Clintoniosides A – C, New Polyhydroxylated Spirostanol Glycosides from the Rhizomes of *Clintonia udensis*

by **Yoshihiro Mimaki***[a]) and **Kazuki Watanabe**[b])

[a]) School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1, Horinouchi, Hachioji, Tokyo 192-0392, Japan

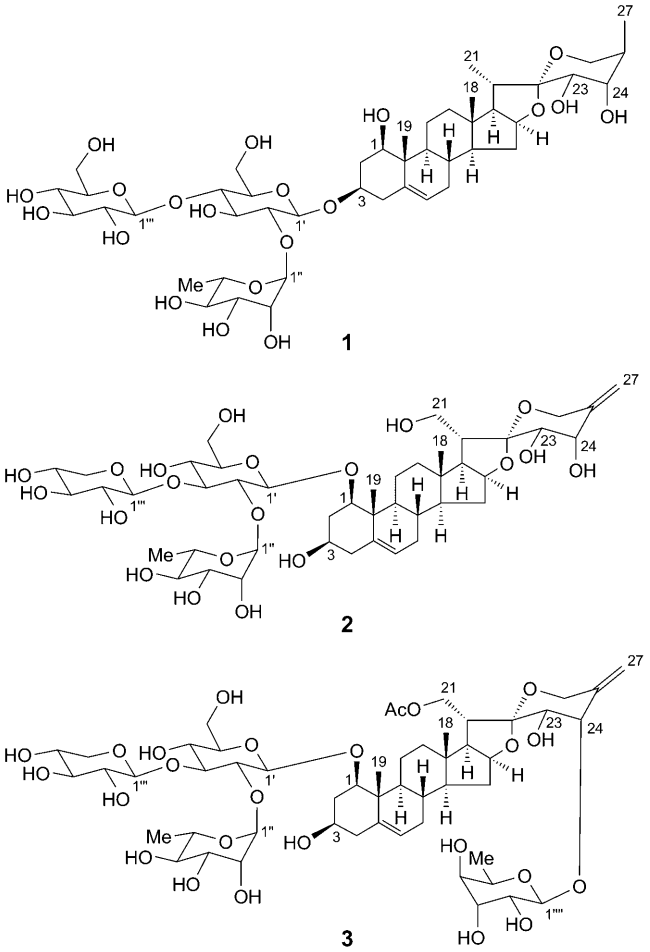
[b]) Faculty of Pharmaceutical Sciences, Aomori University, 2-3-1, Koubata, Aomori, Aomori 030-0943, Japan

Phytochemical analyses were carried out on the rhizomes of *Clintonia udensis* (Liliaceae) with particular attention paid to the steroidal glycoside constituents, resulting in the isolation of three new polyhydroxylated spirostanol glycosides, named clintonioside A (**1**), B (**2**), and C (**3**). On the basis of their spectroscopic data, including 2D-NMR spectroscopy, in combination with acetylation and hydrolytic cleavage, the structures of **1**–**3** were determined to be (1*β*,3*β*,23*S*,24*S*,25*R*)-1,23,24-trihydroxyspirost-5-en-3-yl *O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranoside (**1**), (1*β*,3*β*,23*S*,24*S*)-3,21,23,24-tetrahydroxyspirosta-5,25(27)-dien-1-yl *O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranoside (**2**), and (1*β*,3*β*,23*S*,24*S*)-21-(acetyloxy)-24-[(6-deoxy-*β*-D-gulopyranosyl)oxy]-3,23-dihydroxyspirosta-5,25(27)-dien-1-yl *O*-*α*-L-rhamnopyranosyl-(1 → 2)-*O*-[*β*-D-xylopyranosyl-(1 → 3)]-*β*-D-glucopyranoside (**3**).

Introduction. – *Clintonia udensis* TRAUTV. et C. A. MEY. is a perennial plant belonging to the family Liliaceae, and is distributed in northeast China, the Korean Peninsula, and Japan. The rhizomes of *C. udensis* have long been used in traditional Chinese medicine for the treatment of blow and fatigue [1], and a few steroidal sapogenins such as diosgenin and heloniogenin have been detected in the plant [2][3]. As part of our continuing investigation of plants of the family Liliaceae [4–8], a phytochemical analysis of the rhizomes of *C. udensis* was conducted, special attention being paid to the steroidal glycoside constituents, which resulted in the isolation of three new polyhydroxylated spirostanol glycosides, named clintonioside A (**1**), B (**2**), and C (**3**). This article reports the structure determination of the new compounds on the basis of their spectroscopic data, including 2D-NMR spectroscopy, in combination with acetylation and hydrolytic cleavage.

Results and Discussion. – The MeOH extract of *C. udensis* rhizomes was passed through a porous-polymer polystyrene resin (*Diaion HP-20*) column successively eluted with 30% MeOH, MeOH, EtOH, and AcOEt. The MeOH-eluate fraction was repeatedly subjected to column chromatography (silica gel and octadecylsilanized (ODS) silica gel), as well as to prep. HPLC, to give clintonioside A (**1**; 14.5 mg), B (**2**; 70 mg), and C (**3**; 20.0 mg).

Clintonioside A (**1**) was obtained as amorphous solid which exhibited an $[M + Na]^+$ peak at *m*/*z* 955.4451 in the HR-ESI-TOF-MS, consistent with the molecular formula



$C_{45}H_{72}O_{20}$. The glycosidic nature of **1** was suggested by strong absorption bands at 3378 and 1051 cm^{-1} in the IR spectrum. Acid hydrolysis of **1** with 0.5M HCl in dioxane/H_2O 1:1 gave D-glucose and L-rhamnose, while the labile aglycone was decomposed under acidic conditions. Identification of the monosaccharides, including their absolute configurations, was carried out by direct HPLC analysis of the hydrolysate by using a combination of refractive-index (r.i.) and optical-rotation (o.r.) detectors. Interpretation of the ^{1}H- and ^{13}C-NMR (*Table 1*), ^{1}H,^{1}H-COSY (*Fig. 1*), HMQC, TOCSY, HMBC (*Fig. 1*), and NOESY data (*Fig. 2*) of **1**, and acetylation of **1** with Ac_2O/pyridine 1:1 followed by spectroscopic analysis of the peracetate derivative allowed the structure of the aglycone moiety to be assigned as (1*β*,3*β*,23*S*,24*S*,25*R*)-spirost-5-ene-1,3,23,24-tetrol. These data are consistent with the structure (1*β*,3*β*,23*S*,24*S*,25*R*)-1,23,24-trihydroxyspirost-5-en-3-yl *O*-*β*-D-glucopyranosyl-(1 → 4)-*O*-[*α*-L-rhamnopyranosyl-(1 → 2)]-*β*-D-glucopyranoside, which was given to clintonioside A (**1**).

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of Clintonioside A* (**1**). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.69 (*dd*, J = 11.9, 4.3)	77.9	Glc:		
CH_2(2)	2.60 (*dd*, J = 11.9, 4.3, H_{eq}),	40.8	H–C(1)	4.98 (*d*, J = 7.5)	100.1
	2.31 (*q*-kike, J = 11.9, H_{ax})		H–C(2)	4.20 (*dd*, J = 8.5, 8.1)	77.2
H–C(3)	3.99 (*m*, $w_{1/2}$ = 18.1)	75.1	H–C(3)	4.23 (*t*-like, J = 8.5)	77.7
CH_2(4)	2.75 (*dd*, J = 11.9, 4.9, H_{eq}),	39.4	H–C(4)	4.21 (*t*-like, J = 8.5)	82.0
	2.80 (*t*-like, J = 11.9, H_{ax})		H–C(5)	3.83 (*ddd*, J = 8.5, 3.8, 2.5)	76.2
C(5)		139.0	CH_2(6)	4.50 (*dd*, J = 12.1, 3.8, H_a),	62.1
H–C(6)	5.50 (br. *d*, J = 5.5)	125.1		4.38 (*dd*, J = 12.1, 2.5, H_b)	
CH_2(7)	1.87 (H_{eq}), 1.53 (H_{ax})	32.3			
H–C(8)	1.54–1.62 (*m*)	32.8	Rha:		
H–C(9)	1.31–1.37 (*m*)	51.2	H–C(1)	6.25 (*d*, J = 1.3)	101.8
C(10)		43.7	H–C(2)	4.74 (*dd*, J = 3.2, 1.3)	72.5
CH_2(11)	1.78 (H_{eq}), 1.26 (H_{ax})	24.1	H–C(3)	4.59 (*dd*, J = 9.4, 3.2)	72.8
CH_2(12)	2.86 (H_{eq}), 1.72 (H_{ax})	40.8	H–C(4)	4.33 (*t*-like, J = 9.4)	74.1
C(13)		40.6	H–C(5)	4.95 (*dq*, J = 9.4, 6.2)	69.5
H–C(14)	1.11–1.17 (*m*)	56.8	Me(6)	1.71 (*d*, J = 6.2)	18.6
CH_2(15)	2.00 (H_α), 1.45 (H_β)	32.3			
H–C(16)	4.63 (*q*-like, J = 8.6)	82.9	Glc′:		
H–C(17)	1.81 (*dd*, J = 8.6, 7.1)	61.5	H–C(1)	5.12 (*d*, J = 7.9)	105.2
Me(18)	1.06 (*s*)	16.7	H–C(2)	4.05 (*dd*, J = 8.5, 7.9)	75.0
Me(19)	1.32 (*s*)	13.7	H–C(3)	4.20 (*dd*, J = 9.1, 8.5)	78.3
H–C(20)	2.93–2.99 (*m*)	37.2	H–C(4)	4.27 (*t*-like, J = 9.1)	71.2
Me(21)	1.15 (*d*, J = 7.0)	14.5	H–C(5)	3.96 (*ddd*, J = 9.1, 5.8, 2.4)	78.5
C(22)		113.1	CH_2(6)	4.46 (*dd*, J = 11.9, 2.4, H_a),	61.8
H–C(23)	3.99 (*d*, J = 3.6)	64.8		4.38 (*dd*, J = 11.9, 5.8, H_b)	
H–C(24)	4.13–4.15 (*m*)	73.8			
H–C(25)	2.06–2.10 (*m*)	37.9			
CH_2(26)	3.33 (br. *d*, J = 11.2, H_{eq}),	59.7			
	4.42 (*dd*, J = 11.2, 2.1, H_{ax})				
Me(27)	1.09 (*d*, J = 7.4)	15.6			

The ^{1}H-NMR spectrum of **1** displayed two *s* at δ(H) 1.32 and 1.06 (each 3 H), indicating the presence of two angular Me groups, two *d* at δ(H) 1.15 (J = 7.0 Hz, 3 H) and 1.09 (J = 7.4 Hz, 3 H) assignable to secondary Me groups, and an olefinic H-atom at δ(H) 5.50 (br. *d*, J = 5.5 Hz), as well as signals for three anomeric H-atoms at δ(H) 6.25 (*d*, J = 1.3 Hz), 5.12 (*d*, J = 7.9 Hz), and 4.98 (*d*, J = 7.5 Hz). The Me signals at δ(H) 1.71 (*d*, J = 6.2 Hz) and δ(C) 18.6 were indicative of a 6-deoxyhexopyranosyl unit. These data, along with those of the three anomeric C-atoms (δ(C) 105.2 (CH), 101.8 (CH), and 100.1 (CH)) and of one distinctive acetal C-atom (δ(C) 113.1 (C)) [9] led to the hypothesis that **1** is a spirostanol glycoside with three monosaccharide units. The ^{13}C-NMR spectrum of **1** (*Table 1*) showed a total of 45 resonance lines, 18 of which were attributed to the three monosaccharide units. This implied a molecular formula $C_{27}H_{42}O_6$ for the aglycone moiety, suggesting a highly oxygenated spirostanol derivative. In the ^{1}H,^{1}H-COSY plot of **1**, the *m* centered at δ(H) 3.99 ($w_{1/2}$ = 18.1 Hz, H–C(3)) was coupled to the signals of two CH_2 groups at δ(H) 2.80 (*t*-like, J = 11.9 Hz, H_{ax}–C(4)) and 2.75 (*dd*, J = 11.9, 4.9 Hz, H_{eq}–C(4)), and at δ(H) 2.60 (*dd*, J = 11.9, 4.3 Hz, H_{eq}–C(2)) and 2.31 (*q*-like, J = 11.9 Hz, H_{ax}–C(2)). The CH_2(2) signals exhibited ^{1}H,^{1}H-spin coupling with the *dd* of an oxygenated CH moiety at δ(H) 3.69 (J = 11.9, 4.3 Hz, H–C(1)), while the CH_2(4) signals showed no additional correlations. In the HMBC plot, the Me group at δ(H) 1.32 (Me(19)) showed long-range correlations

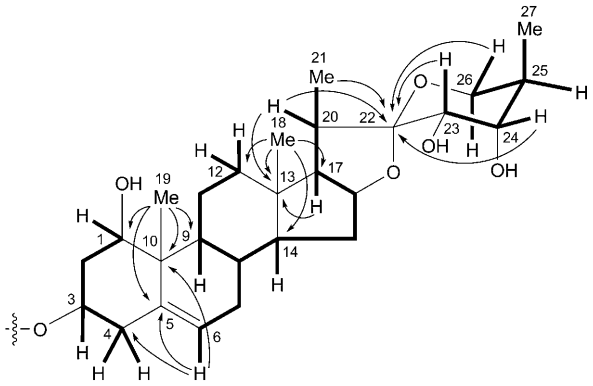


Fig. 1. *^{1}H,^{1}H-COSY* (bold lines) *and HMBC* (arrows) *of the aglycone moiety of* **1**

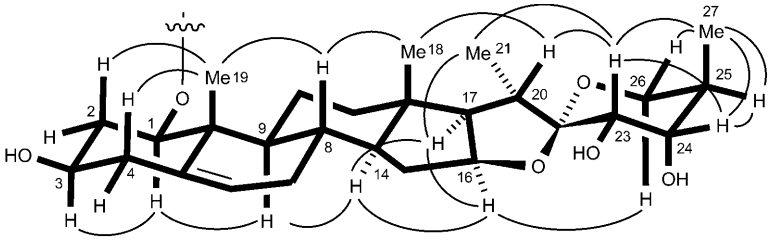


Fig. 2. *NOE Correlations of the aglycone moiety of* **1**

with not only its linked C-atom at δ(C) 43.7 (C(10)) but also with the oxygenated CH moiety at δ(C) 77.9 (C(1)) and the olefinic C-atom at δ(C) 139.0 (C(5)). On the other hand, the olefinic H-atom at δ(H) 5.50 (H–C(6)) exhibited long-range correlations with the CH_2 moiety at δ(C) 39.4 (C(4)) and the olefinic C(5). These findings indicated the presence of an O-atom at C(1) and C(3), a C=C bond between C(5) and C(6), and a Me group at C(10). The ^{1}H,^{1}H-COSY (*Fig. 1*) and 2D-TOCSY cross-peaks were analyzed starting with the olefinic H–C(6) and Me(21) (δ(H) 1.15 (*d*, J = 7.0 Hz)). In the HMBC spectrum, the cross-peaks δ(H) 1.06 (Me(18))/δ(C) 40.6 (C(13)), 40.8 (C(12)), 56.8 (C(14)), and 82.9 (C(17)) were present (*Fig. 1*). These data led to the construction of the rings *B*–*E*, with a Me group at C(13). The ring-*F* portion was established as follows: Me(27) at δ(H) 1.09 (J = 6.8 Hz) showed ^{1}H,^{1}H-spin-coupling with H–C(25) (br. *m* at δ(H) 2.06–2.10). Me(27) also exhibited correlations with the oxygenated CH_2 moiety at δ(H) 4.42 (*dd*, J = 11.2, 2.1 Hz, H_{ax}–C(26)) and 3.33 (br. *d*, J = 11.2 Hz, H_{eq}–C(26)) and with the oxygenated CH group at δ(H) 4.13–4.15 (*m*, H–C(24)). H–C(24), in turn, displayed a correlation with another oxygenated CH moiety at δ(H) 3.99 (*d*, J = 3.6 Hz, H–C(23)). These subsequent correlations allowed the ring-*F* fragment of **1** to be assigned as –CH(23)(O–)–CH(24)(O–)–CH(25)(Me(27))–CH_2(26)–O–. The HMBC cross-peaks C(22) (δ(C) 113.1/H–C(20), Me(21), H–C(23), H–C(24), and H_{eq}–C(26) suggested that ring *E* is linked to ring *F via* the acetal atom C(22).

In the phase-sensitive NOESY experiment, the correlations H–C(8)/Me(18) and Me(19), H–C(14)/H–C(9), H–C(16), and H–C(17), H–C(17)/H–C(16) and Me(21), and Me(18)/H–C(20) provided evidence for the usual steroid ring fusions (*B*/*C trans*, *C*/*D trans*, and *D*/*E cis*) and the (20α) configuration (*Fig. 2*). The β-equatorial orientations at C(1) and C(3) were revealed by the coupling constants of H–C(1) (*dd*, J = 11.9, 4.3 Hz) and H–C(3) (*m*, $w_{1/2}$ = 18.1 Hz), and were supported by the NOEs H–C(1)/H–C(3) and H–C(9). The NOEs H–C(16)/H_{ax}–C(26), H–C(23)/H–C(20), Me(21), H–C(24), and Me(27), and H–C(24)/H–C(23), H–C(25), and Me(27), in addition

to the small J(H–C(23), H–C(24)) (3.6 Hz) and J(H–C(25), H_{ax}–C(26)) (2.1 Hz), supported the (22α,23*S*,24*S*,25*R*) configuration. When the ^{1}H-NMR spectrum of the dodecaacetate derivative of **1** was compared to that of **1**, the H–C(1), H–C(23), and H–C(24) signals were moved downfield by 1.26, 0.79, and 1.34 ppm to δ(H) 4.95, 4.78, and 5.48, respectively, whereas the δ(H) of H–C(3) was almost unaffected. These findings indicated that C(1), C(23), and C(24) of **1** have a free OH group, and that C(3) is glycosylated. The ^{1}H- and ^{13}C-NMR data and the results of the acid hydrolysis implied that the glycoside moiety of **1** is composed of a terminal α-L-rhamnopyranosyl unit (Rha), a terminal β-D-glucopyranosyl unit (Glc′), and a 2,4-disubstituted β-D-glucopyranosyl unit (Glc). In the HMBC spectrum, the cross-peaks H–C(1)(Rha)/C(2)(Glc), H–C(1)(Glc)/C(3)(aglycone), and H–C(1)(Glc′)/C(4)(Glc) established the linkages between the sugar units and their linkage to the aglycone.

Clintonioside B (**2**), obtained as an amorphous solid, exhibited a molecular formula $C_{44}H_{68}O_{20}$ as deduced from the HR-ESI-TOF-MS (*m*/*z* 939.4272 ([M + Na]$^+$)). The ^{1}H- and ^{13}C-NMR spectra of **2** (*Table 2*) were similar to those of **1**; however, slight differences could be recognized in the signals arising from both the aglycone and sugar moieties. Acid hydrolysis of **2** with 0.5M HCl in dioxane/H_2O 1:1 gave D-xylose, D-glucose, and L-rhamnose. Comparison of the NMR data of **2** with those of **1** and the polyoxygenated steroidal saponins from *Helleborus orientalis* [10], and analysis of the ^{1}H,^{1}H-COSY, HMQC, HMBC (*Fig. 3*), and NOESY data (*Fig. 3*) of **2** allowed the structure of the aglycone of **2** to be identified as (1β,3β,23*S*,24*S*)-spirosta-5,25(27)-diene-1,3,21,23,24-pentol. The structure of **2** was finally established as (1β,3β,23*S*,24*S*)-3,21,23,24-tetrahydroxyspirosta-5,25(27)-dien-1-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside.

In the ^{1}H-NMR spectrum of **2**, the *d*s of the two secondary Me groups of **1** at δ(H) 1.15 and 1.09 were replaced by the signals of a CH_2OH (δ(H) 4.20 (*dd*, J = 10.5, 6.9 Hz) and 4.02–4.06 (*m*)) and an exocyclic CH_2=C group (δ(H) 5.06 and 4.96 (each br. *s*)). The C*H*$_2$OH group showed ^{1}H,^{1}H-spin couplings with H–C(20) at δ(H) 3.44 (*q*-like, J = 6.9 Hz) in the ^{1}H,^{1}H-COSY plot, and a long-range correlation with the acetal atom C(22) at δ(C) 112.3 in the HMBC spectrum (*Fig. 3*). The H-atom of the CH_2=C group at δ(H) 5.06 showed long-range correlations with the olefinic C(25) at δ(C) 146.3 and the OH-substituted C(24) at δ(C) 74.1, whereas the H-atom of the CH_2=C group at δ(H) 4.96 was correlated with C(25) and the oxygenated C(26) at δ(C) 60.7. HMBC Cross-peaks C(22)/H–C(20), H–C(23) (δ(H) 4.42), H–C(24) (δ(H) 4.69), and H_{eq}–C(26) (δ(H) 4.02) were also observed. Thus, the presence of an OH group at C(21) and the C(25)=CH_2(27) bond in **2** was established. The NOE correlations H–C(16)/H_{ax}–C(26), H–C(20)/Me(18) and H–C(23), and H–C(23)/H–C(24), as well as a small J(H–C(23), H–C(24)) (4.0 Hz) are consistent with the (20α,22α,23*S*,24*S*) configuration. The δ(C) of C(1) of **1** was moved downfield by 6.4 ppm to δ(C) 84.3 in **2**, whereas that of C(3) was displaced upfield by 7.0 ppm to δ(C) 68.1, suggesting that a triglycoside group is linked to C(1) of the aglycone of **2**. The ^{1}H- and ^{13}C-NMR data and the results of the acid hydrolysis indicated that the glycoside moiety of **2** is composed of a terminal α-L-rhamnopyranosyl unit (Rha), a terminal β-D-xylopyranosyl unit (Xyl), and a 2,3-disubstituted β-D-glucopyranosyl unit (Glc). Their linkages were established by the HMBC cross-peaks H–C(1)(Rha)/C(2)(Glc), H–C(1)(Glc)/C(1)(aglycone), and H–C(1)(Xyl)/C(3)(Glc).

Clintonioside C (**3**) gave an [M + Na]$^+$ ion peak at *m*/*z* 1127.4843 in the HR-ESI-TOF-MS, indicating that **3** has a molecular formula $C_{52}H_{80}O_{25}$. The spectral data of **3** were essentially analogous those of **2**, suggesting the presence of the same aglycone, except for an additional acetyl group. Comparison of the NMR data of **3** (*Table 3*) with those of the structurally related saponins from *Helleborus orientalis* [10], and interpretation of the ^{1}H,^{1}H-COSY, HMQC, TOCSY, HMBC, and NOESY data of **3**, combined with the results of acid hydrolysis followed by chromatographic analysis

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of Clintonioside B* (**2**). δ in ppm, J in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H–C(1)	3.84 (*dd*, J = 12.0, 4.0)	84.3	Glc:		
CH_2(2)	2.64 (*dd*, J = 12.0, 4.0, H_{eq}), 2.41 (*q*-like, J = 12.0, H_{ax})	37.8	H–C(1)	4.77 (*d*, J = 7.7)	100.0
			H–C(2)	4.12 (*dd*, J = 9.1, 7.7)	76.2
H–C(3)	3.76 (*m*, $w_{1/2}$ = 21.7)	68.1	H–C(3)	4.03 (*t*-like, J = 9.1)	88.4
CH_2(4)	2.52 (*dd*, J = 12.0, 4.8, H_{eq}), 2.65 (*t*-like, J = 12.0, H_{ax})	43.7	H–C(4)	3.76–3.80 (*m*)	70.2
			H–C(5)	3.75–3.79 (*m*)	77.7
C(5)		139.3	CH_2(6)	4.45 (br. *d*, J = 10.5, H_a), 4.16 (*dd*, J = 10.5, 4.6, H_b)	63.2
H–C(6)	5.52 (br. *d*, J = 5.8)	124.7			
CH_2(7)	1.84 (H_{eq}), 1.52 (H_{ax})	31.8			
H–C(8)	1.51–1.59 (*m*)	33.1	Rha:		
H–C(9)	1.60–1.66 (*m*)	50.2	H–C(1)	6.40 (br. *s*)	101.6
C(10)		42.7	H–C(2)	4.79 (br. *d*, J = 3.3)	72.4
CH_2(11)	2.84 (H_{eq}), 1.63 (H_{ax})	24.1	H–C(3)	4.58 (*dd*, J = 9.1, 3.3)	72.4
CH_2(12)	1.95 (H_{eq}), 1.52 (H_{ax})	40.4	H–C(4)	4.30 (*dd*, J = 9.5, 9.1)	74.1
C(13)		40.9	H–C(5)	4.81 (*dq*, J = 9.5, 6.1)	69.5
H–C(14)	1.22–1.28 (*m*)	57.1	Me(6)	1.71 (*d*, J = 6.1)	19.2
CH_2(15)	1.95 (H_α), 1.47 (H_β)	32.4			
H–C(16)	4.59 (*q*-like, J = 8.6)	83.6	Xyl:		
H–C(17)	2.04 (*dd*, J = 8.6, 6.9)	58.0	H–C(1)	4.93 (*d*, J = 7.7)	105.2
Me(18)	1.15 (*s*)	17.1	H–C(2)	3.96 (*dd*, J = 8.4, 7.7)	74.7
Me(19)	1.36 (*s*)	15.0	H–C(3)	4.10–4.14 (*m*)	78.3
H–C(20)	3.44 (*q*-like, J = 6.9)	45.9	H–C(4)	4.10–4.14 (*m*)	70.6
CH_2(21)	4.20 (*dd*, J = 10.5, 6.9, H_a), 4.02–4.06 (*m*, H_b)	62.2	CH_2(5)	4.24 (*dd*, J = 10.5, 4.4, H_a), 3.68 (*t*-like, J = 10.5, H_b)	67.2
C(22)		112.3			
H–C(23)	4.42 (*d*, J = 4.0)	71.1			
H–C(24)	4.69 (*d*, J = 4.0)	74.1			
C(25)		146.3			
CH_2(26)	4.02 (*d*, J = 12.2, H_{eq}), 4.85 (*d*, J = 12.2, H_{ax})	60.7			
CH_2(27)	5.06 (br. *s*, H_a), 4.96 (br. *s*, H_b)	112.4			

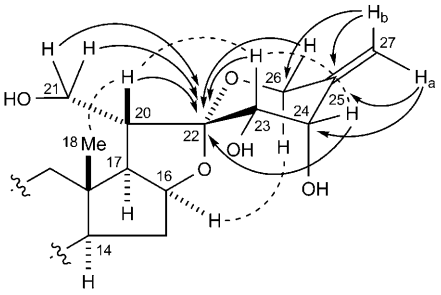


Fig. 3. *Key HMBC* (arrows) *and NOE correlations* (dotted lines) *of the aglycone moiety of* **2**

(→6-deoxy-D-gulose, D-glucose, L-rhamnose, and D-xylose), established the structure of **3** as (1β,3β,23*S*,24*S*)-21-(acetyloxy)-24-[(6-deoxy-β-D-gulopyranosyl)oxy]-3,23-di-

hydroxyspirosta-5,25(27)-diene-1-yl *O*-α-L-rhamnopyranosyl-(1 → 2)-*O*-[β-D-xylopyranosyl-(1 → 3)]-β-D-glucopyranoside.

Table 3. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; (D_5)pyridine) *of Clintonioside C* (**3**). δ in ppm, *J* in Hz.

	δ(H)	δ(C)		δ(H)	δ(C)
H−C(1)	3.81 (*dd*, *J* = 12.0, 4.0)	84.7	Glc:		
CH_2(2)	2.65 (*dd*, *J* = 12.0, 4.0, H_{eq}),	38.0	H−C(1)	4.75 (*d*, *J* = 7.7)	100.3
	2.42 (*q*-like, *J* = 12.0, H_{ax})		H−C(2)	4.11 (*dd*, *J* = 8.8, 7.7)	76.1
H−C(3)	3.77 (*m*, $w_{1/2}$ = 19.9)	68.0	H−C(3)	4.02 (*t*-like, *J* = 8.8)	88.4
CH_2(4)	2.54 (*dd*, *J* = 11.6, 4.2, H_{eq}),	43.7	H−C(4)	3.81 (*dd*, *J* = 9.1, 8.8)	70.0
	2.67 (*t*-like, *J* = 11.6, H_{ax})		H−C(5)	3.76 (*ddd*, *J* = 9.1, 5.5, 1.8)	77.6
C(5)		139.4	CH_2(6)	4.47 (*dd*, *J* = 11.5, 1.8, H_a),	63.1
H−C(6)	5.54 (br. *d*, *J* = 5.7)	124.7		4.20 (*dd*, *J* = 11.5, 5.5, H_b)	
CH_2(7)	1.78 (H_{eq}), 1.51 (H_{ax})	31.7			
H−C(8)	1.41 – 1.49 (*m*)	33.0	Rha:		
H−C(9)	1.59 – 1.65 (*m*)	50.2	H−C(1)	6.38 (br. *s*)	101.6
C(10)		42.7	H−C(2)	4.76 (br. *d*, *J* = 3.3)	72.3
CH_2(11)	2.90 (H_{eq}), 1.61 (H_{ax})	24.0	H−C(3)	4.58 (*dd*, *J* = 9.4, 3.3)	72.3
CH_2(12)	1.85 (H_{eq}), 1.47 (H_{ax})	39.9	H−C(4)	4.29 (*t*-like *J* = 9.4)	74.2
C(13)		41.0	H−C(5)	4.80 (*dq*, *J* = 9.4, 6.2)	69.5
H−C(14)	1.12 – 1.18 (*m*)	57.0	Me(6)	1.70 (*d*, *J* = 6.2)	19.2
CH_2(15)	1.81 (H_a), 1.44 (H_b)	32.3			
H−C(16)	4.53 (*q*-like, *J* = 8.6)	83.6	Xyl:		
H−C(17)	1.85 (*dd*, *J* = 8.6, 6.0)	58.6	H−C(1)	4.92 (*d*, *J* = 7.6)	105.2
Me(18)	1.05 (*s*)	16.8	H−C(2)	3.95 (*dd*, *J* = 9.1, 7.6)	74.7
Me(19)	1.38 (*s*)	15.0	H−C(3)	4.08 – 4.12 (*m*)	78.3
H−C(20)	3.22 – 3.28 (*m*)	42.6	H−C(4)	4.09 – 4.13 (*m*)	70.5
CH_2(21)	4.38 (*dd*, *J* = 10.6, 8.9, H_a),	65.0	CH_2(5)	4.24 (*dd*, *J* = 11.2, 4.3, H_a),	67.2
	4.33 (*dd*, *J* = 10.6, 6.5, H_b)			3.67 (*t*-like, *J* = 11.2, H_b)	
C(22)		111.0			
H−C(23)	4.14 (*d*, *J* = 4.1)	71.4	6-deoxy-Gul:		
H−C(24)	4.76 (*d*, *J* = 4.1)	82.1	H−C(1)	5.68 (*d*, *J* = 8.2)	103.9
C(25)		143.6	H−C(2)	4.54 (*dd*, *J* = 8.2, 3.0)	70.3
CH_2(26)	3.98 (*d*, *J* = 12.1, H_{eq}),	61.5	H−C(3)	4.74 (*dd*, *J* = 3.5, 3.0)	73.5
	4.84 (*d*, *J* = 12.1, H_{ax})		H−C(4)	4.09 (br. *d*, *J* = 3.5)	73.4
CH_2(27)	5.22 (br. *s*, H_a), 5.09 (br. *s*, H_b)	113.9	H−C(5)	4.54 (*q*-like, *J* = 6.5)	69.9
Ac	1.95 (*s*)	170.8, 20.9	Me(6)	1.46 (*d*, *J* = 6.5)	16.8

The molecular formula of **3** was higher than that of **2** by $C_8H_{12}O_5$, and the ^{1}H-NMR spectrum of **3** showed four anomeric H-atoms at δ(H) 6.38 (br. *s*), 5.68 (*d*, *J* = 8.2 Hz), 4.92 (*d*, *J* = 7.6 Hz), and 4.75 (*d*, *J* = 7.7 Hz), as well as two secondary Me groups at δ(H) 1.70 (*d*, *J* = 6.2 Hz) and 1.46 (*d*, *J* = 6.5 Hz), suggesting that **3** structurally corresponded to **2** with two instead of one 6-deoxyhexosyl group. The presence of an Ac group was inferred from the IR (1720 cm^{-1}) and NMR data (δ(H) 1.95 (*s*, 3 H); δ(C) 170.8 and 20.9).

The ^{1}H,^{1}H-COSY and TOCSY data established the ^{1}H,^{1}H-spin-coupling correlations and multiplet patterns of the additional 6-deoxyhexosyl H-atoms which exhibited a large *J*(H−C(1), H−C(2)) (8.2 Hz), a small *J*(H−C(2), H−C(3)) (3.0 Hz) and *J*(H−C(3), H−C(4)) (3.5 Hz), and a very small *J*(H−C(4), H−C(5)) (< 0.5 Hz). The H-atoms of the additional 6-deoxyhexosyl unit were correlated to the one-bond coupled C-atoms in the HMQC spectrum, resulting in the assignments of C(1) – C(6) to

δ(C) 103.9, 70.3, 73.5, 73.4, 69.9, and 16.8, resp. The acid hydrolysis of **3** suggested that the 6-deoxyhexose was 6-deoxy-β-D-gulopyranose (6-deoxy-Gul). In the HMBC spectrum, a cross-peak H–C(1)(6-deoxy-Gul)/C(24)(aglycone) confirmed that the deoxysugar is linked to C(24). The triglycoside moiety attached at C(1) of the aglycone was shown to be the same as that of **2** by the HMBC cross-peaks H–C(1)(Rha)/C(2)(Glc), H–C(1)(Xyl)/C(3)(Glc), and H–C(1)(Glc)/C(1)(aglycone). The position of the acetyl group was determined by the HMBC cross-peaks C=O(Ac)/CH_2(21)(aglycone).

Clintoniosides A–C (**1**–**3**) are new polyhydroxylated spirostanol saponins. Although a number of steroidal glycosides have been isolated from higher plants [11], several polyhydroxylated spirostanol saponins structurally related to clintoniosides A–C have been detected in a limited species of the monocotyledonous plants such as in *Ornithogalum thyrsoides* (Liliaceae) [12], *Polygonatum sibiricum* (Liliaceae) [13], *Dracaena draco* (Agavaceae) [14], *Ruscus aculeatus* (Liliaceae) [15], *Brodiaea californica* (Liliaceae) [16], *Sansevieria trifasciata* (Agavaceae) [17], *Nolina recurvata* (Agavaceae) [18], and *Ophiopogon japonicus* (Liliaceae) [19], except for those isolated from *Helleborus orientalis*, a dicotyledonous plant belonging to the Ranunculaceae family [10].

Experimental Part

General. TLC: precoated silica-gel *60-F_{254}* (SiO_2, 0.25 mm; *Merck*, Darmstadt, Germany), and *RP-18-F_{254}-S* (0.25 mm; *Merck*) plates; visualization by spraying with 10% H_2SO_4 soln., followed by heating. Column chromatography (CC): *Diaion HP-20* (*Mitsubishi-Chemical*, Tokyo, Japan), SiO_2 (*Fuji-Silysia Chemical*, Aichi, Japan), and ODS SiO_2 (*Nacalai Tesque*, Kyoto, Japan). Anal. HPLC: *CCPM* pump (*Tosoh*, Tokyo, Japan), *CCP-PX-8010* controller (*Tosoh*), *RI-8010* detector (*Tosoh*), *Shodex-OR-2* detector (*Showa-Denko*, Tokyo, Japan), and *Rheodyne* injection port; t_R in min; r.i. = refractive index, o.r. = optical rotation. Prep. HPLC: *Capcell-Pak-C_{18}-UG120* column (10 mm i.d. × 250 mm, 5 μm; *Shiseido*, Tokyo, Japan); flow rate 1.0 ml/min; t_R in min. Optical rotations: *Jasco DIP-360* (Tokyo, Japan) automatic digital polarimeter. IR Spectra: *Jasco FT-IR 620* spectrophotometer; ν in cm^{-1}. NMR Spectra: *Bruker DRX-500* spectrometer (Karlsruhe, Germany); at 500 (^1H) or 125 MHz (^{13}C); standard *Bruker* pulse programs; (D_5)pyridine solns.; chemical shifts δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. MS: *Micromass-LCT* spectrometer (*Waters*, Manchester, UK); in *m*/*z*.

Plant Material. The rhizomes of *C. udensis* were collected in Yunnan Province, P. R. China, in October 1999. The plant was identified by Dr. *Yutaka Sashida*, emer. professor of Tokyo University of Pharmacy and Life Sciences. A voucher specimen was deposited with our laboratory (voucher No. 99-10-011-CU).

Extraction and Isolation. The rhizomes of *C. udensis* (1.0 kg of dry weight) was extracted with MeOH (9 l) under reflux for 3 h. After the removal of the solvent, the MeOH extract (150 g) was subjected to CC (*Diaion HP-20* (1.6 kg), 80.0 mm i.d. × 400 mm column), 30% MeOH, MeOH, EtOH, and then AcOEt (each 10 l)). The MeOH-eluted portion (51.0 g) was subjected to CC (SiO_2 (1.0 kg), 80 mm i.d. × 300 mm column, stepwise gradient $CHCl_3$/MeOH 9 : 1, 6 : 1, 4 : 1, 2 : 1, and 0 : 1 (each 2 l)): *Fractions I–VI*. *Fr. VI* was subjected by prep. HPLC (MeCN/H_2O 1 : 2) to give **1**–**3** with a few impurities, which were further purified by prep. HPLC (MeCN/H_2O 1 : 3): **1** (14.5 mg; t_R 82–85), **2** (7.0 mg; t_R 72–74), and **3** (20.0 mg; t_R 60–62).

Clintonioside A (= *(1β,3β,23*S*,24*S*,25*R*)-1,23,24-Trihydroxyspirost-5-en-3-yl* O-*β*-D-*Glucopyranosyl-(1 → 4)*-O-*[α*-L-*rhamnopyranosyl-(1 → 2)]-β*-D-*glucopyranoside* = *(1β,3β,23*S*,24*S*,25*R*)-1,23,24-Trihydroxyspirost-5-en-3-yl* O-*6-Deoxy-α*-L-*mannopyranosyl-(1 → 2)*-O-*[β*-D-*glucopyranosyl-(1 → 4)]-β*-D-*glucopyranoside*; **1**). Amorphous solid. $[\alpha]_D^{22} = -92.0$ ($c = 0.10$, MeOH). IR (film): 3378 (OH), 2967 and 2909 (CH), 1447, 1374, 1269, 1051, 976, 903. ^1H- and ^{13}C-NMR: *Table 1*. HR-ESI-TOF-MS (pos.): 955.4451 ($[M + Na]^+$, $C_{45}H_{72}O_{20}Na^+$; calc. 955.4515).

Acid Hydrolysis of Clintonioside A (**1**). A soln. of **1** (2.0 mg) in 0.5M HCl in dioxane/H_2O 1:1 (3 ml) was heated at 95° for 1 h under Ar. After cooling, the mixture was neutralized by passage through an *Amberlite-IRA-93ZU* (*Organo*, Tokyo, Japan) column and then subjected to CC (*Diaion HP-20*, 40% MeOH, then Me_2CO/EtOH 1:1) to give an aglycone fraction and a sugar fraction. TLC Analysis of the aglycone fraction revealed several unidentified artifactual sapogenols. After the sugar fraction was passed through a *Sep-Pak-C_{18}* cartridge (*Waters*, Milford, MA, USA; with 40% MeOH) and a *Toyopak-IC-SP-M* cartridge (*Tosoh*; with 40% MeOH), it was analyzed by HPLC (*Capcell-Pak-NH_2-UG80* column (4.6 mm i.d. × 250 mm, 5 μm; *Shiseido*), MeCN/H_2O 17:3, flow rate 1.0 ml/min, r.i. and o.r. detection): t_R 6.06 (L-rhamnose; neg. o.r.); 12.92 (D-glucose; pos. o.r.).

Acetylation of Clintonioside A (**1**). Compound **1** (2.0 mg) was treated with Ac_2O/pyridine 1:1 (2 ml) in the presence of *N*,*N*-dimethylpyridin-4-amine (2.0 mg) as catalyst at r.t. for 20 h. The mixture was subjected to CC (SiO_2, hexane/AcOEt 2:3): 1.7 mg of *clintonioside A dodecaacetate*. Amorphous solid. $[\alpha]_D^{24} = -50.0$ ($c = 0.10$, MeOH). IR (film): 2929 (CH), 1745 (C=O), 1443, 1373, 1237, 1128, 1045, 923, 837. ^{1}H-NMR: 5.80 (br. *s*, H−C(1″)); 5.70 (br. *d*, *J* = 4.5, H−C(6)); 5.49–5.47 (*m*, H−C(24)); 5.12 (*d*, *J* = 8.0, H−C(1‴)); 4.95 (*dd*, *J* = 11.5, 4.0, H−C(1)); 4.86 (*d*, *J* = 7.8, H−C(1′)); 4.78 (*d*, *J* = 3.7, H−C(23)); 4.42 (*dd*, *J* = 11.3, 2.3, H_{ax}−C(26)); 4.00–3.92 (*m*, H−C(3)); 3.33 (br. *d*, *J* = 11.3, H_{eq}−C(26)); 2.41, 2.19 (2×), 2.18, 2.12, 2.11 (2×), 2.04, 2.03, 2.02, 1.99 (2×) (12*s*, 12 *Me*CO); 1.46 (*d*, *J* = 6.2, Me(6″)); 1.31 (*s*, Me(19)); 1.16 (*d*, *J* = 6.4, Me(21)); 1.14 (*d*, *J* = 7.0, Me(27)); 0.88 (*s*, Me(18)).

Clintonioside B (= *(1β,3β,23S,24S)-3,21,23,24-Tetrahydroxyspirosta-5,25(27)-dien-1-yl* O-*α*-L-*Rhamnopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranoside* = *(1β,3β,23S,24S)-3,21,23,24-Tetrahydroxyspirosta-5,25(27)-dien-1-yl* O-*6-Deoxy-α*-L-*mannopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranoside*; **2**). Amorphous solid. $[\alpha]_D^{22} = -66.0$ ($c = 0.10$, MeOH). IR (film): 3379 (OH), 2920 and 2849 (CH), 1444, 1373, 1254, 1048, 957, 922. ^{1}H- and ^{13}C-NMR: *Table 2*. HR-ESI-TOF-MS (pos.): 939.4272 ($[M + Na]^+$, $C_{44}H_{68}O_{20}Na^+$; calc. 939.4202).

Acid Hydrolysis of Clintonioside B (**2**). Compound **2** (1.5 mg) was subjected to acid hydrolysis as described for **1**: sugar fraction (0.7 mg). HPLC Analysis (see above) showed the presence of D-glucose, L-rhamnose, and D-xylose; t_R 6.60 (L-rhamnose; neg. o.r.); 8.30 (D-xylose; pos. o.r.); 12.87 (D-glucose; pos. o.r.).

Clintonioside C (= *(1β,3β,23S,24S)-21-(Acetyloxy)-24-[(6-deoxy-β*-D-*gulopyranosyl)oxy]-3,23-dihydroxyspirosta-5,25(27)-dien-1-yl* O-*α*-L-*Rhamnopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranoside* = *(1β,3β,23S,24S)-21-(Acetyloxy)-24-[(6-deoxy-β*-D-*gulopyranosyl)oxy]-3,23-dihydroxyspirosta-5,25(27)-dien-1-yl* O-*6-Deoxy-α*-L-*mannopyranosyl-(1 → 2)*-O-*[β*-D-*xylopyranosyl-(1 → 3)]-β*-D-*glucopyranoside*; **3**). Amorphous solid. $[\alpha]_D^{25} = -64.0$ ($c = 0.10$, MeOH). IR (film): 3398 (OH), 2921 (CH), 1720 (C=O), 1444, 1374, 1257, 1048, 990, 921. ^{1}H- and ^{13}C-NMR: *Table 3*. HR-ESI-TOF-MS (pos.): 1127.4843 ($[M + Na]^+$, $C_{52}H_{80}O_{25}Na^+$; calc. 1127.4886).

Acid Hydrolysis of Clintonioside C (**3**). Compound **3** (5.3 mg) was subjected to acid hydrolysis as described for **1**: sugar fraction (1.5 mg). HPLC Analysis (see above) showed the presence of D-glucose, L-rhamnose and/or 6-deoxy-D-gulose, and D-xylose; t_R 6.58 (L-rhamnose and/or 6-deoxy-D-gulose; neg. o.r.); 8.29 (D-xylose; pos. o.r.); 12.90 (D-glucose; pos. o.r.).

Acid Hydrolysis of Clintonioside C (**3**) *for the Identification of* L-*Rhamnose and 6-Deoxy*-D-*gulose*. A soln. of **3** (5.2 mg) in 0.2M HCl in MeOH/H_2O 1:1 (3 ml) was heated at 65° for 2.5 h under Ar. After cooling, the mixture was neutralized by passage through an *Amberlite-IRA-93ZU* column (*Organo*), and fractionated by using a *Sep-Pak-C_{18}* cartridge (*Waters*; with 10% MeOH): a sugar fraction (0.8 mg). The sugar fraction was analyzed by HPLC (*Capcell-Pak-C_{18}-AQ* column (4.6 mm i.d. × 250 mm, 5 μm; *Shiseido*), H_2O, flow rate 1.0 ml/min, r.i. and o.r. detection): t_R 10.66 (methyl 6-deoxy-*α*-D-guloside; pos. o.r.); 12.09 (methyl *β*-L-rhamnoside; pos. o.r.); 12.43 (methyl 6-deoxy-*β*-D-guloside; neg. o.r.); 14.88 (methyl *α*-L-rhamnoside; neg. o.r.).

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