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Steroidal Saponins from Elephant Garlic, Bulbs of *Allium ampeloprasum* L.

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From bulbs of *Allium ampeloprasum* L. (elephant garlic: Liliaceae), a new spirostane-type saponin, named ampeloside Bs_1 (**7**), and two new furostane-type saponins, named ampelosides-Bf_1 (**8**) and -Bf_2 (**9**), were isolated along with a known spirostane-type saponin, the prosapogenin of aginoside (**1**). The structures of the new saponins (**7**—**9**) were established as agigenin 3-*O*-β-glucopyranosyl(1→3)-β-glucopyranosyl(1→4)-β-galactopyranoside, (25*R*)-26-*O*-β-glucopyranosyl-22-hydroxy-5α-furostane-2α,3β,6β,26-tetraol-3-*O*-β-glucopyranosyl(1→3)-β-glucopyranosyl-(1→4)-β-galactopyranoside and (25*R*)-26-*O*-β-glucopyranosyl-22-hydroxy-5α-furostane-2α,3β,6β,26-tetraol-3-*O*-β-glucopyranosyl(1→4)-β-galactopyranoside, respectively. Antifungal activity of these saponins (**1**, **7** and **8**) is discussed.

Keywords——*Allium ampeloprasum*; elephant garlic; Liliaceae; furostane-type saponin; spirostane-type saponin; ampeloside Bs_1; ampeloside Bf_1; ampeloside Bf_2; agigenin; ^{13}C-NMR; antifungal activity

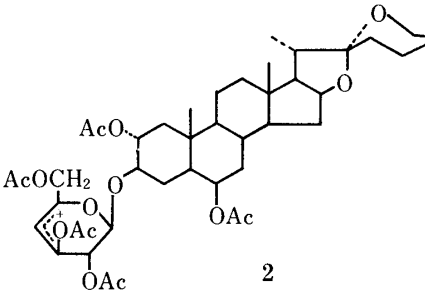
Allium ampeloprasum L. (local name, elephant garlic or great-headed garlic; Liliaceae), which is cultivated mainly in the U.S.A. and Canada, has leek-like tops and flowers, and large garlic-like bulbs. Elephant garlic has a much milder flavor than garlic (*A. sativum* L.; Liliaceae), and is used for domestic cooking. The existence of saponins and hemolytic activity,[1] and the toxicity of an alkaloidal extract[2] of *A. ampeloprasum* were reported. While chemical studies on saponins of *Allium* specimens have been reported,[3] no chemical investigation of *A. ampeloprasum* has been reported as yet.

The present paper deals with the isolation, structure determination, and antifungal activity of steroidal saponins of *A. ampeloprasum*.

The freezing bulbs (5 kg) were homogenized with methanol and then extracted with hot methanol. The extract was concentrated to dryness and the residue was subjected to column chromatography on highly porous polymer, which separated the saponin mixture from other substances in a yield of 0.26%. This saponin mixture was separated by repeated column chromatography to give four saponins, tentatively named I—IV in increasing order of polarities on silica gel thin layer chromatography (TLC).

Saponin I (**1**, 0.005% yield) showed characteristic absorption bands of the (25*R*)-spiroketal moiety in the infrared (IR) spectrum[4] at 976, 919, 894 and 862 cm^{-1} (KBr) (intensity; $919 < 894$, (25*R*)-spiroketal). Acid hydrolysis of **1** yielded glucose and galactose, and the proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **1** in C_5D_5N exhibited two anomeric proton signals at δ 5.26 (1H, d, $J=7.69$ Hz) and 4.98 (1H, d, $J=7.69$ Hz), indicating the presence of β-glycosyl linkages in **1**. The electron impact mass spectrum (EI-MS) of acetylated **1** showed fragment ions at *m*/*z* 619 [(hexosyl-hexose)Ac_7]$^+$ and 331 [(terminal hexose)Ac_4]$^+$ as well as ions at *m*/*z* 803 (fragment **2**) and 515 (fragment **3**) (Chart 1), while the field desorption mass spectrum (FD-MS) of **1** exhibited ions at *m/z* 795 $[M+Na]^+$ and 774 $[M+H]^+$. The permethylate[5] of **1** was subjected to sequence analysis of the sugar moiety by gas chromatography mass spectrum (GC-MS),[6] yielding 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, which originated from hexose linked at C-4-OH and terminal hexose, respectively. Mild hydrolysis of **1** afforded agigenin (**4**) which has already been isolated from *Allium giganteum*[7] and the prosapogenin (**5**), along with the starting material (**1**). Comparison of the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra (Table I) of **5** and **4** showed glycosylation shifts at C-2, C-3 and C-4, demonstrating that the 3-hydroxyl group of **5** was glycosylated. On acid hydrolysis, **5** gave galactose, and the EI-MS of acetylated **5** exhibited ions at *m/z* 862 $[M]^+$ and 331 $[(\text{terminal Gal})Ac_4]^+$ as well as an ion at *m/z* 515 (fragment **3**), while the FD-MS of **5** exhibited an ion at *m/z* 610 $[M]^+$. Compound **5** was shown characteristic absorption bands of the (25*R*)-spiroketal moiety in the IR spectrum[4] at 982, 919, 898 and 860 cm^{-1} (KBr) (intensity; $919 < 898$, (25*R*)-spiroketal). It follows that the structure of **5** can be formulated as the 3-*O*-β-galactopyranoside of **4**. On comparison of the ^{13}C-NMR spectrum (Table I) of the sugar moiety of **1** with that of **5**, an additional set of signals due to a terminal β-glucopyranoside unit appeared in the spectrum of **1** and further, the signals due to the β-galactopyranoside unit linked at C-4-OH of **5** were observed at positions very similar to those in the spectrum of **1**, leading to the formulation of **1** as the 3-*O*-β-glucopyranosyl(1→4)-β-galactopyranoside of **4**. The ^{13}C-NMR spectrum of **1** was consistent with this formulation. Compound **1** was identical with the prosapogenin of aginoside, a spirostane-type saponin corresponding to agigenin (**4**), which was obtained in the partial hydrolysis of aginoside (**6**) from *Allium giganteum*.[8]

On acid hydrolysis, the new saponin II (**7**) (0.007% yield), named ampeloside Bs$_1$, gave glucose and galactose, and the EI-MS of acetylated **7** exhibited ions at *m/z* 619 $[(\text{hexosyl-hexose})Ac_7]^+$, 331 $[(\text{terminal hexose})Ac_4]^+$ as well as ions at *m/z* 803 (fragment **2**) and 515 (fragment **3**). The FD-MS of **7** exhibited ions at *m/z* 974 $[M+K]^+$, 958 $[M+Na]^+$, 936



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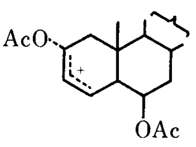

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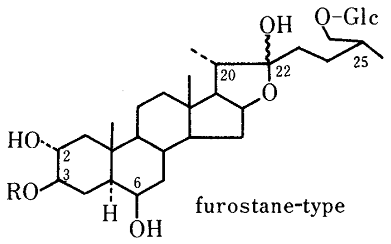
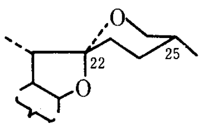
Chart 1



furostane-type



spirostane-type

furostane-type	R	spirostane-type
	–H	:**4**
	–Gal	:**5**
9:	–Gal$\overset{4}{—}$Glc	:**1**
8:	–Gal$\overset{4}{—}$Glc$\overset{3}{—}$Glc	:**7**
	–Gal$\overset{4}{—}$Glc$<^{2}_{3}$ Glc, Xyl	:**6**

Gal: β-D-galactopyranosyl
Glc: β-D-glucopyranosyl
Xyl: β-D-xylopyranosyl

Chart 2

TABLE I. ^{13}C-NMR Chemical Shifts (in C_5D_5N)

	4	5	1	9	7	8
Aglycone moiety						
C-1	47.9	47.3	47.2	47.1	47.2	47.1
C-2	73.1	70.2	70.5	70.4	70.5	70.4
C-3	77.2	85.5	85.2	85.0	85.1	85.0
C-4	34.9	32.0	31.8	31.7	31.8	31.7
C-5	48.4	47.9	47.9	47.8	47.9	47.8
C-6	70.2	70.0	70.0	69.9	70.0	69.9
C-7	40.8	40.9	40.8	40.7	40.8	40.7
C-8	30.0	30.0	30.0	29.8	30.0	29.8
C-9	54.7	54.6	54.6	54.5	54.6	54.5
C-10	37.6	37.0	37.0	36.9	37.0	36.9
C-11	21.4	21.4	21.4	21.3	21.4	21.3
C-12	40.2	40.1	40.1	40.1	40.1	40.1
C-13	40.8	40.9	40.9	41.1	40.8	41.1
C-14	56.3	56.2	56.3	56.0	56.2	56.0
C-15	32.2	32.2	32.2	32.4	32.2	32.3
C-16	81.1	81.1	81.1	81.0	81.1	81.0
C-17	63.0	63.0	63.1	63.8	63.0	63.7
C-18	16.6	16.5	16.6	17.1	16.6	17.1
C-19	17.4	17.3	17.3	16.3	17.3	16.3
C-20	41.9	42.0	42.0	40.5	42.0	40.5
C-21	15.0	15.0	15.0	16.6	15.0	16.6
C-22	109.2	109.2	109.2	110.6	109.2	110.5
C-23	31.7	31.8	31.9	36.9	31.8	36.9
C-24	29.2	29.2	29.2	28.3	29.2	28.2
C-25	30.5	30.6	30.6	34.1	30.6	34.1
C-26	66.8	66.8	66.9	75.2	66.9	75.2
C-27	17.3	17.2	17.2	17.4	17.2	17.4
Sugar moiety						
3-Gal 1	—	103.9	103.3	103.3	103.3	103.2
3-Gal 2	—	72.3	73.0	72.9	72.9	72.8
3-Gal 3	—	75.3	75.7[a]	75.7[a]	75.5[a]	75.4[a]
3-Gal 4	—	70.7	80.1	79.9	80.0	79.8
3-Gal 5	—	77.2	75.9[a]	75.8[a]	75.7[a]	75.6[a]
3-Gal 6	—	62.4	60.9	60.9	60.9	60.9
Glc 1	—	—	107.1	106.9	106.4	106.2
Glc 2	—	—	75.2	75.0	74.6[a]	74.5[a]
Glc 3	—	—	78.5[b]	78.3[b]	88.3	88.1
Glc 4	—	—	72.2	72.1	70.3	70.2
Glc 5	—	—	78.7[b]	78.3[b]	78.5[b]	78.4[b]
Glc 6	—	—	63.1	62.7[c]	62.5[c]	62.4[c]
Glc 1	—	—	—	—	105.5	105.4
Glc 2	—	—	—	—	75.1[a]	75.0[a]
Glc 3	—	—	—	—	78.0[b]	78.2[b]
Glc 4	—	—	—	—	71.6	71.5[d]
Glc 5	—	—	—	—	78.2[b]	78.4[b]
Glc 6	—	—	—	—	62.8[c]	62.7[c]
26-Glc 1	—	—	—	104.7	—	104.7
26-Glc 2	—	—	—	75.0	—	74.9[a]
26-Glc 3	—	—	—	78.4[b]	—	78.1[b]
26-Glc 4	—	—	—	71.6	—	71.6[d]
26-Glc 5	—	—	—	78.5[b]	—	77.8[b]
26-Glc 6	—	—	—	63.0[c]	—	62.7[c]

Gal, β-D-galactopyranosyl; Glc, β-D-glucopyranosyl. *a*—*d*) Assignments in any column may be interchanged, though those given here are preferred.

TABLE II. Antifungal Activity of **1**, **7** and **8**

	C. albicans	*A. niger*
Furostane-type saponin		
8	>800	>800
Spirostane-type saponins		
1	100	>400
7	100	400
Amphotericin B[a]	0.25	2

Minimal inhibitory concentration value, μg/ml; *a*) control drug.

$[M+H]^+$ and 796 $[M+Na-Glc]^+$. Compound **7** showed characteristic absorption bands of the (25*R*)-spiroketal moiety in the IR spectrum[4] at 976, 919, 894 and 866 cm^{-1} (KBr) (intensity; 919<894, (25*R*)-spiroketal). The permethylate[5] of **7** was subjected to sequence analysis of the sugar moiety by GC-MS, yielding 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol, 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylhexitol and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol, which originated from hexose linked at C-4-OH, hexose linked at C-3-OH and terminal hexose, respectively. The anomeric proton signals of **7** at δ 5.26 (1H, d, $J=7.70$ Hz), 5.23 (1H, d, $J=7.69$ Hz) and 4.97 (1H, d, $J=7.69$ Hz) in C_5D_5N indicated that the anomeric configuration of the sugar linkages is β. On comparison of the ^{13}C-NMR spectra (Table I) of the sugar moieties of **7** with those of **1**, an additional set due to a terminal β-glucopyranoside unit appeared in the spectrum of **7** and further, the signals due to the β-glucopyranoside unit linked at C-3-OH of **1** were observed at positions very similar to those in the spectrum of **7**, leading to the formulation of **7** as the 3-*O*-β-glucopyranosyl(1→3)-β-glucopyranosyl(1→4)-β-galactopyranoside of **4**.

The remaining new saponins, saponin IV (0.01% yield) and III (0.003% yield), named ampelosides-Bf$_1$ (**8**) and -Bf$_2$ (**9**) are positive to the Ehrlich reagent.[9] The IR spectra of **8** and **9** does not show characteristic spiroketal absorption bands, and the ^{13}C-NMR spectra show characteristic furostane-type carbon signals as reported by Shoji *et al.*[10] On acid hydrolysis, **8** and **9** gave glucose and galactose, and the EI-MS of their acetates exhibited ions at *m*/*z* 619 $[(\text{hexosyl-hexose})Ac_7]^+$ and 331 $[(\text{terminal hexose})Ac_4]^+$, while the FD-MS of **8** and **9** exhibited ions at *m*/*z* 1137 $[M+Na]^+$ (from **8**), 1119 $[M+Na-H_2O]^+$ (from **8**), 975 $[M+Na]^+$ (from **9**) and 934 $[M-H_2O]^+$ (from **9**). Permethylates[5] of **8** and **9** were subjected to sequence analysis of the sugar moiety by GC-MS,[6] yielding 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methylhexitol (from the permethylates of **8** and **9**), 1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-metylhexitol (from the permethylate of **8**) and 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol (from the permethylates of **8** and **9**) which originated from hexose linked at C-4-OH, hexose linked at C-3-OH and terminal hexose, respectively. The anomeric proton signals of **8** and **9** (**8**, δ 5.26 (1H, d, $J=8.06$ Hz), 5.23 (1H, d, $J=7.32$ Hz), 4.98 (1H, d, $J=7.33$ Hz), 4.81 (1H, d, $J=7.69$ Hz); **9**, δ 5.27 (1H, d, $J=7.69$ Hz), 4.99 (1H, d, $J=7.70$ Hz), 4.82 (1H, d, $J=7.70$ Hz)) in C_5D_5N indicated the anomeric configuration of their sugar linkages to be β. On enzymic hydrolysis with β-glucosidase, **8** and **9** gave glucose and prosapogenins (pro-**8** from **8** and pro-**9** from **9**). In the IR spectra,[4] these prosapogenins (pro-**8** and pro-**9**) showed characteristic absorption bands at 977, 915, 894 and 861 cm^{-1} (KBr) (intensity; 915<894, (25*R*)-spiroketal), 981, 915, 898 and 861 cm^{-1} (KBr) (intensity; 915<898, (25*R*)-spiroketal), respectively. These prosapogenins (pro-**8** and pro-**9**) were identified as **7** and **1** by comparison of TLC behavior, MS (as the acetate or trimethylsilyl ether), IR (KBr), ^{1}H- and ^{13}C-NMR spectra. These results support the proposed structures shown in Chart 2.

The antifungal activity of the three saponins (**1**, **7** and **8**) was investigated in an *in vitro* system. The results are shown in Table II. These saponins did not inhibit the growth of *Aspergillus niger* cultured at 37 °C for 72 h, but the growth of *Candida albicans* was weakly inhibited by **1** and **7**. The spirostane-type saponins (**1** and **7**) appeared to be more active than the furostane-type saponin (**8**).

A study on minor saponins and chemotaxonomical studies on *Allium* spp. collected worldwide are in progress.

Experimental

General Procedures——NMR spectra were taken on a JEOL GX-270 (^{1}H-NMR at 270 MHz and ^{13}C-NMR at 67.80 MHz) spectrometer in C_5D_5N with tetramethylsilane (TMS) as an internal standard.

MS were recorded on a JEOL JMS-DX300 (JMA 3100 data system) mass spectrometer at 70 eV by the direct inlet method. Trimethylsilylation for MS: A sample of saponin (1—2 mg) was heated with *N*-trimethylsilylimidazole (5 drops) in a sealed micro-tube at 80 °C for 2—3 h. The reaction mixture was diluted with H_2O and then extracted with *n*-C_6H_{14}. The *n*-C_6H_{14} layer was washed with H_2O and concentrated to dryness by blowing N_2 gas over it at room temperature. The residue was subjected to MS. Acetylation for MS: A sample (1—2 mg) was heated with $(CH_3CO)_2O$ (2—3 drops) and C_5H_5N (5—6 drops) in a sealed micro-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 the residue was subjected to MS.

The IR spectra were recorded with a Hitachi 215 grating infrared spectrophotometer. Melting points were determined on a Yanaco micro hot stage and are uncorrected. Optical rotations were measured with Union automatic digital polarimeter.

Extraction and Separation of Saponins——Frozen bulbs (5 kg) of *Allium ampeloprasum* (elephant garlic) cultivated in California, U.S.A., were homogenized with MeOH and then extracted with hot MeOH (3 l × 3) to give an MeOH extract (after evaporation). An aqueous solution of this MeOH extract was subjected to column chromatography on ion exchange resin (Amberlite XAD-2, Organo Co., Ltd.) (solvent: 30% MeOH (7 l), MeOH (5 l) and finally $CHCl_3$ (3 l)) to provide a crude saponin fraction (MeOH eluate, 12.9 g). This MeOH eluate (11.9 g) was separated into two fractions, fr. 1 and fr. 2, by column chromatography on silica gel (solvent: $CHCl_2$–MeOH–H_2O (7 : 3 : 0.5→6 : 4 : 1, homogeneous)).

Fraction 1 was separated into two fractions, fr. 1a and fr. 1b, by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, homogeneous)). Fraction 1a was further chromatographed on reversed-phase highly porous polymer (MCI gel CHP20P, Mitsubishi Chemical Ind., Ltd.) (solvent: 73 to 80% MeOH) to give **1** (0.005% yield), colorless needles (from H_2O–MeOH), mp 249—251 °C, $[\alpha]_D^{17}$ $-48.3°$ ($c=1.10$, C_5H_5N). *Anal.* Calcd for $C_{39}H_{64}O_{15} \cdot H_2O$: C, 59.22; H, 8.41. Found: C, 59.47; H, 8.40. Fraction 1b was further chromatographed on reversed-phase highly porous polymer (*vide supra*) (solvent: 73 to 80% MeOH) to give **7** (0.007% yield), colorless needles (from MeOH), mp 300 °C <, $[\alpha]_D^{17}$ $-52.5°$ ($c=1.12$, C_5H_5N). *Anal.* Calcd for $C_{45}H_{74}O_{20} \cdot H_2O$: C, 56.71; H, 8.04. Found: C, 56.55; H, 7.99.

Fraction 2 was separated into two fractions, fr. 2a and fr. 2b, by column chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, homogeneous)). Fraction 2a was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, homogeneous)) and then further chromatographed on reversed-phase highly porous polymer (*vide supra*) (solvent: 80% MeOH) and finally chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5 or 6 : 4 : 1, homogeneous)) to give crude **9**. A solution of crude **9** in H_2O (6 ml) was treated with acetone (3 ml) and the mixture was heated at 100 °C for 20 h to afford **9** (0.003% yield). **9**: a white powder (from H_2O–acetone), $[\alpha]_D^{21}$ $-40.7°$ ($c=1.03$, C_5H_5N). *Anal.* Calcd for $C_{45}H_{76}O_{21} \cdot 3H_2O$: C, 53.66; H, 8.21. Found: C, 53.71; H, 8.07. Fraction 2b was chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)) and then further chromatographed on reversed-phase highly porous polymer (*vide supra*) (solvent: 78% MeOH) and finaly chromatographed on silica gel (solvent: $CHCl_3$–MeOH–H_2O (6 : 4 : 1, homogeneous)) to give crude **8**. A solution of crude **8** in H_2O (20 ml) was treated with acetone (10 ml) and the solution was heated at 100 °C for 20 h to afford **8** (0.01% yield). **8**: a white powder (from H_2O–acetone), $[\alpha]_D^{21}$ $-25.1°$ ($c=1.05$, C_5H_5N). *Anal.* Calcd for $C_{51}H_{86}O_{26} \cdot 5/2H_2O$: C, 52.79; H, 7.91. Found: C, 52.88; H, 7.84.

Enzymic Hydrolysis of 9 and 8——**9**: A solution of **9** (60 mg) and *β*-glucosidase (sweet almonds) (30 mg, P-L Biochemicals, Inc.) in Michaelis buffer (pH 4.35, 20 ml) was incubated at 37 °C for 1 h. The reaction mixture was diluted with H_2O (50 ml) and chromatographed on reversed-phase highly porous polymer (*vide supra*) (solvent: H_2O (120 ml) and then MeOH (150 ml)) to give an H_2O eluate and an MeOH eluate. This MeOH eluate was directly crystallized from H_2O–MeOH to give **1** (20 mg). **8**: A solution of **8** (100 mg) and *β*-glucosidase (*vide supra*) (100 mg) in Michaelis buffer (*vide supra*) (30 ml) was incubated at 37 °C for 1 h. The reaction mixture was diluted with H_2O (100 ml) and chromatographed on highly porous polymer (*vide supra*) (solvent: H_2O (150 ml) and then MeOH (200 ml)) to give an H_2O eluate and an MeOH eluate. This MeOH eluate was subjected to chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (7 : 3 : 0.5, homogeneous)) to give **7** (34 mg).

Partial Hydrolysis of 1——Compound **1** (150 mg) was heated with 2% H_2SO_4 in H_2O for 1 h at 100 °C. The reaction mixture was diluted with H_2O (40 ml) and chromatographed on reversed-phase highly porous polymer (*vide supra*) (solvent: H_2O (240 ml) and then MeOH (400 ml)) to give an H_2O eluate and an MeOH eluate. This MeOH eluate was subjected to chromatography on silica gel (solvent: $CHCl_3$–MeOH–H_2O (8 : 2 : 0.2, homogeneous)) to give **4** (45 mg), **5** (7 mg) and **1** (31 mg). **4**: colorless needles (from $CHCl_3$–MeOH), mp 267—269 °C, $[\alpha]_D^{17}$ $-75.3°$ ($c = 1.20$, $CHCl_3$–MeOH (10 : 1)) (lit.[8] mp 265—267 °C, $[\alpha]_D^{29}$ $-74.2 \pm 2°$ ($c = 1.27$, $CHCl_3$)). *Anal.* Calcd for $C_{27}H_{44}O_5 \cdot 1/2H_2O$: C, 70.86; H, 9.91. Found: C, 70.85; H, 9.67.

Hydrolysis of Saponins and Identification of the Resulting Monosaccharides——Each saponin (a few mg) was heated with 10% HCl in H_2O–dioxane (1 : 1) in a sealed micro-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. For analysis by gas liquid chromatography (GLC), the residue was trimethylsilylated by the same procedure as that used for MS. GLC; On a Shimadzu GC-9AM gas chromatograph equipped with a glass column of 1% Silicone OV-17 (or 2% Silicone OV-1) on Gaschrom Q (80—100 mesh), 3 mm i.d. × 2 m; detector, hydrogen flame ionization detector (FID); injection temperature, 180 °C (or 230 °C); column temperature, 155 °C (or 200 °C); carrier gas, N_2 (40 ml/min).

Permethylation[5] Followed by Alditol Acetate Analysis of Saponins——Permethylation: A solution of each saponin (4—5 mg) in DMSO (0.5 ml) was treated with powdered NaOH (20 mg) and methyl iodide (0.1 ml) and the mixture was stirred for 1 h in a closed vial at room temperature. The reaction mixture was diluted with H_2O (1 ml) and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O (1 ml × 3), dried and concentrated to dryness. Sequence analysis by GC-MS:[6] A solution of the resulting permethylether in 0.5 N H_2SO_4 in 95% AcOH (0.5 ml) was heated for 4 h in a closed vial at 80 °C. The reaction mixture was deionized by passage through a column of Dowex 1-X8 (AcOH form) (5 mm i.d. × 5 cm) ion exchange resin, eluting with H_2O (10 ml) and MeOH (7 ml). The H_2O eluate and MeOH eluate were combined and concentrated to dryness, then $NaBH_4$ (30 mg) in H_2O (2 ml) was added. After standing at room temperature overnight, the mixture was concentrated to dryness. Boric acid in the residue was removed by repeated co-distillation with MeOH. The resulting methylated alditol mixture was acetylated with Ac_2O–C_5H_5N (1 : 1) (1 ml) at 80 °C for 2 h, then toluene was added and the whole was subjected to azeotropic distillation. The residue was extracted with *n*-C_6H_{14} and methylated alditol acetates in the *n*-C_6H_{14} layer were analyzed by GC-MS.

GC-MS was taken on a JEOL JMS-DX300 (JMA 3100 data system) equipped with a 2.0 mm × 2.0 m glass column of 1.5% OV-210 on Chromosorb W (80—100 mesh); injection temperature, 210 °C; column temperature, 180 °C; carrier gas, He (40 ml/min); ionization voltage, 70 eV.

Antifungal Activity——To check the antifungal activity of the saponins (**1**, **7** and **8**), two fungus strains, *Aspergillus niger* ATCC 16404 and *Candida albicans* ATCC 10231, were obtained from the Institute for Fermentation, Osaka, Japan. For the test, spores of *A. niger* formed on Sabouraud agar medium and active *C. albicans* precultured in Sabouraud broth were cultured with or without various concentrations of each saponin at 37 °C with agitation for 72 h and 24 h, respectively. The activity of each saponin against *A. niger* was judged by measuring the dry weight of the fungus at the end of culture. With *C. albicans*, evaluation was done by measuring the turbidity of the medium.

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