Antiyeast Steroidal Saponins from *Yucca schidigera* (Mohave Yucca), a New Anti-Food-Deteriorating Agent

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A saponin fraction from the stems of *Yucca schidigera* (Mohave yucca) exhibited potent growth-inhibitory activities against certain food-deteriorating yeasts, film-forming yeasts, and dermatophytic yeasts and fungi. From this fraction, a number of new anti-yeast monodesmosidic spirostanol saponins, named schidigera-saponins A1 (**1**), A2 (**2**), A3 (**3**), B1 (**4**), C1 (**5**),C2 (**6**); 25(*R* and *S*) schidigera-saponins D1 (**7**), D2 (**8**), E1 (**12**), F1 (**13**); and 25(*S*) schidigera-saponins D3 (**9**), D4 (**10**), D5 (**11**), and F2 (**14**) were isolated, together with several related known saponins, and the structures were elucidated by spectroscopic methods (see Chart 1). The relationship between the antiyeast activities and the structures of these saponins is described.

It is known that the deterioration of cooked foods is mainly caused by infection with yeasts. We have searched for antiyeast natural products that can be safely used as antideteriorating agents in foods. It has been disclosed that a saponin fraction of the stems of *Yucca schidigera* Roezl ex Ortgies (Mohave yucca, Agavaceae) exhibit potent antiyeast activities against brewer's yeast (*Saccharomyces cervisiae*), dermatophytic yeasts and fungi, and food-deteriorating yeasts and film-foaming yeasts that spoil soy sauce.[1]

Mohave yucca is native to the desert of the southwestern United States and northern Baja California, Mexico. In the acid hydrolysate of the extract of this plant, several steroidal sapogenins have been identified.[2−4] As to the saponin constituents of this plant, Kameoka et al. isolated and identified a monodesmoside tentatively named YS-1.[5] We have conducted the isolation and identification of a number of saponins from Mohave yucca, which were reported in preliminary fashion in 1996.[1] The present paper deals with the experimental details of this study together with additional results obtained by further phytochemical investigation. In the previous report,[1] the investigated part was erroneously described as "rhizomes"; it is now revised to "stems".

Results and Discussion

The EtOH extract of the stems was fractionated by column chromatography on a highly porous polymer resin, Diaion HP-20, eluted with H_2O; MeOH 40, 60, 80, 95 and 100%, successively; and finally with Me_2CO. The 95% MeOH eluate, which was mainly composed of monodesmosidic saponins, being named the saponin fraction (SF), was subjected to chromatography on Si gel and then on ODS, affording saponins **1**−**14**.

Saponin **1**, one of the major saponins and named schidigera-saponin A1 (YE-1 earlier[1]), was obtained as a white amorphous powder. Its molecular weight was shown to be 870 Da by negative ion FABMS, and the molecular formula was established as $C_{44}H_{68}O_{18}$ by HRFABMS. The ^{13}C NMR spectrum of **1** indicated signals due to three sugar units and a spirostane-type steroid aglycon. On acid hydrolysis, **1** afforded glucose, xylose, and an aglycon (**15**). To establish the structure of **15**, the ^{13}C NMR signals of **15** (Table 1) were compared with those of sarsasapogenin (**16**)[6] and convallamarogenin (**17**).[7] The ^{13}C NMR signals of **15** were in good agreement with those due to the A/B/C/D rings of **16** and those due to the E/F rings of **17**, permitting the structure of **15** to be assigned as 5β-spirost-25(27)-en-3β-ol. It was apparent that **1** was a 3-*O*-monodesmoside of **15** from the ^{13}C NMR glycosylation shift observed.[8] The FABMS fragment ions of **1** at *m*/*z* 737 [M − Xyl − H]$^-$ and 707 [M − Glc − H]$^-$, suggested that the sugar moiety of **1** is not a linear sugar chain but a branched one. The structure of the sugar moiety was finally established as β-D-xylopyranosyl-(1→3)[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl by means of the HMBC data summarized in Figure 1. Thus, **1** was formulated as 5β-spirost-25(27)-en-3β-ol 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranoside.

The FABMS of saponin **2**, named schidigera-saponin A2, led to the same molecular formula as that of **1**. The ^{13}C NMR spectrum indicated that both compounds have the same aglycon moiety. On acid hydrolysis, **2** gave glucose, xylose, and galactose as sugar components. The FABMS revealed that the sugar moiety of **2** is a branched sugar chain. HMBC correlations obtained for **2** clarified the presence of a 3-*O*-β-D-xylopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranosyl residue (Figure 1). Hence, the structure of **2** was established as 5β-spirost-25(27)-en-3β-ol 3-*O*-β-D-xylopyranosyl-(1→3)[β-D-glucopyranosyl-(1→2)]-β-D-galactopyranoside.

Saponin **3**, schidigera-saponin A3 (YE-5 earlier[1]), exhibited a pseudomolecular ion in the negative FABMS at *m*/*z* 899 and a fragment ion at *m*/*z* 737, ascribable to [M − Glc − H]$^-$. The ^{13}C NMR spectrum of **3** indicated that **3** has the same aglycon as that of **1** and **2**. On acid hydrolysis, **3** gave glucose as the sole sugar component. The ^{13}C NMR signals due to the sugar moiety of **3** were almost super imposable on those of Ys-III,[9] which is 3-*O*-β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→2)]-β-D-glucopyranosyl-(25*R*)-5β-spirostan-3β-ol from the caudex of *Yucca gloriosa*. Thus, saponin **3** was assigned as 5β-spirost-25(27)-en-3β-

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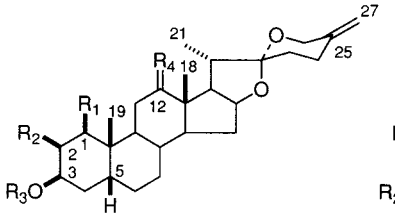
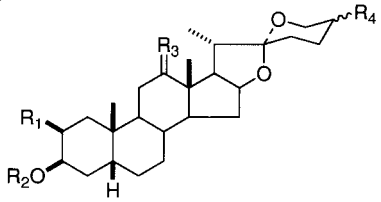
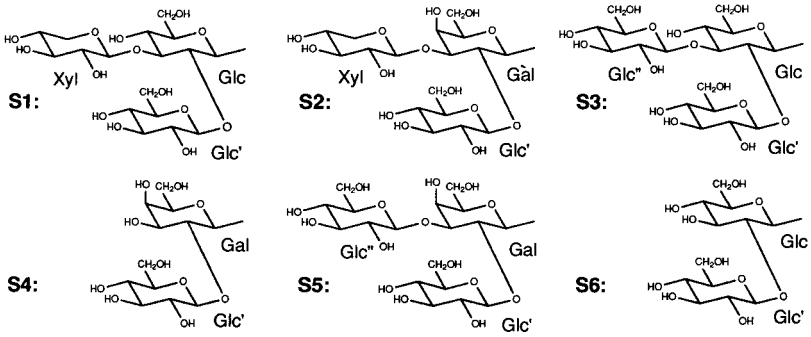
Chart 1



	R_1	R_2	R_3	R_4
1:	H	H	S1	H_2
2:	H	H	S2	H_2
3:	H	H	S3	H_2
4:	H	H	S1	O
5:	H	OH	S2	H_2
6:	H	OH	S4	H_2
15:	H	H	H	H_2
17:	OH	H	H	H_2
18:	H	H	H	O
20:	H	OH	H	H_2



	R_1	R_2	R_3	R_4
7:	H	S1	H_2	eq + ax Me
8:	H	S2	H_2	eq + ax Me
9:	H	S3	H_2	eq + ax Me
10:	H	S5	H_2	eq + ax Me
11:	H	S6	H_2	eq + ax Me
12:	H	S1	O	eq + ax Me
13:	OH	S2	H_2	eq + ax Me
14:	OH	S4	H_2	eq + ax Me
16:	H	H	H_2	ax Me
19:	H	H	O	eq Me
21:	OH	H	H_2	eq Me
22:	H	H	H_2	eq Me
23:	OH	H	H_2	ax Me



ol 3-*O*-*β*-D-glucopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside.

Saponin **4**, schidigera-saponin B1, showed FABMS ion peaks at *m*/*z* 883, 751, and 721, which were, respectively, 14 amu greater than those of **1**. The ^{13}C NMR spectrum of **4** was identical with that of **1** except for the signals due to the C-ring carbons of aglycon moiety. On acid hydrolysis, in the same way as **1**, compound **4** gave glucose, xylose, and a new aglycon (**18**), schidigera-genin B. The ^{13}C NMR signals of **18** were compared with those of **15** and (25*R*)-5*β*-spirostan-3*β*-ol-12-one (**19**),[10] disclosing that **18** has the same A/B/C ring moiety as **19** and the same A/B/D/E/F ring moiety as **15**. Therefore, **18** was formulated as 5*β*-spirost-25(27)-en-3*β*-ol-12-one, and accordingly, **4** was assigned as the 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside of **18**.

The ^{13}C NMR spectrum of saponin **5**, named schidigera-saponin C1 (YE-3 earlier[1]), indicated that **5** has the same sugar moiety as that of **2** but a different aglycon. The negative FABMS of **5** showed significant fragments at *m*/*z* 885 [M − H]$^-$, 753 [M − Xyl − H]$^-$, and 723 [M − Glc − H]$^-$, all of which are 16 amu greater than those of **2**. On acid hydrolysis, **5** gave the same sugar components as those of **2** and a new aglycon (**20**), named schiligera-genin C. The ^{13}C NMR signals of **20** were coincident with those of **15** except for the A/B ring carbons. The ^{13}C NMR signals due to the A/B ring portions of **20** were superimposable on those of samogenin [(25*R*)-5*β*-spirostane-2*β*,3*β*-diol],[9] leading to the structure of the aglycon **20** as 5*β*-spirost-25(27)-ene-2*β*,3*β*-diol. By reference to the ^{13}C NMR signals of 3-*O*-glycosylated 5*β*-spirostane-2*β*,3*β*-diol,[9] **6** was formulated as 3-*O*-*β*-D-xylopyranosyl-(1→3)[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranosyl-5*β*-spirost-25(27)-ene-2*β*,3*β*-diol.

Saponin **6**, schidigera-saponin C2, was concluded to have the same aglycon as that of **5**, by comparing their ^{13}C NMR spectra. On acid hydrolysis, **6** gave glucose and galactose as sugar components. The negative FABMS of **6** exhibited a pseudomolecular ion at *m*/*z* 753 and a fragment ion at *m*/*z* 593 assignable to [M − Glc − H]$^-$, suggesting that **6** is a de-xylopyranosylated analogue of **5**. The ^{13}C NMR signals due to the sugar moiety of **6** were in good agreement with those of saponin An-II, markogenin 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucopyranoside, a compound obtained from *Anemarrhena asphodeloides*.[11] Therefore, the structure of **6** was established as schidigeragenin C 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside.

The ^{1}H and ^{13}C NMR signals due to protons and carbons around C-25 of their aglycon moieties revealed that saponins **7**−**14** were mixtures of C-25 epimers (*R* and *S*) of spirostanol monodesmosides. Generally, the separation of a mixture of such epimeric saponins into each C-25 epimer has been very difficult,[12] so structures of **7**−**14** have been elucidated as C-25 epimeric mixtures.

Comparison of their ^{1}H and ^{13}C NMR spectra indicated that saponins **7**−**11** were formulated as monodesmosides of the C-25 epimers, sarsasapogenin (**16**) and smilagenin (**22**), being different from each other only in the composition of the sugar moieties.

Comparison of the ^{13}C NMR signals due to the sugar moiety of **7** (YE-2 previously[1]) with **1** indicated their co-identity, leading to the formulation of **7** as 25(*R,S*)-5*β*-spirostan-3*β*-ol 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopy-

Table 1. ^{13}C NMR Signals of the Aglycon Moieties of the Schidigera-saponins, their Aglycons and Related Compounds (in C_5D_5N)

position	**1**	**2**	**3**	**4**	**5**	**6**	**15**	**18**	**20**
C-1	30.9	30.8	30.9	30.7	40.3	40.3	30.7	30.3	39.4
C-2	26.8	26.9	26.8	26.8	67.1	67.2	28.7	28.3	67.6
C-3	75.0	75.1	75.3	75.1	81.6	81.6	66.2	65.8	70.4
C-4	30.9	30.8	30.9	30.4	31.8	31.8	34.5	34.2	33.7
C-5	36.9	36.1	36.8	36.5	36.1	36.5	37.1	36.6	36.3
C-6	26.8	26.9	26.8	26.5	26.4	26.3	27.2	26.9	26.4
C-7	26.8	26.9	26.9	26.5	26.9	26.8	27.0	26.6	26.9
C-8	35.7	35.7	35.7	34.8	35.8	35.7	35.7	34.9	35.7
C-9	40.4	40.4	40.5	42.6	41.7	41.6	40.5	42.6	41.6
C-10	35.3	35.3	35.3	35.7	37.1	37.2	35.7	36.0	37.1
C-11	21.3	21.2	21.2	37.8	21.4	21.4	21.3	37.8	21.5
C-12	40.4	40.4	40.5	212.6	40.3	40.3	40.3	212.6	40.4
C-13	41.0	41.0	41.0	55.7	41.0	41.0	41.1	55.7	41.0
C-14	56.6	56.6	56.6	56.2	56.5	56.5	56.7	56.3	56.5
C-15	32.2	32.1	32.1	31.5	32.1	32.1	32.2	31.5	32.1
C-16	81.6	81.6	81.6	79.9	80.5	81.3	81.6	80.0	81.6
C-17	63.3	63.3	63.3	54.5	63.3	63.1	63.3	54.5	63.2
C-18	16.5	16.5	16.9	16.1	16.5	16.5	16.6	16.0	16.6
C-19	24.0	23.9	23.9	23.1	23.8	23.9	24.3	23.4	24.2
C-20	42.0	42.0	42.0	42.1	42.0	42.0	42.0	42.0	42.0
C-21	14.9	14.9	14.9	13.6	14.7	14.9	15.0	13.7	15.0
C-22	109.4	109.4	109.4	109.4	109.4	109.5	109.4	109.0	109.4
C-23	28.9[a]	28.9[a]	28.9[a]	29.0[a]	28.9[a]	28.9[a]	29.1[a]	28.9[a]	29.0[a]
C-24	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.3[a]	33.3[a]
C-25	144.6	144.6	144.6	144.4	144.6	144.5	144.6	144.4	144.6
C-26	65.1	65.1	65.1	65.1	65.1	65.1	65.1	65.0	65.0
C-27	108.4	108.3	108.3	108.4	108.4	108.4	108.5	108.2	108.5

position	**7**		**8**		**9**		**10**		**11**		**12**		**13**		**14**	
C-1	30.9		30.8		30.9		30.8		30.9		30.7		40.3		40.5	
C-2	26.8		26.8		27.0		26.9		26.8		26.8		67.1		67.1	
C-3	75.0		75.1		75.3		75.3		75.3		75.0		81.3		81.5	
C-4	30.9		30.8		30.9		30.8		30.9		30.5		31.3		31.8	
C-5	36.9		36.6		36.8		36.6		37.0		36.4		36.1		36.5	
C-6	26.8		26.8		27.0		26.9		26.8		26.5		26.3		26.2	
C-7	26.8		26.8		27.0		26.9		26.8		26.5		26.8		26.8	
C-8	35.7		35.7		35.7		35.3		35.6		34.8		35.7		35.7	
C-9	40.4		40.4		40.5		40.4		40.4		42.7		41.6		41.5	
C-10	35.3		35.3		35.3		35.3		35.3		35.7		37.0		37.1	
C-11	21.2		21.2		21.3		21.2		21.2		37.7		21.4		21.4	
C-12	40.4		40.4		40.4		40.4		40.4		212.6		40.3		40.3	
C-13	41.0		41.0		41.0		41.0		40.9		55.6		40.9		40.9	
C-14	56.6		56.6		56.6		56.7		56.6		56.1		56.4		56.4	
C-15	32.2		32.1		32.2		32.2		32.1		31.5		32.1		32.1	
C-16	81.3		81.3		81.4		81.3		81.3		79.8		80.6		80.3	
C-17	63.1		63.3		63.2		63.1		63.1		54.5		63.1		63.0	
C-18	16.4		16.5		16.5		16.5		16.5		15.9		16.3		16.4	
C-19	24.0		23.9		24.0		23.9		24.0		23.1		23.7		23.8	
	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*	25*R*	25*S*
C-20	42.0	42.5	42.0	42.5	42.1	42.6	42.0	42.6	42.0	42.5	42.1	43.2	41.8	42.5	42.0	42.5
C-21	14.8	14.8	14.7	14.7	14.9	14.9	14.8	14.8	14.8	14.8	13.7	13.7	14.7	14.7	14.7	14.7
C-22	109.0	109.7	109.3	109.7	109.2	109.7	109.2	109.7	109.0	109.7	109.2	109.6	109.3	109.7	109.0	109.6
C-23	32.2	26.5	32.1	26.5	32.2	26.6	32.2	26.5	32.1	26.5	31.9	26.5	32.1	26.5	32.1	26.5
C-24	29.3	26.2	29.2	26.2	29.3	26.3	29.3	26.2	29.0	26.2	29.2	26.3	29.2	26.3	29.2	26.2
C-25	30.9	27.5	30.7	27.6	30.8	27.6	30.8	27.5	30.9	27.5	30.7	27.5	30.7	27.5	30.8	27.5
C-26	66.9	65.2	67.1	65.1	67.0	65.2	67.0	65.2	66.9	65.1	67.0	65.2	67.1	65.1	67.1	65.1
C-27	17.2	16.3	17.2	16.3	17.2	16.3	17.2	16.3	17.2	16.5	17.2	16.2	17.2	16.3	17.2	16.3

[a] Signals maybe reversed.

ranosyl-(1→2)]-*β*-D-glucopyranoside. In 1993, Kameoka et al. reported the isolation and identification of a saponin tentatively named YS-1 from *Y. schidigera*, which is identical with the 25(*S*)-epimer of **7**.[5] This assignment has not been published in a research article to date. The present authors would like to designate **7** as 25(*R* and *S*)-schidigera-saponin D1. In a similar manner, the ^{13}C NMR spectrum of **8** revealed the identity of the sugar moiety to be the same as that of **2**, leading to the formulation of **8**, 25(*R* and *S*)-schidigera-saponin D2, as 25(*R*,*S*)-5*β*-spirostan-3*β*-ol 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranoside.

The identification of the sugar moieties of **9** (YE-6 previously[1]), **10**, and **11** were established by the respective comparison of their ^{13}C NMR signals due to the sugar moieties of saponins Ys-III, Ys-IV, and Ys-I from the caudex of *Y. gloriosa*,[9] leading to the formulation of these saponins as follows: **9**, an epimeric mixture of saponin Ys-III and its 25(*S*)-epimer, 25(*S*)-schidigera-saponin D3; **10**, an epimeric mixture of saponin Ys-IV and its 25(*S*)-epimer, 25(*S*)-schidigera-saponin D4, and **11**, an epimeric mixture of saponin Ys-I and its 25(*S*)-epimer, 25(*S*)-schidigera-saponin D5.

Inspection of the ^{13}C NMR signals due to the aglycon moiety of **12** in comparison with those of **4** and **7** indicated that the aglycon of **12** is constituted by a mixture of 25(*R*)-spirostan-12-one (gloriogenin)[9] and its 25(*S*)-epimer, schidigera-genin E. The ^{13}C NMR signals due to the sugar

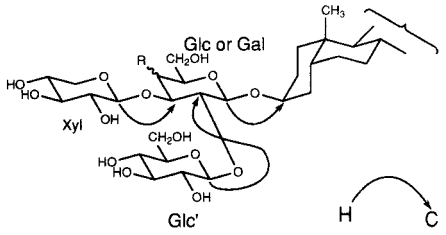


Figure 1. Key HMBC correlations of **1** and **2** (**1**: R = eq OH, **2**: R = ax OH).

moiety of **12** proved that this saponin, 25(*R* and *S*)-schidigera-saponin E1, has the same sugar moiety as that of **1** and **4**, leading to the assignment of the structure of **12** as 25(*R,S*)-5*β*-spirostan-3*β*-ol-12-one 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-glucopyranoside.

Inspection of their ^{13}C NMR spectra revealed the common aglycon of **13** (YE-4 previously[1]) and **14** to be a C-25 epimeric mixture of samogenin (**21**)[9] and markogenin (**23**).[11] The sugar moiety of **13** was proved to be identical with those of **2** and **5** by comparison of their ^{13}C NMR signals, which were further confirmed by means of HMBC correlations. The structure of **13** was thus elucidated as 25(*R,S*)-5*β*-spirostane-2*β*,3*β*-diol 3-*O*-*β*-D-xylopyranosyl-(1→3)-[*β*-D-glucopyranosyl-(1→2)]-*β*-D-galactopyranoside. Isolation of the 25(*R*) epimer **13** from *Yucca recurviflora* has been reported at a meeting by Kameoka et al. in 1993.[5] However, this study has not been published formally as yet. The name 25(*R* and *S*)-schidigera-saponin F1 is proposed for **13**. In the same way, the structure of the sugar moiety of **14** was determined to be identical with that of **6**, proving that **14** is a mixture of saponin Ys-V from *Y. gloriosa*[9] and its 25(*S*)-epimer, 25(*S*)-5*β*-spirostane-2*β*,3*β*-diol 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-galactopyranoside, designated now as 25(*S*)-schidigera-saponin F2.

The antiyeast and antifungal activities of the saponin fraction (SF) of *Y. schidigera* is summarized in Tables 3 and 4. Infection with *Hansenula anomala* and *Kloeckera apiculata* of food products of boiled rice such as "sushi" and "musubi" causes an odor similar to that of an organic solvent. Infection of cooked beans and processed fish meat with *Candida famata* and *Pichia carsonii* results in an odor like that of kerosene. Potent growth-inhibitory activities against these food-deteriorating yeasts were observed for the SF. *Pichia nakazawae*, *Dermatomyces hansenii*, and *Zygosaccharomyces rouxii* are known as film-forming yeasts, damaging oriental fermented seasonings such as the soy sauces "shoyu" and "miso". The SF showed strong antiyeast activities against these yeasts. Remarkable growth-inhibitory effects against dermatophytic yeasts and fungi were also observed for the SF. However, it showed no or only weak growth inhibition against both Gram-positive and Gram-negative bacteria.

The antiyeast activities of each saponin from *Y. schidigera* were determined and are summarized in Table 5. Those saponins having a branched-chain trisaccharide moiety without any oxygen functionalities at C-2 and -12 exhibited potent antiyeast activities, while saponins with 2*β*-hydroxyl (**5**, **6**, **13**, and **14**) or 12-keto (**4** and **12**) groups showed very weak or no activity. A saponin (**11**) with a disaccharide moiety exhibited relatively low activities. The aglycons showed no activity. No antiyeast activity was observed for the 60% and 80% aqueous MeOH eluates from their chromatography on a highly porous polymer, and these seem to contain bisdesmosidic furostanol saponins.

All parts of Mohave yucca have been utilized as a foodstuff and a folk medicine by Native Americans, and the extract is commercially used as a long-lasting foaming agent in beverages and as a food supplement for humans and livestock in the United States and Japan. It is nontoxic and nonmutagenic, being recognized as a safe food adjuvant by the U.S. Food and Drug Administration (FDA) under the Title 21 the Code of Federal Regulations 172.510. (21 CFR 172.510) The extract is tasteless, colorless, and odorless, exerting no influence on the quality of foods. Based on the present study, Mohave yucca extract and its SF are now on sale in Japan as an antideteriorating agent for extending the shelf-life of food products containing cooked rice and beans, pickled vegetables, processed fish meat, and fermented seasonings.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-1000 automatic digital polarimeter. NMR spectra were recorded on JEOL FX-90Q (90 MHz for ^1H, and 22.5 MHz for ^{13}C) or on JEOL Lambda 500 (500 MHz for ^1H, and 125 MHz for ^{13}C) NMR spectrometers. FABMS in a glycerol matrix in the negative-ion mode and EIMS were recorded on a JEOL SX-102A instrument. GC was carried out on a Shimadzu GC-7A apparatus equipped with a FID detector and a glass capillary column precoated with dimethylpolysiloxane (0.25 mm × 30 m) at 170 °C. Si gel (Merck and Fuji-Silysia), Diaion HP-20 (Mitsubishi Chemcal Industries), and ODS (Fuji-Silysia) were used for column chromatography. The solvents for spectral determination were C_5D_5N−TMS (NMR) and MeOH ($[\alpha]_D$), unless otherwise stated. TLC was carried out on precoated Si gel $60F_{254}$ (0.25 mm thick, Merck) and $RP_{18}F_{254}$ (0.25 mm thick, Merck) plates, and spots were visualized by spraying the plates with 10% H_2SO_4 solution, followed by heating.

Plant Material. The stems of *Yucca schidigera* Roezl were obtained from Agro Industrias El Alamo, Baja California, Mexico, in November 1996. A voucher specimen (MZ961101) was deposited at the Biological and Chemical Research Department, Maruzen Pharmaceuticals Co., Ltd.

Extraction and Isolation. The powdered, dried plant material (100 g) was extracted with hot EtOH (300 mL × 3). The EtOH extract was concentrated under reduced pressure to afford a residue (20.6 g). The residue (19.6 g) was suspended in H_2O and passed through a Diaion HP-20 column, eluting with H_2O (7.2 g), 40% MeOH (0.8 g), 60% MeOH (4.1 g), 80% MeOH (4.2 g), 95% MeOH (1.9 g), MeOH (0.2 g), and Me_2CO (0.3 g), sequentially. The 95% MeOH eluate (1.8 g) was chromatographed on a Si gel column ($CHCl_3$−MeOH−H_2O, 30:10:1) to give seven fractions: I (220 mg), II (790 mg), III (119 mg), IV (346 mg), V (96 mg), VI (93 mg), and VII (72 mg), in order of elution. Fraction II was chromatographed on an ODS column eluting with MeCN−H_2O (74:26 and 80:20) to yield six fractions, IIa (13 mg), IIb (**4**: 70 mg), IIc (**12**: 123 mg), IId (18 mg), IIe (**1**: 144 mg), and IIf (422 mg). Fraction IIf (422 mg) was further separated on a Si gel column (EtOAc−EtOH−H_2O, 10:2:1) to afford **11** (19 mg) and **7** (378 mg). Fraction III was chromatographed on an ODS column eluting with MeCN−H_2O (74:26 and 80:20) to provide eight fractions, IIIa (11 mg), IIIb (**4**: 11 mg), IIIc (**12**: 20 mg), IIId (**6**: 11 mg), IIIe (**14**: 20 mg), IIIf (**1**: 22 mg), IIIg (**7**: 28 mg), and IIIh (6 mg). Fraction IV was chromatographed on an ODS column eluting with MeCN−H_2O (74:26 and 80:20) to give nine fractions, IVa (34 mg), IVb (16 mg), IVc (**13**: 12 mg), IVd (11 mg), IVe (**5**: 35 mg), IVf (**13**: 65 mg), IVg (8 mg), IVh (34 mg), and IVi (81 mg). Fraction V was chromatographed on an ODS column eluting with MeCN−H_2O (74:26 and 80:20) to yield nine fractions, Va (8 mg), Vb (4 mg), Vc (5 mg), Vd (7 mg), Ve (3 mg), Vf (**5**: 7 mg), Vg (**13**: 13 mg), Vh (4 mg), Vi (11 mg), Vj (7 mg), Vk (**10**: 16 mg), and Vl (8 mg). Fraction IVh was chromatographed on a Si gel column ($CHCl_3$−MeOH−H_2O, 40:10:1) to afford **2** (11 mg) and **3** (22 mg). Fraction IVi was repeatedly chromatographed over Si gel ($CHCl_3$−MeOH−H_2O, 40:10:1) to give **8** (11 mg), **9** (12 mg), and a mixture of **8** and **9** (11 mg).

Table 2. ^{13}C NMR Signals of the Sugar Moieties of the Schidigera-saponins (in C_5D_5N)

position	**1**	**3**	**4**	**7**	**9**	**11**	**12**	position	**2**	**5**	**6**	**8**	**10**	**13**	**14**
Glc-1	102.0	101.7	101.9	101.9	101.7	101.9	101.9	Gal-1	102.0	102.2	102.9	101.9	101.8	102.3	103.0
2	80.0	79.7	80.2	80.0	79.9	83.2	79.8	2	77.7	77.6	81.3	77.7	77.5	77.5	81.5
3	87.2	88.5	87.3	87.2	88.5	77.9	87.2	3	84.3	84.4	76.7	84.3	84.3	84.4	76.7
4	69.8	70.2	69.8	69.8	70.2	78.2	69.7	4	69.8	69.8	69.9	69.8	69.8	69.7	69.8
5	77.8	77.7	77.8	77.8	77.7	71.8	77.8	5	76.2	76.6	76.7	76.2	76.2	76.7	76.7
6	63.5	63.6	63.5	63.5	63.6	63.1	63.5	6	63.6	63.6	63.1	63.6	63.6	63.5	63.0
Glc′-1	104.0	104.1	104.0	104.0	104.1	105.8	104.0	Glc′-1	104.3	104.2	105.7	104.3	104.3	104.2	105.9
2	76.3	76.3	76.3	76.3	76.3	76.8	76.3	2	76.2	76.2	75.2	76.2	76.2	76.2	75.2
3	78.5	78.4	78.5	78.5	78.5	77.9	78.5	3	78.4	78.6	78.0	78.3	78.3	78.5	78.2
4	72.7	71.7	72.7	72.6	71.8	72.1	72.7	4	72.9	72.9	72.1	72.9	71.8	72.9	72.0
5	78.2	78.4	78.3	78.2	78.5	78.2	78.2	5	77.4	77.2	78.0	77.4	78.1	77.3	78.0
6	62.6	62.7	62.4	62.5	62.6	62.9	62.2	6	62.1	62.0	62.1	62.1	62.7	62.0	62.0
Xyl-1	105.2		105.1	105.2			105.2	Xyl-1	106.1	106.0		106.1		106.0	
2	75.5		75.1	75.5			75.0	2	75.1	75.1		75.1		75.0	
3	77.8		77.8	77.8			77.8	3	78.4	78.3		78.3		78.3	
4	70.8		70.7	70.7			70.7	4	71.0	71.0		71.0		70.9	
5	67.2		67.2	67.1			67.2	5	67.1	67.1		67.1		67.1	
Glc″-1		104.8			104.8			Glc″-1					105.2		
2		75.4			75.5			2					76.2		
3		77.7			77.7			3					77.7		
4		72.7			72.8			4					72.9		
5		78.4			78.5			5					78.1		
6		62.5			62.5			6					62.4		

Table 3. Antiyeast Activities of Saponin Fraction

organism	MIC (μg/mL)
Candida albicans TIMM 0134[a]	62.5
Candida famata IFO 661004[b]	31.3
Cryptococcus laurentii IFO 609	125
Debaryomyces hansenii IFO 18[c]	31.3
Debaryomyces hansenii IFO 27[c]	62.5
Debaryomyces hansenii IFO 47[c]	31.3
Debaryomyces hansenii IFO 7011[c]	125
Hansenula anomala HUT 7083[b]	31.3
Kloeckera apiculata IFO 154	62.5
Pichia nakazawae HUT 1688[c]	31.3
Pichia carsonii IFO 946[b]	31.3
Saccharomyces cerevisiae IFO 203	62.5
Saccharomyces cerevisiae HUT 2075	31.3
Saccharomyces cerevisiae JCM 2223	62.5
Zygosaccharomyces rouxii IFO 845[c]	31.3
Zygosaccharomyces rouxii IFO 1130[c]	31.3

[a] Food-deteriorating yeast. [b] Dermatophytic yeast. [c] Film-forming yeast in soy sauce.

Table 4. Antifungal Activities of Saponin Fraction

organism	MIC (μg/mL)
Aspergillus awamori HUT 2014	>1000
Aspergillus awamori HUT 2015	>1000
Aspergillus niger IFO 4343	>1000
Aspergillus oryzae HUT 2065	>1000
Aspergillus oryzae HUT 2175	125
Aspergillus oryzae HUT 2188	>1000
Aspergillus oryzae HUT 2192	>1000
Aspergillus sydowii HUT 4097	>1000
Epidermophyton floccosum IFO 9045[a]	31.3
Mucor pusillus HUT 1185	15.6
Penicillium expansum IFO 5453	>1000
Rhizopus formosaensis IFO 4756	>1000
Rhizopus nigricans IFO 4731	>1000
Sabouraudites canis IFO 7863[a]	31.3
Trichophyton rubrum IFO 5807[a]	15.6
Trichophyton mentagrophytes IFO 5809[a]	31.3

[a] Dermatophytic fungus.

Schidegera-saponin A1 (1): a white amorphous powder; $[\alpha]^{24}_D$ −44.6° (*c* 1.11, MeOH); ^1H NMR (500 MHz) δ 0.80 (3H, s, H-18), 0.93 (3H, s, H-19), 1.09 (3H, d, J = 7.0 Hz, H-21), 4.77, 4.80 (each 1H, s, H-27), anomeric protons 4.92 (1H, d, J = 7.9 Hz, Glc H-1), 5.29 (1H, d, J = 7.6 Hz, Xyl H-1), 5.67 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 869 [M − H]$^-$, 737 [M − Xyl − H]$^-$, 707 [M − Glc − H]$^-$; HRFABMS *m/z* 869.4500 (calcd for $C_{44}H_{69}O_{17}$, 869.4535).

Table 5. Antiyeast Activities of Schidigera-saponins

	S.c[a]	*C.a*[b]	*H.a*[c]	*P.n*[d]	*K.a*[e]	*D.h*[f]
1	3.13	6.25	3.13	3.13	12.5	6.25
2	12.5	12.5	3.13	3.13	>100	>100
3	12.5	12.5	6.13	3.13	>100	>100
4	>100	>100	>100	>100	>100	>100
5	100	100	>100	100	>100	>100
6	>100	>100	>100	>100	>100	>100
7	6.25	50	3.13	3.13	>100	6.25
8	25	>100	3.13	12.5	>100	50
9	6.25	>100	1.56	3.13	>100	6.25
10	12.5	25	3.13	6.25	50	6.25
11	12.5	12.5	6.25	3.13	>100	>100
12	100	>100	100	>100	>100	>100
13	100	>100	>100	>100	>100	100
14	>100	>100	>100	100	>100	>100

[a] *Saccharomyces cerevisiae* IFO 203. [b] *Candida albicans* TIMM 0134. [c] *Hansenula anomala* HUT 7083. [d] *Pichia nakazawae* HUT 1688. [e] *Kloeckera apiculata* IFO 154. [f] *Debaryomyces hansenii* IFO18.

Schidigera-saponin A2 (2): a white amorphous powder; $[\alpha]^{28}_D$ −55.2° (*c* 0.52, pyridine); ^1H NMR (500 MHz) δ 0.80 (3H, s, H-18), 0.95 (3H, s, H-19), 1.06 (3H, d, J = 6.7 Hz, H-21), 4.77, 4.80 (each 1H, s, H-27), anomeric protons 4.92 (1H, d, J = 7.6 Hz, Gal H-1), 5.24 (1H, d, J = 7.6 Hz, Xyl H-1), 5.57 (1H, d, J = 7.6 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 869 [M − H]$^-$, 737 [M − Xyl − H]$^-$, 707 [M − Glc − H]$^-$; HRFABMS *m/z* 869.4536 (calcd for $C_{44}H_{69}O_{17}$, 869.4535).

Schidegera-saponin A3 (3): a white amorphous powder; $[\alpha]^{28}_D$ −52.2° (*c* 1.71, MeOH); ^1H NMR (500 MHz) δ 0.80 (3H, s, H-18), 0.93 (3H, s, H-19), 1.09 (3H, d, J = 6.7 Hz, H-21), 4.77, 4.80 (each 1H, s, H-27), anomeric protons 4.88 (1H, d, J = 7.6 Hz, Glc H-1), 5.34 (1H, d, J = 7.9 Hz, Glc″ H-1), 5.65 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 899 [M − H]$^-$, 737 [M − Glc − H]$^-$; HRFABMS *m/z* 899.4649 (calcd for $C_{44}H_{71}O_{18}$, 899.4640).

Schidigera-saponin B1 (4): a white amorphous powder; $[\alpha]^{24}_D$ −10.3° (*c* 1.71, MeOH); ^1H NMR (500 MHz) δ 0.94 (3H, s, H-18), 1.07 (3H, s, H-19), 1.31 (3H, d, J = 7.0 Hz, H-21), 4.77, 4.81 (each 1H, br s, H-27), 4.88 (1H, d, J = 7.9 Hz, Glc H-1), 5.29 (1H, d, J = 7.9 Hz, Xyl H-1), 5.66 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 883 [M − H]$^-$, 751 [M − Xyl − H]$^-$, 721 [M − Glc − H]$^-$; HRFABMS *m/z* 883.4318 (calcd for $C_{44}H_{67}O_{18}$, 883.4327).

Schidegera-saponin C1 (5): a white amorphous powder; $[\alpha]^{24}_D$ −56.4° (*c* 0.11, MeOH); ^{1}H NMR (500 MHz) δ 0.78 (3H, s, H-18), 0.94 (3H, s, H-19), 1.07 (3H, d, J = 7.0 Hz, H-21), 4.77, 4.80 (each 1H, s, H-27), anomeric protons 4.92 (1H, overlapped with HDO signal, Glc H-1), 5.24 (1H, d, J = 7.6 Hz, Xyl H-1), 5.57 (1H, d, J = 7.6 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 885 [M − H]$^-$, 753 [M − Xyl − H]$^-$, 723 [M − Glc − H]$^-$; HRFABMS *m/z* 885.4457 (calcd for $C_{44}H_{69}O_{18}$, 885.4484).

Schididera-saponin C2 (6): a white amorphous powder; $[\alpha]^{24}_D$ −38.2° (*c* 0.55, MeOH);^{1}H NMR (500 MHz) δ 0.74 (3H, s, H-18), 0.90 (3H, s, H-19), 1.02 (3H, d, J = 6.8 Hz, H-21), 4.72, 4.75 (each 1H, s, H-27), anomeric protons 4.92 (1H, overlapped with HDO signal, Gal H-1), 5.22 (1H, d, J = 7.8 Hz, Glc H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 753 [M − H]$^-$, 591 [M − Glc − H]$^-$; HRFABMS *m/z*: 753.4068 (calcd for $C_{39}H_{61}O_{14}$, 753.4061).

25(*R* and *S*)-Schidegera-saponin D1 (7): a white amorphous powder; $[\alpha]^{24}_D$ −42.5° (*c* 1.10, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.68 [(3H), d, J = 5.5 Hz, H-27], 0.80 [(3H), s, H-18], 0.92 [(3H), s, H-19], 1.13 [(3H), d, J = 6.7 Hz, H-21], 25*S* 0.79 [(3H), s, H-18], 0.92 [(3H), s, H-19], 1.06 [(3H), d, J = 7.0 Hz, H-27], 1.14 [(3H), d, J = 7.0 Hz, H-21], anomeric protons 4.91 (1H, d, J = 7.6 Hz, Glc H-1), 5.29 (1H, d, J = 7.9 Hz, Xyl H-1), 5.67 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 871 [M − H]$^-$, 739 [M − Xyl − H]$^-$, 709 [M − Glc − H]$^-$; HRFABMS *m/z* 871.4684 (calcd for $C_{44}H_{71}O_{17}$, 871.4691).

25(*R* and *S*)-Schidegera-saponin D2 (8): a white amorphous powder; $[\alpha]^{28}_D$ −56.4° (*c* 0.93, pyridine); ^{1}H NMR (500 MHz) δ 25*R* 0.68 [(3H), d, J = 5.4 Hz, H-27], 0.80 [(3H), s, H-18], 0.94 [(3H), s, H-19], 1.14 [(3H), d, J = 6.7 Hz, H-21], 25*S* 0.79 [(3H), s, H-18], 0.94 [(3H), s, H-19], 1.06 [(3H), d, J = 7.0 Hz, H-27], 1.14 [(3H), d, J = 6.7 Hz, H-21], anomeric protons 4.91 (1H, d, J = 7.6 Hz, Gal H-1), 5.24 (1H d, J = 7.6 Hz, Xyl H-1), 5.57 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 871 [M − H]$^-$, 739 [M − Xyl − H]$^-$, 709 [M − Glc − H]$^-$; HRFABMS *m/z* 871.4689 (calcd for $C_{44}H_{71}O_{17}$, 871.4691).

Mixture of Saponin Ys-III[9] and 25(*S*)-Schidegera-saponin D3 (9): a white amorphous powder; $[\alpha]^{28}_D$ −42.5° (*c* 0.88, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.67 [(3H), d, J = 5.8 Hz, H-27], 0.81 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.14 [(3H), d, J = 6.7 Hz, H-21], 25*S* 0.80 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.06 [(3H), d, J = 7.0 Hz, H-27], 1.14 [(3H), d, J = 7.0 Hz, H-21], anomeric protons 4.88 (1H, d, J = 7.6 Hz, Glc H-1], 5.34 (1H, d, J = 7.9 Hz, Glc″ H-1), 5.65 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 901 [M − H]$^-$, 739 [M − Glc − H]$^-$; HRFABMS *m/z* 901.4778 (calcd for $C_{44}H_{73}O_{18}$, 901.4797).

Mixture of Saponin Ys-IV[9] and 25(*S*)-Schidigera-saponin D4 (10): a white amorphous powder; $[\alpha]^{28}_D$ −35.7° (*c* 0.79, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.68 [(3H), d, 5.4 Hz, H-27], 0.81 [(3H), s, H-18], 0.94 [(3H), s, H-19], 1.14 [(3H), d, J = 7.0 Hz, H-21], 25*S* 0.80 [(3H), s, H-18], 0.94 [(3H), s, H-19], 1.06 [(3H), d, J = 7.0 Hz, H-27], 1.14 [(3H), d, J = 7.0 Hz, H-21], anomeric protons 4.82 (overlapped with HDO signal, Gal H-1), 5.40 (1H, d, J = 7.6 Hz, Glc″ H-1), 5.58 (1H, d, J = 7.6 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 901 [M − H]$^-$, 739 [M − Glc − H]$^-$; HRFABMS *m/z* 901.4822 (calcd for $C_{44}H_{73}O_{18}$, 901.4797).

Mixture of Saponin Ys-I[9] and 25(*S*)-Schidigera-saponin D5 (11): a white amorphous powder; $[\alpha]^{24}_D$ −44.4° (*c* 0.65, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.64 [(3H), d, J = 5.6 Hz, H-27], 0.77 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.09 [(3H), d, J = 6.8 Hz, H-21], 25*S* 0.76 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.02 [(3H), d, J = 6.8 Hz, H-27], 1.08 [(3H),d, J = 7.1 Hz, H-21], anomeric protons 4.91 (overlapped with HDO signal, Glc H-1), 5.34 (1H, d, J = 7.6 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 739 [M − H]$^-$, 577 [M − Glc − H]$^-$, 421 [M − Glc − Glc − H]$^-$; HRFABMS *m/z* 739.4254 (calcd for $C_{39}H_{63}O_{13}$, 739.4269).

25(*R* and *S*)-Schidigera-saponin E1 (12): a white amorphous powder; $[\alpha]^{24}_D$ −12.7° (*c* 1.01, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.67 [(3H), d, J = 5.8 Hz, H-27], 0.93 [(3H), s, H-18], 1.06 [(3H), s, H-19], 1.34 [(3H), d, J = 6.7 Hz, H-21], 25*S* 0.93 [(3H), s, H-18], 1.04 [(3H), d, J = 5.8 Hz, H-27], 1.06 [(3H), s, H-19], 1.35 [(3H), d, J = 7.0 Hz, H-21], anomeric protons 4.87 (1H, d, J = 7.9 Hz, Glc H-1), 5.28 (1H, d, J = 7.6 Hz, Xyl H-1), 5.66 (1H, d, J = 7.6 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 885 [M − H]$^-$, 753 [M − Xyl − H]$^-$, 723 [M − Glc − H]$^-$; HRFABMS *m/z* 885.4449 (calcd for $C_{44}H_{69}O_{18}$, 885.4484).

25(*R* and *S*)-Schidigera-saponin F1 (13): a white amorphous powder; $[\alpha]^{24}_D$ −56.2° (*c* 1.59, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.68 [(3H), d, J = 5.8 Hz, H-27], 0.77 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.12 [(3H), d, J = 7.0 Hz, H-21], 25*S* 0.77 [(3H), s, H-18], 0.93 [(3H), s, H-19], 1.05 [(3H), d, J = 7.0 Hz, H-27], 1.12 [(3H), d, J = 7.0 Hz, H-21], anomeric protons 4.91 (overlapped with HDO signal, Gal H-1), 5.24 (1H, d, J = 7.9 Hz, Xyl H-1), 5.56 (1H, d, J = 7.9 Hz, Glc′ H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 887 [M − H]$^-$, 755 [M − Xyl − H]$^-$, 725 [M − Glc − H]$^-$; HRFABMS *m/z* 887.4589 (calcd for $C_{44}H_{71}O_{18}$, 887.4640).

Mixture of Saponin Ys-V[9] and 25(*S*)-Schidigera-saponin F2 (14): a white amorphous powder; $[\alpha]^{24}_D$ −57.3° (*c* 1.07, MeOH); ^{1}H NMR (500 MHz) δ 25*R* 0.64 [(3H), d, J = 5.3 Hz, H-27], 0.73 [(3H), s, H-18], 0.89 [(3H), s, H-19], 1.07 [(3H), d, J = 7.1 Hz, H-21], 25*S* 0.73 [(3H), s, H-18], 0.89 [(3H), s, H-19], 1.01 [(3H), d, J = 7.0 Hz, H-27], 1.07 [(3H), d, J = 7.1 Hz, H-21], anomeric protons 4.92 (1H, d, J = 8.3 Hz, Gal H-1), 5.22 (1H, d, J = 7.8 Hz, Glc H-1); ^{13}C NMR, see Tables 1 and 2; negative FABMS *m/z* 755 [M − H]$^-$, 593 [M − Glc − H]$^-$; HRFABMS *m/z* 755.4193 (calcd for $C_{39}H_{63}O_{14}$, 755.4218).

Identification of Sugar Components of Compounds 1−14. A solution of each saponin (a few milligrams) in 1 M HCl in 50% 1,4-dioxane (2 mL) was heated at 80 °C for 4 h. Each reaction mixture was neutralized with Amberlite MB-3 (H^+ and ^-OH form, Organo Co., Japan), filtered, and then concentrated to dryness in vacuo. The residue was examined by TLC with $CHCl_3$−MeOH−H_2O (6:4:1) and by GLC as a TMS ether and compared with authentic samples.

Acid Hydrolysis of Compounds 1, 4, and 5. A solution of **4** (51 mg) in EtOH (10 mL) and 1 N HCl (10 mL) was heated at 80 °C for 19 h. On cooling, the reaction mixture was neutralized with 0.5 N aqueous KOH and concentrated to dryness in vacuo. The residue was dissolved in H_2O and extracted with EtOAc. The EtOAc layer was chromatographed on Si gel (EtOAc−*n*-hexane, 1:1) to give **18** (20 mg). In the same manner, **1** (50 mg) gave **15** (23 mg), and **6** (30 mg) gave **20** (14 mg).

Schidigeragenin A (15): a white amorphous powder; $[\alpha]^{23}_D$ −67.0° (*c* 1.00, $CHCl_3$); ^{1}H NMR (90 MHz) δ 0.93 (3H, s, H-18), 1.09 (3H, s, H− 19), 1.18 (3H, d, J = 6.2 Hz, H-21), 4.87 (2H, br s, H-27); ^{13}C NMR, see Table 1; HREIMS *m/z* 414.3176 (calcd for $C_{27}H_{42}O_3$, 414.3134).

Schidegeragenin B (18): a white amorphous powder; $[\alpha]^{23}_D$ +18.1° (*c* 1.00, $CHCl_3$); ^{1}H NMR (90 MHz) δ 1.09 (3H, s, H-18), 1.18 (3H, s, H-19), 1.32 (3H, d, J = 5.9 Hz, H-21), 4.85 (2H, br s, H-27); ^{13}C NMR, see Table 1; HREIMS *m/z* 428.2958 (calcd for $C_{27}H_{40}O_4$, 428.2927).

Schidigeragenin C (20): a white amorphous powder; $[\alpha]^{23}_D$ −85.0° (*c* 0.24, $CHCl_3$); ^{1}H NMR (90 MHz) δ 0.91 (3H, s, H-18), 1.09 (3H, s, H-19), 1.16 (3H, d, J = 6.5 Hz, H-21), 4.85 (2H, br s, H-27); ^{13}C NMR, see Table 1; HREIMS *m/z* 430.3113 (calcd for $C_{27}H_{42}O_4$, 430.3083).

Antimicrobial Assay. Inhibitory activity against each microorganism was determined using an agar dilution method. In brief, 0.5 mL of serial two-fold dilutions of test samples were mixed with 9.5 mL of culture medium. The culture media used were a standard agar medium (Nissui, Tokyo, Japan) for bacteria and glucose−peptone agar medium, which contains glucose (20 g), peptone (10 g), and agar (15 g) in distilled water (1 L), for yeasts and fungi. Each culture medium containing a test sample was poured into a Petri dish, onto which each microorganism was streaked. After an appropriate incubation period (48 h, 30 °C), the inhibitory activity of the sample was assessed as the minimum inhibitory concentration (MIC), the lowest concentration tested at which no growth was observed.

Saponin Fraction. MIC against Gram-positive bacteria, >1000 μg/mL (*Bacillus circulans* IFO 3329, *Enterococcus faecalis* IFO 3971, *Lactobacillus plantarum* IFO 3070, *Lactobacillus rhamnosus* IFO 12521, *Staphylococcus epidermidis* IID 866, *Streptococcus mutans* IFO 13955), 1000 μg/mL (*Bacillus licheniformis* IFO 12200, *Bacillus subtilis* IFO 3007, *Staphylococcus aureus* IFO 3060, *Staphylococcus aureus* IID 671); MIC against Gram-negative bacteria, >1000 μg/mL (*Escherichia coli* HUT 215, *Pseudomonas aeruginosa* JCM 2776, *Pseudomonas fluorescens* JCM 2779), 1000 μg/mL (*Alcaligenes faecalis* IFO 13111, *Klebsiella pneumoniae* IFO 14940, *Proteus vulgaris* IFO 3851).

References and Notes

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