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Saponins from Leaves of *Kalopanax septemlobus* (THUNB.) KOIDZ.: Structures of Kalopanax-saponins La, Lb and Lc

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From leaves of *Kalopanax septemlobus* (THUNB.) KOIDZ., three new saponins named kalopanax-saponins La (6), Lb (7) and Lc (8) were isolated together with five known saponins, kalopanax-saponins A and B, akeboside Stb, eleutheroside K and saponin Pg. The new sapogenin 9 named kalopanax-genin L1, which is common to 6, 7 and 8, was formulated as 22α-hydroxyhederagenin. On the basis of chemical and spectral data, the structures of these saponins were elucidated as follows: 3-*O*-α-L-arabinopyranosyl-22α-hydroxyhederagenin (6), 3-*O*-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-22α-hydroxyhederagenin (7) and 3-*O*-β-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-22α-hydroxyhederagenin (8).

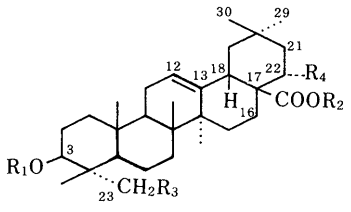
Keywords *Kalopanax septemlobus*; Araliaceae; Chinese folk medicine; ciqiu; saponin; kalopanax-saponin; 22α-hydroxyhederagenin; 3β,22α,23-trihydroxyolean-12-en-28-oic acid

In the previous paper,[1] we reported the isolation and structural determination of three new saponins named kalopanax-saponins C, D and F[2] together with four known saponins from roots of *Kalopanax septemlobus* (THUNB.) KOIDZ (Chinese name: ciqiu 刺楸, Japanese name: harigiri) collected in Jilin district, North-East China. Continuing the investigation of this plant, we have studied saponins of the leaves of this plant. The present report deals with isolation and structure elucidation of three new saponins together with identification of five known saponins from the leaves.

Air-dried leaves of *Kalopanax. septemlobus* were extracted with methanol. A suspension of the methanolic extract in water were washed with ethyl ether and extracted with ethyl acetate and then with 1-butanol saturated with water, successively. The ethyl acetate extract was subjected to chromatography on silica gel followed by high-performance liquid chromatography (HPLC) to give saponins **1**, **2**, **3**, **4**, **6**, **7** and **8** (yields from dried leaves: 0.008, 0.01, 0.21, 0.006, 0.01, 0.002 and 0.04%, respectively). Chromatography of the butanolic extract in a similar manner afforded a saponin **5** (yield from dried leaves: 0.05%).

Based on the proton- and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) evidence and the result of acid hydrolysis, as well as comparison of the optical rotations, saponin **1** was shown to be identical with akeboside Stb from *Akebia quinata* (=β2-fatsin from *Fatsia japonica*).[3] In the same manner, saponin **2** was found to be identical with eleutheroside K from *Acanthopanax senticosus*[4] and saponins **3** and **5** were proved to be identical with kalopanax-saponins A and B,[1,5] respectively. Saponin **4** was identical with saponin Pg from *Akebia quinata*.[6]

A new saponin **6** named kalopanax-saponin La, on acid hydrolysis, afforded arabinose and a new sapogenin **9** named kalopanax-genin L1. The positive and negative fast atom bombardment mass spectrum (FAB-MS) of **9** exhibited $(M+Na)^+$ at *m/z* 511 (488+23) and $(M-H)^-$ at *m/z* 487 (488−1), respectively. The ^{1}H- and ^{13}C-NMR spectra (in C_5D_5N) of **9** showed six quaternary methyl signals [proton signals at δ 0.93, 0.94, 1.10, 1.11, 1.12, 1.14 (each



	R_1	R_2	R_3	R_4
1:	-Ara	H	OH	H
2:	-Ara^{2}-Rha	H	H	H
3:	-Ara^{2}-Rha	H	OH	H
4:	-Ara^{2}-Rha^{3}-Xyl	H	OH	H
5:	-Ara^{2}-Rha	-Glc^{6}-Glc^{4}-Rha	OH	H
6:	-Ara	H	OH	OH
7:	-Ara^{2}-Rha	H	OH	OH
8:	-Ara^{2}-Rha^{3}-Xyl	H	OH	OH
9:	H	H	OH	OH
10:	H	H	OH	H
11:	Ac	CH_3	OAc	OAc
12:	H	CH_3	OH	OH

Chart 1

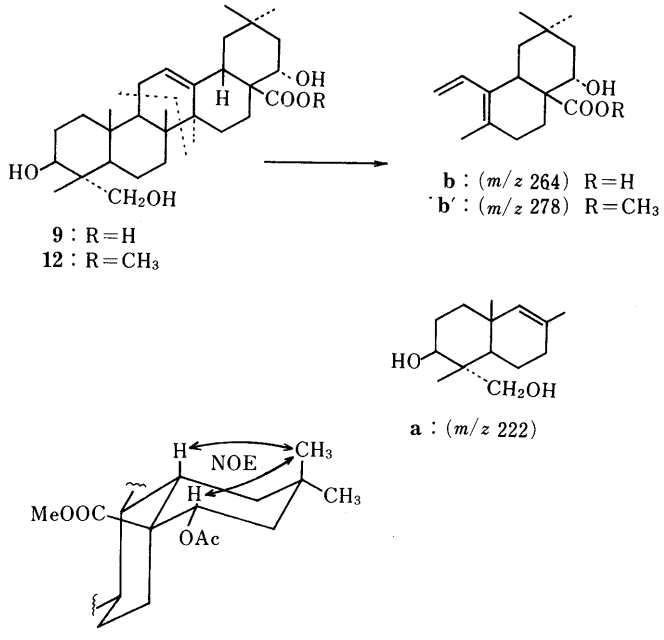


Chart 2

TABLE I. ^{13}C-NMR Chemical Shifts of Kalopanax Saponins in C_5D_5N

	Aglycone moieties						Sugar moieties			
	9	**11**[a]	**6**	**7**	**8**	**10**[7]		**6**	**7**	**8**
							Ara			
C-1	38.80	37.74	38.79	38.65	39.23	38.53	1	106.63	104.30	104.25
C-2	27.97	23.40	26.10	26.22	26.37	27.40	2	73.14	75.79	75.88
C-3	73.59	74.51	81.91	81.05	81.44	73.32	3	74.68	74.67	74.32
C-4	42.60	40.53	42.56	42.55	42.89	42.59	4	69.58	69.69	69.16
C-5	48.64	47.61	47.61	47.67	48.01	48.48	5	66.92	65.78	65.36
C-6	18.62	17.93	18.19	18.52	18.47	18.34	Rha			
C-7	32.85	32.16	32.77	32.80	33.04	32.74	1		101.67	101.52
C-8	39.96	39.43	40.02	39.96	40.16	39.54	2		72.52	71.94
C-9	48.15	47.86	48.15	48.15	48.39	47.92	3		72.38	83.10
C-10	37.19	36.72	36.94	36.85	37.14	37.00	4		74.13	73.01
C-11	23.88	22.93	23.89	23.75	23.93	23.50	5		69.30	69.79
C-12	122.82	123.27	122.88	122.77	122.62	122.23	6		18.52	18.47
C-13	144.21	142.17	144.19	144.26	144.51	144.57	Xyl			
C-14	42.09	41.69	42.56	42.55	42.88	41.95	1			107.17
C-15	27.69	26.86	27.98	27.98	28.27	28.10	2			75.54
C-16	16.90	16.82	16.90	17.01	17.49	23.45	3			78.27
C-17	53.08	50.99	53.10	53.08	53.17	46.21	4			71.16
C-18	43.52	42.39	43.53	43.52	43.67	41.75	5			67.31
C-19	46.20	44.90	45.95	45.98	46.70	46.40				
C-20	31.53	31.42	31.53	31.48	31.58	30.67				
C-21	43.23	39.10	43.28	43.52	43.77	33.96				
C-22	71.54	72.72	71.52	71.55	71.94	32.96				
C-23	67.99	65.45	64.53	64.50	64.53	67.92				
C-24	13.11	13.07	13.59	13.99	14.04	12.78				
C-25	15.94	15.74	16.07	16.03	16.28	15.69				
C-26	17.50	16.75	17.44	17.45	17.79	17.23				
C-27	26.71	26.23	26.71	26.71	26.66	25.88				
C-28	179.50	175.04	179.40	179.60	180.05	179.86				
C-29	33.39	32.88	33.38	33.39	33.53	32.96				
C-30	25.15	24.67	25.16	25.20	25.29	23.59				
OCH_3		51.94								
CH_3CO		170.94								
		170.69								
		170.13								
CH_3CO		21.21								
		21.01								
		20.90								

a) Determined in $CDCl_3$.

3H, s), carbon signals see Table I] and exhibited signals due to the following groups at very similar positions to those observed for hederagenin (**10**)[7]; a carboxyl group (a carbon signal at δ 179.5), a trisubstituted double bond [carbon signals at δ 122.8 and 144.2, a proton signal at δ 5.51 (1H, t-like)], a primary alcoholic group [a carbon signal at δ 68.0, proton signals at δ 3.75, 4.20 (each 1H, d, J=10.8 Hz)] and a secondary alcoholic group [a carbon signal at δ 73.6, a proton signal at δ 4.25 (1H, dd, coupling constant could not be determined due to partial signal overlap)]. A proton signal due to 18-H which is characteristic of olean-12-en-28-oic acid type triterpenes[7] was observed for **9** at δ 3.56 (1H, dd, J=4.4, 10.2 Hz) and the presence of an additional secondary hydroxyl group was indicated by a carbon signal at δ 71.5 and a proton signal at δ 4.58 (1H, dd, J=5.9, 10.8 Hz). The ^{1}H-NMR spectrum (in $CDCl_3$) of a methyl ester acetate (**11**) of **9** showed signals due to six quaternary methyls [δ 0.73, 0.87, 1.09, 1.17 (each 3H, s), 0.99 (6H, s)], three acetoxyl groups [δ 2.04, 2.06, 2.16 (each 3H, s)], a carbomethoxy group [δ 3.62 (3H, s)], the 18-H [δ 2.99 (1H, dd, J=4.4, 10.3 Hz)], a primary acetoxyl group [δ 3.73, 3.92 (each 1H, d, J=11.6 Hz)] and two secondary acetoxyl groups [δ 4.82 (1H, dd, J=5.1, 11.2 Hz), 5.22 (1H, dd, J=7.0, 10.3 Hz)]. The fragment-ions which are characteristic of the retro Diels–Alder cleavage of olean-12-en-28-oic acid derivatives were observed as follows (Chart 2); the positive FAB-MS of **9** showed ions at m/z 223 $(a+H)^+$ and 265 $(b+H)^+$ and the electron impact mass spectrum (EI-MS) of a methyl ester (**12**) of **9** exhibited ions at m/z 278 (b′), 219 (b′$-COOCH_3$) and 201 ($219-H_2O$). These results suggested that **9** is a hydroxyhederagenin with an additional hydroxyl group located in the D or E ring.

In the ^{13}C-NMR (Table I), on going from **10** to **9**, signals due to 17-, 21- and 22-C were displaced downfield by 6.9, 9.2 and 38.6 ppm, respectively and that due to 16-C was significantly shifted upfield by 6.6 ppm, while other carbon signals remained at almost unchanged positions. Similar displacement of carbon signals was reported from erythrodiol (olean-12-ene-3β,28-diol) to 22α-hydroxyerythrodiol.[8] Taking into account the hydroxylation shift rule and the γ-gauche effect[9] as well as the coupling constants of the carbinyl proton signal of **9** (and **11**) (*vide supra*), it follows that the additional secondary hydroxyl group of **9** must be located at 22α (equatorial). Assignment of proton signals due to the 29- and 30-*gem*-dimethyl groups of **11** was substantiated by ^{1}H–^{1}H and ^{1}H–^{13}C-correlation spec-

troscopy (COSY) as well as by the COLOC procedure. Irradiation of a signal at δ 1.09 (3H, s, 30-CH_3)[10] resulted in nuclear Overhauser effect (NOE) at signals due to 18-H (5.5%) and 22β-H (4.4%), confirming the location of the secondary hydroxyl group at 22α. Based on these results, **9** can be formulated as 22α-hydroxyhederagenin.

The positive FAB-MS of **6** showed ions at *m/z* 643 $(M+Na)^+$, 621 $(M+H)^+$, 489 $(M+H-Ara)^+$. On comparison of the ^{13}C-NMR spectrum of **6** with that of **9** (Table I), the signal ascribable to 3-C of the aglycone moiety was displaced downfield by 8.3 ppm and the signal due to 2-C was displaced upfield by 1.9 ppm (glycosylation shift),[11-13] while other signals remained almost unshifted. Further, signals due to the sugar moiety of **6** (Table I) appeared at almost the same positions as those of α-L-arabinopyranosides[13] such as **1**.[1] It follows that **6** can be formulated as the 3-*O*-α-L-arabinopyranoside of **9**, as shown in Chart 1.

A new saponin **7** named kalopanax-saponin Lb, exhibited ions at *m/z* 789 $(M+Na)^+$ and 767 $(M+H)^+$ in the positive FAB-MS and on acid hydrolysis, **7** afforded **9**, arabinose and rhamnose. Comparison of the ^{13}C-NMR spectrum of **7** with that of **9** demonstrated the same glycosylation shift around 3-C as observed for **6** (Table I). Carbon signals of **7** at δ 104.3 and 101.7 indicated the presence of two sugar units. The observation of a fragment ion at *m/z* 621 $(M+H-Rha)^+$ in the positive FAB-MS of **7** indicated the presence of a terminal rhamnose unit. Carbon signals due to the sugar moiety of **7** (Table I) appeared at almost the same positions as those of 2-*O*-α-L-rhamnopyranosyl-α-L-arabinopyranosides[14] such as **3**.[1] Based on these results, **7** can be formulated as the 3-*O*-α-L-rhamnopyranosyl(1$\rightarrow$2)-α-L-arabinopyranoside of **9**.

Another new saponin **8**, named kalopanax-saponin Lc, exhibited ions at *m/z* 921 $(M+Na)^+$ and 899 $(M+H)^+$ in the positive FAB-MS and afforded **9**, arabinose, rhamnose and xylose on acid hydrolysis. The glycosylation shift around 3-C as well as three anomeric carbon signals at δ 107.2, 104.3 and 101.5 in the ^{13}C-NMR spectrum of **8** (Table I) disclosed that **8** is a 3-*O*-glycoside of **9** which has three monosaccharide units. In the positive FAB-MS of **8**, ions at *m/z* 767 $[M+H-pentose]^+$, 621 $[M+H-pentose-Rha]^+$ and 489 $[M+H-pentose-Rha-pentose]^+$ indicated that the sugar moiety of **8** consists of a linear pentose–rhamnose–pentose unit. On partial hydrolysis, **8** afforded **6** and **7** which were identified by comparison of the ^{1}H- and ^{13}C-NMR spectra with those of respective authentic samples. Further, carbon signals due to the sugar moiety of **8** (Table I) were observed at almost the same positions as those of **4**.[1] From these results, the structure of **8** was established as the 3-*O*-β-D-xylopyranosyl-(1$\rightarrow$3)-α-L-rhamnopyranosyl-(1$\rightarrow$2)-α-arabinopyranoside of **9**.

Experimental

Melting points were measured on a micro hot-stage and are uncorrected. Optical rotations were taken on a Union PM-101 automatic digital polarimeter. Infrared (IR) spectra were taken on a Shimadzu FTIR-4200 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a JEOL GX-400 spectrometer using tetramethylsilane (TMS) as an internal standard. MS were taken on a JEOL JMS-SX 102 spectrometer. Preparative HPLC was carried out on a column of TSK-gel ODS-120T (21.5 mm i.d. $\times$ 30 cm) with a Toyo Soda HLC 803D pump and a Toyo Soda RI-8 differential refractometer as a detector (flow rate of mobile phase: 6 ml/min). For column chromatography, Kieselgel 60 (70—230 mesh, Merck) was used. All solvent systems for chromatography were homogeneous.

Extraction and Separation of Saponins The air-dried leaves of *Kalopanax septemlobus* (1.5 kg), collected in Jilin, China, was extracted with hot MeOH. A suspension of the MeOH extract (139.5 g) in H_2O was washed with Et_2O and extracted with AcOEt and then with 1-BuOH saturated with H_2O. The AcOEt layer and BuOH layer were each concentrated to dryness to give the AcOEt extract (34 g) and BuOH extract (11.4 g), respectively. The AcOEt extract was chromatographed on silica gel first with $CHCl_3$–MeOH (6:1) and then with $CHCl_3$–MeOH–H_2O (40:10:1) to give three fractions (frs.), frs. I, II and III in order of elution, and each of these was separated and purified by HPLC: fr. I (mobile phase 85% MeOH) gave **1** (yield from dried leaves: 0.008%) and **2** (0.01%); fr. II (mobile phase 77% MeOH) afforded **3** (0.21%) and **6** (0.006%); fr. III (mobile phase 75% MeOH) gave **4** (0.01%), **7** (0.002%) and **8** (0.004%). The BuOH extract was chromatographed on silica gel with $CHCl_3$–MeOH–H_2O (30:10:1 and 70:30:5, successively) to give frs. IV and V, the latter was purified by HPLC with 60% MeOH to give **5** (yield, 0.05%).

1: Colorless needles (MeOH), mp 228—230 °C (dec.), $[\alpha]_D^{20}$ +48.0° (c = 0.60, MeOH).

2: A white powder, $[\alpha]_D^{20}$ +11.3° (c = 0.52, MeOH).

3: Colorless needles (MeOH), mp 250—251 °C, $[\alpha]_D^{20}$ +16.8° (c = 0.50, MeOH).

4: A white powder, $[\alpha]_D^{20}$ +9.6° (c = 0.46, MeOH).

5: A white powder, $[\alpha]_D^{20}$ −8.3° (c = 0.80, MeOH).

6: A white powder, $[\alpha]_D^{20}$ +40.4° (c = 0.42, MeOH). *Anal.* Calcd for $C_{35}H_{56}O_9 \cdot H_2O$: C, 65.80; H, 9.15. Found: C, 65.75; H, 9.12. ^{1}H-NMR (C_5D_5N) δ: 0.93, 0.94, 1.00, 1.07, 1.12, 1.29 (each 3H, s), 5.49 (1H, t-like, 12-H), 4.99 (1H, d, J = 7.0 Hz, anomeric H).

7: A white powder, $[\alpha]_D^{20}$ +49.3° (c = 0.55, MeOH). *Anal.* Calcd for $C_{41}H_{66}O_{13} \cdot 2H_2O$: C, 61.32; H, 8.79. Found: C, 61.42; H, 8.64. ^{1}H-NMR (in C_5D_5N) δ: 0.94, 0.99, 1.12, 1.28 (each 3H, s), 1.07 (6H, s), 1.64 (3H, d, J = 6.0 Hz, CH_3 of Rha), 5.16 (1H, d, J = 7.0 Hz, anomeric H of Ara), 6.27 (1H, s, anomeric H of Rha), 5.48 (1H, t-like, 12-H).

8: A white powder, $[\alpha]_D^{20}$ +45.6° (c = 0.40, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{17} \cdot 2H_2O$: C, 59.08; H, 8.41. Found: C, 59.49; H, 8.69. ^{1}H-NMR (C_5D_5N) δ: 0.92, 1.03, 1.06, 1.27 (each 3H, s), 0.97 (6H, s), 1.66 (3H, d, J = 6.0 Hz, CH_3 of Rha), 5.01, 5.12 (each 1H, d, J = 7.0 Hz, anomeric H of Ara and Xyl), 5.98 (1H, s, anomeric H of Rha), 5.38 (1H, t-like, 12-H).

Acid Hydrolysis of 6, 7 and 8 On heating with 1.5% aqueous H_2SO_4 for 24 h at 70 °C followed by work-up in the usual way, **6** afforded **9** and arabinose; **7** afforded arabinose, rhamnose and **9**; **8** afforded arabinose, rhamnose, xylose and **9**. Identification of the resulting monosaccharides was carried out as described in the previous paper.[15]

9: A white powder, $[\alpha]_D^{20}$ +67.3° (c = 0.32, EtOH). *Anal.* Calcd for $C_{30}H_{48}O_5 \cdot 2H_2O$: C, 68.67; H, 9.99. Found: C, 68.70; H, 9.77. IR (Nujol) cm^{-1}: 3360 (OH), 1696 (COOH).

Methyl Ester Acetate (11) of 9 The aglycone **9** (20 mg) was treated with CH_2N_2 in MeOH–Et_2O and the product was purified by column chromatography on silica gel [solvent: $CHCl_3$–MeOH (10:1)] to give a methyl ester (**12**) as a white powder, which was acetylated by heating with anhydrous $(CH_3CO)_2O$ (1.5 ml) and C_5H_5N (1.5 ml) in a sealed tube at 80 °C for 2—3 h. The reaction mixture was concentrated to dryness by blowing N_2 gas over it at room temperature and then purified by column chromatography on silica gel [solvent: benzene–acetone (8:1)] to give **11** (15 mg) as a white powder. Positive FAB-MS $(M+H)^+$ Calcd for $C_{37}H_{56}O_8$ + H: 629.4053; Found: 629.4027.

Partial Hydrolysis of 8 A solution of **8** (45 mg) in aqueous 1.5% H_2SO_4 (15 ml) was heated at 70 °C for 8 h. The reaction mixture was diluted with H_2O and then extracted with 1-BuOH saturated with H_2O. The BuOH layer was concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$–MeOH (4:1) to give **6** (10 mg) and **7** (12 mg), identification of which was achieved by comparison of the ^{1}H- and ^{13}C-NMR spectra with those of respective authentic samples.

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References and Notes

1) C.-J. Shao, R. Kasai, J.-D. Xu, and O. Tanaka, *Chem. Pharm. Bull.*, **37**, 311 (1989).
2) Kalopanax-saponin E in the previous report[1] is identical with

spinasaponin A which has already been isolated from *Spinacia oleracea* not as a free form, but as a methyl ester [R. Tschesche, H. Rehkaemper, and G. Wulff, *Justus Liebigs. Ann. Chem.*, **726**, 125 (1969)].
3) M. Fujita, H. Itokawa, and Y. Kumekawa, *Yakugaku Zasshi*, **94**, 194 (1974); M. Tanemura and K. Takamura, *ibid.*, **95**, 10 (1975).
4) G. M. Frolova, Yu. S. Ovadov, and N. I. Suprunov, *Khim. Prir. Soedin.*, **7**, 614, 618 (1971); C.-J. Shao, R. Kasai, J.-D. Xu, and O. Tanaka, *Chem. Pharm. Bull.*, **36**, 601 (1988).
5) A. Ya. Khorlin, A. G. Ven'yaminova, and N. K. Kochetkov, *Dokl. Akad, Nauk. SSSR Ser. Khim.*, **1966**, 1588 (1966).
6) R. Higuchi and T. Kawasaki, *Chem. Pharm. Bull.*, **24**, 1021 (1976); R. Encarnacion, L. Kenne, G. Samuelsson, and F. Sandberg, *Phytochemistry*, **20**, 1939 (1981).
7) A. Inada, M. Yamada, H. Murata, M. Kobayashi, H. Toya, Y. Kato, and T. Nakanishi, *Chem. Pharm. Bull.*, **36**, 4269 (1988).
8) K. Tori, S. Seo, A. Shimaoka, and Y. Tomita, *Tetrahedron Lett.*, **48**, 4227 (1974).
9) H. Beierbeck and J. K. Sanders, *Can. J. Chem.*, **54**, 2985 (1976).
10) H. Kojima and H. Ogura, *Phytochemistry*, **28**, 1703 (1989).
11) R. Kasai, M. Suzuo, J. Asakawa, and O. Tanaka, *Tetrahedron Lett.*, **1977**, 175.
12) R. Kasai, M. Okihara, J. Asakawa, K. Mizutani, and O. Tanaka, *Tetrahedron*, **35**, 1427 (1979).
13) K. Mizutani, R. Kasai, and O. Tanaka, *Carbohydr. Res.*, **87**, 19 (1980).
14) K. Mizutani, A. Hayashi, R. Kasai, O. Tanaka, N. Yoshida, and T. Nakajima, *Carbohydr. Res.*, **126**, 177 (1984).
15) K. Mizutani, K. Ohtani, J.-X. Wei, R. Kasai, and O. Tanaka, *Planta Medica*, **1984**, 327.