Biologically Active Triterpenoid Saponins from *Acanthopanax senticosus*

Wenhong Jiang,[†,‡] Wei Li,[†] Likun Han,[§] Lijuan Liu,[‡] Qingbo Zhang,[‡] Shujie Zhang,[‡] Tamotsu Nikaido,[†] and Kazuo Koike*[,†]

Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan, Department of Traditional Chinese Medicine, Heilongjiang Provincial Institute for Drug Control, Harbin 150001, People's Republic of China, and Department of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Tsukide 3-1-100, Kumamoto City, Kumamoto 862-8502, Japan

Received May 1, 2006

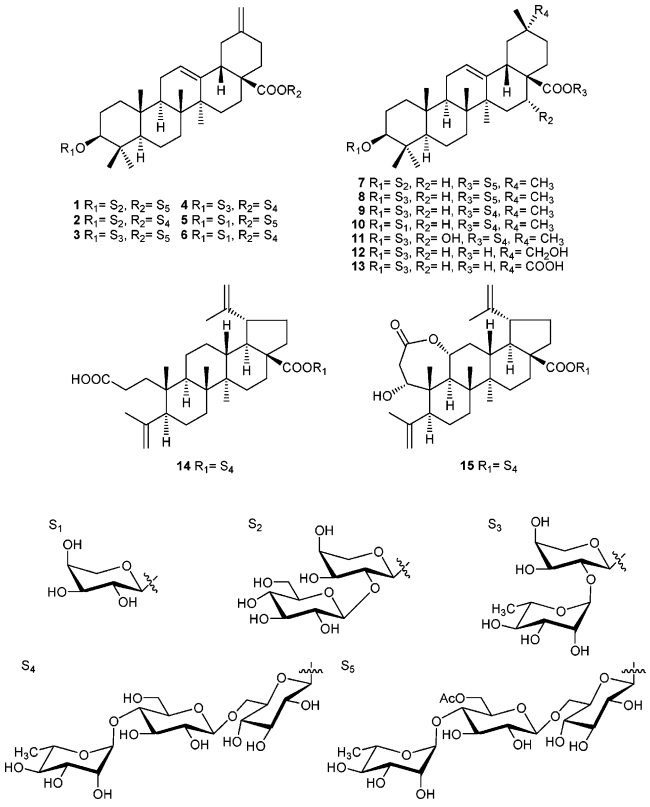
Three new triterpenoid saponins, acanthopanaxosides A (**1**), B (**7**), and C (**13**), were isolated from the leaves of *Acanthopanax senticosus*, together with 12 known saponins. The structures of these new saponins were established as 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-30-nor-olean-12,20(29)-dien-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**1**), 3-*O*-*β*-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester (**7**), and 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-3*β*-hydroxyolean-12-ene-28,29-dioic acid (**13**), on the basis of spectroscopic analysis and chemical degradation. Among the known compounds, sessiloside and tauroside H_1 are reported for the first time from *A. senticosus*. The biological activity of compounds **1**−**15** was examined against pancreatic lipase. Ciwujianoside C_1 (**6**), tauroside H_1 (**11**), 3-*O*-α-rhamnopyranosyl-(1→2)-α-arabinopyranosyl mesembryanthemoidigenic acid (**12**), acanthopanaxoside C (**13**), sessiloside (**14**), and chiisanoside (**15**) inhibited pancreatic lipase activity in vitro. In turn, ciwujianosides C_2 (**3**), D_2 (**5**), C_4 (**8**), and C_3 (**10**) and hederasaponin B (**9**) enhanced this enzyme.

Acanthopanax senticosus (Rupr. Maxim) Harms. is a shrub, belonging to the family Araliaceae, which is commonly found in forests 500−2000 m in elevation in the northeast of Asia. It usually grows up to 2 m in height and generally has prickly stems bearing five leaflets (palmate) and umbel-shaped flowers. This species has been used widely in Traditional Chinese Medicine for the treatment of neurasthenia, hypertension, and ischemic heart disease.[1] The presence of triterpenoid saponins in the leaves of this plant has been known since 1971.[2] So far, 25 triterpenoid saponins have been isolated from the leaves of *A. senticosus*.[3] On the basis of the type of aglycon, these saponins can be classified into the oleanane, noroleanane, lupane, and 3,4-secolupane classes. The saponin-containing fraction from the leaves of *A. senticosus* has been reported to possess several important pharmacological effects, as exemplified by the reduction of acute myocardial infarction in acute ischemic dogs.[4] As a part of our ongoing investigation on the medicinal plants in northeast mainland China[5] and our interest in the chemistry of bioactive triterpenoid saponins,[5b,6] we have carried out a chemical investigation on the leaves of *A. senticosus*. We describe herein the isolation and the structure elucidation of three new triterpenoid saponins, named acanthopanaxosides A (**1**), B (**7**), and C (**13**), from the leaves of *A. senticosus*, along with an evaluation of the pancreatic lipase inhibitory activity of the isolated saponins **1**−**15**.

Results and Discussion

A 70% EtOH extract of the leaves of *A. senticosus* was partitioned between *n*-BuOH and H_2O. The *n*-BuOH-soluble fraction was subjected to passage over a Diaion HP-20 column, followed by washing with MeOH and H_2O mixtures in different ratios. Further purification using combinations of silica gel column chromatography, ODS column chromatography, and preparative HPLC afforded 15 triterpenoid saponins, namely, three new compounds, acanthopanaxosides A (**1**), B (**7**), and C (**13**), and 12 known saponins. The known saponins were identified as ciwujianosides A_2 (**2**), C_2 (**3**), B (**4**), D_2 (**5**), C_1 (**6**), and C_4 (**8**),[3d,e] hederasaponin B (**9**),[3d] ciwujianoside C_3 (**10**),[3d] tauroside H_1 (**11**),[3b] 3-*O*-α-rhamnopyranosyl-(1→2)-α-arabinopyranosyl mesembryanthemoidigenic acid (**12**),[3e] sessiloside (**14**),[3a] and chiisanoside (**15**)[3c] by detailed NMR analysis and comparison with literature data. This is the first report of tauroside H_1 (**11**) and sessiloside (**14**) from *A. senticosus*.

Chart 1



Acanthopanaxoside A (**1**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ +11.8 (*c* 1.0, MeOH). The negative-ion HRFABMS of **1** showed an accurate $[M - H]^-$ ion peak at *m*/*z* 1245.5963, in accordance with an empirical molecular formula of $C_{60}H_{94}O_{27}$. The fragmentation patterns in the negative-ion FABMS of **1** indicated losses of an acetyl moiety (*m*/*z* 1203 $[M - C_2H_3O]^-$) and ester-linked sugars (*m*/*z* 733 $[M - C_{20}H_{33}O_{15}]^-$). On acid hydrolysis of **1** with 1 M HCl (dioxane−H_2O, 1:1, v/v), 3*β*-hydroxy-30-nor-

* Corresponding author. Tel: +81-47-4721396. Fax: +81-47-4721404. E-mail: koike@phar.toho-u.ac.jp.
† Toho University.
‡ Heilongjiang Provincial Institute for Drug Control.
§ Prefectural University of Kumamoto.

10.1021/np060195+ CCC: $33.50
Published on Web 10/17/2006

oleana-12,20(29)-dien-28-oic acid (akebonic acid) was obtained as the aglycon, which was identified by direct comparison of the ^{1}H and ^{13}C NMR data with those in the literature.[7] When the ^{13}C NMR data of the aglycon part of **1** were compared with those of akebonic acid, glycosylation shifts were observed at C-3 (+10.9 ppm) and C-28 (−3.4 ppm), which indicated **1** to be a 3,28-bisdesmoside. Acid hydrolysis of **1** afforded the component sugars L-arabinose, D-glucose, and L-rhamnose in the ratio 1:3:1, which were identified by GLC analysis of their trimethylsilyl thiazolidine derivatives.[8] The ^{1}H NMR spectra showed five anomeric proton signals at δ 4.97 (d, J = 3.9 Hz), 4.98 (d, J = 8.1 Hz), 5.18 (d, J = 7.7 Hz), 5.53 (br s), and 6.18 (d, J = 8.2 Hz), which give correlations in the HMQC spectrum with the carbon anomeric signals in the ^{13}C NMR spectrum at δ 104.8, 104.8, 106.0, 102.9, and 95.7, respectively. The assignments of the proton and the carbon resonances by further analysis of 2D NMR data, involving the DQF-COSY, TOCSY, NOESY, HMQC, and HMBC experiments, revealed the presence of an α-arabinopyranosyl, an α-rhamnopyranosyl, and three β-glucopyranosyl moieties. The positions of the sugar moieties were defined unambiguously by a HMBC experiment. Observation of the correlations between δ_H 4.97 (H-1, Ara-H-1) and δ_C 88.9 (C-3), and δ_H 5.18 (H-1, Glc-H-1) and δ_C 80.9 (Ara-C-2), indicated that the β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl moiety was linked to C-3 of the aglycon. Similarly, the linkages of the trisaccharide chain at C-28 were established as an α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl unit by the HMBC correlations between δ_H 6.18 (Glc′-H-1) and δ_C 175.7 (C-28), δ_H 4.98 (Glc″-H-1) and δ_C 69.5 (Glc′-C-6), and δ_H 5.53 (Rha-H-1) and δ_C 79.2 (Glc″-C-4). Besides the signals due to the aglycon and the component sugars, the ^{1}H and ^{13}C NMR data also showed the presence of an acetyl group [δ_H 1.93 (3H, s); δ_C 170.6, 20.6]. On comparing the ^{13}C NMR spectrum of **1** with that of ciwujianoside A_2 (**2**),[3d] the downfield shift observed at Glc″-C-6 (+2.3 ppm) and the upfield shift at Glc″-C-5 (−3.4 ppm) indicated that the acetyl group was attached to Glc″-C-6, which was confirmed by the HMBC correlation between δ_H 4.53, 4.63 (Glc″-H-6) and δ_C 170.6. On the basis of the above evidence, the structure of **1** was concluded to be 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosyl-30-nor-oleana-12,20-(29)-dien-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Acanthopanaxoside B (**7**) was obtained as an amorphous powder, $[\alpha]^{22}_D$ −4.4 (*c* 0.8, MeOH). The HRFABMS of **7** showed an accurate $[M - H]^-$ ion peak at *m*/*z* 1261.6210, in accordance with an empirical molecular formula of $C_{61}H_{98}O_{27}$. The fragmentation patterns in the negative-ion FABMS of **7** indicated losses of an acetyl moiety (*m*/*z* 1219 $[M - C_2H_3O]^-$) and ester-linked sugars (*m*/*z* 749 $[M - C_{20}H_{33}O_{15}]^-$). Acid hydrolysis of **7** afforded 3β-hydroxyolean-12-en-28-oic acid (oleanolic acid) as the aglycon, and L-arabinose, D-glucose, and L-rhamnose as component sugars. In the ^{13}C NMR spectrum, the shift values of C-3 at δ 88.9 and C-28 at δ 176.5 suggested that **7** is a 3,28-bisdesmoside of oleanolic acid. Comparison of the ^{1}H and ^{13}C NMR data of **7** with those of **1** revealed that the signals of the sugar moieties were superimposable, suggesting that the sugar structures at C-3 and C-28 were the same as those in **1**. This conclusion was confirmed by the HMBC correlations between δ_H 4.96 (Ara-H-1) and δ_C 88.9 (C-3), δ_H 5.19 (Glc-H-1) and δ_C 81.0 (Ara-C-2), δ_H 6.24 (Glc′-H-1) and δ_C 176.5 (C-28), δ_H 5.00 (Glc″-H-1) and δ_C 69.4 (Glc′-C-6), δ_H 5.54 (Rha-H-1) and δ_C 79.2 (Glc″-C-4), and δ_H 4.54, 4.63 (Glc″-H-6) and δ_C 170.6 (*C*OCH_3). On the basis of the above results, the structure of **7** was concluded to be 3-*O*-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranosylolean-12-en-28-oic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-6-*O*-acetyl-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

Acanthopanaxoside C (**13**) was obtained as an amorphous powder, $[\alpha]^{25}_D$ −3.2 (*c* 0.8, MeOH). The HRFABMS of **13** showed an accurate $[M - H]^-$ ion peak at *m*/*z* 763.4297, in accordance with an empirical molecular formula of $C_{41}H_{64}O_{13}$. The ^{13}C NMR spectrum of **13** revealed the signals of two free carboxyl carbons at δ 180.0 and 181.1 and six tertiary methyl carbons at δ 15.5, 17.0, 17.4, 20.1, 26.1, and 28.1. Comparison of the ^{1}H and ^{13}C NMR data of the aglycon part in **13** with those in **7** revealed superimposable signals for the protons and carbons in rings A−D, but differences were observed in the E ring, suggesting the replacement of a methyl group at C-30 in **7** by a free carboxyl group in **13** and the loss of an ester-linked sugar chain at C-28. Further analysis of the 2D NMR data was used to identify the aglycon as 3β-hydroxyolean-12-ene-28,29-dioic acid (serratagenic acid).[9] The ^{1}H and ^{13}C NMR spectra of **13** displayed two sugar anomeric proton signals at δ 4.91 (d, J = 5.3 Hz) and 6.13 (d, J = 0.9 Hz) and the corresponding carbon signals at δ 104.8 and 101.7, respectively. Assignment of the sugar signals, in combination with the result of acid hydrolysis, suggested the presence of α-L-rhamnopyranosyl and α-L-arabinopyranosyl moieties in **13**. The connectivity of the α-L-rhamnopyranosyl moiety to C-2 of the α-L-arabinopyranosyl moiety and the linkage of the disaccharide chain at C-3 were defined unambiguously by the HMBC correlations between δ_H 6.13 (Rha-H-1) and δ_C 75.9 (Ara-C-2), and δ_H 4.91 (Ara-H-1) and δ_C 88.8 (C-3). On the basis of the above results, the structure of **13** was concluded to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-3β-hydroxyolean-12-ene-28,29-dioic acid.

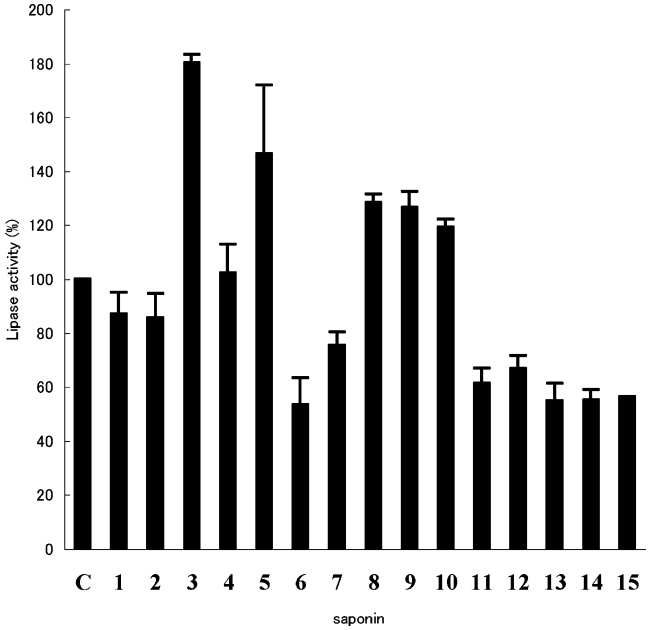


Figure 1. Effects of various saponins isolated from *A. senticosus* on pancreatic lipase activity. Results are expressed as means ± SEM of three experiments. (C: control; **1**−**15**: saponins **1**−**15**; final concentration: 1 mg/mL).

The number of persons suffering from obesity is continuously increasing, and being overweight is recognized as a risk factor for related diseases such as type II diabetes, hyperlipidemia, and hypertension. Pancreatic lipase is well known to play a crucial role in the digestion of long-chain triglycerides, and thus, inhibition of pancreatic lipase activities is an effective way to reduce fat absorption. It has been reported that oleanane-type and lupane-type triterpenoid saponins exhibit inhibitory effects upon pancreatic lipase and suppress the increase of body weight due to a high-fat diet.[3d,5b,10] To the best of our knowledge, the inhibition activities on pancreatic lipase of saponins from *A. senticosus* have not been studied. The pancreatic lipase inhibitory action of compounds **1**−**15** was evaluated in an assay system using triolein emulsified with phosphatidylcholine (Figure 1). The nor-oleanane-type saponin **6**

Table 1. ^{13}C and ^{1}H NMR Spectroscopic Data of the Aglycon Moieties of Compounds **1**, **7**, and **13** (in Pyridine-d_5)

	1		**7**		**13**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.8	0.85 m	38.8	0.89 m	38.9	0.96 m
		1.47 m		1.50 dt (12.1, 3.4)		1.50 dt (14.0, 3.8)
2	26.5	1.81 m	26.5	1.84 m	26.5	1.82 br t (13.3)
		2.06 m		2.07 m		2.08 dd (13.3, 3.8)
3	88.9	3.22 dd (11.6, 4.0)	88.9	3.20 dd (11.5, 4.6)	88.8	3.24 dd (11.7, 4.3)
4	39.5		39.5		39.5	
5	55.8	0.72 d (11.4)	55.9	0.72 d (11.5)	55.9	0.77 d (11.5)
6	18.5	1.29 br d (12.0)	18.6	1.29 m	18.5	1.30 m
		1.43 m		1.43 m		1.47 m
7	33.1	1.30 br d (11.9)	33.2	1.31 br d (12.2)	33.2	1.28 m
		1.43 m		1.45 m		1.47 m
8	39.9		39.9		39.8	
9	48.0	1.57 dd (10.1, 7.3)	48.1	1.60 dd (10.1, 7.5)	48.0	1.66[a]
10	37.0		37.0		37.1	
11	23.8	1.85 m (2H)	23.8	1.89 m (2H)	23.8	1.91 m (2H)
12	123.3	5.44 t (3.5)	122.9	5.41 t (3.4)	123.1	5.54 t (3.4)
13	143.4		144.1		144.3	
14	42.1		42.2		42.2	
15	28.3	1.16 br d (13.7)	28.3	1.15 br d (14.0)	28.3	1.20 br d (13.3)
		2.29 br t (11.8)		2.30 td (14.0, 3.2)		2.17 m
16	23.5	2.04 m	23.4	1.96 br d (14.0)	23.8	2.06 br d (12.3)
		2.18 m		2.09 m		2.26 td (13.8, 3.9)
17	47.5		47.1		46.7	
18	47.4	3.10 dd (13.4, 4.7)	41.7	3.17 dd (9.8, 4.1)	41.1	3.45 dd (13.8, 4.1)
19	41.7	2.19 m	46.3	1.78 br t (10.6)	41.1	1.94 dt (13.8, 4.1)
		2.58 t (13.4)		1.22[a]		2.61 t (13.8)
20	148.4		30.8		42.6	
21	30.1	2.11 m	34.1	1.15 br d (14.0)	29.3	1.86 br d (12.6)
		2.20 m		1.36 td (14.0, 3.9)		2.35 td (13.6, 3.7)
22	37.7	1.74 td (13.8, 4.6)	32.6	1.80 br d (10.6)	32.4	1.98 dt (13.8, 3.5)
		2.06 m		1.94 m		2.17 m
23	28.3	1.21 s	28.3	1.22 s	28.1	1.17 s
24	16.8	1.05 s	16.8	1.05 s	17.0	1.06 s
25	15.6	0.88 s	15.6	0.89 s	15.5	0.85 s
26	17.5	1.07 s	17.5	1.09 s	17.4	1.01 s
27	26.0	1.19 s	26.1	1.23 s	26.1	1.31 s
28	175.7		176.5		180.0	
29	107.4	4.73 br s, 4.77 br s	33.2	0.91 s	181.1	
30			23.8	0.93 s	20.1	1.59 s

[a] Overlapped signals.

inhibited the pancreatic lipase activity by 46.5%, and compounds **3** and **5** enhanced the activity by 80.5% and 46.7%, at a concentration of 1 mg/mL. It is worthy of note that acetylation at Glc″-C-6 has major effects on activity, as suggested from the differences in lipase activity between **6** (inhibited by 46.5%) and **5** (enhanced by 80.5%), and **4** (no inhibition activity) and **3** (enhanced by 46.7%). Although the saponins with oleanolic acid as the aglycon exhibited no inhibition effect on pancreatic lipase, saponin **11**, with 16α-hydroxyoleanolic acid (echinocystic acid) as the aglycon, inhibited lipase activity by 38.3% at a concentration of 1 mg/mL. Further, oleanane-type saponins **12** and **13**, bearing a free carboxyl moiety, inhibited the activity of pancreatic lipase by 33.0% and 44.9%. Both the seco-lupane-type saponins **14** (44.6% inhibition) and **15** (56.1% inhibition) exhibited effects in good agreement with a recently published report about lupane-type saponins from the leaves of *A. sessiliflorus*.[3d]

Experimental Section

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. FABMS and HRFABMS 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, Japan), silica gel (silica gel 60, Kanto Chemical Co., Inc., Tokyo, Japan), 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 instrument.

Plant Material. The leaves of *A. senticosus* were collected in October 2004 at Yagou, Acheng, Heilongjiang Province, People's Republic of China. Plant identification was confirmed by one of the authors (T.N.). The specimen (CWJY-051016) has been deposited at the Department of Chinese Traditional Medicine, Heilongjiang Provincial Institute for Drug Control, People's Republic of China.

Extraction and Isolation. Dried leaves (2.7 kg) of *A. senticosus* were extracted twice with 70% EtOH at room temperature. Evaporation of the solvent under reduced pressure gave an extract (423.5 g) that was then partitioned between *n*-BuOH and H_2O. The *n*-BuOH layer was evaporated under reduced pressure below 40 °C to give a residue (94.1 g), which was subjected to passage over a Diaion HP-20 column and eluted with H_2O and 30%, 60%, 80%, and 100% MeOH, successively. The 80% MeOH and MeOH eluates were concentrated (25.6 and 10.3 g) and chromatographed separately on silica gel, with a gradient of $CHCl_3$−MeOH−H_2O (60:20:3, 60:29:6, 6:4:1) and ODS, with 60−70% MeOH, to give several saponin fractions. Further HPLC purification (30−37% MeCN in H_2O) afforded **1** (34 mg), **2** (72 mg), **3** (210 mg), **4** (46 mg), **5** (227 mg), **6** (57 mg), **7** (46 mg), **8** (240 mg), **9** (224 mg), **10** (44 mg), **11** (29 mg), **12** (54 mg), **13** (8 mg), **14** (38 mg), and **15** (758 mg).

Acanthopanaxoside A (1): amorphous powder, $[\alpha]^{22}_D$ +11.8 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3408, 1736, 1632, 1383, 1261, 1073 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; negative-ion FABMS *m*/*z* 1245 $[M - H]^-$, 1203 $[M - C_2H_3O]^-$, 765 $[M - C_{31}H_{45}O_4]^-$, 733 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m*/*z* 1245.5963 (calcd for $C_{60}H_{93}O_{27}$ $[M - H]^-$, 1245.5904).

Table 2. ^{13}C and ^{1}H NMR Spectroscopic Data of Sugar Moieties of Compounds **1**, **7**, and **13** (in Pyridine-d_5)

	1		**7**		**13**	
position	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
3-*O*-sugar moieties						
ara 1	104.8	4.97 d (3.9)	104.8	4.96 d (5.7)	104.8	4.91 d (5.3)
2	80.9	4.59 t (6.4)	81.0	4.60 dd (7.1, 5.7)	75.9	4.56 t (5.5)
3	73.4	4.35[a]	73.4	4.37[a]	73.8	4.29[a]
4	68.3	4.35[a]	68.3	4.37[a]	68.6	4.28 m
5	64.9	3.79 br d (10.1)	64.9	3.79 dd (11.4, 1.6)	64.6	3.84 dd (12.2, 2.5)
		4.29[a]		4.30[a]		4.30 dd (12.2, 5.3)
glc 1	106.0	5.18 d (7.7)	106.0	5.19 d (7.7)		
2	76.4	4.10 t (8.9)	76.4	4.08[a]		
3	78.2	4.19 t (8.9)	78.2	4.20 t (9.0)		
4	71.6	4.31[a]	71.7	4.30[a]		
5	78.1	3.81 m	78.1	3.82 m		
6	62.6	4.42 br s	62.6	4.43 br s		
rha 1					101.7	6.13 d (0.9)
2					72.4	4.74 dd (3.2, 1.4)
3					72.6	4.63 dd (9.3, 3.2)
4					74.1	4.29 t (9.3)
5					69.9	4.58 dq (9.3, 6.2)
6					18.6	1.64 d (6.2)
28-*O*-sugar moieties						
glc inner 1	95.7	6.18 d (8.2)	95.6	6.24 d (8.0)		
2	73.4	4.10 t (8.9)	73.9	4.10 t (8.4)		
3	78.7	4.18 t (8.9)	78.8	4.20 t (9.0)		
4	70.9	4.25 t (9.3)	71.0	4.25 t (9.6)		
5	78.0	4.08 m	78.2	4.10 m		
6	69.5	4.33 dd (9.6, 5.1)	69.4	4.35 dd (11.7, 5.0)		
		4.65 br d (9.6)		4.68 dq (11.7, 1.9)		
glc outer 1	104.8	4.98 d (8.1)	104.8	5.00 d (7.8)		
2	75.0	3.95 t (8.1)	75.1	3.95 t (7.8)		
3	76.4	4.13 t (8.7)	76.4	4.12 t (8.3)		
4	79.2	4.09 t (8.9)	79.2	4.10 t (8.7)		
5	73.7	3.83 m	73.7	3.83 m		
6	63.7	4.53 dd (11.9, 4.6)	63.7	4.54 dd (11.9, 4.6)		
		4.63 dd (11.9, 1.8)		4.63 dd (11.9, 2.1)		
rha 1	102.9	5.53 br s	103.0	5.54 d (1.4)		
2	72.4	4.62[a]	72.4	4.62 br s		
3	72.7	4.50 dd (9.1, 3.2)	72.7	4.50 dd (8.7, 1.8)		
4	73.8	4.30[a]	73.8	4.30[a]		
5	70.6	4.84 dq (10.9, 6.2)	70.7	4.85 dq (9.3, 6.2)		
6	18.5	1.71 d (6.2)	18.5	1.70 d (6.2)		
CH_3*C*O	20.6	1.93 s	20.6	1.93 s		
CH_3*C*O	170.6		170.6			

[a] Overlapped signals.

Acanthopanaxoside B (7): amorphous powder, $[\alpha]^{22}_D$ −4.4 (*c* 0.8, MeOH); IR (KBr) ν_{max} 3405, 1733, 1632, 1591, 1383, 1256, 1071 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; negative-ion FABMS *m*/*z* 1261 $[M - H]^-$, 1219 $[M - C_2H_3O]^-$, 749 $[M - C_{20}H_{33}O_{15}]^-$; HRFABMS *m*/*z* 1261.6210 (calcd for $C_{61}H_{97}O_{27}$ $[M - H]^-$, 1261.6218).

Acanthopanaxoside C (13): amorphous powder, $[\alpha]^{25}_D$ −3.2 (*c* 0.8, MeOH); IR (KBr) ν_{max} 3430, 1700, 1547, 1463, 1386, 1257, 1237, 1054 cm^{-1}; ^{1}H NMR (pyridine-d_5, 500 MHz) and ^{13}C NMR (pyridine-d_5, 125 MHz), see Tables 1 and 2; negative-ion FABMS *m*/*z* 763 $[M - H]^-$; HRFABMS *m*/*z* 763.4297 (calcd for $C_{41}H_{63}O_{13}$ $[M - H]^-$, 763.4269).

Acid Hydrolysis of 1, 7, and 13 and Determination of the Absolute Configuration of the Monosaccharide Units. A solution of **1**, **7**, and **13** (each 0.5 mg) in 1 M HCl (dioxane−H_2O, 1:1, 200 μL) was heated at 100 °C for 1 h under an Ar atmosphere. After dioxane was removed, each solution was extracted with EtOAc (1 mL × 3) to obtain the aglycons **1a**, **7a**, and **13a**. The aglycons were identified as akebonic acid (**1a**),[7] oleanolic acid (**7a**), and serratagenic acid (**13a**),[9] respectively, by comparing their ^{1}H and ^{13}C NMR data with those in the literature. Each aqueous layer was neutralized by passing through an ion-exchange resin (Amberlite MB-3, Organo, Tokyo, Japan) column and concentrated under reduced pressure to dryness, to give a residue of the sugar fraction. Each 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. Each mixture was kept at 60 °C for 1.5 h. After the reaction mixture was dried in vacuo, each residue was trimethylsilylated with 1-trimethylsilylimidazole (0.1 mL) for 2 h. Each 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-1 (30 m × 0.25 mm × 0.25 μm, Supelco), column temperature, 230 °C; injection temperature, 250 °C; carrier gas, N_2. In the acid hydrolyzate of **1**, **7**, and **13**, D-glucose, L-arabinose, and L-rhamnose were confirmed by comparison of the retention times of their derivatives with those of the D-glucose, L-glucose, L-arabinose, and L-rhamnose derivatives prepared in a similar way, which showed retention times of 10.80, 11.20, 6.31, and 7.51 min, respectively.

Measurement of Pancreatic Lipase Activity. Lipase activity was determined by measuring the rate of release of oleic acid from triolein. Briefly, a suspension of triolein (80 mg), phosphatidylcholine (10 mg), and taurocholic acid (5 mg), in 9 mL of 0.1 M *N*-tris(hydroxymethyl)-methyl-2-aminoetheanesulfonic acid (TES) buffer (pH 7.0) containing 0.1 M NaCl, was sonicated for 5 min. This sonicated substrate suspension (100 μL) was incubated with 50 μL (10 units) of pancreatic lipase and 100 μL of various sample solutions for 30 min at 37 °C in a final volume of 250 μL. The amount of released oleic acid was determined by the method of Zapf et al.,[11] with a slight modification.[12] The incubation mixture was added to 3 mL aliquots of a 1:1 (v/v) mixture of $CHCl_3$ and *n*-heptane containing 2% (v/v) MeOH and extracted by shaking the tubes horizontally for 10 min in a shaker. The mixture was centrifuged at 2000*g* for 10 min, and the upper aqueous phase was removed by suction. A copper reagent (1 mL) was then added to the lower organic phase. The tube was shaken for 10 min, the mixture was centrifuged at 2000*g* for 10 min, and 0.5 mL of the upper organic phase, which contained copper salts of the extracted free fatty acids, was treated with 0.5 mL of 0.1% (w/v) bathocuproine in

$CHCl_3$ containing 0.05% (w/v) 3(2)-*tert*-butyl-4-hydroxyanisole. The absorbance was then measured at 480 nm. Orlistat was used as the positive control with an IC_{50} value of 0.04 mg/mL. Lipase activity was expressed as micromoles of oleic acid released per milliliter of reaction mixture per hour.

Acknowledgment. This research was partially supported by the Ministry of Education, Science, Sports and Culture of Japan, Grant-in-Aid for Young Scientists (B), 18790102, 2006.

References and Notes

(1) *Zhong Hua Ben Cao*; Shanghai Scientific Technologic Publisher: Shanghai, 1999; Vol. 5, pp 765−775.
(2) Frolova, G.; Ovodov, Y.; Suprunov, N. *Khimiya Prirodnykh Soedinenii* **1971**, *7*, 614−618. (*Chem. Abstr.* **1971**, *104*, 165302).
(3) (a) Shao, C.; Kasai, R.; Xu, J.; Tanaka, O. *Chem. Pharm. Bull.* **1988**, *36*, 601−608. (b) Shao, C.; Kasai, R.; Xu, J.; Tanaka, O. *Chem. Pharm. Bull.* **1989**, *37*, 42−45. (c) Melek, F.; Miyase, T.; Abdel, S.; Hetta, M.; Mahmoud, I. *Phytochemistry* **2002**, *60*, 185−195. (d) Yoshizumi, K.; Hirano, K.; Ando, H.; Hirai, Y. *J. Agric. Food Chem.* **2006**, *54*, 335−341. (e) Jin, J.; Lee, S.; Lee, Y.; Kim, J.; Heo, J.; Hye, S. *Planta Med.* **2004**, *70*, 564−566.
(4) (a) Ma, L.; Lu, Z.; Lu, W.; Sui, D.; Cheng, H. *Zhongguo Yaoxue Zazhi* **1994**, *29*, 654−657. (b) Sui, D.; Lu, Z.; Li, S.; Cai, Y. *Zhongguo Zhongyao Zazhi* **1994**, *19*, 683−685. (c) Sui, D.; Lu, Z.; Ma, L.; Fan, Z. *Zhongguo Zhongyao Zazhi* **1994**, *19*, 746−747. (d) Sui, D.; Han, C.; Yu, X.; Qu, S. *Jilin Daxue Xuebao Yixueban* **2004**, *30*, 56−59. (e) Liu, L.; Sui, D.; Qu, S.; Yu, X.; Wang, Z.; Chen, Y. *Jilin Daxue Xuebao Yixueban* **2004**, *30*, 66−70.
(5) (a) Liu, L.; Li, W.; Koike, K.; Zhang, S.; Nikaido, T. *Chem. Pharm. Bull.* **2004**, *52*, 566−569. (b) Zheng, Q.; Zheng, Q.; Koike, K.; Han, L.; Okuda, H.; Nikaido, T. *J. Nat. Prod.* **2004**, *67*, 604−613. (c) Koike, K., Li, W., Liu, L., Hata, E., Nikaido, T. *Chem. Pharm. Bull.* **2005**, *53*, 225−228. (d) Chang, X.; Li, W.; Koike, K.; Wu, L.; Nikaido, T. *Chem. Pharm. Bull.* **2006**, *54*, 748−750.
(6) (a) Li, W.; Asada, Y.; Koike, K.; Nikaido, T.; Furuya, T.; Yoshikawa, T. *Tetrahedron* **2005**, *61*, 2921−2929. (b) Fu, H.; Koike, K.; Li, W.; Nikaido, T.; Lin, W.; Guo, D. *J. Nat. Prod.* **2005**, *68*, 754−758.
(7) Wu, S.; Yang, S.; Wu, A.; Cheng, Y.; Peng, Q. *Helv. Chim. Acta* **2005**, *88*, 259−265.
(8) Oshima, R.; Yamauchi, Y.; Kumanotani, J. *Carbohydr. Res.* **1982**, *107*, 169−176.
(9) Yu, S.; Yu, D.; Liang, X. *Phytochemistry* **1995**, *38*, 695−698.
(10) Kasai, R.; Matsumoto, K.; Taniyasu, S.; Tanaka, O.; Kim, J. H.; Hahn, D. R. *Chem. Pharm. Bull.* **1986**, *34*, 3284−3289.
(11) Zapf, J.; Schoenle, E.; Waldvoge, M.; Sand, M.; Foresch, E. *Eur. J. Biochem.* **1981**, *113*, 605−610.
(12) Tsujita, T.; Okuda, H. *Eur. J. Biochem.* **1983**, *133*, 215−220.

NP060195+