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NEW TRITERPENOID SAPONINS FROM *STENOCEREUS ERUCA*

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Abstract – Eight new triterpenoid saponins, stellatosides C (**1**), D (**2**), and E (**3**), stellatoside B methyl ester (**4**), stellatoside C methyl ester (**5**), thurberoside A (**6**), phillyriside A (**7**), and treleaseside A (**8**) were isolated from *Stenocereus eruca* A. C. Gibson & K. E. Horak (*Machaerocereus eruca* Br. & R.). The structures of these compounds were elucidated on the basis of spectroscopic evidence and their physicochemical properties.

INTRODUCTION

The cactus plant is a succulent plant in North and South America which grows wild during the dry season and during breaks in the rainy season. Cacti are classified by their forms into tree-form cactus (Pereskioideae), bushy cactus and columnar cactus (Cactioideae), and globular cactus (Opuntioideae). After screening numerous cacti, we discovered that some bushy or columnar cacti (Cactioideae) contained many saponins. Further research led us to isolate seventeen new and twenty known triterpene sapogenins,[1-16] in addition to fifteen new saponins and two known ones from several cacti.[18-21] Some triterpene sapogenins from cacti showed antinociceptive[22] and antitumor-promotion activities.[23] In the previous study, we have reported the structures of stellatoside B and erucasaponin A from *Stenocereus eruca* A. C. Gibson & K. E. Horak (*Machaerocereus eruca* Br. & R.).[20] In this study, newly we isolated eight new triterpenoid saponins, stellatosides C (**1**), D (**2**), and E (**3**), stellatoside B methyl ester (**4**), stellatoside C methyl ester (**5**), thurberoside A (**6**), phillyriside A (**7**), and treleaseside A (**8**) from *Stenocereus eruca*. Thurberoside A (**6**), phillyriside A (**7**) and treleaseside A (**8**) were the first saponins with thurberogenin, 27-desoxyphillyrigenin, and treleasegenic acid as aglycons. This paper discusses the isolation and structure elucidation of these new saponins (**1**-**8**).

RESULTS AND DISCUSSION

Dried *Stenocereus eruca* was extracted repeatedly with chloroform and then with methanol. The methanol extract was passed through a Diaion HP-20 column to adsorb the saponins. The methanolic eluate was separated by silica gel and octadecyl silyl silica gel (ODS) column chromatography, yielding stellatosides C, D, and E (**1**-**3**), stellatosides B and C methyl esters (**4** and **5**), thurberoside A (**6**), phillyriside A (**7**), and treleaseside A (**8**). The structures of compounds **1**-**8** were determined spectroscopically using DEPT, HMQC, HMBC, DQF-COSY, HSQC-TOCSY, phase sensitive TOCSY and phase sensitive NOESY experiments (Figure 1).

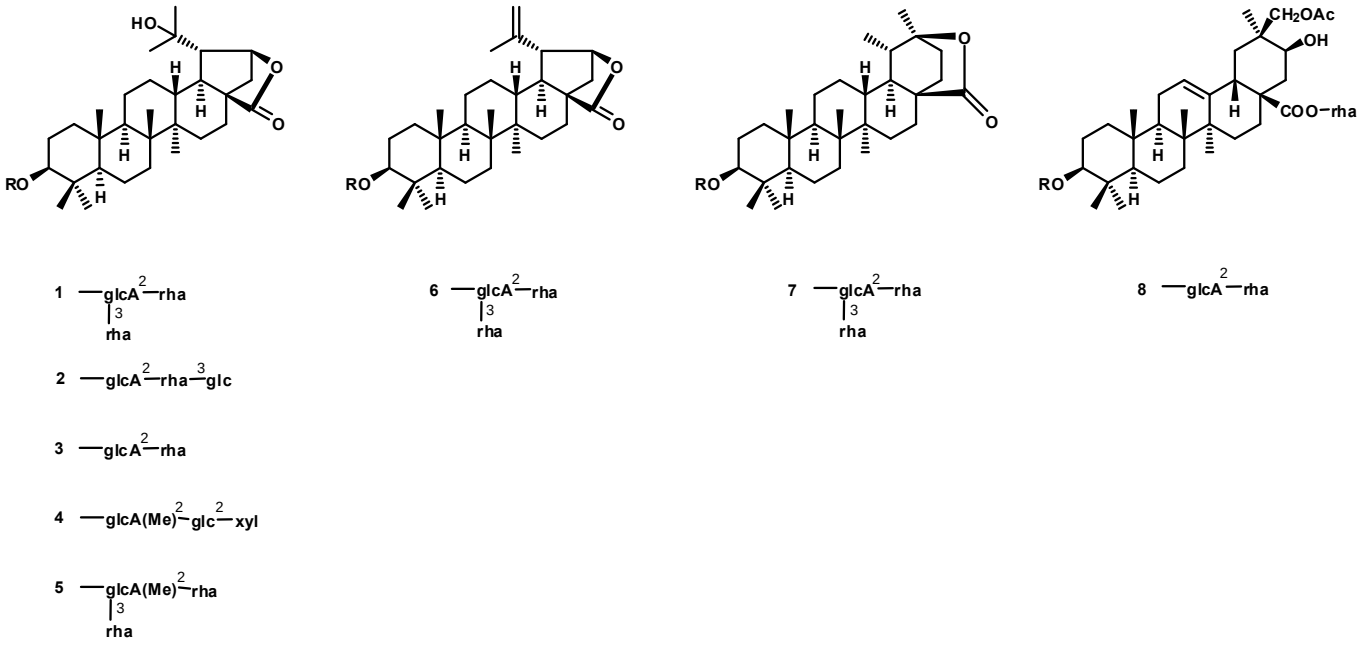

Figure 1. Structures of **1**-**8**

Stellatoside C (**1**), a colorless powder, has a lupane-type triterpene skeleton, stellatogenin as the aglycon and three sugars, glucuronic acid and two rhamnoses. Its molecular formula $C_{48}H_{76}O_{18}$ was determined with its positive ion HRFABMS (*m*/*z* 941.5127, $[M+H]^+$). The IR spectrum of **1** showed absorptions at 3410 cm^{-1} (hydroxy) and 1740 cm^{-1} (lactone). In the aglycon moiety, ^{1}H-NMR spectrum indicated the presence of seven methyl groups characterized by singlets at δ_H 0.70-1.16. Since **1** did not have an olefinic proton, had two methine carbon signals, the aglycon moiety of compound **1** was predicted to be a lupane-type triterpene. From the dara of HMQC, HMBC, DQF-COSY, and phase sensitive NOESY spectral correlations, revealed that –OH was in the C-20 and lactone ring was existed between C-21 and C-28, the aglycon moiety of **1** was determined to be stellatogenin (Figure 1, 2). Addition, the aglycon was obtained by 3.5%HCl hydrolysis of **1** and was identified by ^{1}H- and ^{13}C-NMR spectral data.[13,26] About the sugar unit, in the ^{1}H- and ^{13}C-NMR spectra, three anomeric proton signals [δ_H 4.35 (1H, d, *J* =

7.3 Hz), 4.73, 4.88 (each 1H, br s)] and carbon signals [δ_C 100.6 (CH), 100.9 (CH), 103.3 (CH)] were recognized. Detailed analysis of **1** was conducted with HMQC, HMBC, DQF-COSY, phase sensitive NOESY, TOCSY, and phase sensitive HSQC-TOCSY experiments, each sugar units were determined as one β-glucuronic acid and two α-rhamnoses. About the linkage of the each sugar units, between H-1' and C-3 correlated in the HMBC experiment indicated that the glucuronic acid unit binds at C-3 of the aglycon moiety. From the anomeric proton of rhamnose-1 to C-2' of glcuronic acid HMBC correlation indicates that rhamnose-1 unit binds at C-2' of the glucuronic acid unit. Similarly, the HMBC correction from anomeric proton of rhamnose-2 to C-3' of glcuronic acid indicated that rhamnose-2 unit binds at C-3' of glucuronic acid unit (Figure 2). The absolute configuration of each sugar units as D-glucuronic acid and L-rhamnose were deternined by measuring ODS HPLC retention times of each sugar derivatives.[24] Thus, the structure of **1** was determined to be stellatogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranoside.

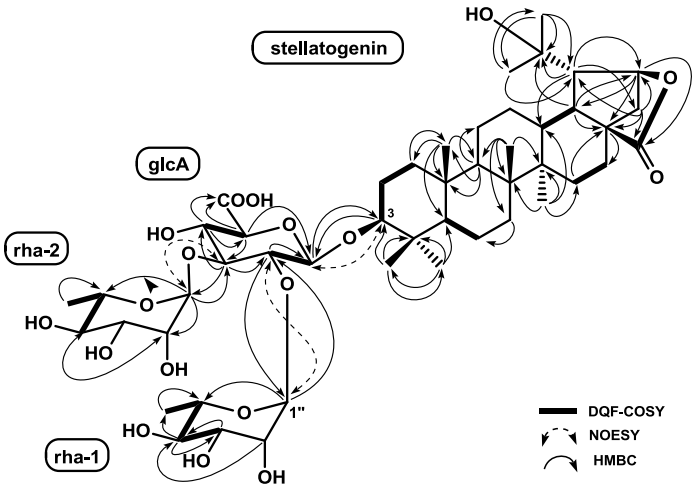

Figure 2. 2D-NMR Correlations of Stellatoside C (**1**)

Stellatoside D (**2**) was isolated as a colorless powder. Its molecular formula $C_{48}H_{76}O_{19}$ was determined from its negative ion HRFABMS (*m*/*z* 955.4916, $[M-H]^-$). The IR spectrum of **2** showed absorptions at 3400 cm^{-1} (hydroxy) and 1760 cm^{-1} (lactone). About the aglycon moiety, the ^1H- and ^{13}C-NMR spectrum data was confortable with **1**, the aglycon moiety of **2** was determined to be stellatogenin. Regarding the sugar moieties, three anomeric proton signals [δ_H 4.28 (1H, d, *J* = 7.6 Hz), δ_H 4.29 (1H, d, *J* = 6.1 Hz), δ_H 5.26 (1H, br s)] and carbon signals [δ_C 99.7 (CH), 103.3 (CH), 104.8 (CH)] suggested the presence of three sugar units. In addition, carbonyl carbon signal (δ_C 174.2), methylene signal (δ_C 60.8) and methyl signal (δ_C 17.8) indicated the presence of glucuronic acid, glucose and rhamnose units, respectively. From the analysis of the HMQC, HMBC, DQF-COSY, Phase sensitive NOESY, TOCSY

and Phase sensitive TOCSY experiments all the protons and carbons of the sugar moiety were assigned as shown as Table 1 and Table 2-1. The linkage of the sugar units at C-3 of stellatogenin and the sequence of the sugar chains were established by the following HMBC and NOESY spectra. Analysis of the HMBC spectrum of **2** established that these were cross-peaks between anomeric proton of glucuronic acid at δ 4.29 and C-3 of stellatogenin at δ 88.0. This result indicated that the glucuronic acid was connected to C-3 of the aglycon. The linkage of the rhamnose and glucuronic acid was indicated by HMBC cross-peak between anomeric proton of rhamnose at δ 5.26 and C-2' of glucuronic acid at δ 75.8. Similarly, the linkage of the glucose and rhamnose was indicated by HMBC cross-peak between anomeric proton of glucose at δ 4.28 and C-3"" of rhamnose at δ 81.7. In the NOESY spectrum, NOE correlations between the methine proton at H-3 of the aglycon (δ 3.00) and the anomelic proton of glucuronic acid (δ 4.29), between H-2' of glucuronic acid (δ 3.20) and the anomelic proton (δ 5.26) of rhamnose, and between H-3"" (δ 3.65) of rhamnose and the anomelic proton of glucose (δ 4.28). On the basis of the above, the structure of **2** was determined as stellatogenin 3-*O*-β-D-glucopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranoside.

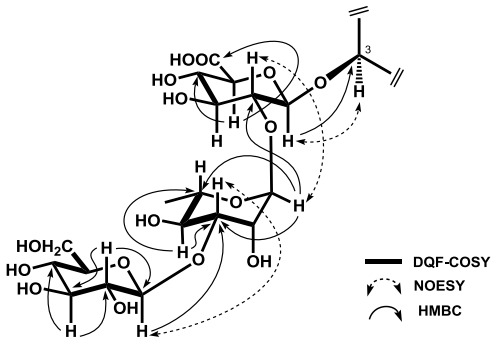

Figure 3. 2D Correlations of Stellatoside D (**2**)

Stellatoside E (**3**) was obtained as a colorless powder. Its molecular formula $C_{42}H_{66}O_{14}$ was determined from its negative ion HRFABMS (*m*/*z* 793.4370 $[M-H]^-$). The IR spectrum of **3** showed absorptions at 3410 cm^{-1} (hydroxy) and 1760 cm^{-1} (lactone). The ^1H- and ^{13}C-NMR spectrum data were similar with compound **2** (stellatoside D). Although compound **2** had a glucuronic acid, rhamnose and glucose, compound **3** had glucuronic acid and rhamnose as sugar moiety. The linkage of the oligosaccharide moiety was determined by HMBC correlations between anomeric proton of glucuronic acid (δ 4.25) and C-3 of aglycon (δ 87.9), and anomeric proton of rhamnose (δ 5.24) and C-2' of glucuronic acid (δ 76.3). In conclusion, the structure of **3** was determined as stellatogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranoside.

Stellatoside B methyl ester (**4**) was obtained as a colorless powder. Its molecular formula $C_{48}H_{76}O_{19}$ was determined from its negative ion HRFABMS (*m/z* 955.4915, $[M-H]^-$). The IR spectrum of **5** showed absorptions at 3410 cm^{-1} (hydroxy) and 1750 cm^{-1} (lactone). From the ^{1}H- and ^{13}C-NMR signal patterns, the aglycon moiety was determined to be stellatogenin. The ^{1}H- and ^{13}C-NMR spectrum data showed that compound **4** has three sugars, glucuronic acid methyl ester, glucose, and xylose. Since resonance C-6' of glucuronic acid was upfield shifted and a new methyl proton signal at δ_H 3.64 (3H, s) had HMBC correlation with carbonyl carbon of glucuronic acid, the structure of **4** was determined as methyl ester of stellatoside B.[20] Thus, **4** was elucidated as stellatogenin 3-*O*-β-D-xylopyranosyl-(1→2)-β-D-glucopyranosyl-(1→2)-6-*O*-methyl-β-D-glucuronopyranoside.

Stellatoside C methyl ester (**5**) was obtained as a colorless powder. Its molecular formula $C_{49}H_{78}O_{18}$ was determined from its positive ion HRFABMS (*m/z* 955.5283, $[M+H]^+$). The IR spectrum of **5** showed absorptions at 3410 cm^{-1} (hydroxy) and 1760 cm^{-1} (lactone). Compared to the ^{1}H- and ^{13}C-NMR data of **1**, a proton signal at δ_H 3.66 (3H, s) and two carbon signals at δ_C 51.9 (CH_3) and δ_C 169.4 (C) were different. Since these signals indicated that **5** has a methyl ester in its structure, the oligosaccharide moiety of **5** was methyl ester of **1**. Thus, **5** was determined to be stellatogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-6-*O*-methyl-β-D-glucuronopyranoside. Although **5** was methyl ester of **1**, we considered compound **5** is not artifact from compound **1**. Because if compound **5** was artifact, probably, we had isolated methyl esters of saponins from cacti. In our previous study, we have isolated same saponins from cacti[17-21] as same methods, and this is first isolation as methyl ester of glucuronic acid on saponin from cacti. Then, we considered this methyl ester of stellatoside C was new compound from *S. eruca.*

Thurberoside A (**6**) was isolated as a colorless powder. Its molecular formula $C_{48}H_{74}O_{17}$ was determined from its positive ion HRFABMS (*m/z* 923.4998, $[M+H]^+$). The IR spectrum of **6** showed absorptions at 3400 cm^{-1} (hydroxy) and 1760 cm^{-1} (lactone). The compound displayed 48 signals in its ^{13}C-NMR spectrum. Almost all the signals in the ^{13}C-NMR spectrum were similar with **1**, however, exomethylene signals [δ_H 4.87, 4.88 (each 1H, br s), δ_C 112.3 (CH_2), 150.3 (C)] were newly appeared in **6**. Then, aglycon moiety of **6** was considered to be thurberogenin.[25] The sugar moiety was same with its of **1**. From the data of 1D and 2D NMR data, the structure of **6** was determined to be thurberogenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranoside.

Phirylliside A (**7**) was obtained as a colorless powder. Its molecular formula $C_{48}H_{76}O_{17}$ was determined from its positive ion HRFABMS (*m/z* 925.5161, $[M+H]^+$). The IR spectrum showed absorptions at 3410 cm^{-1} (hydroxy) and 1740 cm^{-1} (lactone). In the ^{1}H-NMR spectrum, six methyl signals as singlet (δ_H 0.71–1.21) and one methyl signal as doublet [δ_H 0.91 (3H, d, J = 6.7 Hz)] were observed. The aglycon

of **7** was identified as 27-desoxyphillyrigenin by comparison with our NMR data.[16] The sugar moieties were determined same as **1**. Finally, the structure of **7** was determined to be 27-desoxyphillyrigenin 3-*O*-α-L-rhamnopyranosyl-(1→2)-[α-L-rhamnopyranosyl-(1→3)]-β-D-glucuronopyranoside.

Treleaseside A (**8**) was isolated as a colorless powder. Its molecular formula $C_{50}H_{78}O_{20}$ was determined from its negative ion HRFABMS (*m*/*z* 997.5016, $[M-H]^-$). The IR spectrum showed absorptions at 3400 cm^{-1} (hydroxy) and 1720 cm^{-1} (carbonyl). The compound displayed 50 signals in its ^{13}C-NMR spectrum. Its ^{1}H-NMR spectrum indicated the presence of six methyl groups characterized by singlets at δ_H 0.65, 0.73, 0.84, 1.09 (each 3H, s), and 0.94, (6H, s). The signal patterns of ^{1}H- and ^{13}C-NMR spectrum data of aglycon moiety were similar with treleasegenic acid which had already reported as triterpene sapogenin from *S. eruca*.[16] The different part of the aglycon of **8** and treleasegenic acid was **8** had newly acetyl group [δ_c 20.7 (CH_3) and δ_c 170.6 (C=O)]. The position of the acetyl group was determined by HMBC correlations between H_2-30 of aglycon (δ_H 3.91 and 4.26, each 1H, d, J = 11.3 Hz) and δ_C 170.6 (carbonyl carbon of acethyl group). From this information, the acetyl group was considered to be bound to position 30 of treleasegenic acid.

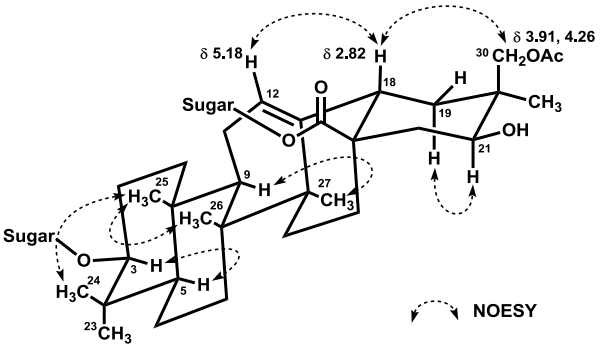

Figure 4. NOESY Correlations of Treleaseside A (**8**)

The structure of treleasegenic acid 30 acetate[16] as aglycon was identified by NOESY correlations. In NOESY spectrum, the methine proton of H-18 (δ 2.82) had cross-peaks with the methine proton of H-12 (δ 5.18) and the methylene protons of H_2-30 (δ 3.91 and 4.26). The three anomeric carbons of the sugar moieties [δ_C 93.7 (CH), 99.8 (CH), 103.7 (CH)] had HMQC correlations with anomeric protons at δ_H 5.72 (1H, br s), δ_H 5.26 (1H, br s) and 4.25 (1H, d, J = 7.6 Hz), respectively. The sugar moiety at C-3 was same with **3**, and the rhamnose unit was bound at C-28 of treleasegenic acid 30-acetate. In conclusion, the structure of **8** was determined to be treleasegenic acid 30-acetate 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-glucuronopyranosyl 28-*O*-α-L-rhamnopyranoside. The absolute configuration of each sugar units of compounds **2-8** as D-glucuronic acid, D-xylose and L-rhamnose were deternined by measuring ODS HPLC retention times of each sugar derivatives.[24]

Table 1. ^{13}C-NMR Spectral Data of **1-8** (125 MHz, in DMSO-d_6)

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
C-1	38.7	38.6	38.7	38.2	38.5	38.5	38.8	38.4
C-2	25.5	25.5	25.5	25.6	25.6	25.7	25.5	25.4
C-3	87.8	88.0	87.9	88.4	88.1	88.1	87.8	87.8
C-4	38.6	38.7	38.6	38.7	38.6	38.7	38.6	38.5
C-5	55.2	55.2	55.2	54.9	55.0	55.2	55.3	55.2
C-6	17.6	17.7	17.6	17.6	17.6	17.6	17.6	17.7
C-7	34.0	34.0	34.0	34.0	34.0	33.7	33.5	32.4
C-8	40.5	40.5	40.5	40.5	40.5	40.5	40.0	38.8
C-9	49.7	49.7	49.7	49.6	49.6	49.7	49.9	46.9
C-10	36.7	36.3	36.3	36.3	36.4	36.4	36.4	36.2
C-11	20.7	20.7	20.7	20.7	20.7	20.2	20.5	22.9
C-12	27.8	27.8	27.8	27.8	27.8	25.7	24.5	122.6
C-13	40.2	40.2	40.2	40.1	40.1	40.3	42.5	142.1
C-14	42.5	42.5	42.5	42.5	42.5	41.9	40.6	41.2
C-15	26.0	26.0	26.0	26.0	26.0	26.0	26.6	27.2
C-16	23.3	23.4	23.3	23.3	23.3	22.7	27.1	23.8
C-17	51.4	51.4	51.4	51.4	51.4	50.9	41.4	47.9
C-18	41.6	41.6	41.6	41.6	41.6	44.0	47.3	40.1
C-19	54.7	54.8	54.7	54.7	54.7	52.5	41.5	40.0
C-20	68.6	68.6	68.9	68.6	68.5	150.3	83.8	40.0
C-21	81.0	81.0	81.0	81.0	81.0	81.0	26.7	70.6
C-22	43.9	43.9	43.9	43.9	43.9	43.8	31.2	39.8
C-23	27.3	27.4	27.3	27.3	27.2	27.2	27.2	16.2
C-24	15.9*	16.0*	16.0	15.9	15.9*	15.9	15.9	27.4
C-25	16.1*	16.1*	16.0	15.9	16.0*	16.0	16.2	15.2
C-26	15.7	15.7	15.7	15.7	15.7	15.5	15.3	16.9
C-27	13.8	13.8	13.8	13.9	13.8	13.6	13.9	25.3
C-28	178.7	178.7	178.7	178.7	178.7	177.7	175.9	173.6
C-29	29.9	29.9	29.9	29.9	29.9	112.3	18.3	23.9
C-30	30.2	30.2	30.2	30.1	30.1	23.0	23.6	63.4
30-$CH_3\underline{C}O$								170.6
30-$\underline{C}H_3CO$								20.7
glcA								
1’	103.3	103.3	103.7	103.5	102.8	103.3	103.4	103.7
2’	77.2	75.8	76.3	79.2	76.2	76.2	77.1	76.4
3’	82.0	77.6	72.3	76.2	81.0	82.0	82.1	73.7
4’	70.9	72.5	77.8	71.1	70.1	70.5	71.0	78.0
5’	74.1	74.0	74.1	75.1	74.9	75.2	74.1	73.7
$\underline{C}OOH$	172.6	174.2	172.7			170.2	173.3	173.6
$\underline{C}OOCH_3$				169.4	169.4			
$COO\underline{C}H_3$				51.8	51.9			
glc								
1’’		104.8		100.5				
2’’		73.9		82.9				
3’’		76.3		76.5				
4’’		76.8		70.0				
5’’		69.8		76.0				
6’’		60.8		61.1				
xyl								
1’’’				104.5				
2’’’				74.1				
3’’’				75.7				
4’’’				69.3				
5’’’				65.8				
C-3 rha 1								
1’’’’	100.6	99.7	99.8		100.1	100.3	100.6	99.8
2’’’’	70.0	69.3	70.3		70.5	70.4	70.0	70.3
3’’’’	70.4	81.7	70.3		70.4	70.4	70.4	70.4
4’’’’	71.7	70.8	72.0		71.6	71.8	71.7	72.5
5’’’’	68.8	67.8	68.9		68.9	68.8	68.8	68.0
6’’’’	17.7	17.8	17.8		17.7	17.7	17.7	17.8*
C-3 rha 2								
1’’’’’	100.9				100.6	101.0	101.0	
2’’’’’	70.4				70.4	70.2	70.3	
3’’’’’	70.6				70.0	70.1	70.3	
4’’’’’	71.9				71.8	71.6	71.9	
5’’’’’	68.5				68.8	68.5	68.5	
6’’’’’	17.7				17.7	17.7	17.7	
C-28 rha								
1’’’’’’								93.7
2’’’’’’								70.5
3’’’’’’								71.1
4’’’’’’								71.4
5’’’’’’								69.5
6’’’’’’								17.9*

*: may be interchanged

Table 2-1. ^{1}H-NMR Spectroscopic Data [δ_H, mult. (*J* in Hz)] of **1**-**4** (500 MHz, in DMSO-d_6)

Position	**1**	**2**	**3**	**4**
1	0.87 (1H, m), 1.53 (1H, m)	0.87, 1.54 (each 1H, m)	0.82, 1.58 (each 1H, m)	0.82, 1.51 (each 1H, m)
2	α 1.92 (1H, br d, 11.5)	α 1.99 (1H, br d, 11.3)	α 1.92 (1H, br d, 10.4)	α 1.62 (1H, m)
	β 1.46 (1H, m)	β 1.47 (1H, m)	β 1.49 (1H, m)	β 1.49 (1H, m)
3	3.03 (1H, dd, 11.5, 3.6)	3.00 (1H, dd, 11.3, 3.7)	3.00 (1H, dd, 10.4, 3.7)	3.01 (1H, m)
5	0.65 (1H, br d, 11.3)	0.65 (1H, br d, 10.7)	0.66 (1H, br d, 11.3)	0.64 (1H, br d, 11.3)
6	α 1.47 (1H, m)	α 1.44 (1H, m)	α 1.44 (1H, m)	α 1.46 (1H, m)
	β 1.32 (1H, m)	β 1.33 (1H, m)	β 1.32 (1H, m)	β 1.30 (1H, m)
7	1.35 (2H, m)	1.33 (2H, m)	1.33 (2H, m)	1.32 (2H, m)
9	1.21 (1H, m)	1.21 (1H, m)	1.22 (1H, m)	1.17(1H, m)
11	α 1.15 (1H, m)	α 1.14 (1H, m)	α 1.15 (1H, m)	α 1.14 (1H, m)
	β 1.42 (1H, m)	β 1.40 (1H, m)	β 1.41 (1H, m)	β 1.40 (1H, m)
12	1.15, 1.47 (each 1H, m)	1.17, 1.47 (each 1H, m)	1.15, 1.47 (each 1H, m)	1.14, 1.49 (each 1H, m)
13	1.21 (1H, m)	1.22 (1H, m)	1.22 (1H, br d, 11.5)	1.22 (1H, m)
15	α 0.99 (1H, m)	α 0.97 (1H, m)	α 0.99 (1H, m)	α 0.99 (1H, m)
	β 2.28 (1H, ddd, 13.0, 12.8, 4.7)	β 2.29 (1H, dt, 11.9, 3.7)	β 2.29 (1H, dt, 13.1, 4.7)	β 2.29 (1H, dt, 13.1, 4.6)
16	α 1.61 (1H, m)	α 1.60 (1H, br dd, 12.5, 3.7)	α 1.60 (1H, br dd, 13.1, 4.7)	α 1.60 (1H, m)
	β 1.81 (1H, m)	β 1.83 (1H, m)	β 1.81 (1H, m)	β 1.82 (1H, m)
18	1.84 (1H, dd, 11.2, 5.6)	1.86 (1H, dd, 11.2, 5.6)	1.86 (1H, dd, 11.5, 5.8)	1.86 (1H, dd, 11.9, 5.7)
19	1.56 (1H, br d, 5.6)	1.57 (1H, br d, 5.6)	1.57 (1H, br d, 5.8)	1.57 (1H, br d, 5.7)
21	4.66 (1H, br s)	4.67 (1H, br s)	4.67 (1H, br s)	4.68 (1H, br s)
22	α 2.04 (1H, d, 10.4)	α 2.05 (1H, d, 10.1)	α 2.05 (1H, d, 10.2)	α 2.06 (1H, d, 10.2)
	β 1.77 (1H, d, 10.4)	β 1.78 (1H, br d, 10.1)	β 1.78 (1H, d, 10.2)	β 1.79 (1H, d, 10.2)
23	0.91 (3H, s)	0.93 (3H, s)	0.91 (3H, s)	0.92 (3H, s)
24	0.70 (3H, s)	0.72 (3H, s)	0.71 (3H, s)	0.71 (3H, s)
25	0.74 (3H, s)	0.75 (3H, s)	0.75 (3H, s)	0.74 (3H, s)
26	0.85 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.86 (3H, s)
27	0.93 (3H, s)	0.94 (3H, s)	0.94 (3H, s)	0.94 (3H, s)
29	1.16 (3H, s)	1.17 (3H, s)	1.17 (3H, s)	1.17 (3H, s)
30	1.07 (3H, s)	1.08 (3H, s)	1.08 (3H, s)	1.07 (3H, s)
glcA				
1’	4.35 (1H, d, 7.3)	4.29 (1H, d, 6.1)	4.25 (1H, d, 7.3)	4.38 (1H, d, 5.2)
2’	3.24 (1H, dd, 7.9, 7.3)	3.20 (1H, m)	3.22 (1H, br t, 7.3)	3.43 (1H, m)
3’	3.44 (1H, m)	3.34 (1H, m)	3.16 (1H, m)	3.36 (1H, m)
4’	3.30 (1H, dd, 9.7, 8.9)	3.14 (1H, br t, 9.8)	3.29 (1H, dd, 9.5, 8.9)	3.35 (1H, m)
5’	3.37 (1H, d, 9.7)	3.28 (1H, d, 9.8)	3.32 (1H, d, 9.5)	3.75 (1H, d, 9.5)
$COOCH_3$				3.64 (3H, s)
glc				
1’’		4.28 (1H, d, 7.6)		4.74 (1H, d, 6.0)
2’’		3.04 (1H, m)		3.16 (1H, m)
3’’		3.13 (1H, m)		3.06 (1H, m)
4’’		3.06 (1H, m)		3.06 (1H, m)
5’’		3.07 (1H, m)		3.42 (1H, m)
6’’		3.45 (1H, br d, 10.7)		3.41, 3.63 (each 1H, m)
		3.62 (1H, dd, 10.7, 5.2)		
xyl				
1’’’				4.45 (1H, d, 6.4)
2’’’				3.01 (1H, m)
3’’’				3.11 (1H, m)
4’’’				3.27 (1H, m)
5’’’				3.05 (1H, m)
				3.73 (1H, dd, 11.3, 5.2)
C-3 rha 1				
1’’’’	4.88 (1H, br s)	5.26 (1H, br s)	5.24 (1H, br s)	
2’’’’	3.62 (1H, br s)	3.93 (1H, br s)	3.67 (1H, br s)	
3’’’’	3.40 (1H, m)	3.65 (1H, dt, 9.2, 2.1)	3.46 (1H, br d, 9.2)	
4’’’’	3.15 (1H, m)	3.40 (1H, dt, 9.2, 2.1)	3.16 (1H, br t, 9.2)	
5’’’’	3.71 (1H, dq, 9.5, 6.1)	3.83 (1H, dq, 9.2, 6.1)	3.88 (1H, dq, 9.2, 6.1)	
6’’’’	1.04 (1H, d, 6.1)	1.07 (1H, d, 6.1)	1.04 (1H, d, 6.1)	
C-3 rha 2				
1’’’’’	4.73 (1H, br s)			
2’’’’’	3.66 (1H, br s)			
3’’’’’	3.40 (1H, m)			
4’’’’’	3.15 (1H, m)			
5’’’’’	3.91 (1H, dq, 9.2, 6.1)			
6’’’’’	1.04 (1H, d, 6.1)			

Table 2-2. ^{1}H-NMR Spectroscopic Data [d_H, mult. (*J* in Hz)] of **5**-**8** (500 MHz, in DMSO-d_6)

Position	**5**	**6**	**7**	**8**
1	0.81, 1.55 (each 1H, m)	0.88 (1H, m) 1.54 (1H, d, 12.5)	α 0.86 (1H, m) β 1.53 (1H, m)	0.88, 1.46 (each 1H, m)
2	α 1.70 (1H, m) β 1.49 (1H, m)	1.04, 1.73 (each 1H, m)	α 1.93 (1H, br d, 11.3) β 1.49 (1H, m)	α 1.47 (1H, m) β 1.51 (1H, m)
3	3.04 (1H, dd, 11.6, 4.0)	3.03 (1H, dd, 11.3, 4.0)	3.03 (1H, dd, 11.3, 3.4)	3.00 (1H, dd, 11.0, 4.0)
5	0.66 (1H, br d, 11.3)	0.67 (1H, br d, 10.7)	0.66 (1H, br d, 8.2)	0.70 (1H, m)
6	α 1.44 (1H, m) β 1.33 (1H, m)	α 1.43 (1H, d, 8.5) β 1.34 (1H, m)	α 1.28 (1H, m) β 1.43 (1H, m)	1.30, 1.46 (each 1H, m)
7	1.33 (2H, m)	α 1.34 (1H, m) β 1.02 (1H, m)	1.29 (2H, m)	1.41, 1.43 (each 1H, m)
9	1.22 (1H, m)	1.25 (1H, m)	1.27 (1H, m)	1.50 (1H, m)
11	α 1.13 (1H, m) β 1.40 (1H, m)	1.15, 1.39 (each 1H, m)	1.45 (2H, m)	1.76 (2H, m)
12	1.15, 1.46 (each 1H, m)	0.99, 1.22 (each 1H, m)	1.60 (2H, m)	5.18 (1H, br t, 3.6)
13	1.22 (1H, br d, 11.6)	1.26 (1H, dd, 12.0, 4.5)	1.00 (1H, br d, 11.3)	
15	α 0.99 (1H, m) β 2.29 (1H, dt, 13.1, 5.1)	1.00 (1H, m) 2.19 (1H, dt, 13.0, 4.2)	α 0.99 (1H, m) β 1.83 (1H, dd, 11.3, 3.4)	1.01, 1.51 (each 1H, m)
16	α 1.62 (1H, br dd, 13.1, 4.6) β 1.83 (1H, m)	α 1.69 (1H, m) β 1.83 (1H, br d, 11.3)	α 1.20 (1H, m) β 1.67 (1H, m)	α 1.59 (1H, m) β 1.64 (1H, m)
18	1.86 (1H, dd, 11.6, 6.0)	1.90 (1H, dd, 12.0, 6.9)	1.08 (1H, m)	2.82 (1H, dd, 13.7, 4.0)
19	1.57 (1H, br d, 6.0)	2.09 (1H, br d, 6.9)	1.47 (1H, m)	1.59 (1H, m)
21	4.68 (1H, br s)	4.56 (1H, br s)	α 1.47 (1H, m) β 1.89 (1H, d, 10.4)	3.46 (1H, m)
22	α 2.05 (1H, d, 10.1) β 1.78 (1H, d, 10.1)	1.72 (1H, d, 10.1) 1.95 (1H, d, 10.1)	α 1.60 (1H, m) β 1.47 (1H , m)	α 1.38 (1H, m) β 1.61 (1H, m)
23	0.90 (3H, s)	0.91 (3H, s)	0.91 (3H, s)	0.73 (3H, s)
24	0.70 (3H, s)	0.71 (3H, s)	0.71 (3H, s)	0.94 (3H, s)
25	0.75 (3H, s)	0.74 (3H, s)	0.75 (3H, s)	0.84 (3H, s)
26	0.86 (3H, s)	0.85 (3H, s)	0.81 (3H, s)	0.65 (3H, s)
27	0.94 (3H, s)	0.94 (3H, s)	0.87 (3H, s)	1.09 (3H, s)
29	1.18 (3H, s)	4.87, 4.88 (each 1H, br s)	0.91 (3H, d, 6.7)	0.94 (3H, s)
30	1.05 (3H, s)	1.78 (3H, s)	1.21 (3H, s)	3.91, 4.26 (each 1H, d, 11.3)
30-C$\underline{H}_3$CO				2.03 (3H, s)
glcA				
1’	4.57 (1H, d, 6.7)	4.35 (1H, d, 7.2)	4.35 (1H, d, 7.5)	4.25 (1H, d, 7.6)
2’	3.38 (1H, t, 6.7)	3.35 (1H, t, 7.2)	3.25 (1H, t, 7.5)	3.17 (1H, m)
3’	3.54 (1H, t, 6.7)	3.52 (1H, m)	3.47 (1H, m)	3.25 (1H, m)
4’	3.61 (1H, m)	3.30 (1H, m)	3.31 (1H, t, 9.6)	3.30 (1H, m)
5’	3.90 (1H, d, 9.2)	3.69 (1H, m)	3.38 (1H, d, 9.6)	3.21 (1H, m)
6-COOC$\underline{H}_3$	3.66 (3H,s)			
C-3 rha 1				
1’’’’	4.85 (1H, br s)	4.88 (1H, br s)	4.89 (1H, br s)	5.26 (1H, br s)
2’’’’	3.64 (1H, br s)	3.64 (1H, br s)	3.62 (1H, br s)	3.68 (1H, m)
3’’’’	3.40 (1H, m)	3.40 (1H, m)	3.40 (1H, m)	3.45 (1H, m)
4’’’’	3.17 (1H, t, 9.3)	3.18 (1H, m)	3.16 (1H, m)	3.14 (1H, m)
5’’’’	3.67 (1H, m)	3.69 (1H, m)	3.73 (1H, dq, 9.4, 6.1)	3.79 (1H, m)
6’’’’	1.07 (1H, d, 6.1)	1.07 (1H, d, 6.1)	1.05 (1H, d, 6.1)	1.05 (1H, d, 6.1)
C-3 rha 2				
1’’’’’	4.71 (1H, br s)	4.72 (1H, br s)	4.74 (1H, br s)	
2’’’’’	3.64 (1H, br s)	3.67 (1H, br s)	3.67 (1H, br s)	
3’’’’’	3.40 (1H, m)	3.39 (1H, m)	3.40 (1H, m)	
4’’’’’	3.19 (1H, t, 9.1)	3.19 (1H, m)	3.16 (1H, m)	
5’’’’’	3.74 (1H, dq, 9.1, 6.1)	3.76 (1H, dq, 9.2, 6.1)	3.92 (1H, dq, 9.5, 6.1)	
6’’’’’	1.07 (1H, d, 6.1)	1.04 (1H, d, 6.1)	1.04 (1H, d, 6.1)	
C-28 rha				
1’’’’’’				5.72 (1H, br s)
2’’’’’’				3.44 (1H, m)
3’’’’’’				3.47 (1H, m)
4’’’’’’				3.23 (1H, m)
5’’’’’’				3.55 (1H, m)
6’’’’’’				1.10 (1H, d, 6.4)

EXPERIMENTAL

General experimental procedures

The $[\alpha]_D$ values were determined with a JASCO DIP-140 digital polarimeter. IR spectra were measured with a JASCO A-102 IR spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were recorded using a JNM LA-500 spectrometer in DMSO-d_6 with tetramethylsilane as an internal standard. Kieselgel-60F_{254} (MERCK) or RP-18F_{254S} (MERCK)-precoated plates were employed for thin-layer chromatography (TLC). Column chromatography was carried out on 70-230 mesh silica gel (MERCK) and prep ODS-7515-12A. HREIMS and HRFABMS were obtained using a JEOL JMS-700 spectrometer.

Plant materal

Stenocereus eruca A.C. Gibson & K. E. Horak was cultivated originally at the Hokoen (Iga City, Mie, Japan).

Extraction and isolation

The dried and powdered whole plants of *S. eruca* (432.7 g) were extracted three times with $CHCl_3$ and then extracted three times with MeOH. The MeOH extract (83.0 g, 19.2%) was applied to a column of Diaion HP-20 and eluted with 30% MeOH in H_2O (1.4 g), 70% MeOH in H_2O (20.4 g), and 100% MeOH (20.8 g). Since the same spot was observed in the 70% MeOH and 100% MeOH eluted fractions, these fractions were mixed (41.2 g, 9.5%). The mixed fraction was separated by silica gel column chromatography using a stepwise gradient [Silica gel: 800 g, $CHCl_3$–MeOH–H_2O 90:12:1→70:10:1→60:10:1→50:10:1→40:10:1→35:10:1→30:10:1→25:10:1→20:10:1→15:6:1→13:6:1→10:5:1→8:5:1→6:4:1→MeOH] to give seven fractions [Fr. A (1.3 g), Fr. B (8.6 g), Fr. C (6.0 g), Fr. D (7.8 g), Fr. E (6.8 g), Fr. F (6.1 g), and Fr. G (3.6 g)].

Fraction C (6.0 g) was further subjected to silica gel column chromatography using a stepwise gradient [Silica gel: 220 g, $CHCl_3$–MeOH–H_2O 70:10:1→60:10:1→50:10:1→40:10:1→30:10:1→20:10:1→15:10:1→MeOH] to give seven fractions [Fr. C1 (39.1 mg), Fr. C2 (775.8 mg), Fr. C3 (2031.6 mg), Fr. C4 (1170.0 mg), Fr. C5 (1024.6 mg), Fr. C6 (752.2 mg), and Fr. C7 (42.6 mg)]. Fraction C2 (775.8 mg) was chromatographed on silica gel column chromatography using a stepwise gradient [Silica gel: 70 g, $CHCl_3$–MeOH–H_2O 80:10:1→70:10:1→60:10:1→50:10:1→MeOH] to give four fractions [Fr. C2-1 (140.8 mg), Fr. C2-2 (132.8 mg), Fr. C2-3 (138.7 mg) and Fr. C2-4 (358.0 mg)]. Fraction C2-4 (358.0 mg) was separated by octadecyl silylated silica gel (ODS) column chromatography using a stepwise gradient [50% MeOH→55% MeOH→65% MeOH→80% MeOH→MeOH] to afford stellatoside C (**1**, 109.9 mg, 0.03%).

Fraction C6 (752.2 mg) was separated by ODS column chromatography using a stepwise gradient [30% MeOH→40% MeOH→50% MeOH→60% MeOH→MeOH] to afford stellatoside D (**2**, 64.1 mg 0.01%).

Fraction B (8.6 g) was separated by silica gel column chromatography using a stepwise gradient [Silica

gel: 400 g, $CHCl_3$–MeOH–H_2O 60:10:1→55:10:1→45:10:1→40:10:1→30:10:1→20:10:1→MeOH] to afford four fractions [Fr. B1 (155.9 mg), Fr. B2 (696.4 mg), Fr. B3 (2217.6 mg), and Fr. B4 (5487.6 mg)]. Fraction B2 (696.4 mg) was separated by ODS column chromatography using a stepwise gradient [60% MeOH→70% MeOH→80% MeOH→MeOH] to give twelve fractions [Fr. B2-1 (323.1 mg), Fr. B2-2 (26.9 mg), Fr. B2-3 (21.1 mg), Fr. B2-4 (17.7 mg), Fr. B2-5 (4.4 mg), Fr. B2-6 (3.4 mg), Fr. B2-7 (13.4 mg), Fr. B2-8 (19.2 mg), Fr. B2-9 (155.2 mg), Fr. B2-10 (50.1 mg), Fr. B2-11 (3.3 mg), and Fr. B2-12 (65.9 mg)]. Fr. B2-9 (155.2 mg) was subjected to silica gel column chromatography using a stepwise gradient [Silica gel: 50 g, $CHCl_3$–MeOH–H_2O 60:10:1→MeOH] to afford stellatoside C methyl ester (**5**, 73.6 mg, 0.02%). Furthermore, fraction B4 (5487.6 mg) was separated on silica gel column chromatography using a stepwise gradient [Silica gel: 250 g, $CHCl_3$–MeOH–H_2O 55:10:1→50:10:1→ 45:10:1→40:10:1→30:10:1→20:10:1→15:10:1→MeOH] to give four fractions [B4-1 (48.3 mg), B4-2 (4228.0 mg), B4-3 (282.8 mg), and B4-4 (977.2 mg)]. Fr. B4-2 (4228.0 mg) was chromatographed on silica gel column chromatography using a stepwise gradient [Silica gel: 150 g, $CHCl_3$–MeOH–H_2O 70:10:1→60:10:1→50:10:1→40:10:1→30:10:1→MeOH] to give five fractions [B4-2-1 (49.4 mg), B4-2-2 (1389.0 mg), B4-2-3 (1860.2 mg), B4-2-4 (479.0 mg), and B4-2-5 (276.3 mg)]. Fraction B4-2-4 (479.0 mg) was separated by ODS column chromatography to afford five fractions [B4-2-4-1 (20.6 mg), B4-2-4-2 (79.2 mg), B4-2-4-3 (161.9 mg), B4-2-4-4 (129.9 mg), and B4-2-4-5 (42.0 mg)]. Fraction B4-2-4-3 (161.9 mg) was identified as stellatoside C (**1**, 161.9 mg, 0.04%).

Fraction B3 (2217.6 mg) was separated by silica gel column chromatography using a stepwise gradient [Silica gel: 80 g, $CHCl_3$–MeOH–H_2O 70:10:1→60:10:1→50:10:1→40:10:1→30:10:1→20:10:1→ MeOH] to afford three fractions [Fr. B3-1 (184.7 mg), Fr. B3-2 (1848.4 mg) and Fr. B3-3 (21.3 mg)]. Fraction B3-2 (1848.4 mg) was separated by ODS column chromatography using a stepwise gradient [60% MeOH→80% MeOH→MeOH] to give three fractions [Fr. B3-2-1 (348.7 mg), Fr. B3-2-2 (1523.0 mg) and Fr. B3-2-3 (45.8 mg)]. Fraction B3-2-2 (1523.0 mg) underwent ODS column chromatography using a stepwise gradient [60% MeOH→80% MeOH→MeOH] to give seven fractions [Fr. B3-2-2-1 (86.0 mg), Fr. B3-2-2-2 (124.3 mg), Fr. B3-2-2-3 (74.8 mg), Fr. B3-2-2-4 (184.1 mg), Fr. B3-2-2-5 (203.8 mg) Fr. B3-2-2-6 (327.9 mg), and Fr. B3-2-2-7 (385.9 mg)]. Fraction B3-2-2-3 (74.8 mg) was purified by ODS column chromatography using a stepwise gradient [60% MeOH→MeOH] to give stellatoside E (**3**, 21.9 mg, 0.005%) and stellatoside B methyl ester (**4**, 49.8 mg, 0.01%).

Fraction B3-2-2-7 (385.9 mg) was separated by ODS column chromatography using a stepwise gradient [70% MeOH→MeOH] to give four fractions [Fr. B3-2-2-7-1 (49.0 mg), Fr. B3-2-2-7-2 (8.0 mg), Fr. B3-2-2-7-3 (151.9 mg), and Fr. B3-2-2-7-4 (126.8 mg)] and thurberoside A (**6**, 71.1 mg, 0.02%).

Fraction B3-2-2-7-3 (151.9 mg) was repeatedly chromatographed on silica gel using a stepwise gradient [Silica gel: 50 g, $CHCl_3$–MeOH–H_2O 60:10:1→50:10:1→40:10:1→30:10:1→MeOH] to afford four

fractions [Fr. 3-2-2-7-3-1 (4.6 mg), Fr. B3-2-2-7-3-2 (8.7 mg), Fr. B3-2-2-7-3-3 (120.5 mg), and Fr. B3-2-2-7-3-4 (9.6 mg)]. Fraction B2-2-2-7-3-3 (120.5 mg) was subjected to ODS column chromatography using a stepwise gradient [50% MeOH→60% MeOH→65% MeOH→75% MeOH→ MeOH] to obtain phillyriside A (**7**, 20.3 mg, 0.005%).

Fraction E (6.85 g) was separated by silica gel column chromatography using a stepwise gradient [Silica gel: 240 g, $CHCl_3$–MeOH–H_2O 40:10:1→30:10:1→25:10:1→20:10:1→15:10:1→10:6:1→6:4:1→ MeOH] to give eight fractions [Fr. E1 (81.3 mg), Fr. E2 (272.4 mg), Fr. E3 (714.8 mg), Fr. E4 (1158.2 mg), Fr. E5 (882.7 mg), Fr. E6 (689.4 mg), Fr. E7 (1906.7 mg), and Fr. E8 (3.6 g)]. Fraction E3 (714.8 mg) was repeatedly chromatographed on silica gel using a stepwise gradient [Silica gel: 77 g, $CHCl_3$–MeOH–H_2O 30:10:1→25:10:1→20:10:1→MeOH] to give four fractions [Fr. E3-1 (5.6 mg), Fr. E3-2 (88.0 mg), Fr. E3-3 (429.9 mg), and Fr. B3-4 (22.0 mg)]. Fraction E3-3 (429.9 mg) was separated by ODS column chromatography using a stepwise gradient [50% MeOH→60% MeOH→MeOH] to give four fractions [Fr. E3-3-1 (92.8 mg), Fr. E3-3-2 (144.2 mg), Fr. E3-3-3 (22.8 mg), and Fr. B3-3-4 (161.4 mg)]. Fraction E3-3-1 (92.8 mg) was separated by silica gel column chromatography using a stepwise gradient [Silica gel: 25 g, $CHCl_3$–MeOH–H_2O 30:10:1→20:10:1→MeOH] to give two fractions [Fr. E3-3-1-1 (26.5 mg) and Fr. E3-3-1-2 (39.0 mg)]. Fraction E3-3-1-2 (39.0 mg) was separated by ODS column chromatography using a stepwise gradient [40% MeOH→50% MeOH→MeOH] to give treleaseside A (**8**, 13.8 mg, 0.003%).

Stellatoside C (1)

Colorless powder (279.9 mg); $[\alpha]_D^{27}$ -110.4 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3410, 2950, 1740, 1600, 1450, 1390, 1040 cm^{-1}; positive FABMS *m/z*: 941 $[M+H]^+$, 795 $[M\text{-rha}+H]^+$; negative FABMS *m/z*: 939 $[M-H]^-$, 793 $[M\text{-rha-H}]^-$; positive HRFABMS *m/z*: 941.5127 (calcd. for $C_{48}H_{77}O_{18}$ $[M+H]^+$, 941.5110); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Stellatoside D (2)

Colorless powder (64.1 mg); $[\alpha]_D^{27}$ -114.1 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3400, 2950, 1760, 1630, 1040 cm^{-1}; negative FABMS *m/z*: 955 $[M-H]^-$, 793 $[M\text{-glc-H}]^-$, 647 $[M\text{-glc-rha-H}]^-$; negative HRFABMS *m/z*: 955.4916 (calcd. for $C_{48}H_{75}O_{19}$ $[M-H]^-$, 955.4903); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Stellatoside E (3)

Colorless powder (21.9 mg); $[\alpha]_D^{22}$ -32.2 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3410, 2950, 1760, 1600, 1450, 1390, 1040 cm^{-1}; positive FABMS *m/z*: 795 $[M+H]^+$; negative FABMS *m/z*: 793 $[M-H]^-$, 647 $[M\text{-rha-H}]^-$; negative HRFABMS *m/z*: 793.4370 (calcd. for $C_{42}H_{65}O_{14}$ $[M-H]^-$, 793.4374); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Stellatoside B methyl ester (4)

Colorless powder (49.8 mg); $[\alpha]_D^{23}$ -16.1 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3410, 2950, 1750, 1640, 1450, 1360, 1040 cm^{-1}; positive FABMS *m/z*: 957 $[M+H]^+$, 825 $[M\text{-xyl}+H]^+$; negative FABMS *m/z*: 955 $[M-H]^-$, 823 $[M\text{-xyl-H}]^-$; negative HRFABMS *m/z*: 955.4915 (calcd. for $C_{48}H_{75}O_{19}$ $[M-H]^-$, 955.4903); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Stellatoside C methyl ester (5)

Colorless powder (73.6 mg); $[\alpha]_D^{27}$ -144.5 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3410, 2950, 1760, 1640, 1450, 1390, 1040 cm^{-1}; positive FABMS *m/z*: 955 $[M+H]^+$, 794 $[M\text{-glc}+H]^+$, 647 $[M\text{-glc-rha}+H]^+$; positive HRFABMS *m/z*: 955,5283 (calcd. for $C_{49}H_{79}O_{18}$ $[M+H]^+$, 955.5266); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Thurberoside A (6)

Colorless powder (71.1 mg); $[\alpha]_D^{27}$ -31.7 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3400, 2950, 1760, 1640, 1450, 1390, 1050 cm^{-1}; positive FABMS *m/z*: 923 $[M+H]^+$; negative FABMS *m/z*: 921 $[M-H]^-$, 775 $[M\text{-rha-H}]^-$; positive HRFABMS *m/z*: 923.4998 (calcd. for $C_{49}H_{79}O_{18}$ $[M+H]^+$, 923.5004); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Phillyriside A (7)

Colorless powder (20.3 mg); $[\alpha]_D^{23}$ -18.5 (*c* 1.00, MeOH); IR ν_{max} (KBr): 3410, 2950, 1740, 1600, 1450, 1400, 1040 cm^{-1}; positive FABMS *m/z*: 925 $[M+H]^+$, 779 $[M\text{-rha}+H]^+$; negative FABMS *m/z*: 923 $[M-H]^-$, 778 $[(M\text{-rha-H})^-$; positive HRFABMS *m/z*: 925.5161 (calcd. for $C_{48}H_{77}O_{17}$ $[M+H]^+$, 925.5161); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Treleaseside A (8)

Colorless powder (13.8 mg); $[\alpha]_D^{23}$ -8.7 (*c* 0.30, MeOH); IR ν_{max} (KBr): 3400, 2930, 1720, 1610, 1460, 1390, 1250, 1140, 1040 cm^{-1}; negative FABMS *m/z*: 997 $[M-H]^-$, 851 $[M\text{-rha-H}]^-$; negative HRFABMS *m/z*: 997.5016 (calcd. for $C_{50}H_{77}O_{20}$ $[M-H]^-$, 997.5008); ^{1}H- and ^{13}C-NMR, see Tables 1 and 2.

Acidic hydrolysis of 1

1 (20 mg) was hydrolyzed with 5 mL of 3.5 % HCl and heated at 110 ^oC for 3 h. The reaction mixture was neutralized with 1M NaOH, extracted with $CHCl_3$. $CHCl_3$ extract was chromatographed on silica gel column chromatography ($CHCl_3$:MeOH = 100:1), to afford aglycon of **1** (7.6 mg). The aglycon was identified as stellatogenin by TLC with authentic sample and comparison with ^{1}H- and ^{13}C-NMR.[13,26]

Acidic hydrolysis of 6, 7

6, 7 (8 mg) were hydrolyzed and separated with similar way as in the case of **1**, to afford thurberogenin[25] (2.3 mg) and 27-desoxyphillyrigenin[16] (2.8 mg). These aglycons were identified by TLC with authentic samples and comparison with ^{1}H- and ^{13}C-NMR, respectively.[16,25]

Determination of sugar configuration

Saponins (**1**-**8**) (each 2 mg) were hydrolyzed by 3.5% HCl (0.4 mL) at 110 °C for 2.5 h. The reaction mixture was neutralized with 0.5 M NaOH. After drying under vacuum, the residue was dissolved in 0.4 mL pyridine containing 2 mg of L-cysteine methyl ester hydrochloride and heated at 60 °C for 1 h. *O*-Tolylisothiocyanate (2 μL) was then added and the mixture was heated at 60 °C for 1 h. The reaction mixture was directly analyzed by reversed-phase HPLC using a SSC-3461 HPLC Pump with a SSC-5410 UV/VIS detector (Senshu Scientific Co., Ltd.). A Senshu Pak PEGASIL 4.6 ϕ × 250 mm HPLC column was used (temp, 35 °C; flow, 0.8 mL/min; eluate, MeCN-H_2O 25:75 containing 50 mM H_3PO_4). The HPLC column was washed with MeOH after each injection. The reaction conditions for D, L-glucose, D-glucuronic acid, D, L-xylose and L-rhamnose were the same as described above. In the case of the L-glucuronic acid and D-rhamnose, D-glucuronic acid or L-rhamnose dissolved in 0.4 mL pyridine containing 2 mg D-cysteine methyl ester hydrochloride was used for the reaction, respectively. The derivative was used for determining the retention time instead of L-glucuronic acid, D-rhamnose. The retention times for the authentic sugar derivatives, D-glucose (19.49), L-glucose (17.98), D-xylose (22.66), L-xylose (21.10), D-glucuronic acid (20.34), L-glucuronic acid (derived from D-glucuronic acid and D-cysteine methyl ester, 19.51), L-rhamnose (33.10) and D-rhamnose (derived from L-rhamnose and D-cysteine methyl ester, 17.69) were used for comparison with the retention times from the reaction mixtures for each saponin.[24] The peaks at 20.28 and 33.09 of the sugar derivatives from **8** coincided with derivatives of D-glucuronic acid and L-rhamnose, respectively.[21] For the other saponins (**1**-**7**), the same results as for compound **8** were obtained.

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25. ^{1}H-NMR data of thurberogenin in C_5D_5N δ ppm; 0.83 (3H, s, H_3-25), 0.98 (3H, s, H_3-27), 1.03 (6H, s, H_3-24, H_3-26), 1.23 (3H, s, H_3-23), 1.80 (3H, s, H_3-30), 3.45 (1H, br t, J = 8.1 Hz, H-3), 4.59 (1H. d, J = 1.9 Hz, H-21), 4.91, 4.97 (each 1H, d, J = 1.1 Hz, H_2-29).
^{13}C-NMR data of thurberogenin in C_5D_5N δ ppm; 39.1 (C-1), 28.2 (C-2), 78.0 (C-3), 39.4 (C-4), 55.8 (C-5), 18.6 (C-6), 34.5 (C-7), 40.9 (C-8), 50.6 (C-9), 37.4 (C-10), 20.8 (C-11), 26.9* (C-12),

40.4 (C-13), 42.5 (C-14), 26.5* (C-15), 23.7 (C-16), 51.5 (C-17), 44.9 (C-18), 53.1 (C-19), 144.2 (C-20), 81.7 (C-21), 44.4 (C-22), 28.6 (C-23), 16.0 (C-24), 16.3 (C-25), 16.3(C-26), 14.0 (C-27), 178.2 (C-28), 112.5 (C-29), 23.3 (C-30), (*: may be interchanged).

26. ^{1}H-NMR data of stellatogenin in C_5D_5N δ ppm; 3.43 (1H, brt, J = 8.0 Hz, H-3), 5.01 (1H. brs, H-21), 1.21 (3H, s, H_3-23), 1.01 (3H, s, H_3-24), 0.83 (3H, s, H_3-25), 1.08 (3H, s, H_3-26), 0.95 (3H, s, H_3-27), 1.48 (3H, s, H_3-29), 1.39 (3H, s, H_3-30).