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Stachyssaponins I—VIII, New Oleanane-Type Triterpene Saponins from *Stachys riederi* CHAMISSO

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Water extracts from the whole plant of *Stachys riederi* gave eight new bisdesmosides of echinocystic acid. The structures of these compounds were elucidated by chemical and spectroscopic evidence.

Keywords *Stachys riederi*; stachyssaponin; Labiatae; oleanane-type triterpene saponin; echinocystic acid bisdesmoside

Phytochemical investigations of *Stachys* species (Labiatae) have shown the presence of flavonoids,[1] iridoids,[2-4] diterpenoids[5] and phenylethanoid glycosides.[2,4,6,7] We also reported structures of phenylethanoid glycosides, a lignan diglycoside and a 1-octen-3-ol triglycoside from the whole plant of *S. riederi* CHAMISSO.[8] In this paper, we report the isolation and structure elucidation of eight new oleanane-type triterpene glycosides from the water extract of the whole plant of *S. riederi* CHAMISSO.

The water extract of the whole plant was passed through a Mitsubishi Diaion HP-20 column and the adsorbed materials were eluted with 60% methanol aq. and methanol, successively. The methanol eluate was chromatographed on silica gel, ODS and a phenyl alkyl (PhA) column to give **1**—**8**.

Stachyssaponin I (**1**) revealed a pseudo molecular ion peak $[M+Na]^+$ at m/z 921 in the FAB-MS, and elemental analysis data was consistent with $C_{46}H_{74}O_{17}\cdot 3/2H_2O$. On acid hydrolysis, **1** afforded echinocystic acid[9] as an aglycone moiety and D-glucose and L-arabinose in the ratio of 1:2 as a sugar moiety. Sugar proton signals in the ^{1}H-NMR spectrum were assigned by detailed proton spin decoupling experiments starting from irradiation at each anomeric proton signal (Table II). Nuclear Overhauser effect (NOE)s were observed at H-3 [δ 3.34 (dd, $J=12.5$, 4 Hz)] of the aglycone moiety, on irradiating at H-1 [δ 4.86 (d, $J=8$ Hz)] of the glucose moiety and at H_2-6 [δ 4.27 (dd, $J=11.5$, 5 Hz), 4.84 (br d, $J=11.5$ Hz)] of the glucose moiety on irradiating at H-1 [δ 4.96 (d, $J=6.5$ Hz)] of the arabinose moiety in the difference NOE spectrum. Also, H-1 of another arabinose moiety was observed downfield [δ 6.33 (d, $J=6$ Hz)], suggesting that this arabinose moiety is attached to C-28 of the echinocystic acid moiety. The anomeric configurations of glucose and arabinose moieties were determined to be β and α, respectively, from the J values of their anomeric proton signals. From the above mentioned results, the structure of stachyssaponin I was elucidated to be 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-α-L-arabinopyranosyl ester. The glycosylation shifts in the ^{13}C-NMR spectrum also supported this structure.

Stachyssaponins II (**2**), $C_{52}H_{84}O_{21}\cdot H_2O$ and III (**3**), $C_{52}H_{84}O_{21}\cdot 3H_2O$ showed four anomeric proton signals in the ^{1}H-NMR spectra. On acid hydrolysis, **2** gave D-glucose, L-arabinose, L-rhamnose (1:2:1) and **3** gave D-glucose, L-arabinose, D-xylose, L-rhamnose (1:1:1:1) as a sugar moiety in addition to echinocystic acid as an aglycone moiety. Sugar proton signals were assigned by spin decoupling experiments as shown in Table II. All carbon signals observed for **2** were assigned from heteronuclear multiple bond connectivity (HMBC) spectrum and heteronuclear multiple quantum coherence (HMQC) spectrum, and those of **3** from ^{13}C–^{1}H correlation spectroscopy (COSY) spectrum as shown in Table I. By comparison of ^{1}H- and ^{13}C- NMR chemical shifts of **2** with those of **1**, **1** and **2** proved to be similar to each other in aglycone moiety and 3-*O*-sugar moiety. When the signal at δ 5.74 (H-1 of rhamnose moiety) was irradiated, an NOE was observed at the signal [δ 4.54 (t, $J=3$ Hz)], which was assigned to H-2 of the ester-linked arabinose moiety from its coupling pattern in the difference NOE spectrum of **2**. On the other hand, ^{1}H- and ^{13}C-NMR chemical shifts of **3** were similar to those of **2**, except for a terminal xylose moiety. NOEs were observed at H_2-6 [δ 4.33 (dd, $J=11$, 5 Hz), 4.85 (br d, $J=11$ Hz)] of the glucose moiety on irradiation at H-1 [δ 5.01 (d, $J=7$ Hz)] of the xylose moiety in **3**. These results supported that a xylose moiety is attached to C-6 of the glucose moiety. Considering coupling constants and the chemical shifts of each anomeric signal in the NMR spectra,[10] the structures of stachyssaponins II and III were characterized as 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester and 3-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, respectively.

The FAB-MS and elemental analysis of stachyssaponin IV (**4**) gave the molecular formula $C_{57}H_{92}O_{25}\cdot 3/2H_2O$. Compound **4** showed the presence of five anomeric proton and carbon signals [δ 4.84 (d, $J=8$ Hz), 4.94 (d, $J=7$ Hz), 5.15 (d, $J=7.5$ Hz), 5.76 (s), 6.44 (d, $J=2.5$ Hz): 93.7, 101.1, 105.3, 106.9, 107.0] in the NMR spectra. All carbon signals were assigned from HMBC and HMQC spectra after assignment of most proton signals by detailed spin decoupling experiments. The ^{1}H- and ^{13}C-NMR chemical shifts of **4** were similar to those of **2** except for the signals due to the terminal xylose moiety. For the purpose of investigating the binding site of five monosaccharides, we employed a difference NOE. When the signals at δ 5.76 (H-1 of the rhamnose moiety), 5.15 (H-1 of the xylose moiety), 4.94 (H-1 of the arabinose moiety) and 4.84 (H-1 of the glucose moiety) were irradiated, NOEs were observed at signals at δ 4.54 (t, $J=2.5$ Hz, H-2 of

TABLE I. ^{13}C-NMR Chemical Shifts of Sugar Moieties of Compounds **1**—**8** in Pyridine-d_5 at 35 °C

Carbon No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Sugar moiety at C-3								
Glucose								
1	107.0	107.0	107.0	107.0	107.0	107.0	106.9	107.0
2	75.7	75.7	75.7	75.7	75.6	75.7	75.7	75.7
3	78.7	78.7	78.7	78.7	78.7	78.7	78.7	78.7
4	72.1	72.1	72.4	72.1	72.1	72.1	72.1	71.9
5	76.7	76.7	76.9	76.7	76.7	76.7	76.7	76.9
6	70.0	70.0	70.1	70.0	70.0	70.0	70.0	70.3
Arabinose								
1	105.3	105.3		105.3	105.3	105.3	105.3	
2	72.3	72.3		72.3	72.3	72.3	72.3	
3	74.2	74.3		74.3	74.3	74.3	74.3	
4	69.0	69.0		69.0	69.0	69.0	69.0	
5	66.3	66.3		66.3	66.3	66.3	66.3	
Xylose								
1			105.8					105.8
2			75.4					75.1
3			78.2					78.1
4			71.2					71.2
5			67.1					67.1
Sugar moiety at C-28								
Arabinose								
1	95.9	93.6	93.6	93.7	93.7	93.7	93.7	93.7
2	71.4	75.4	74.9	75.1	75.0	75.0	75.5	74.9
3	73.7	70.5	70.5	70.3	70.7	70.4	69.8	70.3
4	67.6	66.1	66.1	66.3	66.5	66.3	66.1	66.2
5	65.7	63.0	63.0	63.3	63.6	63.3	63.2	63.3
Rhamnose								
1		101.5	101.5	101.1	101.1	101.1	101.1	101.1
2		72.4	71.9	72.0	72.0	72.0	71.2	72.0
3		72.6	72.6	72.7	72.6	72.7	82.8	72.7
4		73.9	73.9	83.7	83.0	83.3	78.3	83.7
5		70.1	69.0	68.6	68.5	68.4	68.8	68.6
6		18.4	18.6	18.4	18.4	18.4	18.6	18.4
Xylose								
1				106.9	106.3	106.4	105.3	106.9
2				76.1	73.7	76.0	75.7	76.1
3				78.6	79.3	75.2	79.0	78.6
4				71.1	69.0	72.9	71.3	71.1
5				67.5	67.1	63.5	67.3	67.5
Ac					21.1	20.8		
					170.7	170.5		
Glucose								
1							105.1	
2							75.5	
3							78.4	
4							71.7	
5							78.1	
6							62.7	

Recorded at 125.65 MHz.

ester-linked arabinose moiety), 4.36 (t, $J=9$ Hz, H-4 of rhamnose moiety), 4.25/4.82 (dd, $J=11$, 5 Hz/br d, 11 Hz, H_2-6 of glucose moiety) and 3.32 (dd, $J=12$, 4 Hz, H-3 of aglycone moiety), respectively. Consequently, the structure of stachyssaponin IV was elucidated to be 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.

In the ^{1}H-NMR spectra of stachyssaponins V (**5**), $C_{59}H_{94}O_{26}\cdot 13/2H_2O$ and VI (**6**), $C_{59}H_{94}O_{26}\cdot 4H_2O$, each five anomeric proton signals (δ 4.86, 4.95, 5.20, 5.81, 6.39; 4.85, 4.95, 5.19, 5.76, 6.44, respectively) and an acetyl signal (δ 1.93; 1.90, respectively) were observed. These anomeric signals were similar to those of **4** in their coupling constants and chemical shifts. On acid hydrolysis, both compounds afforded echinocystic acid and sugar in the same ratio as in the case of **4**. By comparing the ^{13}C-NMR chemical shifts of **5** and **6** with those of **4**, we assumed that **5** or **6** was a monoacetate of **4**. Most proton signals were assigned by spin decoupling experiments. Proton signals assigned to H-3 in **5** and H-4 in **6** of the xylose moiety were shifted downfield at δ 5.64 (t, $J=9.5$ Hz) and 5.29 (overlapping with other signals), respectively, suggesting that an acetyl group is attached to C-3 and C-4 of the xylose moiety in **5** and **6**, respectively. Acylation shifts in the ^{13}C-NMR spectrum also support these results.[11] From the results of NOE experiments of **5** and **6**, it was clarified in **5** and **6** that the rhamnose moiety is attached to C-2 of the ester-linked arabinose moiety, the xylose moiety is attached to C-4 of the rhamnose moiety, the glucose moiety is attached to C-3 of the aglycone moiety and the arabinose moiety is attached to C-6 of the glucose moiety. From this evidence, the structures of stachyssaponins V and VI were characterized as 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-L-glucopyranosyl] echinocystic acid 28-*O*-[β-D-3-*O*-acetyl-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester and 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-[β-D-4-*O*-acetyl-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester, respectively. The glycosylation shifts in the ^{13}C-NMR spectra also supported these

Table II. ^{1}H-NMR Chemical Shifts of Compounds **1**—**8** in Pyridine-d_5 at 35 °C

Proton No.	**1**	**2**	**3**	**4**
Aglycon moiety				
3	3.34 (dd, J=12.5, 4 Hz)	3.30 (dd, J=11.5, 4 Hz)	3.34 (dd, J=11.5, 4 Hz)	3.32 (dd, J=12, 4 Hz)
5	0.78 (br d, J=11.5 Hz)	0.74 (br d, J=11.5 Hz)	0.75 (br d, J=11.5 Hz)	0.76 (br d, J=12 Hz)
12	5.60 (t-like, J=3 Hz)	5.57 (t-like, J=3 Hz)	5.58$^{a)}$	5.59 (t-like, J=3 Hz)
16	5.24 (br s)	5.22 (br s)	5.25 (br s)	5.25 (br s)
18	3.58 (dd, J=14, 4 Hz)	3.55 (dd, J=14, 4.5 Hz)	3.58 (dd, J=15, 4 Hz)	3.56 (br dd, J=14, 4 Hz)
19α	2.78 (t, J=14 Hz)	2.75 (t, J=13.5 Hz)	2.78 (t, J=15 Hz)	2.77 (t, J=14 Hz)
23	1.25 (s)	1.20 (s)	1.22 (s)	1.24 (s)
24	0.90 (s)	0.86 (s)	0.88 (s)	0.88 (s)
25	0.99 (s)	0.94 (s)	0.97 (s)	0.98 (s)
26	1.06 (s)	1.04 (s)	1.06 (s)	1.08 (s)
27	1.79 (s)	1.75 (s)	1.77 (s)	1.77 (s)
29	1.04 (s)	1.01 (s)	1.05 (s)	1.04 (s)
30	1.15 (s)	1.13 (s)	1.16 (s)	1.15 (s)
Sugar moiety at C-3				
Glucose				
1	4.86 (d, J=8 Hz)	4.82 (d, J=8 Hz)	4.87 (d, J=8 Hz),	4.84 (d, J=8 Hz)
2	3.98 (t, J=8 Hz)	3.94 (t, J=8 Hz)	3.97 (t, J=8.5 Hz)	3.96 (t, J=8.5 Hz)
3	4.18$^{a)}$	4.15 (t, J=8 Hz)	4.16$^{a)}$	4.17$^{a)}$
4	4.09$^{a)}$	4.09$^{a)}$	4.09$^{a)}$	4.09$^{a)}$
5	4.09$^{a)}$	4.09$^{a)}$	4.09$^{a)}$	4.09$^{a)}$
6	4.27 (dd, J=11.5, 5 Hz)	4.23 (dd, J=11, 5 Hz)	4.33 (dd, J=11, 5 Hz)	4.25 (dd, J=11, 5 Hz)
	4.84 (br d, J=11.5 Hz)	4.80 (br d, J=11 Hz)	4.85 (br d, J=11 Hz)	4.82 (br d, J=11 Hz)
Arabinose				
1	4.96 (d, J=6.5 Hz)	4.92 (d, J=6.5 Hz)		4.94 (d, J=7 Hz)
2	4.46 (t, J=7 Hz)	4.42 (t, J=6.5 Hz)		4.44 (dd, J=8, 7 Hz)
3	4.18$^{a)}$	4.14$^{a)}$		4.16 (dd, J=8, 4.5 Hz)
4	4.32$^{a)}$	4.28$^{a)}$		4.31$^{a)}$
5	3.77 (d, J=11 Hz)	3.73 (d, J=10 Hz)		3.76 (d, J=11 Hz)
	4.30$^{a)}$	4.25$^{a)}$		4.29$^{a)}$
Xylose				
1			5.01 (d, J=7 Hz)	
2			4.02 (t, J=7.5 Hz)	
3			4.12$^{a)}$	
4			4.19$^{a)}$	
5			3.68 (t, J=11 Hz)	
			4.32$^{a)}$	
Sugar moiety at C-28				
Arabinose				
1	6.33 (d, J=6 Hz)	6.48 (d, J=3 Hz)	6.51 (d, J=1.5 Hz)	6.44 (d, J=2.5 Hz)
2	4.56 (t, J=6 Hz)	4.54$^{a)}$	4.57$^{a)}$	4.54$^{a)}$
3	4.39 (dd, J=6, 3 Hz)	4.53 (t, J=3.5 Hz)	4.56$^{a)}$	4.50$^{a)}$
4	4.49$^{a)}$	4.42$^{a)}$	4.42$^{a)}$	4.38$^{a)}$
5	3.93 (dd, J=11, 3 Hz)	3.90 (dd, J=11, 4.5 Hz)	3.93 (dd, J=11, 4 Hz)	3.93 (dd, J=11, 3.5 Hz)
	4.43$^{a)}$	4.48$^{a)}$	4.51$^{a)}$	4.49$^{a)}$
Rhamnose				
1		5.74 (s)	5.78 (s)	5.76 (s)
2		4.54$^{a)}$	4.57$^{a)}$	4.54$^{a)}$
3		4.45 (dd, J=9.5, 3 Hz)	4.49 (dd, J=10, 3 Hz)	4.56 (dd, J=9, 3 Hz)
4		4.21 (t, J=9.5 Hz)	4.25 (t, J=9.5 Hz)	4.36 (t, J=9 Hz)
5		4.41$^{a)}$	4.44$^{a)}$	4.38$^{a)}$
6		1.65 (d, J=6.5 Hz)	1.68 (d, J=6.5 Hz)	1.73 (d, J=6 Hz)
Xylose				
1				5.15 (d, J=7.5 Hz)
2				4.00 (t, J=7.5 Hz)
3				4.05 (t, J=8 Hz)
4				4.12$^{a)}$
5				3.47 (t, J=10.5 Hz)
				4.19 (dd, J=10.5, 5 Hz)

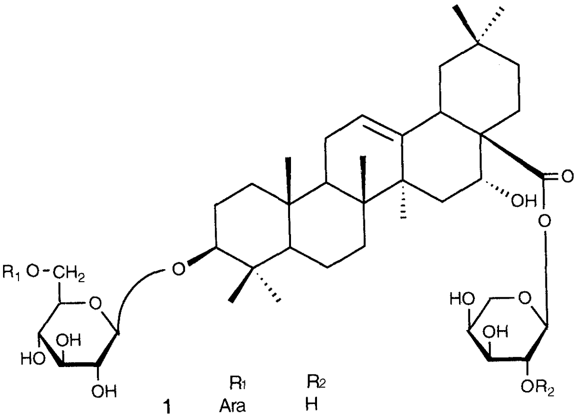
Table II. (continued)

Proton No.	**5**	**6**	**7**	**8**
Aglycon moiety				
3	3.33 (dd, *J*=11.5, 4 Hz)	3.32 (dd, *J*=11.5, 4 Hz)	3.33 (dd, *J*=11.5, 4 Hz)	3.33 (dd, *J*=11.5, 4 Hz)
5	0.76 (br d, *J*=11.5 Hz)	0.77 (d, *J*=11.5 Hz)	0.78 (d, *J*=12 Hz)	0.75 (d, *J*=13 Hz)
12	5.59a)	5.59 (t-like, *J*=3 Hz)	5.59 (t-like, *J*=3 Hz)	5.58 (t-like, *J*=4 Hz)
16	5.25 (br s)	5.24 (br s)	5.22 (br s)	5.25 (br s)
18	3.55 (dd, *J*=15, 5 Hz)	3.56 (dd, *J*=13.5, 4.5 Hz)	3.56 (dd, *J*=14, 4 Hz)	3.56 (dd, *J*=15, 4 Hz)
19α	2.77 (t, *J*=14.5 Hz)	2.78 (t, *J*=13.5 Hz)	2.77 (t, *J*=13.5 Hz)	2.78 (t, *J*=14.5 Hz)
23	1.26 (s)	1.25 (s)	1.25 (s)	1.23 (s)
24	0.90 (s)	0.89 (s)	0.90 (s)	0.87 (s)
25	1.00 (s)	0.99 (s)	0.99 (s)	0.98 (s)
26	1.08 (s)	1.07 (s)	1.07 (s)	1.07 (s)
27	1.77 (s)	1.77 (s)	1.78 (s)	1.77 (s)
29	1.04 (s)	1.04 (s)	1.04 (s)	1.04 (s)
30	1.14 (s)	1.15 (s)	1.16 (s)	1.15 (s)
Sugar moiety at C-3				
Glucose				
1	4.86a)	4.85 (d, *J*=7.5 Hz)	4.85 (d, *J*=8 Hz)	4.85 (d, *J*=8.5 Hz)
2	3.97 (t, *J*=10 Hz)	3.96 (t, *J*=7.5 Hz)	3.98 (t, *J*=8.5 Hz)	3.97 (t, *J*=9 Hz)
3	4.17a)	4.16a)	4.16a)	4.18a)
4	4.10a)	4.09a)	4.10a)	4.09a)
5	4.10a)	4.09a)	4.10a)	4.09a)
6	4.26 (dd, *J*=11.5, 5 Hz)	4.26a)	4.27a)	4.33 (dd, *J*=11.5, 5.5 Hz)
	4.83a)	4.83 (br d, *J*=11 Hz)	4.83 (br d, *J*=10 Hz)	4.84 (br d, *J*=11.5 Hz)
Arabinose				
1	4.95 (d, *J*=7 Hz)	4.95 (d, *J*=7 Hz)	4.96 (d, *J*=6.5 Hz)	
2	4.45 (t, *J*=7.5 Hz)	4.44 (t, *J*=6.5 Hz)	4.45a)	
3	4.17a)	4.17a)	4.18a)	
4	4.32a)	4.31a)	4.32 (br s)	
5	3.76 (d, *J*=11.5 Hz)	3.76 (d, *J*=10 Hz)	3.76 (d, *J*=11 Hz)	
	4.30a)	4.30a)	4.29a)	
Xylose				
1				5.00 (d, *J*=7 Hz)
2				4.01 (t, *J*=7 Hz)
3				4.16a)
4				4.29a)
5				3.68 (t, *J*=11 Hz)
				4.31a)
Sugar moiety at C-28				
Arabinose				
1	6.39 (d, *J*=3.5 Hz)	6.44 (br d, *J*=1.5 Hz)	6.42 (br s)	6.44 (d, *J*=2.5 Hz)
2	4.54a)	4.54a)	4.47a)	4.55a)
3	4.52a)	4.50a)	4.47a)	4.50a)
4	4.35a)	4.37a)	4.42a)	4.39a)
5	3.92 (dd, *J*=11.5, 4 Hz)	3.93 (dd, *J*=12, 4 Hz)	3.92 (dd, *J*=11, 3.5 Hz)	3.93 (dd, *J*=11.5, 4 Hz)
	4.49a)	4.49a)	4.50a)	4.49a)
Rhamnose				
1	5.81 (s)	5.76 (s)	5.62 (br s)	5.76 (s)
2	4.54a)	4.54a)	4.88 (m)	4.55a)
3	4.52a)	4.57a)	4.65 (dd, *J*=9, 3 Hz)	4.58 (dd, *J*=9, 2.5 Hz)
4	4.37a)	4.35a)	4.48a)	4.36 (t, *J*=9 Hz)
5	4.47a)	4.39a)	4.42a)	4.38a)
6	1.71 (d, *J*=5 Hz)	1.70 (d, *J*=6 Hz)	1.73 (d, *J*=6 Hz)	1.74 (d, *J*=6 Hz)
Xylose				
1	5.20 (d, *J*=8.5 Hz)	5.19 (d, *J*=7 Hz)	5.42 (d, *J*=8 Hz)	5.15 (d, *J*=8.5 Hz)
2	4.01 (dd, *J*=9.5, 8.5 Hz)	4.01 (t, *J*=7.5 Hz)	3.95a)	4.00 (t, *J*=8.5 Hz)
3	5.64 (t, *J*=9.5 Hz)	4.16a)	4.10a)	4.05 (t, *J*=9 Hz)
4	4.15a)	5.29a)	4.14a)	4.12a)
5	3.44 (t, *J*=10 Hz)	3.32 (t, *J*=9.5 Hz)	3.44 (t, *J*=10 Hz)	3.48 (t, *J*=11.5 Hz)
	4.19a)	4.20a)	4.18a)	4.20a)
Ac	1.93 (s)	1.90 (s)		
Glucose				
1			5.21 (d, *J*=8 Hz)	
2			3.99 (t, *J*=8.5 Hz)	
3			4.13a)	
4			4.14a)	
5			3.78 (m)	
6			4.21 (dd, *J*=12, 5.5 Hz)	
			4.39 (dd, *J*=12, 2 Hz)	

Recorded at 500 MHz. *a*) Overlapping with other signals.

structures. An acyl group in the sugar moiety can migrate easily, therefore, we cannot deny the possibility that the structures are artifactual.

The ^{1}H-NMR spectrum of stachyssaponin VII (**7**), $C_{63}H_{102}O_{30}\cdot 5H_2O$, showed six anomeric proton signals at δ 4.85 (d, $J=8$ Hz), 4.96 (d, $J=6.5$ Hz), 5.21 (d, $J=8$ Hz), 5.42 (d, $J=8$ Hz), 5.62 (br s) and 6.42 (br s). Proton signals of the sugar moiety and all carbon signals were assigned by spin decoupling experiments and ^{13}C–^{1}H COSY spectrum, respectively. Detailed comparisons of the ^{1}H- and ^{13}C-NMR spectra of **7** with those of **4** have shown that those of **7** have signals due to a terminal glucose moiety. NOEs were observed at H-2 [δ 4.47 (br s)] of the ester-linked arabinose moiety on irradiation at H-1 (δ 5.62) of the rhamnose moiety, at H-3 [δ 4.65 (dd, $J=9$, 3 Hz)] of the rhamnose moiety on irradiation at H-1 (δ 5.21) of the terminal glucose moiety, and at H-4 [δ 4.48 (t, $J=9$ Hz)] of the rhamnose moiety on irradiation at H-1 (δ 5.42) of the xylose moiety. Therefore, the structure of stachyssaponin VII was determined to be 3-*O*-[α-L-arabinopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-{β-D-glucopyranosyl-(1→3)-[β-D-xylopyranosyl-(1→4)]-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl} ester.



	R₁	R₂
1	Ara	H
2	Ara	Rham
3	Xyl	Rham
4	Ara	Rham –4– Xyl
5	Ara	Rham –4– Xyl –3– Ac
6	Ara	Rham –4– Xyl –4– Ac
7	Ara	Rham –4– Xyl I 3 Glc
8	Xyl	Rham –4– Xyl

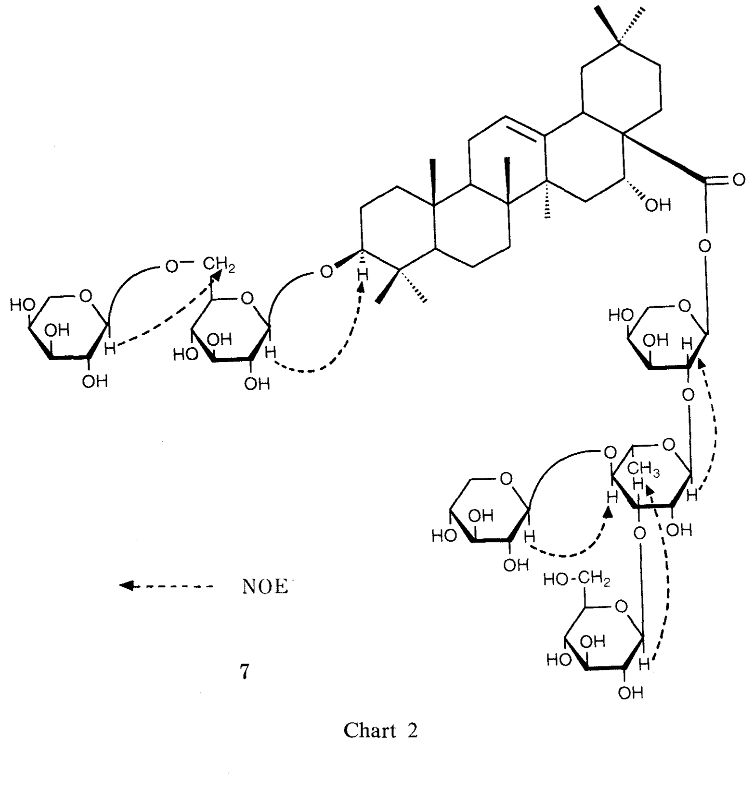
Chart 1

The ^{1}H- and ^{13}C-NMR spectra of stachyssaponin VIII (**8**), $C_{57}H_{92}O_{25}\cdot 2H_2O$, were similar to those of **4** except for a terminal xylose moiety. Acid hydrolysis liberated D-glucose, L-arabinose, D-xylose, and L-rhamnose (1 : 1 : 2 : 1) as a sugar moiety. Sugar proton signals were assigned by spin decoupling experiments. NOEs were observed at H_2-6 [δ 4.33 (dd, $J=11.5$, 5.5 Hz), 4.84 (br d, $J=11.5$ Hz)] of the glucose moiety, denoting that the xylose moiety is linked to C-6 of the glucose moiety. Consequently, the structure of stachyssaponin VIII could be represented as 3-*O*-[β-D-xylopyranosyl-(1→6)-β-D-glucopyranosyl] echinocystic acid 28-*O*-[β-D-xylopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl] ester.

This is the first report on the isolation of saponins from *stachys* species (Labiatae).

Experimental

General $[\alpha]_D$ were measured with a JASCO DIP-360 digital polarimeter, and FAB-MS were measured on a JEOL JMS-SX102 mass spectrometer. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GSX-500. Chemical shifts are given on the δ scale with tetramethylsilane as an internal standard. HPLC was done on a JASCO model 800



7

Chart 2

instrument. Gas chromatography (GC) was run on a Hitachi G-3000 gas chromatagraph.

Extraction and Isolation The powdered whole plant of *S. riederi* CHAMISSO (2 kg), collected in Fujinomiya, Shizuoka, Japan in July, 1992, were extracted twice with hot H_2O. The H_2O extract was passed through a Mitsubishi Diaion HP-20 column (9 cm × 40 cm). After the content of the column was washed with H_2O, the adsorbed materials were eluted with 60% MeOH aq. and MeOH, successively. The MeOH eluate (16 g) was chromatographed on a silica gel column (300 g) using $CHCl_3$–MeOH–EtOAc–H_2O (45 : 15 : 38 : 2), increasing a portion of MeOH to give eight frac tions (frs. 1—8). From frs. 5 and 6 (2.1 g), compounds **1**—**8** were isolated by preparative HPLC [Develosil Lop-ODS 50 mm × 50 cm × 2, MeCN–H_2O (1 : 4–7 : 13) linear gradient]. **1** (19 mg), **2** (38 mg), **3** (8 mg), **4** (72 mg), **5** (7 mg), **6** (32 mg), **7** (20 mg) and **8** (13 mg).

Stachyssaponin I (1) Amorphous powder. $[\alpha]_D^{26}$ −8.1 (c=0.74, MeOH). *Anal.* Calcd for $C_{46}H_{74}O_{17} \cdot 3/2H_2O$: C, 57.97; H, 8.46. Found: C, 58.02; H, 8.60. FAB-MS m/z: 921 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR δ: aglycone moiety: 15.8 (C-24), 17.1 (C-25), 17.6 (C-26), 18.6 (C-6), 23.9 (C-11), 24.9 (C-30), 26.8 (C-2), 27.3 (C-27), 28.3 (C-23), 31.0 (C-20), 32.1 (C-22), 33.3 (C-29), 33.5 (C-7), 36.0 (C-21), 36.2 (C-15), 37.1 (C-10), 38.9 (C-1), 39.6 (C-4), 40.1 (C-8), 41.3 (C-18), 42.1 (C-14), 47.2 (C-9, C-19), 49.5 (C-17), 56.0 (C-5), 74.1 (C-16), 89.1 (C-3), 122.9 (C-12), 144.4 (C-13), 176.2 (C-28). ^{13}C-NMR data of aglycone moiety of **2**—**8** are neary the same with this data; sugar moiety: shown in Table I.

Stachyssaponin II (2) Amorphous powder. $[\alpha]_D^{24}$ −38.2° (c=1.40, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{21} \cdot H_2O$: C, 58.25; H, 8.18. Found: C, 58.28; H, 8.49. FAB-MS m/z: 1068 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin III (3) Amorphous powder. $[\alpha]_D^{24}$ −15.6° (c=0.48, MeOH). *Anal.* Calcd for $C_{52}H_{84}O_{21} \cdot 3H_2O$: C, 55.90; H, 8.11. Found: C, 55.86; H, 8.04. FAB-MS m/z: 1068 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin IV (4) Amorphous powder. $[\alpha]_D^{24}$ −41.3° (c=1.50, MeOH). *Anal.* Calcd for $C_{57}H_{92}O_{25} \cdot 3/2H_2O$: C, 56.84; H, 7.95. Found: C, 56.67; H, 8.14. FAB-MS m/z: 1199 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin V (5) Amorphous powder. $[\alpha]_D^{23}$ −60.0° (c=0.70, MeOH). *Anal.* Calcd for $C_{59}H_{94}O_{26} \cdot 13/2H_2O$: C, 53.02; H, 8.07; Found: C, 52.85; H, 7.65. FAB-MS m/z: 1242 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin VI (6) Amorphous powder. $[\alpha]_D^{26}$ −46.3° (c=1.23, MeOH). *Anal.* Calcd for $C_{59}H_{94}O_{26} \cdot 4H_2O$: C, 54.87; H, 8.43. Found: C, 54.61; H, 8.23. FAB-MS m/z: 1242 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin VII (7) Amorphous powder. $[\alpha]_D^{23}$ −58.3° (c=1.60, MeOH). *Anal.* Calcd for $C_{63}H_{102}O_{30} \cdot 5H_2O$: C, 52.93; H, 7.90. Found: C, 52.99; H, 7.95. FAB-MS m/z: 1362 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Stachyssaponin VIII (8) Amorphous powder. $[\alpha]_D^{24}$ −33.2° (c=0.98, MeOH). *Anal.* Calcd for $C_{57}H_{92}O_{25} \cdot 2H_2O$: C, 56.42; H, 7.97. Found: C, 56.32; H, 8.06. FAB-MS m/z: 1199 $[M+Na]^+$. ^1H-NMR: shown in Table II. ^{13}C-NMR: sugar moiety: shown in Table I.

Acid Hydrolysis of 1 A solution of **1** (10 mg) in 5% H_2SO_4 aq. (0.5 ml) and dioxane (0.5 ml) was heated in a boiling water bath for 1 h. The reaction mixture was diluted with H_2O and was passed through a Diaion HP-20 column (1.5 cm × 5 cm). After the content of the column was washed with H_2O, the adsorbed materials were eluted with MeOH. The MeOH eluate was chromatographed to give echinocystic acid (3 mg) (conditions: column; Develosil ODS 20 mm × 25 cm, flow rate; 6.5 ml/min, solvent; 60% MeCN aq. +0.05% trifluoroacetic acid). Colorless needles, mp 300—303 °C (from MeOH). $[\alpha]_D^{24}$ + 40.3° (c= 0.36, MeOH). The melting point, $[\alpha]_D$ and ^1H-NMR data were the same as previously reported.[9]

Acid Hydrolysis of 1—8 A solution of each glycoside (1 mg) in 5% H_2SO_4 aq. (3 drops) and dioxane (3 drops) was heated in a boiling water bath for 1 h. The reaction mixture was diluted with H_2O and extracted with EtOAc twice. The EtOAc layer was concentrated to dryness and was subjected to HPLC (conditions: column; YMC R-ODS-7 4.6 mm × 25 cm, flow rate; 1.3 ml/min, solvent; 80% MeOH aq., detection; 205 nm). As a result, aglycone moieties of **1**—**8** were shown to be echinocystic acid, t_R; 9.4 min. The H_2O layer was passed through an Amberlite IRA-60E column and the eluate was concentrated. The residue was dissolved in pyridine (0.2 ml). After addition of D-cysteine hydrochloride (0.5 mg), the mixture was warmed at 60 °C for 1 h. The solvent was blown off under an air stream and the residue was trimethylsilylated and checked by GC.[12] The GC conditions for the determination of the absolute configurations of the component mono-saccharides were as follows: column, Supelco capillary column SPB™-1, 0.25 mm × 27 m; column temperature, 220 °C, carrier gas, N_2; t_R, D-arabinose (12.8 min), L-xylose (13.0 min), D-xylose (13.7 min), L-arabinose (14.0 min), D-rhamnose (15.6 min),13) L-rhamnose (16.3 min), L-glucose (24.0 min), D-glucose (24.7 min). From **1** D-glucose, L-arabinose (1 : 2), from **2**, D-glucose, L-arabinose, L-rhamnose (1 : 2 : 1), from **3**, D-glucose, L-arabinose, D-xylose, L-rhamnose (1 : 1 : 1 : 1), from **4**—**6**, D-glucose, L-arabinose, D-xylose, L-rhamnose (1 : 2 : 1 : 1), from **7**, D-glucose, L-arabinose, D-xylose, L-rhamnose (2 : 2 : 1 : 1) and from **8**, D-glucose, L-arabinose, D-xylose, L-rhamnose (1 : 1 : 2 : 1) were detected.

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References and Notes

1) A. Lenherr, M. F. Lahloub, O. Sticher, *Phytochemistry*, **23**, 2343 (1984).
2) I. Calis, A. A. Basaran, I. Saracoglu, O. Sticher, *Phytochemistry*, **31**, 167 (1992).
3) M. Jeker, O. Sticher, *Helv. Chim. Acta*, **72**, 1787 (1989).
4) T. Miyase, A. Ueno, T. Kitani, H, Kobayashi, U. Kawahara, J. Yamahara, *Yakugaku Zasshi*, **110**, 652 (1990).
5) F. Piozzi, G. Savona, J. R. Hanson, *Phytochemistry*, **19**, 1237 (1980); C. Fazio, S. Passannanti, M. P. Paternostro, F. Piozzi, *ibid.*, **31**, 3147 (1992).
6) A. A. Basaran, I. Calis, C. Anklin, S. Nishibe, O. Sticher, *Helv. Chim. Acta*, **71**, 1483 (1988).
7) H. Nishimura, H. Sasaki, N. Inagaki, M. Chin, H. Mitsuhashi, *Phytochemistry*, **30**, 965 (1991).
8) T. Ikeda, T. Miyase, A. Ueno, *Natural Medicines*, **48**, 32 (1994).
9) T. Warashina, T. Miyase, A. Ueno, *Chem. Pharm. Bull.*, **39**, 388 (1991).
10) A. Tanaka, *Yakugaku Zasshi*, **105**, 323 (1985).
11) K. Yoshimoto, Y. Itatani, Y. Tsuda, *Chem. Pharm. Bull.*, **28**, 2065 (1980).
12) S. Hara, H. Okabe, K. Mihashi, *Chem. Pharm. Bull.*, **34**, 1843 (1986).
13) The retention time was substituted for that of the thiazolidine derivative from L-cysteine and L-rhamnose for D-rhamnose.