	3	4.26 (t, 8.7)	78.5
19	ax 2.80 (t, 13.8)	47.2	4	4.27 (t, 8.7)	71.2
	eq 1.37 (dd, 13.8, 2.9)		5	4.02 (m)	79.4
20		30.8	6	4.44 (dd, 11.8, 2.1)	62.3
21	ax 2.44 (m)	36.0		4.37 (dd, 11.8, 4.4)	
	eq 1.26 (m)				

a) Overlapped signals.

data in the literature. Although known in other genera, this is the first report of the isolation of the known compounds **2** and **4**—**12** from *Acanthopanax*.

Acanthopanaxoside E (**1**) was obtained as an amorphous powder, $[\alpha]_D^{22}$ −20.8° (c=1.0, MeOH). The molecular formula was established as $C_{42}H_{66}O_{15}$ from data of the positive-ion high-resolution (HR)-FAB-MS (*m/z* 833.4259 [M+Na]$^+$). The ^{13}C-NMR spectrum showed 42 carbon signals, of which 30 were assigned to the aglycon, 12 to the sugar moieties. The six sp^3 carbons at δ 15.7, 17.0, 17.6, 24.6, 27.3, 28.2 and 33.5, and the two sp^2 carbons at 122.8 (d) and 144.4 (s), coupled with information from the ^{1}H-NMR (six methyl proton singlets at δ 0.87, 0.99, 1.01, 1.04, 1.12, 1.29 and 1.86, and a broad triplet-like vinyl proton signal at δ 5.59), indicated that the aglycon had an olean-12-ene skeleton. Furthermore, the ^{13}C-NMR spectrum showed an ester carbonyl signal at δ_C 176.0 (C-28). In the ^{1}H-NMR spectrum, signals assignable to carbinylic protons of the aglycon were observed at δ 3.42 (dd, J=11.7, 3.9 Hz) and 5.30 (br s), suggesting the carbinylic protons could be placed at 3α and 16β, respectively.[25)] Thus, the aglycon was identified as 3β,16α-dihydroxyolean-12-en-28-oic acid (echinocystic acid).[26,27)] The aglycon was isolated after acid hydrolysis of **1** and confirmed by comparison of the spectral and physical data with those of an authentic sample. On acid hydrolysis, **1** also afforded D-glucuronic acid and D-glucose as component sugars, which were identified by GC-MS analysis following conversion to the trimethylsilyl thiazolidine derivatives.[28)] The ^{1}H- and ^{13}C-NMR spectra of **1** (Table 1) revealed signals assignable to β-glucopyranosyl and β-glucuronopyranosyl moieties, with the anomeric proton signals at δ 5.04 (d, J=7.8 Hz, GlcA-H-1) and 6.32 (d, J=8.0 Hz, Glc-H-1), and the corresponding anomeric carbon signals at δ 107.2 and 95.9. When the ^{13}C-NMR data of **1** were compared with that of echinocystic acid, glycosylation shifts were observed at C-2 (−1.3 ppm), C-3 (+11.1 ppm) and C-28 (−3.8 ppm), which suggested **2** was a 3,28-bisdesmoside. The glucuronopyranosyl moiety attached to C-3 was established by the heteronuclear multiple bond connectivity (HMBC) between δ_H 5.04 (H-1, GlcA-1) and δ_C 89.1 (C-3). The glucopyranosyl moiety attached to C-28 was determined by HMBC correlation between δ_H 6.32 (H-1, Glc-1) and δ_C 176.0 (C-28). Thus, the structure of acanthopanaxoside E (**1**) was determined to be 3-*O*-β-D-glucuronopyranosyl echinocystic acid 28-*O*-β-D-glucopyranoside.

Table 2. Inhibitory Activities of Compounds **2**—**5** from *A. senticosus* Fruits against Pancreatic Lipase

Substance	IC_{50} (mM)
2	0.22
3	0.25
4	0.26
5	0.29
Orlistat	0.04

The results are expressed as mean±S.E.M., n=4.

The biological activities of compounds **1**—**16** against pancreatic lipase were examined. When the percentage inhibition was higher than 30% at a concentration of 1 mg/ml, the IC_{50} values were evaluated. Silphioside F (**2**),[11)] copteroside B (**3**),[11)] hederagenin 3-*O*-β-D-glucuronopyranoside 6′-*O*-methyl ester (**4**)[12)] and gypsogenin 3-*O*-β-D-glucuronide (**5**)[13)] showed inhibitory activities (IC_{50} values: 0.22, 0.25, 0.26 and 0.29 mM, respectively), which were five to seven

times lower than the positive control, olistat (IC_{50} value: 0.04 mM) (Table 2). From these results, we postulated that the free carboxylic acid group at position 28 in the structure is required for the enhancement of inhibition of pancreatic lipase activity, which is in good agreement with the findings of a previous study.[29)]

Experimental

General Experimental Procedures Optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5 dm length cell. IR spectra were measured with a JASCO FT/IR-300E (by a KBr disk method) spectrometer. ^{1}H- and ^{13}C-NMR were measured with a JEOL ECP-500 spectrometer in δ (ppm), with reference to TMS. FAB-MS and HR-FAB-MS were taken on a JEOL JMS-700 MStation. Preparative HPLC was performed on a JASCO model PU-2080 HPLC system, equipped with a Shodex RI-101 differential refractometer detector and a YMC-Pack RP-C_{18} column (150×20 mm i.d.). Diaion HP-20 (Mitsubishi Chemical Corporation, Tokyo), silica gel (silica gel 60, Kanto Chemical Co., Inc., Tokyo) and ODS (Chromatorex DM1020TM, 100—200 mesh, Fuji Silysia Chemical, Ltd., Aichi, Japan) were used for column chromatography. TLC was conducted with Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin-Elmer Clarus 500 GC-MS.

Plant Material The fruits of *A. senticosus* were collected in October 2004 at Mudanjiang, Heilongjiang Province, P. R. of China, and confirmed by one of the authors (Q.Z.). A specimen has been deposited at the Department of Chinese Traditional Medicine, Heilongjiang Provincial Institute for Drug Control, P. R. of China.

Extraction and Isolation The air-dried fruits of *A. senticosus* (1.39 kg) were extracted twice with 70% EtOH at room temperature. Evaporation of the solvent under reduced pressure gave the 70% EtOH extract (501.27 g). The extract was suspended in H_2O and then extracted with EtOAc and *n*-BuOH. The *n*-BuOH layer was evaporated under reduced pressure at below 40 °C to yield an *n*-BuOH fraction (61.87 g) which was subjected to a Diaion HP-20 column and eluted with H_2O, 50% MeOH in H_2O and MeOH. The MeOH eluate was concentrated (18.41 g) and chromatographed on silica gel with a gradient of $CHCl_3$–MeOH–H_2O (60 : 20 : 3, 60 : 29 : 6, 6 : 4 : 1) to give two fractions. Further separation of Fr. 1 was achieved by HPLC using 60—90% MeOH in 3% AcOH to give **2** (357 mg), **3** (1304 mg), **4** (40 mg), **5** (10 mg), **6** (531 mg), **7** (1928 mg) and **9** (27 mg). Fraction 2 was further purified by HPLC using 33—40% MeCN in 3% AcOH to afford **1** (25 mg), **8** (8 mg), **10** (5 mg), **11** (66 mg), **12** (17 mg), **13** (12 mg), **14** (113 mg), **15** (27 mg) and **16** (5 mg).

Acanthopanaxoside E (**1**): Amorphous powder. $[\alpha]_D^{22}$ −20.8° (c=1.0, MeOH). IR (KBr) ν_{max} cm^{-1}: 3404, 2942, 1731, 1636, 1380, 1229, 1073, 1032. ^{1}H-NMR (pyridine-d_5, 500 MHz) and ^{13}C-NMR (pyridine-d_5, 125 MHz): see Table 1. FAB-MS (positive) m/z 832.95 $[M+Na]^+$. HR-FAB-MS (positive) m/z 833.4259 $[M+Na]^+$ (Calcd for $C_{42}H_{66}O_{15}Na$, 833.4299).

Acid Hydrolysis and Determination of the Absolute Configuration of the Monosaccharides A solution of **1** (10 mg) in 1 M HCl (dioxane–H_2O, 1 : 1, 2 ml) was heated at 100 °C for 2 h. After dioxane was removed, the solution was extracted with EtOAc (2 ml×3). The extract was washed with H_2O, and evaporated to give echinocystic acid (3 mg), which was identified by comparison of its spectral and physical data with those of an authentic sample.[25)] The H_2O layer was concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. The residue was dissolved in pyridine (0.1 ml), to which 0.08 M L-cysteine methyl ester hydrochloride in pyridine (0.15 ml) was added. The mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried *in vacuo*, the residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 ml) for 2 h. The mixture was partitioned between *n*-hexane and H_2O (0.3 ml each) and the *n*-hexane extract was analyzed by GC-MS under the following conditions: capillary column, EQUITY^{TM}-1 (30 m×0.25 mm×0.25 μm, Supelco); column temperature, 230 °C; injection temperature, 250 °C; carrier N_2 gas; detection in EI mode, ionization potential, 70 eV; ion-source temperature, 280 °C. D-Glucose and D-glucuronic acid were confirmed by comparison of the retention times of their derivatives with those of L-glucose, D-glucose and D-glucuronic acid derivatives prepared in a similar way which showed retention times of 11.17, 10.71, and 11.30 min, respectively.

Measurement of Pancreatic Lipase Activity Lipase activity was determined by measuring the rate of release of oleic acid from triolein. A suspension of triolein (80 mg), lecithin (9 mg) and taurocholic acid (5 mg) in 9 ml of 0.1 M *N*-tris-(hydroxymethyl)-methyl-2-aminoethanesulfonic acid buffer (pH 7.0) containing 0.1 M NaCl was sonicated for 5 min. The sonicated substrate suspension (0.05 ml) was incubated with 0.05 ml of pancreatic lipase and 0.1 ml of various concentrations of the sample solution for 30 min at 37 °C in a final volume of 0.2 ml. The amount of release of oleic acid produced was determined based on the method described by Zapf *et al.* with a minor modification.[30,31)] The lipase activity was expressed as moles of oleic acid released per liter of reaction mixture per hour.

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