

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

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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,<sup>1)</sup> iridoids,<sup>2-4)</sup> diterpenoids<sup>5)</sup> and phenylethanoid glycosides.<sup>2,4,6,7)</sup> We also reported structures of phenylethanoid glycosides, a lignan diglycoside and a 1-octen-3-ol triglycoside from the whole plant of *S. riederi* CHAMISO.<sup>8)</sup> 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* CHAMISO.

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<sup>9)</sup> 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 <sup>1</sup>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 [ $\delta$  3.34 (dd,  $J=12.5$ , 4 Hz)] of the aglycone moiety, on irradiating at H-1 [ $\delta$  4.86 (d,  $J=8$  Hz)] of the glucose moiety and at H<sub>2</sub>-6 [ $\delta$  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 [ $\delta$  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 [ $\delta$  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  $\beta$  and  $\alpha$ , 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*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*- $\alpha$ -L-arabinopyranosyl ester. The glycosylation shifts in the <sup>13</sup>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 <sup>1</sup>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 <sup>13</sup>C-<sup>1</sup>H correlation spectroscopy (COSY) spectrum as shown in Table I. By comparison of <sup>1</sup>H- and <sup>13</sup>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  $\delta$  5.74 (H-1 of rhamnose moiety) was irradiated, an NOE was observed at the signal [ $\delta$  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, <sup>1</sup>H- and <sup>13</sup>C-NMR chemical shifts of **3** were similar to those of **2**, except for a terminal xylose moiety. NOEs were observed at H<sub>2</sub>-6 [ $\delta$  4.33 (dd,  $J=11$ , 5 Hz), 4.85 (br d,  $J=11$  Hz)] of the glucose moiety on irradiation at H-1 [ $\delta$  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,<sup>10)</sup> the structures of stachyssaponins II and III were characterized as 3-*O*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl] ester and 3-*O*-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-[ $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -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 [ $\delta$  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 <sup>1</sup>H- and <sup>13</sup>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  $\delta$  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  $\delta$  4.54 (t,  $J=2.5$  Hz, H-2 of

TABLE I.  $^{13}\text{C}$ -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<sub>2</sub>-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*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl] ester.

In the  $^1\text{H}$ -NMR spectra of stachyssaponins V (**5**), C<sub>59</sub>H<sub>94</sub>O<sub>26</sub>·13/2H<sub>2</sub>O and VI (**6**), C<sub>59</sub>H<sub>94</sub>O<sub>26</sub>·4H<sub>2</sub>O, each five anomeric proton signals ( $\delta$  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 ( $\delta$  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  $^{13}\text{C}$ -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  $\delta$  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  $^{13}\text{C}$ -NMR spectrum also support these results.<sup>11)</sup> 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*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -L-glucopyranosyl]

echinocystic acid 28-*O*-[ $\beta$ -D-3-*O*-acetyl-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl] ester and 3-*O*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-[ $\beta$ -D-4-*O*-acetyl-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -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*<sub>5</sub> 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 <sup>a)</sup>	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)
19 $\alpha$	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 <sup>a)</sup>	4.15 (t, $J=8$ Hz)	4.16 <sup>a)</sup>	4.17 <sup>a)</sup>
4	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>
5	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>	4.09 <sup>a)</sup>
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 <sup>a)</sup>	4.14 <sup>a)</sup>		4.16 (dd, $J=8$ , 4.5 Hz)
4	4.32 <sup>a)</sup>	4.28 <sup>a)</sup>		4.31 <sup>a)</sup>
5	3.77 (d, $J=11$ Hz)	3.73 (d, $J=10$ Hz)		3.76 (d, $J=11$ Hz)
	4.30 <sup>a)</sup>	4.25 <sup>a)</sup>		4.29 <sup>a)</sup>
Xylose				
1			5.01 (d, $J=7$ Hz)	
2			4.02 (t, $J=7.5$ Hz)	
3			4.12 <sup>a)</sup>	
4			4.19 <sup>a)</sup>	
5			3.68 (t, $J=11$ Hz)	
			4.32 <sup>a)</sup>	
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 <sup>a)</sup>	4.57 <sup>a)</sup>	4.54 <sup>a)</sup>
3	4.39 (dd, $J=6$ , 3 Hz)	4.53 (t, $J=3.5$ Hz)	4.56 <sup>a)</sup>	4.50 <sup>a)</sup>
4	4.49 <sup>a)</sup>	4.42 <sup>a)</sup>	4.42 <sup>a)</sup>	4.38 <sup>a)</sup>
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 <sup>a)</sup>	4.48 <sup>a)</sup>	4.51 <sup>a)</sup>	4.49 <sup>a)</sup>
Rhamnose				
1		5.74 (s)	5.78 (s)	5.76 (s)
2		4.54 <sup>a)</sup>	4.57 <sup>a)</sup>	4.54 <sup>a)</sup>
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 <sup>a)</sup>	4.44 <sup>a)</sup>	4.38 <sup>a)</sup>
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 <sup>a)</sup>
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	5.59 <sup>a)</sup>	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)
19 $\alpha$	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	4.86 <sup>a)</sup>	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	4.17 <sup>a)</sup>	4.16 <sup>a)</sup>	4.16 <sup>a)</sup>	4.18 <sup>a)</sup>
4	4.10 <sup>a)</sup>	4.09 <sup>a)</sup>	4.10 <sup>a)</sup>	4.09 <sup>a)</sup>
5	4.10 <sup>a)</sup>	4.09 <sup>a)</sup>	4.10 <sup>a)</sup>	4.09 <sup>a)</sup>
6	4.26 (dd, $J=11.5$ , 5 Hz)	4.26 <sup>a)</sup>	4.27 <sup>a)</sup>	4.33 (dd, $J=11.5$ , 5.5 Hz)
	4.83 <sup>a)</sup>	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)	4.45 <sup>a)</sup>	
3	4.17 <sup>a)</sup>	4.17 <sup>a)</sup>	4.18 <sup>a)</sup>	
4	4.32 <sup>a)</sup>	4.31 <sup>a)</sup>	4.32 (br s)	
5	3.76 (d, $J=11.5$ Hz)	3.76 (d, $J=10$ Hz)	3.76 (d, $J=11$ Hz)	
	4.30 <sup>a)</sup>	4.30 <sup>a)</sup>	4.29 <sup>a)</sup>	
Xylose				
1				5.00 (d, $J=7$ Hz)
2				4.01 (t, $J=7$ Hz)
3				4.16 <sup>a)</sup>
4				4.29 <sup>a)</sup>
5				3.68 (t, $J=11$ Hz)
				4.31 <sup>a)</sup>
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	4.54 <sup>a)</sup>	4.54 <sup>a)</sup>	4.47 <sup>a)</sup>	4.55 <sup>a)</sup>
3	4.52 <sup>a)</sup>	4.50 <sup>a)</sup>	4.47 <sup>a)</sup>	4.50 <sup>a)</sup>
4	4.35 <sup>a)</sup>	4.37 <sup>a)</sup>	4.42 <sup>a)</sup>	4.39 <sup>a)</sup>
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)
	4.49 <sup>a)</sup>	4.49 <sup>a)</sup>	4.50 <sup>a)</sup>	4.49 <sup>a)</sup>
Rhamnose				
1	5.81 (s)	5.76 (s)	5.62 (br s)	5.76 (s)
2	4.54 <sup>a)</sup>	4.54 <sup>a)</sup>	4.88 (m)	4.55 <sup>a)</sup>
3	4.52 <sup>a)</sup>	4.57 <sup>a)</sup>	4.65 (dd, $J=9$ , 3 Hz)	4.58 (dd, $J=9$ , 2.5 Hz)
4	4.37 <sup>a)</sup>	4.35 <sup>a)</sup>	4.48 <sup>a)</sup>	4.36 (t, $J=9$ Hz)
5	4.47 <sup>a)</sup>	4.39 <sup>a)</sup>	4.42 <sup>a)</sup>	4.38 <sup>a)</sup>
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)	3.95 <sup>a)</sup>	4.00 (t, $J=8.5$ Hz)
3	5.64 (t, $J=9.5$ Hz)	4.16 <sup>a)</sup>	4.10 <sup>a)</sup>	4.05 (t, $J=9$ Hz)
4	4.15 <sup>a)</sup>	5.29 <sup>a)</sup>	4.14 <sup>a)</sup>	4.12 <sup>a)</sup>
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)
	4.19 <sup>a)</sup>	4.20 <sup>a)</sup>	4.18 <sup>a)</sup>	4.20 <sup>a)</sup>
Ac				
Glucose				
1			5.21 (d, $J=8$ Hz)	
2			3.99 (t, $J=8.5$ Hz)	
3			4.13 <sup>a)</sup>	
4			4.14 <sup>a)</sup>	
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  $^1\text{H-NMR}$  spectrum of stachyssaponin VII (7),  $\text{C}_{63}\text{H}_{102}\text{O}_{30} \cdot 5\text{H}_2\text{O}$ , showed six anomeric proton signals at  $\delta$  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 (brs) and 6.42 (brs). Proton signals of the sugar moiety and all carbon signals were assigned by spin decoupling experiments and  $^{13}\text{C-NMR}$  COSY spectrum, respectively. Detailed comparisons of the  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectra of 7 with those of 4 have shown

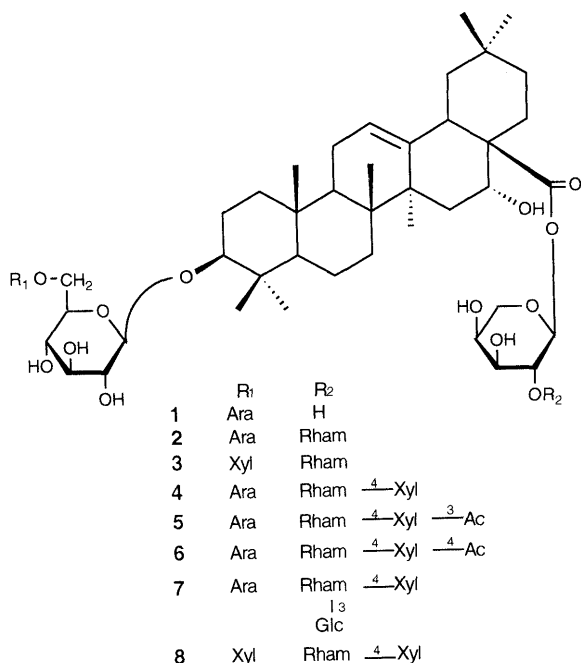


Chart 1

that those of 7 have signals due to a terminal glucose moiety. NOEs were observed at H-2 [ $\delta$  4.47 (brs)] of the ester-linked arabinose moiety on irradiation at H-1 ( $\delta$  5.62) of the rhamnose moiety, at H-3 [ $\delta$  4.65 (dd,  $J=9, 3$  Hz)] of the rhamnose moiety on irradiation at H-1 ( $\delta$  5.21) of the terminal glucose moiety, and at H-4 [ $\delta$  4.48 (t,  $J=9$  Hz)] of the rhamnose moiety on irradiation at H-1 ( $\delta$  5.42) of the xylose moiety. Therefore, the structure of stachyssaponin VII was determined to be 3-*O*-[ $\alpha$ -L-arabinopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-{ $\beta$ -D-glucopyranosyl-(1 $\rightarrow$ 3)-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)]- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -L-arabinopyranosyl} ester.

The  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectra of stachyssaponin VIII (8),  $\text{C}_{57}\text{H}_{92}\text{O}_{25} \cdot 2\text{H}_2\text{O}$ , 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<sub>2</sub>-6 [ $\delta$  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*-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-glucopyranosyl] echinocystic acid 28-*O*-[ $\beta$ -D-xylopyranosyl-(1 $\rightarrow$ 4)- $\alpha$ -L-rhamnopyranosyl-(1 $\rightarrow$ 2)- $\alpha$ -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.  $^1\text{H-}$  and  $^{13}\text{C-NMR}$  spectra were recorded on a JEOL GSX-500. Chemical shifts are given on the  $\delta$  scale with tetramethylsilane as an internal standard. HPLC was done on a JASCO model 800

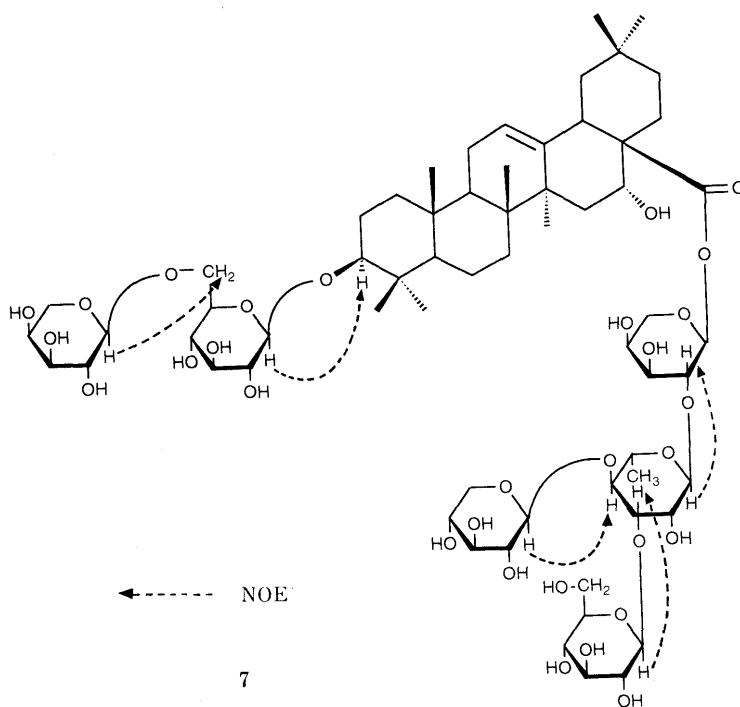


Chart 2

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

**Extraction and Isolation** The powdered whole plant of *S. riederi* CHAMISO (2 kg), collected in Fujinomiya, Shizuoka, Japan in July, 1992, were extracted twice with hot H<sub>2</sub>O. The H<sub>2</sub>O extract was passed through a Mitsubishi Diaion HP-20 column (9 cm × 40 cm). After the content of the column was washed with H<sub>2</sub>O, 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<sub>3</sub>-MeOH-EtOAc-H<sub>2</sub>O (45:15:38:2), increasing a portion of MeOH to give eight fractions (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<sub>2</sub>O (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<sub>46</sub>H<sub>74</sub>O<sub>17</sub>·3/2H<sub>2</sub>O: C, 57.97; H, 8.46. Found: C, 58.02; H, 8.60. FAB-MS  $m/z$ : 921 [M+Na]<sup>+</sup>. <sup>1</sup>H-NMR: shown in Table II. <sup>13</sup>C-NMR  $\delta$ : 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). <sup>13</sup>C-NMR data of aglycone moiety of **2–8** are nearly the same with this data; sugar moiety: shown in Table I.

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

**Stachyssaponin III (3)** Amorphous powder.  $[\alpha]_D^{24} -15.6^\circ$  ( $c=0.48$ , MeOH). *Anal.* Calcd for C<sub>52</sub>H<sub>84</sub>O<sub>21</sub>·3H<sub>2</sub>O: C, 55.90; H, 8.11. Found: C, 55.86; H, 8.04. FAB-MS  $m/z$ : 1068 [M+Na]<sup>+</sup>. <sup>1</sup>H-NMR: shown in Table II. <sup>13</sup>C-NMR: sugar moiety: shown in Table I.

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

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

**Stachyssaponin VI (6)** Amorphous powder.  $[\alpha]_D^{23} -46.3^\circ$  ( $c=1.23$ , MeOH). *Anal.* Calcd for C<sub>59</sub>H<sub>94</sub>O<sub>26</sub>·4H<sub>2</sub>O: C, 54.87; H, 8.43. Found: C, 54.61; H, 8.23. FAB-MS  $m/z$ : 1242 [M+Na]<sup>+</sup>. <sup>1</sup>H-NMR: shown in Table II. <sup>13</sup>C-NMR: sugar moiety: shown in Table I.

**Stachyssaponin VII (7)** Amorphous powder.  $[\alpha]_D^{23} -58.3^\circ$  ( $c=1.60$ , MeOH). *Anal.* Calcd for C<sub>63</sub>H<sub>102</sub>O<sub>30</sub>·5H<sub>2</sub>O: C, 52.93; H, 7.90. Found: C, 52.99; H, 7.95. FAB-MS  $m/z$ : 1362 [M+Na]<sup>+</sup>. <sup>1</sup>H-NMR: shown in Table II. <sup>13</sup>C-NMR: sugar moiety: shown in Table I.

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

**Acid Hydrolysis of 1** A solution of **1** (10 mg) in 5% H<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>O and was passed through a Diaion HP-20 column (1.5 cm × 5 cm). After the content of the column was washed with H<sub>2</sub>O, 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).  $[\alpha]_D^{24} + 40.3^\circ$  ( $c=0.36$ , MeOH). The melting point,  $[\alpha]_D$  and <sup>1</sup>H-NMR data were the same as previously reported.<sup>9)</sup>

**Acid Hydrolysis of 1–8** A solution of each glycoside (1 mg) in 5% H<sub>2</sub>SO<sub>4</sub> aq. (3 drops) and dioxane (3 drops) was heated in a boiling water bath for 1 h. The reaction mixture was diluted with H<sub>2</sub>O 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<sub>2</sub>O 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.<sup>12)</sup> The GC conditions for the determination of the absolute configurations of the component mono-saccharides were as follows: column, Supelco capillary column SPB<sup>TM</sup>-1, 0.25 mm × 27 m; column temperature, 220 °C, carrier gas, N<sub>2</sub>;  $t_R$ , 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), 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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