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Studies on the Constituents of *Clematis* Species. VI.[1] The Constituents of *Clematis stans* SIEB. *et* ZUCC.[2]

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From the roots of *Clematis stans* three new oleanane-type triterpenoid saponins named clemastanoside A, B and C, and two new lignan glycosides named clemastanin A and B, have been isolated together with three known triterpenoid saponins, huzhangoside B, C and D, and three known lignan glycosides, (+)-lariciresinol 4-*O*-β-D-glucopyranoside, (+)-lariciresinol 4′-*O*-β-D-glucopyranoside and (+)-pinoresinol 4,4′-*O*-bis-β-D-glucopyranoside. In addition, from the leaves, four new oleanane-type triterpenoid saponins, named clemastanoside D, E, F and G, have been isolated together with five known triterpenoid saponins, hederasaponin B, kizutasaponin K_{12}, huzhangoside B, sieboldianoside B and huzhangoside D, and three known flavonoids, isoquercitrin, rutin and quercetin 3-*O*-β-D-glucuronopyranoside. The structures of the new compounds were elucidated based on chemical and physicochemical evidence as follows: clemastanoside A, 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-(4-*O*-acetyl)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester (terminal rhamnosyl 4-*O*-acetate of huzhangoside B); clemastanoside B and C, 3-*O*-β-D-xylopyranosyl- and 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-β-D-galactopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, respectively; clemastanoside D, 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-β-D-glucopyranosyl ester; clemastanoside E, F and G, terminal rhamnosyl 4-*O*-, 3-O- and 2-*O*-acetate of 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl hederagenin 28-*O*-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester, respectively; clemastanin A, (7*S*,8*R*)-3-methoxy-3′,4,9,9′-tetrahydroxy-4′,7-epoxy-5′,8-lignan 3′-*O*-β-D-glucopyranoside; clemastanin B, (+)-lariciresinol 4,4′-*O*-bis-β-D-glucopyranoside.

Key words *Clematis stans*; oleanolic acid bisdesmoside; hederagenin bisdesmoside; lignan glycoside; quercetin glycoside; Ranunculaceae

Clematis stans (Kusabotan in Japanese) is a plant of the Ranunculaceae found throughout Japan.[3] The literature contains no report of its constituents. As a continuation of our study of the constituents from *Clematis* species,[1] the roots and leaves of this plant were examined. This paper deals with the structural elucidation of the constituents of plants collected in the Ishikawa prefecture, Japan.

The water-soluble portion of a hot MeOH ext. from the roots was successively extracted with ether, AcOEt and BuOH. The BuOH-soluble part was subjected to repeated chromatography to give eleven compounds (**1**—**11**). In a similar manner, twelve compounds (**2**, **6**, **12**—**21**) were isolated from the leaves as described in the experimental section.

Compounds **2**, **3**, **6**, **7**, **8**, **10**, **13**, **17**, **18**, **19**, **20** and **21** were identified as huzhangoside B,[4] huzhangoside C,[4] huzhangoside D,[4] (+)-lariciresinol 4-*O*-β-D-glucopyranoside,[5] (+)-lariciresinol 4′-*O*-β-D-glucopyranoside,[5] (+)-pinoresinol 4,4′-*O*-bis-β-D-glucopyranoside,[6] hederasaponin B,[7] kizutasaponin K_{12}[8] sieboldianoside B,[9] isoquercitrin,[10] rutin[10] and quercetin 3-*O*-β-D-glucuronopyranoside,[11] respectively, based on chemical data, ^{13}C-NMR spectra and specific rotation data.

Compound **1** named clemastanoside A was subjected to acid-hydrolysis to give oleanolic acid, arabinose, glucose, rhamnose and ribose. On alkaline hydrolysis with 1 N NaOH, **1** gave prosapogenin (**1p**) which was identified as CP_4 (oleanolic acid 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside) by direct comparison.[12] The molecular formula of **1** was determined as $C_{66}H_{106}O_{30}$ from high resolution (HR) FAB-MS [quasi-molecular ion at *m/z* 1401.6656 $(M+Na)^+$] and ^{13}C-NMR spectral data. The ^{13}C-NMR spectrum of **1** exhibited signals due to an acetyl group along with six anomeric carbons. **1** was treated with 0.2 N KOH at room temperature to afford a deacetyl product, which was identified as compound **2** by direct comparison. Consequently, **1** is a monoacetate of **2**. ^{1}H and ^{13}C signals due to both sugar parts were assigned from ^{1}H–^{1}H shift correlation (COSY) and ^{1}H–^{13}C COSY spectral data, and then the data for **1** were compared with those for **2**. H-4 and C-4 signals of the terminal rhamnose unit in **1** were observed at a lower field by 1.51 and 2.1 ppm, and C-3 and C-5 at a higher field by 2.4 and 2.8 ppm, respectively, than the corresponding signals in **2**. These results show that the acetyl group in **1** is connected to the C-4 position of the terminal rhamnose unit.

Therefore, the structure of clemastanoside A (**1**) is concluded to be 3-*O*-β-D-ribopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl oleanolic acid 28-*O*-(4-*O*-acetyl)-α-L-rhamnopyranosyl-(1→4)-β-D-glucopyranosyl-(1→6)-β-D-glucopyranosyl ester.

On acid hydrolysis, clemastanoside B (**4**) gave oleanolic acid, galactose, glucose, rhamnose and xylose and exhibited six anomeric carbon signals in its ^{13}C-NMR spectrum. The molecular formula of **4** was determined as $C_{65}H_{106}O_{30}$ from HR-FAB-MS [quasi-molecular ion at *m/z* 1389.6665 $(M+Na)^+$] and ^{13}C-NMR spectral data. Alkaline hydrolysis of **4** afforded prosapogenin (**4p**), which showed three anomeric carbon signals in the ^{13}C-NMR spectrum and afforded galactose, rhamnose and xylose

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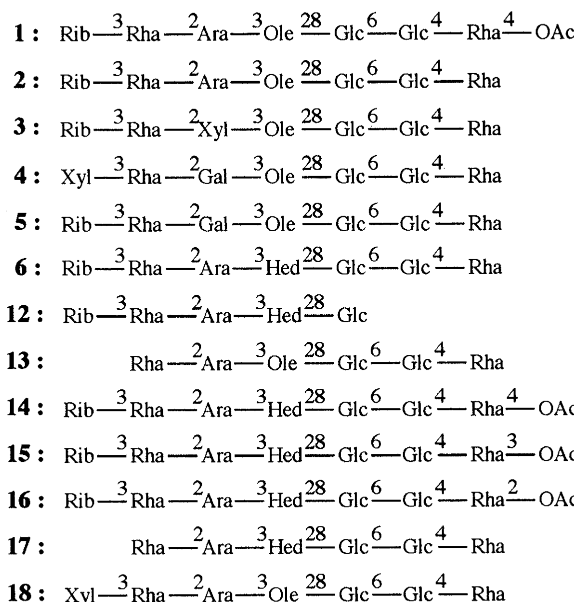
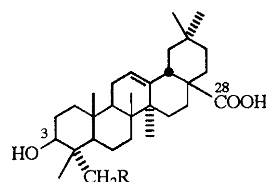
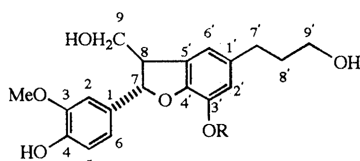


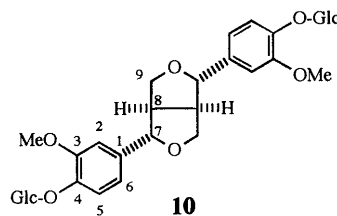


Ole : oleanolic acid (R = H)
Hed : hederagenin (R = OH)

Rib : β-D-ribopyranosyl
Xyl : β-D-xylopyranosyl
Rha : α-L-rhamnopyranosyl
Ara : α-L-arabinopyranosyl
Glc : β-D-glucopyranosyl
Gal : β-D-galactopyranosyl



9: R = Glc
9a: R = H



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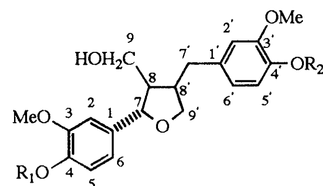
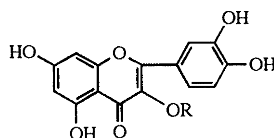


7: R_1 = Glc R_2 = H
8: R_1 = H R_2 = Glc
11: $R_1 = R_2$ = Glc
11a: $R_1 = R_2$ = H



19: R = Glc
20: R = β-rutinosyl
21: R = β-D-glucuronopyranosyl

Chart 1

as sugars on acid hydrolysis, denoting that each sugar in **4p** represented 1 mol. Comparison of the ^{13}C-NMR spectrum of **4** with those of **4p** and **2** revealed that the 28-*O*-glycosyl moiety was α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranose. The ^{1}H and ^{13}C signals due to the sugar moiety in **4** were assigned based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY spectral data and then the sequence of 3-*O*-sugar units was identified as xylopyranosyl-(1→3)-rhamnopyranosyl-(1→2)-galactopyranosyl-(1→3)-oleanolic acid by the ^{1}H detected heteronuclear multiple bond connectivity (HMBC) spectrum, in which ^{1}H–^{13}C long-range correlations between galactosyl H-1/C-3, galactosyl H-2/rhamnosyl C-1, rhamnosyl H-3/xylosyl C-1 and xylosyl H-1/rhamnosyl C-3 were observed. The configurations of both galactosyl and xylosyl anomeric positions were determined as *β* based on the $J_{\text{H-1,H-2}}$ value [8 (galactosyl unit), 7.5 Hz (xylosyl unit)], and that of the rhamnosyl C-1 as α by comparison of the rhamnosyl carbon signal pattern with that of CP_3[12] (oleanolic acid 3-*O*-*β*-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside).

From these results, the structure of **4** is 3-*O*-*β*-D-xylopyranosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl oleanolic acid 28-*O*-α-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

Clemastanoside C (**5**) was subjected to acid-hydrolysis to give oleanolic acid, galactose, glucose, rhamnose and ribose. The molecular formula of **5** was determined as $C_{65}H_{106}O_{30}$ from HR-FAB-MS [quasi-molecular ion at *m/z* 1389.6663 $(M+Na)^+$] and ^{13}C-NMR spectral data. Compound **5** was suggested to have a terminal ribose unit because its ^{13}C-NMR spectrum displayed a carbon signal pattern similar to that of **4** except for the terminal xylose part. This was also supported by comparison of its ^{13}C-NMR spectral data with those of **2**. Furthermore, the sequence of the 3-*O*-sugar units was confirmed based on the results of the nuclear Overhauser effect (NOE) experiment; *i.e.* NOEs were observed between ribosyl H-1/rhamnosyl H-3, rhamnosyl H-1/galactosyl H-2 and

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galactosyl H-1/H-3. The assignment of these protons was accomplished by analysis of the ^{1}H–^{1}H COSY spectral data for **5**.

On the basis of these findings, the structure of **5** was concluded to be 3-*O*-*β*-D-ribopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*β*-D-galactopyranosyl oleanolic acid 28-*O*-*α*-L-rhamnopyranosyl-(1→4)-*β*-D-glucopyranosyl-(1→6)-*β*-D-glucopyranosyl ester.

On acid hydrolysis, clemastanoside D (**12**) gave hederagenin, arabinose, glucose, rhamnose and ribose, and alkaline hydrolysis of **12** afforded prosapogenin (**12p**), which was identified as CP_6 (hederagenin 3-*O*-*β*-D-ribopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranoside) by direct comparison.[13] The molecular formula of **12** was determined as $C_{52}H_{84}O_{21}$ from HR-FAB-MS [quasi-molecular ion at *m*/*z* 1067.5388 $(M+Na)^+$] and ^{13}C-NMR spectral data. Compound **12** exhibited the four anomeric carbon signals in the ^{13}C-NMR spectrum, and was considered to be 28-*O*-glucosyl ester of **12p**. The configuration of the glucosyl C-1 was regarded as *β* from the *J* value (8 Hz) of its anomeric proton signal. Consequently, the structure of **12** is 3-*O*-*β*-D-ribopyranosyl-(1→3)-*α*-L-rhamnopyranosyl-(1→2)-*α*-L-arabinopyranosyl hederagenin 28-*O*-*β*-D-glucopyranosyl ester.

Each of the clemastanosides E (**14**), F (**15**) and G (**16**) was considered to have the molecular formula $C_{66}H_{106}O_{31}$ from HR-FAB-MS [quasi-molecular ion at *m*/*z* 1417.6603, 1417.6608, 1417.6609, respectively, each $(M+Na)^+$] and ^{13}C-NMR spectral data. Each of these showed signals due to an acetyl group in the ^{1}H- and ^{13}C-NMR spectra and gave **6** on treatment with mild alkali, 0.2 N KOH, at room temperature as well as **12p** (CP_6) on alkaline hydrolysis with 1 N NaOH. Therefore, these saponins are considered to be the monoacetate of **6**. Comparison of their ^{13}C-NMR spectral data with those of **6** led to the supposition that an acetyl group in **14**, **15** and **16** was present in the terminal rhamnose unit (Table 2). Proton and carbon signals due to the sugar moiety in **6**, **14**, **15** and **16** were assigned based on ^{1}H–^{1}H and ^{1}H–^{13}C COSY [or ^{1}H detected heteronuclear multiple quantum coherence (HMQC) in the case of **15** and **16**] spectral data, and each linking position of an acetyl group in **14**, **15** and **16** was confirmed to be the hydroxy group of C-4, C-3 and C-2 of the terminal rhamnose unit, respectively, by considering the acylation shift.

Accordingly, the structures of clemastanoside E, F and G are given by **14**, **15** and **16**, respectively.

On acid hydrolysis, clemastanin A (**9**) gave glucose as the sugar moiety and was considered to have the molecular formula $C_{25}H_{32}O_{11}$ from HR-FAB-MS [quasi-molecular ion at *m*/*z* 531.1844 $(M+Na)^+$] and ^{13}C-NMR spectral data. The ^{1}H-NMR spectrum of **9** showed the presence of a 1,2,4-trisubstituted benzene [δ_H 7.20, d ($J=8$ Hz), 7.26, dd, ($J=8$, 1 Hz), 7.33, d ($J=1$ Hz)], a 1,2,3,5-tetrasubstitued benzene (δ_H 7.07, br s, 7.49, br s) and a methoxy group (δ_H 3.59, s), and the presence of two C_3 units [CH(O)–CH–CH_2(O) and CH_2–CH_2–CH_2(O)] were revealed by ^{1}H–^{1}H COSY spectral data. Each carbon, except the quaternary one, was assigned by ^{1}H–^{13}C COSY

Table 1. ^{13}C-NMR Spectral Data for the Aglycone Part of Saponins in Pyridine-d_5

C No.	Ole[a]	1	2	3	4	4p	5	18	13	Hed[b]	6	14	15	16	12	17
C-1	39.0	38.9	38.9	39.0	39.0	39.0	39.0	38.9	38.9	38.8	39.1	39.0	39.1	39.1	39.0	39.0
2	28.1	26.7	26.6	26.9	26.9	26.9	26.9	26.7	26.5	27.7	26.3	26.3	26.4	26.4	26.3	26.1
3	78.1	88.7	88.7	88.6	88.6	88.7	88.8	88.7	88.7	73.4	81.0	81.0	81.1	81.1	81.0	80.9
4	39.4	39.6	39.5	39.6	39.6	39.6	39.6	39.6	39.4	42.9	43.5	43.5	43.6	43.6	43.5	43.4
5	55.8	56.0	56.0	56.1	56.0	56.1	56.1	56.0	55.9	48.1	47.6	47.6	47.8	47.8	47.6	47.6
6	18.8	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.6	18.1	18.0	18.2	18.2	18.1	18.1
7	33.29	33.05	33.0	33.0	33.0	33.17	33.07	33.1	33.05	33.19	32.7	32.7	32.8	32.8	32.7	32.7
8	39.8	39.9	39.8	39.9	39.8	39.7	39.9	39.9	39.8	39.7	39.8	39.8	39.9	39.9	39.9	39.8
9	48.1	48.0	48.0	48.0	48.0	48.0	48.0	48.1	48.0	48.6	48.1	48.1	48.2	48.2	48.1	48.1
10	37.4	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.0	37.2	36.8	36.8	36.9	36.9	36.8	36.8
11	23.83	23.8	23.7	23.7	23.7	23.8	23.74	23.8	23.8	23.8	23.7	23.8	23.8	23.8	23.8	23.8
12	122.6	122.9	122.8	122.8	122.8	122.6	122.9	122.9	122.8	122.6	122.8	122.9	122.9	123.0	122.9	122.8
13	144.8	144.1	144.1	144.1	144.1	144.8	144.1	144.1	144.1	144.8	144.0	144.0	144.1	144.1	144.0	144.0
14	42.2	42.1	42.0	42.1	42.1	42.2	42.1	42.1	42.1	42.2	42.0	42.0	42.1	42.0	42.0	42.0
15	28.3	28.3	28.22	28.3	28.3	28.3	28.3	28.3	28.2	28.3	28.2	28.2	28.3	28.3	28.2	28.2
16	23.7	23.4	23.3	23.7	23.3	23.7	23.4	23.4	23.3	23.67	23.3	23.2	23.4	23.4	23.3	23.3
17	46.7	47.0	47.0	47.0	47.0	46.7	47.0	47.0	47.0	46.6	46.9	46.9	47.0	47.0	46.9	46.9
18	42.0	41.7	41.6	41.6	41.6	42.0	41.7	41.7	41.6	42.0	41.6	41.6	41.7	41.7	41.6	41.6
19	46.5	46.2	46.2	46.2	46.2	46.5	46.2	46.2	46.2	46.4	46.1	46.1	46.2	46.2	46.1	46.1
20	31.0	30.8	30.7	30.7	30.7	31.0	30.8	30.7	30.7	30.9	30.7	30.6	30.7	30.8	30.7	30.6
21	34.3	34.0	33.9	34.0	34.0	34.2	34.0	34.0	33.9	34.2	33.9	33.9	34.0	34.0	33.9	33.9
22	33.2	32.5	32.5	32.5	32.5	33.21	32.5	32.5	32.5	33.0	32.4	32.5	32.5	32.6	32.5	32.5
23	28.8	28.2	28.16	28.2	28.2	28.2	28.3	28.2	28.0	67.8	63.9	63.9	64.0	64.0	63.9	63.9
24	16.6	17.2	17.1	17.2	17.39	17.4	17.3	17.2	16.9	13.1	14.1	14.1	14.1	14.1	14.1	13.9
25	15.6	15.7	15.6	15.7	15.6	15.6	15.7	15.7	15.6	16.0	16.1	16.1	16.2	16.2	16.1	16.1
26	17.4	17.5	17.4	17.5	17.42	17.4	17.5	17.5	17.4	17.5	17.5	17.5	17.5	17.6	17.5	17.5
27	26.2	26.1	26.0	26.1	26.1	26.2	26.1	26.1	26.0	26.1	26.0	26.0	26.1	26.0	26.0	26.0
28	180.1	176.5	176.5	176.5	176.5	180.2	176.5	176.5	176.7	180.2	176.5	176.5	176.5	176.5	176.4	176.5
29	33.32	33.11	33.1	33.1	33.1	33.3	33.11	33.1	33.10	33.23	33.0	33.0	33.1	33.1	33.0	33.0
30	23.80	23.7	23.6	23.7	23.6	23.8	23.65	23.7	23.6	23.74	23.6	23.6	23.7	23.7	23.6	23.6

a) Ole: oleanolic acid. *b*) Hed:hederagenin.

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Table 2. ^{13}C-NMR Spectral Data for the Sugar Moiety of Saponins in Pyridine-d_5

C No.	1	2	3	4	4p	5	18	13	6	14	15	16	12	17
3-*O*-Glycosyl moiety														
			(Xyl)	(Gal)	(Gal)	(Gal)								
Ara-1	105.3	105.3	106.1	105.8	105.8	105.8	105.3	104.8	104.7	104.7	104.7	104.8	104.6	104.3
(Xyl) 2	75.3	75.3	77.0	74.8	75.1	75.0	75.1	75.8	75.0	75.1	75.2	75.4	75.1	75.7
(Gal) 3	74.9	74.8	79.9	77.0	77.0	77.0	74.8	73.8	75.32	75.3	75.4	75.3	75.3	74.6
4	69.5	69.4	71.5	71.0	71.0	71.0	69.4	68.6	69.8	69.7	69.8	69.8	69.8	69.3
5	65.8	65.8	67.0	76.7	76.7	76.7	65.8	64.7	66.4	66.4	66.4	66.4	66.4	65.6
6				62.1	62.1	62.1								
Pha-1	101.4	101.3	101.4	101.2	101.4	101.3	101.4	101.7	101.2	101.2	101.4	101.4	101.2	101.6
2	72.1	72.0	72.0	72.1	72.1	72.2	72.0	72.3	72.0	72.0	72.1	72.1	72.0	72.2
3	81.3	81.2	81.2	82.9	83.0	81.3	83.0	72.5	81.1	81.2	81.3	81.3	81.2	72.5
4	72.9	72.8	72.9	73.0	73.1	72.9	73.0	74.0	72.8	72.8	72.9	72.9	72.8	74.0
5	69.9	69.8	69.9	69.4	69.4	69.7	69.7	69.8	69.7	69.8	69.8	69.8	69.7	69.6
6	18.4	18.5	18.6	18.4	18.4	18.4	18.4	18.5	18.4	18.4	18.5	18.5	18.4	18.5
				(Xyl)	(Xyl)		(Xyl)							
Rib-1	104.7	104.6	104.6	107.6	107.7	104.8	107.6		104.6	104.6	104.7	104.8	104.6	
(Xyl) 2	72.8	72.7	72.7	75.6	75.6	72.8	75.7		72.7	72.7	72.8	72.8	72.7	
3	69.0	68.8	68.9	78.5	78.5	69.0	78.5		68.8	68.8	68.9	68.9	68.9	
4	70.3	70.1	70.2	71.1	71.2	70.3	71.2		70.1	70.2	70.3	70.3	70.2	
5	65.3	65.2	65.2	67.5	67.5	65.3	67.5		65.2	65.2	65.3	65.3	65.2	
28-*O*-Glycosyl moiety														
Glc-1	95.6	95.6	95.6	95.6		95.7	95.7	95.6	95.5	95.5	95.7	95.6	95.7	95.6
(Inner) 2	73.9	73.8	73.8	73.9		73.9	73.9	73.8	73.8	73.8	73.9	73.9	74.1	73.8
3	78.7	78.7	78.7	78.7		78.7	78.8	78.7	78.6	78.6	78.8	78.8	78.8	78.6
4	70.9	70.8	70.8	70.8		70.9	70.9	70.8	70.7	70.8	70.8	70.9	71.0	70.7
5	78.1	78.0	78.0	78.0		78.1	78.1	78.0	78.0	78.1	78.0	78.1	79.2	78.0
6	69.2	69.1	69.2	69.2		69.2	69.2	69.1	69.1	69.0	69.3	69.2	62.1	69.1
Glc-1	104.9	104.8	104.8	104.8		104.9	104.9	104.8	104.7	104.7	105.1	104.9		104.8
(Outer) 2	75.5	75.3	75.3	75.3		75.4	75.4	75.3	75.25	75.4	75.5	75.5		75.3
3	76.4	76.4	76.5	76.5		76.5	76.5	76.4	76.4	76.3	76.3	76.3		76.4
4	77.4	78.1	78.2	78.2		78.2	78.2	78.2	78.1	77.3	77.7	76.9		78.1
5	77.2	77.1	77.1	77.1		77.2	77.2	77.1	77.0	77.1	77.1	77.2		77.1
6	61.2	61.2	61.2	61.2		61.3	61.3	61.2	61.2	61.1	61.2	61.0		61.2
Rha-1	102.1	102.6	102.7	102.7		102.8	102.8	102.7	102.6	102.0	102.4	99.0		102.6
2	72.5	72.5	72.6	72.6		72.6	72.6	72.5	72.5	72.4	70.2	74.4		72.5
3	70.3	72.7	72.7	72.7		72.8	72.8	72.7	72.7	70.2	76.4	70.5		72.7
4	76.0	73.9	74.0	74.0		74.0	74.0	73.9	73.9	75.9	70.8	74.2		73.9
5	67.4	70.2	70.3	70.3		70.3	70.3	70.2	70.2	67.3	70.4	70.1		70.2
6	17.9	18.4	18.5	18.5		18.5	18.5	18.5	18.4	17.9	18.4	18.5		18.5
$CH_3\underline{CO}$	170.7									170.7	170.8	170.8		
$\underline{CH_3}CO$	21.1									21.1	21.1	21.0		

spectral data, and then the sequence of these units was examined based on HMBC spectral data. The observed ^{1}H–^{13}C long-range correlations are shown in Fig. 1 by dashed arrows, revealing the gross planar structure. The 7,8-*trans* configuration was shown by the difference NOE spectra of **9**, in which obvious NOEs were observed between H-7/H_2-9, H-8/H-2 and H-6. The absolute configuration of 7*S*, 8*R* was determined based on circular dichroism (CD) spectral data ($[\theta]_{295\,nm} = +5650$)[14] of the aglycone (**9a**) which was obtained following enzymatic hydrolysis of **9**. The configuration of the glucosyl C-1 was regarded as β from the J value (7.5 Hz) of an anomeric proton signal, and the constituent glucose was considered to be in the D form based on the molecular rotation difference between **9** and **9a** ($\Delta[M]_D = -211°$).[15]

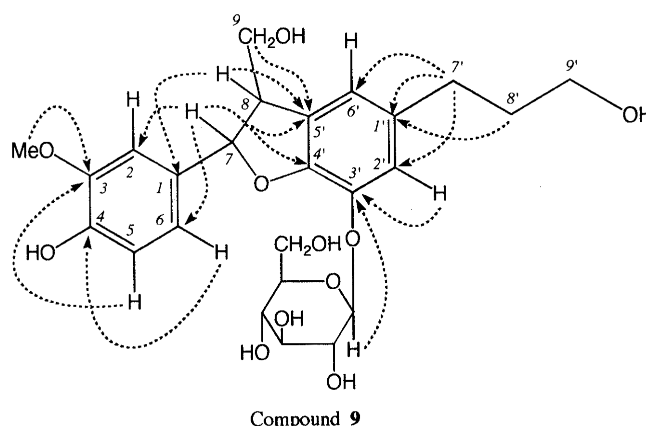

Fig. 1. ^{1}H-→^{13}C Long-Range Correlations Observed in the HMBC Spectrum

On the basis of these results, the structure of **9** was concluded to be 3-methoxy-3′,4,9,9′-tetrahydroxy-4′,7-epoxy-5′,8-lignan 3′-*O*-β-D-glucopyranoside.

On methanolysis, clemastanin B (**11**) afforded an artificial aglycone (**11b**) and a methyl glucoside, and gave a genuine aglycone (**11a**) together with **11b** on enzymatic hydrolysis. Compounds **11a** and **11b** were identified as (+)-lariciresinol[5] and (+)-cyclolariciresinol (isolariciresinol),[16] respectively, by comparison of their NMR spectral data and specific rotations with those described in the literature. The molecular formula of **11** was determined as $C_{32}H_{44}O_{16}$ from HR-FAB-MS [quasi-

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Table 3. ^{1}H-NMR Spectral Data for Saponins in Pyridine-d_5[a)]

	6[b)]	1[c)]	3	18	4	4p	5
Aglycone moiety							
H-3	4.26 overlap	3.27 dd (12, 4)	3.31 dd (11.5, 4)	3.29 dd (12, 4)	3.36 dd (12, 4)	3.38 dd (11.5, 4)	3.35 dd (12, 4)
H-23	3.91 m	—	—	—	—	—	—
	4.28 overlap						
H_3-23	—	1.30 s	1.36 s	1.31 s	1.39 s	1.41 s	1.39 s
H_3-24	1.12 s	1.15 s	1.22 s	1.17 s	1.24 s	1.23 s	1.23 s
H_3-25	0.94 s	0.87 s	0.86 s	0.88 s	0.86 s	0.83 s	0.85 s
H_3-26	1.08 s	1.07 s	1.06 s	1.08 s	1.06 s	1.01 s	1.06 s
H_3-27	1.16 s	1.25 s	1.23 s	1.25 s	1.25 s	1.32 s	1.25 s
H_3-29	0.84 s	0.89 s	0.88 s	0.89 s	0.88 s	0.96 s	0.88 s
H_3-30	0.85 s	0.89 s	0.88 s	0.89 s	0.88 s	0.98 s	0.88 s
3-*O*-Glycosyl moiety							
			(Xyl)		(Gal)	(Gla)	(Gal)
Ara-1	5.03 d (6.5)	4.83 d (6)	4.83 d (7.5)	4.85 d (6)	4.87 d (8)	4.88 d (8)	4.88 d (8)
(Xyl) 2	4.56 dd (8, 6.5)	4.58 dd (7, 6)	4.26 overlap	4.60 dd (7, 6)	4.66 overlap	4.65 dd (9, 8)	4.66 dd (9, 8)
(Gal) 3	3.99 dd (8, 4)	4.25 overlap	4.20 overlap	4.24 overlap	4.24 dd (9, 3)	4.24 dd (9, 3.5)	4.27 dd (9, 2.5)
4	4.11 overlap	4.22 overlap	4.17 overlap	4.24 overlap	4.47 br s	4.47 br d (3.5)	4.48 br s
5	3.64 br d (9.5)	3.80 br d (10.5)	3.71 t (10.5)	3.81 br d (11)	4.09 overlap	4.08 overlap	4.09 overlap
	4.22 br d (9.5)	4.28 overlap	4.33 overlap	4.32 overlap			
6					4.42 overlap	4.42 dd (11, 6)	4.43 m
					4.42 overlap	4.44 dd (11, 6)	4.43 m
Rha-1	6.33 br s	6.29 br s	6.60 br s	6.32 br s	6.56 br s	6.54 d (2)	6.56 br s
2	4.87 br s	4.91 br s	4.98 br s	4.93 br s	4.98 br s	4.99 dd (3.5, 2)	4.98 br s
3	4.74 dd (9.5, 3)	4.74 dd (9.5, 3)	4.80 dd (9.5, 3)	4.77 dd (9.5, 3.5)	4.82 dd (9.5, 3)	4.81 dd (9, 3.5)	4.82 dd (9.5, 3)
4	4.40 t (9.5)	4.43 t (9.5)	4.48 t (9.5)	4.49 t (9.5)	4.49 t (9.5)	4.49 t (9)	4.45 dq (9.5, 7)
5	4.68 dq (9.5, 6)	4.63 dq (9.5, 6)	4.77 dq (9.5, 6)	4.64 dq (9.5, 6)	4.74 dq (9.5, 6)	4.76 dq (9, 6)	1.51 d (7)
6	1.50 d (6)	1.53 d (6)	1.63 d (6)	1.53 d (6)	1.50 d (6)	1.52 d (6)	
				(Xyl)	(Xyl)	(Xyl)	
Rib-1	5.93 d (4.5)	5.95 d (4.5)	5.98 d (4.5)	5.37 d (7.5)	5.40 d (7.5)	5.40 d (7.5)	5.99 d (5)
(Xyl) 2	4.28 overlap	4.31 overlap	4.34 overlap	4.07 dd (9, 7.5)	4.08 overlap	4.08 overlap	4.33 overlap
3	4.50 m	4.51 m	4.54 br s	4.17 overlap	4.19 overlap	4.18 t (8.5)	4.54 br s
4	4.14 m	4.16 overlap	4.18 m	4.19 overlap	4.21 overlap	4.20 m	4.19 m
5	4.14 m	4.16 overlap	4.18 m	3.71 t (10.5)	3.76 t (10.5)	3.76 dd (11.5, 9.5)	4.19 m
	4.27 overlap	4.33 m	4.33 m	4.33 overlap	4.40 overlap	4.37 dd (11.5, 5)	4.37 m

	6[d)]	1[e)]	15	16	12
28-*O*-Glycosyl moiety					
Glc-1	6.20 d (8)	6.23 d (8)	6.25 d (8)	6.23 d (8)	6.33 d (8)
(Inner) 2	4.09 overlap	4.11 dd (9, 8)	4.13 overlap	4.11 overlap	4.19 dd (9, 8)
3	4.18 t (19)	4.19 overlap	4.22 overlap	4.19 t (9)	4.73 t (9)
4	4.28 overlap	4.29 overlap	4.34 t (9)	4.26 overlap	4.36 t (9)
5	4.07 overlap	4.11 overlap	4.09 overlap	4.11 overlap	4.02 ddd (9, 4.5, 2.5)
6	4.30 overlap	4.32 overlap	4.32 overlap	4.32 overlap	4.40 dd (12, 4.5)
	4.63 br d (9.5)	4.67 br d (9.5)	4.66 br d (10)	4.67 overlap	4.46 dd (12, 2.5)
Glc-1	4.97 d (8)	5.01 d (7.5)	4.94 d (7.5)	5.00 d (7.5)	
(Outer) 2	3.91 dd (9, 8)	3.92 dd (9, 7.5)	3.91 dd (9, 7.5)	3.93 dd (9, 7.5)	
3	4.12 overlap	4.15 overlap	4.11 overlap	4.15 t (9)	
4	4.38 t (9)	4.45 t (9.5)	4.46 t (9.5)	4.52 t (9)	
5	3.62 m	3.65 br d (9.5)	3.58 br d (9.5)	3.66 overlap	
6	4.06 overlap	4.06 dd (12, 3)	4.09 overlap	4.29 overlap	
	4.17 overlap	4.19 overlap	4.20 overlap	4.29 overlap	
Rha-1	5.82 br s	5.89 br s	5.87 br s	5.79 br s	
2	4.65 br s	4.65 br s	4.84 br s	5.88 br d (4)	
3	4.54 dd (9.5, 3.5)	4.57 dd (9.5, 3)	5.88 dd (9.5, 3)	4.70 dd (10, 4)	
4	4.30 overlap	5.81 t (9.5)	4.53 t (9.5)	4.23 t (10)	
5	4.93 dq (9.5, 6)	5.04 dq (9.5, 6)	5.14 dq (9.5, 6)	5.12 dq (10, 6)	
6	1.66 d (6)	1.43 d (6)	1.71 d (6)	1.74 d (6)	
CH_3CO		2.01 s	1.94 s	1.93 s	

a) Coupling constants (*J*) in Hz are given in parentheses. *b)* Data for **12**, **13** and **14**—**16** are almost the same as for **6**. *c)* Data for **2** are almost the same as for **1**. *d)* Data for **2**—**5**, **13**, **17** and **18** are almost the same as for **6**. *e)* Data for **14** are almost the same as for **1**.

molecular ion at m/z 707.2532 $(M+Na)^{+}$] and ^{13}C-NMR spectral data. The ^{13}C-NMR spectrum of **11** revealed the presence of 2 mol of β-glucopyranose, whose linking positions were considered to be C_4- and $C_{4'}$-OH by comparison of the ^{13}C-NMR spectral data with those of **11a**. The molecular rotation difference between **11** and **7** (or **8**) suggested the glucose unit to be the D form.

From these results, the structure of **11** was concluded

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Table 4. ^{13}C-NMR Spectral Data for Lignans

C No.	**11a**[a)]	**7**[b)]	**8**[b)]	**11**[b)]	**9**[c)]	**9a**[d)]	**10**[b)]
C-1	134.8	137.6	134.8	137.5	133.7	135.2	135.2
2	108.3	110.0	109.9	110.0	110.8	111.0	110.6
3	146.6	148.6	147.4	148.6	148.8	148.8	149.0
4	145.0	145.4	145.6	145.4	148.1	147.7	145.9
5	114.2	114.9	115.1	114.9	116.4	116.1	115.2
6	118.8	117.6	118.3	117.6	119.9	120.1	118.2
7	82.8	81.5	81.8	81.5	88.5	88.6	84.9
8	52.6	52.4	52.5	52.3	55.1	55.8	53.7
9	60.9	58.5	58.7	58.5	64.3	65.3	71.1
1′	132.3	131.6	134.7	134.5	136.1	136.8	135.2
2′	111.2	112.5	113.1	112.9	116.4	117.3	110.6
3′	146.5	147.3	148.8	148.6	142.5	142.1	149.0
4′	144.0	144.4	144.9	144.7	147.3	145.5	145.9
5′	114.4	115.2	115.3	115.1	130.5	130.3	115.2
6′	121.2	120.4	120.4	120.2	118.4	116.8	118.2
7′	33.3	32.0	32.2	32.0	32.5	32.9	84.9
8′	42.4	41.8	41.9	41.7	35.7	36.4	53.7
9′	72.9	71.8	71.8	71.7	61.3	62.3	71.1
OMe	55.9	55.4	55.6	55.5	55.7	56.8	55.7
	55.9	55.5	55.7	55.5	—	—	55.7
Glc-1		100.0		100.04	102.1		100.2
2		73.1		73.1	74.8		73.2
3		76.7		76.7	78.5		76.9
4		69.6		69.5	71.3		69.7
5		76.9		76.8	78.8		77.0
6		60.5		60.5	62.4		60.7
Glc′-1			100.2	99.98			100.2
2			73.3	73.1			73.2
3			76.9	76.7			76.9
4			69.7	69.5			69.7
5			77.0	76.8			77.0
6			60.7	60.5			60.7

a) Measured in $CDCl_3$. *b)* Measured in DMSO-d_6. *c)* Measured in pyridine-d_5. *d)* Measured in acetone-d_6.

to be (+)-lariciresinol 4,4′-*O*-bis-β-D-glucopyranoside.

As described above, the constituents of *Clematis stans* were examined and six saponins (**1**—**6**) and five lignan glycosides (**7**—**11**) from the roots and nine saponins (**2**, **6**, **12**—**18**) and three quercetin glycosides (**19**—**21**) from the leaves were isolated and characterized. A saponin like **4** or **5**, possessing a galactosyl unit at the C-3 position of oleanolic acid, is unique. This is the first example of the isolation of lignans from *Clematis* species.

In the purification procedure of a mixture of **14**, **15** and **16**, migration and/or elimination of an acetyl group was observed. Compound **14** was relatively stable. Compounds **15** and **16**, however, were unstable even when dissolved in MeOH, especially in the presence of silica gel, but were stable in pyridine solution in the absence of silica gel. Compounds **15** and **16** exhibited very similar chromatographic behavior and their isolation was accomplished by preparative normal phase high-performance (HP) TLC using a solvent system consisting of PrOH–$CHCl_3$–CH_3CN–AcOEt–H_2O (1.3 : 1.0 : 3.0 : 0.2 : 1.1).

Experimental

General Procedures All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL JNM-GSX-400 spectrometer (^{1}H-NMR, 400 MHz. ^{13}C-NMR, 100 MHz) using tetramethylsilane or a residual signal of the solvent as an internal standard: pyridine-d_5, δ_C 123.5, δ_H 7.20 (β-CH); dimethylsulfoxide (DMSO)-d_6, δ_C 39.5, δ_H 2.50; acetone-d_6, δ_C 30.3, δ_H 2.00. The following instruments were used for other physical data: Optical rotation, JASCO DIP-370 digital polarimeter; IR spectra, Hitachi 270-30 spectrometer; UV spectra, Shimadzu UV-3000 double-beam automatic spectrometer; FAB-MS (positive ion mode; matrix, magic bullet), JEOL JMS-SX-102A mass spectrometer; CD spectra, JASCO J-720 CD dispersion spectrometer. HPLC was conducted using a Shimadzu LC-6AD pump system with a Shimadzu SPD-6A UV detector. Gas-liquid chromatography (GLC) was

Table 5. ^{1}H-NMR Spectral Data for Lignans[a)]

	11a[b)]	**7**[c)]	**8**[c)]	**11**[c)]	**9**[d)]	**9a**[e)]	**10**[c)]
H-2	6.86 d (1.5)	6.88 d (1.5)	6.82 overlap	6.90 d (2)	7.33 d (1)	7.01 d (2)	6.95 br s
5	6.84 d (8.5)	7.02 d (8.5)	6.71 d (8)	7.04 d (8.5)	7.20 d (8)	6.76 d (8)	7.04 br d (8.5)
6	6.80 dd (8.5, 1.5)	6.78 dd (8.5, 1.5)	6.69 overlap	6.81 dd (8.5, 2)	7.26 dd (8, 1)	6.84 dd (8, 2)	6.86 br d (8.5)
7	4.79 d (6.5)	4.72 d (6)	4.65 d (6)	4.74 d (6)	6.02 d (7)	5.45 d (6.5)	4.67 br d (4)
8	2.41 br quintet (7)	2.20 m	2.19 br quintet (7)	2.22 br quintet (6)	3.99 br q (6)	3.45 br q (6.5)	3.05 m
9	3.77 dd (11, 6)	3.49 m	3.47 m	3.49 m	4.18 br dd (10.5, 6.5)	3.74 br dd (11, 7.5)	4.15 dd (8.5, 6.5)
	3.91 dd (11, 7)	3.67 m	3.67 m	3,68 m	4.25 br dd (10.5, 5.5)	3.82 br dd (11, 5.5)	overlap
2′	6.69 d (1.5)	6.75 d (1.5)	6.82 overlap	6.84 d (1.5)	7.49 br s	6.55 br s	6.95 br s
5′	6.87 d (8)	6.57 dd (8, 1.5)	6.98 dd (8.5, 1.5)	6.71 dd (8.5, 2)	—	—	7.04 br d (8.5)
6′	6.70 dd (8, 1.5)	6.67 d (8)	6.69 overlap	7.00 d (8.5)	7.07 br s	6.58 br s	6.86 br d (8.5)
7′	2.55 dd (13.5, 10.5)	2.42 dd (13, 11)	2.47 br t (13)	2.49 dd (12.5, 11)	2.82 dt (14, 7)	2.51 (2H) br t (7.5)	4.67 br d (4)
	2.91 dd (13.5, 5)	2.81 dd (13, 5)	2.86 dd (13, 5)	2.87 dd (12.5, 4.5)	2.84 dt (14, 7)		—
8′	2.73 m	2.58 m	2.61 m	2.63 m	2.03 (2H) br quint (7)	1.71 (2H) m	3.05 m
9′	3.75 (β-H) dd (9, 6)	3.58 t (7.5)	3.56 dd (8, 7)	3.60 t (7.5)	3.84 (2H) br t (6)	3.51 (2H) br t (6.5)	4.15 dd (8.5, 6.5)
	4.05 (α-H) dd (9, 6.5)	3.89 t (7.5)	3.88 dd (8, 7)	3.92 t (7.5)	—		overlap
OMe	3.89 s	3.75 s	3.74 s	3.77 s	3.59 s	3.77 s	3.77 s
OMe′	3.87 s	3.74 s	3.74 s	3.76 s	—	—	3.77 s
Glc-H-1		4.87 d (7)	—	4.88 d (7.5)	5.85 d (7.5)		4.88 d (7)
Glc′-H-1			4.85 d (7)	4.87 d (7.5)	—		4.88 d (7)

a) Coupling constants (*J*) in Hz are given in parentheses. *b)* Measured in $CDCl_3$. *c)* Measured in DMSO-d_6. *d)* Measured in pyridine-d_5. *e)* Measured in acetone-d_6.

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performed on a Shimadzu GC-6AM instrument with a flame ionization detector, using a glass column (3 m × 3 mm i.d.) packed with 2% silicone ECNSS-M on Uniport HP (60—80 mesh) (GL Sciences). For column chromatography, Wako-gel C-200 (100—200 mesh), Wako-gel C-300 (200—300 mesh) (Wako Pure Chemical Indus.), Silica gel 60H (15 μm, Merck TA726595), Cosmosil 140C18-OPN (140 μm, Nacalai Tesque), Amberlite XAD-2 (Organo) and Toyopearl HW 40 (Tosoh) were used. For TLC, pre-coated plates of Silica gel $60F_{254}$, RP-18, HP Silica gel $60F_{254}$ and HPRP-18WF_{254s} (Merck) were used and spots were detected under UV_{254} light and/or by spraying with dil. H_2SO_4 followed by heating.

Isolation 1. From the Roots: Dried roots (6.9 kg) of *Clematis stans* collected in the Ishikawa Prefecture, Japan, were extracted three times with hot MeOH. The MeOH extract was concentrated under reduced pressure to give a residue (365 g). The water-soluble part of the residue was partitioned succesively with ether, AcOEt and BuOH. The BuOH-soluble part (38 g), following adsorption on celite (100 g), was chromatographed on silica gel, eluting with $CHCl_3$–MeOH–H_2O (25 : 3 : 0.3→25 : 4 : 0.4→25 : 5 : 0.5→25 : 6 : 0.6→25 : 8 : 1.2→25 : 10 : 1.6 →25 : 11 : 2), to give fr. 1—7. Fraction 1 (1.2 g) was submitted to octadecyl silica (ODS) (50 g) column chromatography (solv., 30% MeOH) and then purified by HPLC [column, YMC-Pack-ODS-A (5 μm, 250 mm × 20 mm i.d.); solv., 32% MeOH] to give **7** (31 mg) and **8** (20 mg). Fraction 2 (0.5 g) was chromatographed on a silica gel (80 g) column (solv., BuOH : AcOEt : H_2O = 2 : 8 : 0.4) and on an ODS (20 g) column (solv., 30% MeOH) and then purified by preparative TLC (RP-18 pre-coated plate, solv., 50% MeOH) to give **9** (25 mg). Fraction 3 (2 g) was chromatographed on an ODS (200 g) column (solv., 50% MeOH) and then purified by medium pressure liquid chromatography (MPLC) [Silica gel 60H (50 g), solv., $CHCl_3$: MeOH : H_2O = 25 : 8 : 1.2] to give **10** (31 mg). Fraction 4 (0.5 g) was purified on an ODS (30 g) column (solv., 70% MeOH) to give **1** (24 mg). Fraction 5 (2 g) was chromatographed on silica-gel (40 g) eluting with $CHCl_3$–MeOH–H_2O (25 : 11 : 2) to give **11** (43 mg). Fraction 6 (0.9 g) was separated by MPLC [Silica gel 60H (50 g), solv., $CHCl_3$: MeOH : H_2O = 25 : 10 : 1.6] to give **2** (60 mg) and 3 (119 mg). Fraction 7 (0.8 g) was chromatographed on a silica gel (100 g) column (solv., $CHCl_3$: MeOH : H_2O = 25 : 10 : 1.6) to give **6** (36 mg) and a mixture of **4** and **5**. This mixture was separated on an ODS (50 g) column (solv., 70% MeOH) and then purified by HPLC [column: YMC-Pack-ODS-A (5 μm, 250 mm × 20 mm i.d.); solv., 28% CH_3CN] to give **4** (19 mg) and **5** (5 mg).

2. From the Leaves: Dried leaves (8 kg) were extracted three times with hot MeOH and treated in the same way as described for the roots to give a BuOH-soluble fraction (90.8 g). The MeOH solution of this was poured into AcOEt to give a precipitate (33.4 g), which was adsorbed on celite (100 g) and submitted to silica gel (2 kg) column chromatography eluting with $CHCl_3$–MeOH–H_2O (25 : 6 : 0.7→25 : 8 : 1.2) to give fr. 1′—7′. Fraction 1′ (4.2 g) was purified repeatedly on XAD-2 (solv., 60% MeOH), Toyopearl (solv., 60% MeOH) and ODS (50 g, solv., 50% MeOH) columns to give **19** (34 mg). Fraction 2′ (2.5 g) was chromatographed on an ODS (250 g) column (solv., 60% MeOH) to give **12** (71 mg). Fraction 3′ (1.2 g) was purified on an ODS (100 g) column (solv., 30% MeOH) to give **20** (14 mg). Fraction 4′ (2.6 g) was chromatographed on an ODS (250 g) column (solv., 60% MeOH) to give **13** (58 mg) and a mixture of **14**, **15** and **16**. This mixture was separated by MPLC [Silica gel 60H (50 g), solv., $CHCl_3$: MeOH : H_2O = 25 : 10 : 1.6] to give **14** (68 mg) and a mixture of **15** and **16** together with a deacetylated product (**6**). The mixture of **15** and **16** was submitted to preparative HPTLC (HP Silica gel $60F_{254}$, solv., PrOH : $CHCl_3$: CH_3CN : AcOEt : H_2O = 1.3 : 1.0 : 3.0 : 0.2 : 1.1) to give **15** (8 mg) and **16** (4 mg). Fraction 5′ (3.5 g) was chromatographed on an ODS (350 g) column (solv., 50% MeOH) to give **17** (108 mg) and a mixture of **2** and **18**. The mixture was separated by HPLC [column: Merck Purospher RP-18 (250 mm × 20 mm i.d.); solv., 36% CH_3CN] to give **2** (30 mg) and **18** (12 mg). Fraction 6′ (12 g) was purified on an ODS (500 g) column (solv., 60% MeOH) to give **6** (7.2 g). Fraction 7′ (0.9 g) was purified on an ODS (100 g) column (solv., 70% MeOH) to give **21** (40 mg).

Acid-Hydrolysis of Saponins A few milligrams of each sample (**1—6**, **12—18**) was dissolved in 2 N H_2SO_4–50% dioxane (0.5—1 ml) and heated on a boiling water bath for 3 h. The reaction mixture was added to 2 ml of water and extracted with $CHCl_3$. The $CHCl_3$ layer was concentrated and the residue was crystallized from MeOH or a mixture of MeOH and $CHCl_3$ to give the aglycone as colorless needles (**1a—5a**, **13a**, **18a**) or colorless prisms (**6a**, **12a**, **14a—17a**). The former was identified as oleanolic acid and the latter as hederagenin by direct comparison (TLC, IR) with authentic specimens. An aqueous solution of the hydrolysate was neutralized with saturated $Ba(OH)_2$ aq. and centrifuged. The supernatant was evaporated and the residue was dissolved in water (1 ml) and treated with $NaBH_4$ (4 mg) for 1 h at room temperature. The reaction mixture was neutralized with AcOH and then concentrated. The residue was acetylated in the usual manner and analyzed by GLC, which revealed the presence of constituent monosaccharides as follows: **1**, **2**, **6**, **12**, **14—16**, rhamnitol (t_R 12′24″), ribitol (t_R 17′17″), arabinitol (t_R 18′50″) and sorbitol (t_R 57′17″); **3**, rhamnitol, ribitol, xylitol (t_R 25′10″) and sorbitol; **4**, rhamnitol, xylitol, sorbitol and galactitol (t_R 49′38″); **5**, rhamnitol, ribitol, sorbitol and galactitol; **13**, **17**, rhamnitol, arabinitol and sorbitol; **18**, rhamnitol, arabinitol, xylitol and sorbitol.

Alkaline-Hydrolysis of Saponins A few milligrams of each sample (**1—3**, **6**, **12—18**) was dissolved in 1 N NaOH aq. (1 ml) and heated on a boiling water bath for 0.5 h. After cooling, a reaction mixture was neutralized with 1 N H_2SO_4 and extracted with BuOH (1 ml × 2). The BuOH layer was washed with water (0.5 ml) and concentrated to give each prosapogenin (**1p—3p**, **6p**, **12p—18p**). Compounds **1p** and **2p** were identified as CP_4[12] by direct comparison. The ^{13}C-NMR signal pattern of 3p coincided with that of huzhangoside A.[4] Compounds **6p**, **12p** and **14p—16p** were identified as CP_6[13] by direct comparison while **13p**, **17p** and **18p** were identified as CP_2,[13] CP_{3b}[17] and CP_3,[12] respectively, by direct comparison.

Clemastanoside A (1) White amorphous powder, $[\alpha]_D^{26}$ −39.3° (c=0.61, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3420, 1740, 1064. HR-FAB-MS m/z: 1401.6656 [M+Na]$^+$ (Calcd for $C_{66}H_{106}NaO_{30}$ 1401.6667). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3.

Deacetylation of 1 A solution of **1** (6 mg) in 0.2 N KOH aq. was left standing overnight at room temperature. The reaction mixture was neutralized with 0.1 N H_2SO_4 and extracted with BuOH. The BuOH layer was washed with water and evaporated to dryness. The residue was dissolved in MeOH and added to AcOEt to give deacetylated **1** (3.1 mg) as a white amorphous powder, which was identified as **2** by direct comparison.

Clemastanoside B (4) White amorphous powder, $[\alpha]_D^{26}$ −29.8° (c=1.26, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3416, 1735, 1062. HR-FAB-MS m/z: 1389.6665 [M+Na]$^+$ (Calcd for $C_{65}H_{106}NaO_{30}$ 1389.6667). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3. Compound **4** (9 mg) was submitted to alkaline hydrolysis with 1 N NaOH aq. as described above to give the prosapogenin (**4p**, 5 mg). Compound **4p**, white amorphous powder, $[\alpha]_D^{28}$ +0.4° (c=0.37, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3412, 1700, 1056. FAB-MS m/z: 935 [M+Na]$^+$. ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3. Compound **4p** (1 mg) was subjected to acid-hydrolysis as described above to give oleanolic acid, galactose, rhamnose and xylose.

Clemastanoside C (5) White amorphous powder, $[\alpha]_D^{27}$ −38.6° (c=0.17, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3436, 1740, 1062. HR-FAB-MS m/z: 1389.6663 [M+Na]$^+$ (Calcd for $C_{65}H_{106}NaO_{30}$ 1389.6667). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3.

Clemastanoside D (12) White amorphous powder, $[\alpha]_D^{22}$ −16.2° (c=2.61, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3448, 1736, 1060. HR-FAB-MS m/z: 1067.5388 [M+Na]$^+$ (Calcd for $C_{52}H_{84}NaO_{21}$ 1067.5403). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3.

Clemastanoside E (14) White amorphous powder, $[\alpha]_D^{21}$ −30.1° (c=1.46, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3448, 1734, 1040. HR-FAB-MS m/z: 1417.6603 [M+Na]$^+$ (Calcd for $C_{66}H_{106}NaO_{31}$ 1417.6616). ^{13}C-NMR: Tables 1, 2. ^1H-NMR : Table 3.

Deacetylation of 14 Compound **14** (5 mg) was deacetylated in the same way as **1** to give deacetylated **14** (2.4 mg) as a white amorphous powder, which was identified as **6** by direct comparison.

Clemastanoside F (15) White amorphous powder, $[\alpha]_D^{28}$ −24.0° (c=0.38, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3450, 1735, 1040. HR-FAB-MS m/z: 1417.6608 [M+Na]$^+$ (Calcd for $C_{66}H_{106}NaO_{31}$ 1417.6616). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3.

Deacetylation of 15 Compound **15** (4 mg) was deacetylated in the same way as **1** to give deacetylated **15** (2 mg) as a white amorphous powder, which was identified as **6** by direct comparison.

Clemastanoside G (16) White amorphous powder, $[\alpha]_D^{30}$ −23.4° (c=0.33, pyridine). IR ν_{max}^{KBr} cm^{-1}: 3450, 1736, 1042. HR-FAB-MS m/z: 1417.6609 [M+Na]$^+$ (Calcd for $C_{66}H_{106}NaO_{31}$ 1417.6616). ^{13}C-NMR: Tables 1, 2. ^1H-NMR: Table 3.

Deacetylation of 16 Compound **16** (2 mg) was deacetylated in the same way as **1** to give deacetylated **16** (*ca.* 1 mg) as a white amorphous powder, which was identified as **6** by direct comparison.

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Identification of Compounds 2, 3, 6, 13, 17 and 18 Compound **2** [white amorphous powder, $[\alpha]_D^{25}$ −41.3° (c=3.63, MeOH)], **3** [white amorphous powder, $[\alpha]_D^{26}$ −38.5° (c=1.29, MeOH)], **6** [white amorphous powder, $[\alpha]_D^{30}$ −31.3° (c=1.47, MeOH)], $[\alpha]_D^{28}$ −24.1° (c=1.39, pyridine)], **13** [white amorphous powder, $[\alpha]_D^{25}$ −28.7° (c=2.63, MeOH)], **17** [white amorphous powder, $[\alpha]_D^{20}$ −20.3° (c=2.73, MeOH)] and **18** [white amorphous powder, $[\alpha]_D^{23}$ −29.5° (c=0.76, MeOH)] were identified as huzhangoside B,[3] huzhangoside C,[3] huzhangoside D,[3] hederasaponin B,[6] kizutasaponin K_{12}[7] and sieboldianoside B,[8] respectively, by comparison of ^{13}C-NMR spectra and specific rotation data with those in the literature. ^{13}C-NMR: Tables 1, 2. ^{1}H-NMR: Table 3.

Clemastanin A (9) White amorphous powder, $[\alpha]_D^{20}$ −44.3° (c=1.19, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 1610, 1520, 1274, 1210. UV λ_{max}^{MeOH} nm (log ε): 216 (4.13), 225 (4.11), 282 (3.74). CD (c=5.39×10^{-5}, MeOH) [θ] (nm): −2810 (287), +10240 (241), −9060 (223). HR-FAB-MS m/z: 531.1844 [M+Na]$^+$ (Calcd for $C_{25}H_{32}NaO_{11}$ 531.1842). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 5. $\Delta[M]_D$: **9**−**9a**=−211°.

Methanolysis of 9 A solution of **9** (1 mg) in 2 N HCl–MeOH (1 ml) was heated under reflux on a water bath for 2 h. After cooling, the reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated and examined by normal-phase silica gel TLC [solv. 1, $CHCl_3$–MeOH–H_2O (25:11:2); solv. 2, PrOH–acetone–H_2O (5:3:1)], which revealed the presence of a methyl glucoside.

Enzymatic Hydrolysis of 9 To a solution of **9** (8 mg) in H_2O (3 ml) was added cellulase (Sigma C-2415, 8 mg) and the mixture stirred for 2 d at 37 °C. The reaction mixture was diluted with H_2O (25 ml) and extracted with AcOEt (30 ml × 3). The AcOEt solution was washed with H_2O, evaporated, and the residue treated with a mixture of acetone and benzene to give **9a** (4.6 mg) as a white amorphous powder. **9a**, $[\alpha]_D^{23}$ +4.2° (c=0.31, acetone). IR ν_{max}^{KBr} cm^{-1}: 3380, 1612, 1520, 1274, 1130, 1032, 854, 806, 682. UV λ_{max}^{MeOH} nm (log ε): 222 sh (4.09), 228 (4.10), 282 (3.75). CD (c=5.39×10^{-5}, MeOH) [θ] (nm): +5650 (295), +5060 (242), −6170 (227). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 5.

Clemastanin B (11) White amorphous powder, $[\alpha]_D^{24}$ −42.7° (c=1.99, H_2O). IR ν_{max}^{KBr} cm^{-1}: 3400, 1590, 1518, 1264, 1226, 1072. UV λ_{max}^{MeOH} nm (log ε): 209 (4.21), 226 (4.20), 276 (3.70). CD (c=4.81×10^{-5}, MeOH) [θ] (nm): −5230 (278), −16680 (228). HR-FAB-MS m/z: 707.2532 [M+Na]$^+$ (Calcd for $C_{32}H_{44}NaO_{16}$ 707.2527). ^{13}C-NMR: Table 4. ^{1}H-NMR: Table 5. $\Delta[M]_D$: **11**−**7**=−160°. $\Delta[M]_D$: **11**−**8**=−119°.

Methanolysis of 11 Compound **11** (1 mg) was subjected to methanolysis and treated in the same manner as **9**. The product was partitioned between AcOEt and H_2O and the H_2O layer was examined by TLC under the same conditions as **9**, which revealed the presence of a methyl glucoside. The AcOEt layer was examined by TLC (solv., $CHCl_3$: MeOH : H_2O = 9 : 1 : 0.1) which showed the presence of **11b**.

Enzymatic Hydrolysis of 11 To a solution of **11** (10.5 mg) in H_2O (3 ml) was added β-glucosidase (P-L-Biochemical Inc. 0211, 9.5 mg) followed by stirring for 43 h at 37 °C. The reaction mixture was treated in the same manner as **9**. The resulting hydrolysate was separated by HPLC [column: YMC-Pack-ODS-A (5 μm, 250 mm × 20 mm i.d.); solv., 25% MeOH] to give **11a** (1.9 mg) and **11b** (2.8 mg). Compound **11a**, a white amorphous powder, $[\alpha]_D^{21}$ +9.5° (c=0.48, acetone). ^{13}C-NMR: Table 4. ^{1}H-NMR : Table 5. Compound **11b**, white amorphous powder, $[\alpha]_D^{23}$ +40.7° (c=0.18, acetone). ^{1}H-NMR (in $CDCl_3$): 1.85 (1H, tdd, J=2.6, 5.5, 10.5 Hz, H-8′), 2.03 (1H, m, H-8), 2.72 (1H, dd, J=4.8, 15.8 Hz, H-7), 2.82 (1H, dd, J=11.4, 15.8 Hz, H′-7), 3.52 (1H, dd, J=5.5, 11.0 Hz, H-9′), 3.726 (1H, dd, J=6.0, 11.0 Hz, H-9), 3.727 (1H, br d, J=10.5 Hz, H-7′), 3.78 (1H, dd, J=2.6, 11.0 Hz, H′-9′), 3.83, 3.85 (each 3H, s, OMe × 2), 3.88 (1H, dd, J=3.0, 11.0 Hz, H′-9), 6.29 (1H, br s, H-3), 6.58 (1H, br s, H-6), 6.59 (1H, d, J=1.8 Hz, H-2′), 6.65 (1H, dd, J=1.8, 8.1 Hz, H-6′), 6.84 (1H, d, J=8.1 Hz, H-5′) . The NMR spectral data and specific rotations of **11a** and **11b** were consistent with those of (+)-lariciresinol[5] and (+)-cyclolariciresinol (isolariciresinol),[16] respectively.

Identification of Known Lignans and Flavonoids Compound **7** [white amorphous powder, $[\alpha]_D^{25}$ −25.3° (c=1.33, MeOH), ^{13}C-NMR: Table 4, ^{1}H-NMR: Table 5], **8** [white amorphous powder, $[\alpha]_D^{22}$ −33.1° (c=1.71, MeOH), ^{13}C-NMR: Table 4, ^{1}H-NMR: Table 5], **10** [white amorphous powder, $[\alpha]_D^{22}$ −30.0° (c=1.11, MeOH), ^{13}C-NMR: Table 4, ^{1}H-NMR: Table 5], **19** [yellow amorphous powder, $[\alpha]_D^{22}$ −11.8° (c=0.11, MeOH)], **20** [yellow amorphous powder, $[\alpha]_D^{20}$ −38.9° (c=0.24, 50% pyridine)] and **21** [yellow amorphous powder, $[\alpha]_D^{20}$ −48.9° (c=0.15, 50% pyridine)] were identified as (+)-lariciresinol 4-O-β-D-glucopyranoside,[5] (+)-lariciresinol 4′-O-β-D-glucopyranoside,[5] (+)-pinoresinol 4,4′-O-bis-β-D-glucopyranoside,[6] isoquercitrin,[10] rutin[10] and quercetin 3-O-β-D-glucuronopyranoside,[11] respectively, by comparison of NMR spectra and specific rotation data with those in the literatures.

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