Calysolins X–XIII, Resin Glycosides from *Calystegia soldanella*, and Their Antiviral Activity toward Herpes Simplex Virus

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Four new resin glycosides having macrolactone structures (jalapins), named calysolins X (1)–XIII (4), were isolated from the leaves, stems, and roots of *Calystegia soldanella* ROEM. et SCHULT. (Convolvulaceae). Their structures were determined on the basis of spectroscopic data as well as chemical evidence. The sugar moieties of 1–4 were partially acylated by some organic acids, including tiglic acid, 2S-methylbutyric acid, and 2S,3S-nilic acid. Additionally, the antiviral activity of 1–4 toward herpes simplex virus type 1 was evaluated. All the compounds showed antiviral activity.

Key words resin glycoside; jalapin; *Calystegia soldanella*; Convolvulaceae; calysolin; anti-herpes simplex virus activity

Calystegia soldanella ROEM. et SCHULT. (Convolvulaceae) is commonly found on the sandy beaches of seas and lakes located the temperate regions of the world. The roots of this plant are used in the treatment of arthritis.¹⁾ In our previous paper, we reported the isolation and structural elucidation of four new glycosidic acids, calysolic acids A–D, which were obtained along with the known glycosidic acid, soldanellic acid B, and three organic acids, 2S-methylbutyric, tiglic, and 2S,3S-nilic acids, upon alkaline hydrolysis of the crude resin glycoside fraction of the leaves, stems, and roots of *C. soldanella*.²⁾ Further, we isolated nine genuine resin glycosides namely calysolins I–IX, all of which possessed characteristic macrolactone structures similar to those of the already known jalapins,³⁾ along with one known jalapin, soldanellic B, and their antiviral activities toward herpes simplex virus type 1 (HSV-1) were evaluated.^{4,5)} As a part of an ongoing study of the resin glycosides from this plant, the current study deals with the isolation and structural elucidation of four new resin glycosides named calysolins X (1)–XIII (4). Additionally, the antiviral activity of 1–4 toward HSV-1 is described herein.

The fresh leaves, stems, and roots of *C. soldanella* were extracted using methanol (MeOH). This extract was suspended in H₂O and then extracted successively using ethyl acetate (EtOAc) and *n*-butanol (BuOH). The EtOAc-soluble fraction was subjected to silica gel column chromatography and HPLC on octadecyl silica (ODS) and silica gel to yield 1–4.

Calysolin X (1) was obtained as an amorphous powder. The negative-ion FAB-MS and positive-ion FAB-MS of 1 exhibited an [M–H][–] ion peak at *m/z* 1051 and an [M+Na]⁺ ion peak at *m/z* 1075, respectively, indicating its molecular weight to be 1052. The molecular formula of 1 was found to be C₅₀H₈₄O₂₃ using high-resolution (HR)-positive-ion FAB-MS. Upon alkaline hydrolysis, 1 yielded a glycosidic acid and an organic acid fraction. The gas chromatography (GC) analysis of the organic acid fraction revealed the presence of tiglic and nilic acids. The glycosidic acid was identified as soldanellic acid B (5) by comparing with an authentic sample²⁾ using ¹H-NMR spec-

troscopy. The ¹H-NMR spectrum of 1 exhibited signals due to one H-2 of the niloyl residue, one H-3 of the tigloyl residue, and four anomeric protons. The ¹³C-NMR spectrum showed signals corresponding to three carboxyl carbons and four anomeric carbons. These data indicated that 1 was composed of 1 mol each of tiglic acid, nilic acid, and 5 and that three ester groups exist in the molecule; hence the carboxyl group of the aglycone, 11S-hydroxyhexadecanoic (11S-jalapinolic) acid, of 1 is also linked intramolecularly with a hydroxy group of the sugar moiety to form a macrocyclic ester structure, as in already known jalapins.³⁾ The presence of the macrocyclic ester structure was supported by the non-equivalent signals due to H₂-2 of jalapinoloyl unit (Jla) of 1 observed at δ 2.79 (1H, ddd, *J*=2.5, 12.0, 16.5 Hz) and δ 2.71 (1H, ddd, *J*=4.0, 4.0, 16.5 Hz), whereas the methyl ester (5a)²⁾ of 5 exhibited the equivalent signal due to H₂-2 of Jla at δ 2.32 (2H, t, *J*=7.5 Hz).²⁾

In order to clarify the positions of the ester linkages, the ¹H-NMR signals due to the sugar moiety of 1 were assigned on the basis of ¹H–¹H correlation spectroscopy (COSY), heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond correlation (HMBC) spectra (Tables 1, 2). By comparing the chemical shifts of the ¹H-NMR signals between 1 and 5a,²⁾ the downfield shifts ($\Delta\delta$ = δ 1– δ 5a) due to acylation were observed for signals assignable to H-2 ($\Delta\delta$ =1.21) and H-4 ($\Delta\delta$ =1.46) of the rhamnosyl residue (Rha) and H-2 ($\Delta\delta$ =ca. 1.61) of the second glucosyl residue (Glc'). Consequently, the ester linkages were located at OH-2 and OH-4 of Rha and OH-2 of Glc'. The sites of each ester linkage of the organic acids and Jla were determined from the HMBC spectrum of 1, with key cross-peaks observed between H-4 of Rha and C-1 of the tigloyl residue (Tig) and H-2 of Glc' and C-1 of Jla. Though no cross-peak between the H-2 of Rha and C-1 of niloyl residue (Nla) was observed in the HMBC spectrum of 1, the above data suggested that Nla, Tig, and Jla were attached to OH-2 of Rha, OH-4 of Rha, and OH-2 of Glc', respectively. These linkages were supported by the fragment ion peaks observed at *m/z* 907 [1051 (M–H)–144 (162 (hexosyl residue)–H₂O)][–], 723 [1051–146 (6-deoxyhexosyl residue)–100 (niloyl residue)–82 (tigloyl residue)][–], 579 [723–144][–], 417

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Table 1. ¹H-NMR Spectral Data for **1–4** (in Pyridine-*d*₅, 500 MHz)

	1	2	3	4
Qui-1	4.69 ^{a)}	4.63 d (7.5)	4.68 d (7.5)	4.68 d (7.5)
2	4.24 ^{b)}	4.10 ^{b)}	4.14 dd (7.5, 9.0)	4.14 ^{b)}
3	4.24 ^{b)}	4.21 ^{b)}	4.25 dd (9.0, 9.0)	4.24 dd (9.0, 9.0)
4	3.57 ^{b)}	3.52 dd (9.0, 9.0)	3.54 dd (9.0, 9.0)	3.53 dd (9.0, 9.0)
5	3.60 ^{b)}	3.59 ^{b)}	3.59 dq (9.0, 6.5)	3.59 dq (9.0, 6.5)
6	1.58 d (6.0)	1.61 d (6.5)	1.57 d (6.5)	1.56 d (6.5)
Glc-1	5.69 d (7.5)	5.52 d (8.0)	5.50 d (7.5)	5.50 d (8.0)
2	4.13 dd (7.5, 9.0)	4.08 ^{b)}	4.12 dd (7.5, 9.0)	4.12 ^{b)}
3	4.30 dd (9.0, 9.0)	4.08 ^{b)}	4.23 ^{b)}	4.23 dd (9.0, 9.0)
4	3.82 dd (9.0, 9.0)	3.75 ^{a)}	3.75 dd (9.0, 9.0)	3.75 dd (9.0, 9.0)
5	3.76 ^{b)}	3.61 ^{b)}	3.64 ^{b)}	3.65 m
6	4.35 dd (2.0, 11.5)	4.30 br d (11.5)	4.32 dd (2.0, 11.5)	4.32 ^{b)}
6	4.08 ^{b)}	4.08 ^{b)}	4.05 dd (5.0, 11.5)	4.04 dd (6.5, 11.5)
Glc'-1	5.26 d (8.0)	5.20 d (8.0)	5.30 d (8.0)	5.30 d (8.0)
2	5.62 dd (8.0, 9.5)	5.56 dd (8.0, 9.5)	5.59 dd (8.0, 9.0)	5.59 dd (8.0, 9.0)
3	4.52 dd (9.5, 9.5)	4.54 dd (9.5, 9.5)	4.59 dd (9.0, 9.0)	4.57 dd (9.0, 9.0)
4	4.14 dd (9.5, 9.5)	4.12 ^{b)}	4.16 ^{b)}	4.14 ^{b)}
5	4.08 ^{b)}	4.13 ^{b)}	4.19 ^{b)}	4.18 ^{b)}
6	4.49 dd (1.5, 12.0)	4.52 dd (2.0, 11.5)	4.50 dd (2.0, 12.5)	4.50 br d (11.5)
6	4.28 dd (5.5, 12.0)	4.27 dd (5.0, 11.5)	4.29 ^{b)}	4.28 dd (5.5, 11.5)
Glc''-1		5.29 d (8.0)	4.97 d (7.5)	4.90 d (8.0)
2		5.50 dd (8.0, 9.0)	3.86 dd (7.5, 9.5)	3.82 dd (8.0, 9.5)
3		4.06 ^{b)}	5.66 dd (9.5, 9.5)	5.62 dd (9.5, 9.5)
4		3.88 dd (9.0, 9.0)	4.27 dd (9.5, 9.5)	4.21 dd (9.5, 9.5)
5		4.10 ^{b)}	3.66 ^{b)}	3.53 ^{b)}
6		4.76 br d (11.5)	4.46 br d (11.5)	4.41 br d (11.5)
6		4.20 ^{b)}	4.30 ^{b)}	4.32 ^{b)}
Glc'''-1		4.97 d (8.0)	4.95 d (7.5)	4.92 d (8.0)
2		3.96 dd (8.0, 9.5)	3.79 dd (7.5, 8.5)	3.86 dd (8.0, 9.0)
3		4.21 ^{b)}	4.09 ^{b)}	4.12 ^{b)}
4		4.15 ^{b)}	4.08 ^{b)}	3.99 dd (9.0, 9.0)
5		3.99 ^{b)}	3.72 ^{b)}	3.86 ^{b)}
6		4.48 dd (1.5, 11.5)	4.24 ^{b)}	4.75 br d (11.5)
6		4.21 ^{b)}	4.20 ^{b)}	4.62 dd (5.5, 11.5)
Rha-1	5.90 d (1.0)	5.89 d (1.0)	5.92 s	5.92 s
2	6.03 dd (1.0, 3.0)	5.93 dd (1.0, 3.5)	6.18 br d (3.5)	6.21 br d (3.5)
3	4.80 dd (3.0, 9.5)	5.17 dd (3.5, 10.0)	5.01 dd (3.5, 10.0)	4.95 dd (3.5, 9.5)
4	5.74 dd (9.5, 9.5)	5.79 dd (10.0, 10.0)	5.90 dd (10.0, 10.0)	5.90 dd (9.5, 9.5)
5	5.21 dq (9.5, 6.5)	5.14 dq (10.0, 6.5)	5.30 ^{b)}	5.32 ^{b)}
6	1.57 d (6.5)	1.56 d (6.5)	1.62 d (6.5)	1.62 d (6.5)
Jla-2	2.79 ddd (2.5, 12.0, 16.5)	2.71 ddd (2.5, 11.5, 16.0)	2.70 ddd (2.5, 12.0, 16.5)	2.71 ^{b)}
2	2.71 ddd (4.0, 4.0, 16.5)	2.63 ddd (4.0, 4.0, 16.0)	2.66 ddd (4.5, 4.5, 16.5)	2.66 ^{b)}
11	3.74 ^{b)}	3.69 m	3.73 ^{b)}	3.73 ^{b)}
16	0.85 t (7.0)	0.85 t (7.5)	0.84 t (7.0)	0.85 t (7.0)
Nla-2	2.98 dq (7.0, 7.0)			
3	4.23 ^{b)}			
4	1.39 d (7.0)			
5	1.32 d (7.0)			
Tig-3	7.22 qq (1.5, 7.5)	6.96 qq (1.5, 7.0)	7.40 qq (1.5, 7.0)	7.39 qq (1.0, 7.0)
4	1.69 br d (7.5)	1.76 dd (1.5, 7.0)	1.70 d (7.0)	1.68 d (7.0)
5	1.86 brs	1.94 brs	1.99 brs	1.97 brs
Tig'-3		7.48 qq (1.5, 7.0)	7.05 qq (1.5, 7.0)	7.02 qq (1.0, 7.0)
4		1.87 dd (1.5, 7.0)	1.63 d (7.0)	1.64 d (7.0)
5		2.07 brs	1.89 brs	1.86 brs
Tig''-3				6.91 qq (1.0, 7.0)
4				1.51 d (7.0)
5				1.78 br s
Mba-2		2.55 ddq (7.0, 7.0, 7.0)	2.57 ddq (7.0, 7.0, 7.0)	2.60 ddq (7.0, 7.0, 7.0)
3		1.86 ^{b)}	1.76 m	1.76 ^{b)}
3		1.58 ^{b)}	1.49 m	1.51 ^{b)}
4		1.02 dd (7.5, 7.5)	0.85 dd (7.0, 7.0)	0.88 dd (7.5, 7.5)
5		1.23 d (7.0)	1.19 d (7.0)	1.20 d (7.0)

δ in ppm from tetramethylsilane (TMS) (coupling constants (*J*) in Hz are given in parentheses). Qui, quinovopyranosyl; Glc, glucopyranosyl; Rha, rhamnopyranosyl; Jla, jalapinoyl; Nla, niloyl; Tig, tigloyl; Mba, 2-methylbutyryl. *a*) Signal was deformed by virtual coupling. *b*) Signals were overlapped with other signals.

Table 2. ^{13}C -NMR Spectral Data for **1–4** (in Pyridine- d_5 , 125 MHz)

	1	2	3	4		1	2	3	4
Qui-1	103.7	103.5	103.4	103.4	Jla-1	173.2	173.3	173.2	173.2
2	80.2	80.6	80.5	80.6	2	34.0	33.7	33.9	34.0
3	79.2	79.3	79.0	78.9	11	82.3	82.1	82.0	81.9
4	76.7	76.8	76.8	76.7	16	14.2	14.2	14.2	14.2
5	72.5	72.2	72.3	72.2	Nla-1	175.4			
6	18.4	18.4	18.3	18.3	2	47.9			
Glc-1	101.5	101.6	101.7	101.7	3	70.4			
2	76.8	76.6	76.5	76.5	4	21.2			
3	85.1	85.7	84.9	85.1	5	13.1			
4	69.9	69.9	70.0	70.0	Tig-1	167.6	166.7	167.7	167.7
5	77.4	77.2	77.0	77.1	2	128.5	128.8	128.7	128.9
6	63.0	63.0	63.1	63.1	3	138.5	139.2	138.6	138.5
Glc'-1	100.6	100.3	100.3	100.4	4	14.4	14.7	14.4	14.4
2	74.9	75.0	75.0	75.0	5	12.4	12.7	12.6	12.6
3	75.3	74.9	74.9	75.0	Tig'-1		166.6	167.3	167.3
4	72.3	72.5	72.5	72.6	2		128.8	129.4	129.5
5	78.8	78.9	78.9	79.0	3		137.0	137.1	136.6
6	62.2	62.3	62.2	62.2	4		14.3	14.1	14.2
Glc''-1		101.6	105.1	105.3	5		12.4	12.3	12.3
2		73.8	72.8	72.9	Tig''-1				167.9
3		74.4	76.6	76.5	2				128.7
4		82.7	76.6	76.7	3				137.3
5		75.5	76.3	76.1	4				14.1
6		62.3	61.6	61.4	5				12.0
Glc'''-1		105.0	104.7	104.9	Mba-1		176.4	176.6	176.6
2		74.9	75.2	75.0	2		40.8	40.9	40.9
3		78.4	78.1	78.2	3		27.2	27.1	27.3
4		71.5	71.6	71.2	4		11.7	11.4	11.4
5		78.6	77.7	75.1	5		16.0	16.1	16.0
6		62.5	62.7	64.7					
Rha-1	97.7	96.8	97.3	97.4					
2	72.7	72.7	72.2	72.3					
3	68.5	72.8	77.1	77.4					
4	75.5	74.4	73.0	72.9					
5	66.9	67.2	67.0	66.9					
6	17.7	17.7	17.7	17.7					

δ in ppm from TMS. Qui, quinvopyranosyl; Glc, glucopyranosyl; Rha, rhamnopyranosyl; Jla, jalapinoyl; Nla, niloyl; Tig, tigloyl; Mba, 2-methylbutyryl.

[579–162][−], and 271 [417–146][−] in the negative-ion FAB-MS of **1**. Taking into account the J values of signals attributable to the anomeric and methine protons of the sugar moiety, the conformations of the quinvopyranosyl and glucopyranosyl residues were found to be $^4\text{C}_1$ and that of the rhamnopyranosyl residue was concluded to be $^1\text{C}_4$. The configuration of the component nilic acid of the crude resin glycoside fraction of this plant had been previously determined to be 2*S*,3*S*.²⁾ Thus, the structure of **1** was defined as 11*S*-jalapinic acid 11-*O*-(2-*O*-2*S*,3*S*-niloyl,4-*O*-tigloyl)- α -L-rhamnopyranosyl-(1→2)-[*O*- β -D-glucopyranosyl-(1→3)]-*O*- β -D-glucopyranosyl-(1→2)- β -D-quinvopyranoside, intramolecular 1,2'''-ester, as shown in Fig. 1.

Calysolin XI (**2**) was obtained as an amorphous powder, and **2** yielded 2-methylbutyric acid, tiglic acid, and calysolic acid D (**6**)²⁾ upon alkaline hydrolysis. The ^1H - and ^{13}C -NMR spectra of **2** indicated that **2** was composed of 1 mol each of 2-methylbutyric acid and **6** and 2 mol of tiglic acid. Furthermore, the molecular formula ($\text{C}_{67}\text{H}_{110}\text{O}_{33}$) of **2**, analyzed using HR-positive-ion FAB-MS, and the nonequivalent signals due to H_2 -2 of Jla in the ^1H -NMR spectrum of **2** suggested that **2**, akin

to **1**, possessed a macrolactone structure. To determine the positions of the ester linkages, the ^1H -NMR signals due to the sugar moiety of **2** were assigned using 2D-NMR spectra including total correlation spectroscopy (TOCSY) spectrum. By comparing the chemical shifts of signals due to the sugar moiety between **2** and the methyl ester (**6a**)²⁾ of **6**, acylation shifts ($\Delta\delta = \delta\text{2} - \delta\text{6a}$) were observed for signals due to H-2 ($\Delta\delta = 0.91$) and H-4 ($\Delta\delta = ca. 1.29$) of Rha, H-2 ($\Delta\delta = ca. 1.60$) of Glc', and H-2 ($\Delta\delta = ca. 1.43$) of the third glucosyl residue (Glc''). Therefore, the ester linkages of **2** were located at OH-2 and OH-4 of Rha, OH-2 of Glc', and OH-2 of Glc''. In the HMBC spectrum of **2**, key cross-peaks were observed between H-4 of Rha and C-1 of the first tigloyl residue (Tig), H-2 of Glc' and C-1 of Jla, and H-2 of Glc'' and C-1 of the second tigloyl residue (Tig'). Therefore, the 2-methylbutyryl residue (Mba), Tig, Tig', and Jla were attached to OH-2 of Rha, OH-4 of Rha, OH-2 of Glc'', and OH-2 of Glc', respectively. These inferences were supported by the fragment ion peaks in the negative-ion FAB-MS of **2**, in which the fragment ion peaks were observed at 1035 [1441 (M-H)–82–162×2][−], 723 [1035–82–84–146][−], 579 [723–144][−], 417 [579–162][−], and 271 [417–146][−]. The configura-

Fig. 1. Structures of 1–4

tion of the component 2-methylbutyric acid of the crude resin glycoside fraction of this plant had been previously determined to be *S*.²⁾ Thus, the structure of **2** was defined as 11*S*-jalapinoic acid 11-*O*-β-D-glucopyranosyl-(1→4)-*O*-(2-*O*-tigloyl)-β-D-glucopyranosyl-(1→3)-*O*-(2-*O*-2*S*-methylbutyryl,4-*O*-tigloyl)-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2'''-ester, as shown in Fig. 1.

Calysolin XII (**3**) was obtained as an amorphous powder and its molecular formula was found to be the same as that of **2** using HR-positive-ion FAB-MS. Upon alkaline hydrolysis, **3** yielded the same organic acids and glycosidic acid as those obtained from **2**. Furthermore, the ¹H- and ¹³C-NMR spectra of **3** indicated that the component units of **3** were same as those of **2** (Tables 1, 2). Based on these data, **3** was considered to be a positional isomer of **2** as to the ester linkage. When compared with the ¹H-NMR signals of **2**, a downfield shift of 1.60 ppm for the signal due to H-3 of Glc'' along with an upfield shift of 1.64 ppm for the signal assignable to H-2 of Glc'' were observed, whereas the signals due to H-2 of Glc' and H-2 and H-4 of Rha resonated at positions similar to those of **2**. In addition, the HMBC spectrum of **3** showed key cross peaks between H-4 of Rha and C-1 of Tig, H-2 of Glc' and C-1 of Jla, and H-3 of Glc'' and C-1 of Tig'. Accordingly, **3** was concluded to be a positional isomer of **2**, wherein Tig' of **2** was at OH-3 of Glc'' rather than at OH-2 of Glc'' (Fig. 1).

Calysolin XIII (**4**) was obtained as an amorphous powder. Upon alkaline hydrolysis, **4** also furnished the same organic acids and glycosidic acid as those obtained from **2** and **3**. The molecular weight of **4** was found to be 1524, which was 82 mass units (tigloyl residue) larger than that of **3**, using positive-ion FAB-MS and negative-ion FAB-MS. The ¹H- and ¹³C-NMR spectra of **4** were superimposable on those of **3**, except for the appearance of signals due to an additional tigloyl residue (Tables 1, 2). On comparing the chemical shifts

of the signals between **4** and **3**, remarkable downfield shifts ($\Delta\delta = \delta\mathbf{4} - \delta\mathbf{3}$) of the signals assignable to H-6a ($\Delta\delta = ca. 0.24$) and H-6b ($\Delta\delta = ca. 0.37$) of the fourth glucosyl residue (Glc''') were observed; in contrast, the signals due to H-2 and H-4 of Rha, H-2 of Glc', and H-3 of Glc'' were resonated at the chemical shifts similar to those of **3**, respectively. Based on these data, **4** was considered to be a derivative of **3**, wherein the third tigloyl residue (Tig'') was attached to OH-6 of Glc'''. This assumption was confirmed as follows: in the HMBC spectrum of **4**, key cross-peaks were observed between H-4 of Rha and C-1 of Tig, H-2 of Glc' and C-1 of Jla, and H-3 of Glc'' and C-1 of Tig'. In addition, the HR-positive-ion FAB-MS of the peracetate (**4a**) of **4** revealed a fragment ion peak at *m/z* 371.1337, which was assigned to the fragment ion of the 6-*O*-tigloyl-2,3,4-*O*-triacyetyl-glucopyranosyl unit.⁴⁾ Consequently, **4** was determined to be a 11*S*-jalapinoic acid 11-*O*-(6-*O*-tigloyl)-β-D-glucopyranosyl-(1→4)-*O*-(3-*O*-tigloyl)-β-D-glucopyranosyl-(1→3)-*O*-(2-*O*-2*S*-methylbutyryl,4-*O*-tigloyl)-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2'''-ester, as shown in Fig. 1.

Compounds **1–4** were evaluated for their anti-HSV-1 activity,⁶⁾ because a previous examination of calysolins I–IX and soldanelline B had indicated such activity.⁵⁾ The 50% effective concentration (EC₅₀) values of all compounds were *ca.* 2.4-fold to 6.8-fold of that of acyclovir, which has been used for the treatment of HSV infections (Table 3). However, all compounds showed relatively strong cytotoxic activity (IC₅₀). Among the tested compounds, **3** showed the highest selectivity index (IC₅₀/EC₅₀). In addition, the results suggested that the migration of the tigloyl residue at OH-2 of Glc''' to OH-3 of Glc'' might contribute to the anti-HSV-1 activity (see, **2** vs. **3**) while the tigloyl residue at C-6 of Glc''' might enhance the cytotoxic activity (see, **3** vs. **4**).

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Table 3. Anti-herpes Activity (EC_{50}), Cytotoxic Activity (IC_{50}), and Selectivity Index (IC_{50}/EC_{50}) of **1–4** and Acyclovir

Sample	EC_{50} (M)	IC_{50} (M)	IC_{50}/EC_{50}
1	7.5	32.6	4.3
2	5.9	24.1	4.1
3	2.6	28.3	10.9
4	3.8	14.6	3.8
Acyclovir	1.1	>44.4	>400

Each value is the mean of duplicate assays.

resulted in the isolation and structural elucidation of four new resin glycosides. All the compounds have intramolecular cyclic ester structures and show anti-HSV-1 activity.

Experimental

The instruments and materials used were as cited in the preceding report^{2,4)} unless otherwise specified.

Isolation of 1–4 The cut fresh leaves, stems, and roots of *C. soldanella* (916.9 g) were extracted with MeOH (10 L) at room temperature for 1 month, and the solvent was removed under reduced pressure to afford a MeOH extract (126.9 g). The MeOH extract was suspended in H₂O (3.5 L) and then successively extracted with EtOAc (1.6 L) and BuOH (0.6 L) to afford an EtOAc-soluble fraction (fr.) (48.57 g) and a BuOH-soluble fr. (8.15 g). A part (46.87 g) of the EtOAc-soluble fr. was chromatographed on silica gel column (Merck Art. 1.07734) using gradient mixtures of CHCl₃–MeOH–H₂O (20:1:0, 10:2:0.1, 8:2:0.2, 7:3:0.5, 6:4:1, 0:1:0) as eluents to furnish fractions (frs.) 1–17. HPLC [column, COSMOSIL 5C18 AR-II, Nacalai Tesque Inc., 20 mm i.d.×250 mm (column 1)] of fr. 11 (3.800 g) eluted with 95% MeOH gave calysolin IX (220 mg), calysolin IV (133 mg), calysolin VIII (131 mg), calysolin I (18 mg), **1** (9 mg), and frs. 11–1–11–7. HPLC (column 1) of a part (2.540 g) of fr. 15 (2.966 g) using 95% MeOH as eluent gave calysolin IX (273 mg) and frs. 15–1–15–10. Fractions 15–7 (74 mg) and fr. 15–9 (162 mg) were each subjected to HPLC (column 1) eluted with 90% MeOH to afford frs. 15–7–1–15–7–4 from fr. 15–7 and **4** (30 mg) and frs. 15–9–1–5–9–2 from fr. 15–9. HPLC (COSMOSIL 5SL-II, Nacalai Tesque Inc., 20 mm i.d.×250 mm) of fr. 15–7–1 (31 mg) using CHCl₃–MeOH–H₂O (8:2:0.2) as eluent gave **3** (16 mg). Fraction 16 (3.044 g) was subjected to HPLC (column 1) eluted with 90% MeOH to afford frs. 16–1–16–8. HPLC (column 1) of fr. 16–8 (1.973 g) using 90% MeOH as eluent gave **2** (34 mg) and frs. 16–8–1–16–8–5.

1: Amorphous powder. $[\alpha]_D^{25}$ –24.3° ($c=0.8$, MeOH). Positive-ion FAB-MS m/z : 1075 [M+Na]⁺. HR-positive-ion FAB-MS m/z : 1075.5287 (Calcd for C₅₀H₈₄O₂₃Na⁺, 1075.5301). Negative-ion FAB-MS m/z : 1051 [M–H][–], 951 [1051–100][–], 907 [1051–144][–], 869 [951–82][–], 723 [869–146][–], 579 [723–144][–], 417 [579–162][–], 271 [417–146][–]. ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

2: Amorphous powder. $[\alpha]_D^{25}$ –17.3° ($c=0.7$, MeOH). Positive-ion FAB-MS m/z : 1465 [M+Na]⁺. HR-positive-ion FAB-MS m/z : 1465.6830 (Calcd for C₆₇H₁₁₀O₃₃Na⁺, 1465.6827). Negative-ion FAB-MS m/z : 1441 [M–H][–], 1359 [1441–82][–], 1279 [1441–162][–], 1277 [1359–82][–], 1197 [1359–162][–], 1035 [1197–162][–], 953 [1035–82][–], 723 [953–84–146][–], 579 [723–144][–], 417 [579–162][–], 271 [417–146][–]. ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

3: Amorphous powder. $[\alpha]_D^{24}$ –15.6° ($c=3.1$, MeOH). Positive-ion FAB-MS m/z : 1465 [M+Na]⁺. HR-positive-ion FAB-MS m/z : 1465.6810 (Calcd for C₆₇H₁₁₀O₃₃Na⁺, 1465.6827). Negative-ion FAB-MS m/z : 1441 [M–H][–], 1359 [1441–82][–], 1279 [1441–162][–], 1197 [1359–162][–], 1035 [1197–162][–], 953 [1035–82][–], 723 [953–84–146][–], 579 [723–144][–], 417 [579–162][–], 271 [417–146][–]. ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

4: Amorphous powder. $[\alpha]_D^{25}$ –12.2° ($c=2.4$, MeOH). Positive-ion FAB-MS m/z : 1547 [M+Na]⁺. HR-positive-ion FAB-MS m/z : 1547.7256 (Calcd for C₇₂H₁₁₆O₃₄Na⁺, 1547.7246). Negative-ion FAB-MS m/z : 1523 [M–H][–], 1441 [1523–82][–], 1359 [1441–82][–], 1279 [1441–162][–], 1197 [1359–162][–], 1035 [1197–162][–], 953 [1035–82][–], 723 [953–146–84][–], 579 [723–144][–], 417 [579–162][–], 271 [417–146][–]. ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

Alkaline Hydrolysis of 1–4 Solutions of **1** (4 mg), **2** (5 mg), **3** (4 mg), and **4** (5 mg) in 1 M KOH–1,4-dioxane (2:1, 1.5 mL) were each heated at 95°C for 1 h. The reaction mixture was adjusted to pH 3 with 1 M HCl and then diluted with H₂O (10 mL) and extracted with ether (3×5 mL). The ether layer was dried over MgSO₄ and evaporated to furnish an organic acid fraction, which was analyzed by using GC [column, Unisole 30T (5%), 3.2 mm i.d.×2 m glass column (column 2); carrier gas N₂, 1.0 kg/cm²; column temperature, 120°C; t_R (min): 4.63 (2-methylbutyric acid) for **2–4**, 10.80 (tiglic acid) for **1–4**]. A part of the organic acid fraction was methylated with diazomethane–ether and then the reaction mixture was analyzed by GC [column 2; column temperature, 100°C; carrier gas, N₂ 1.2 kg/cm²; t_R (min): 4.20 (methyl nilate) for **1**].

The aqueous layer was desalted by MCI gel CHP20 column chromatography (H₂O, acetone) to give a glycosidic acid as an amorphous powder (2 mg from **1**, 3 mg from **2**, 2 mg from **3**, 3 mg from **4**). The glycosidic acids derived from **1–4** were identical with soldanellic acid **B** (**5**), calysolic acid **D** (**6**), calysolic acid **D** (**6**), and calysolic acid **D** (**6**), respectively, by comparison of ¹H-NMR spectra with those of authentic samples.²⁾

Acetylation of 4 Compound **4** (1 mg) was dissolved in acetic anhydride–pyridine (1:1, 0.5 mL), and the solution was left to stand at room temperature overnight. After removal of the reagent under an N₂ stream, the residue was partitioned between ether (1 mL×3) and H₂O (1 mL). The ether layer was concentrated to afford an acetate (**4a**, 1 mg) of **4**.

Compound **4a**: Amorphous powder. Positive-ion FAB-MS m/z : 1011, 699, 371. HR-positive-ion FAB-MS m/z : 371.1337 (Calcd for C₁₇H₂₃O₉⁺, 371.1342). ¹H-NMR spectral data (in pyridine-*d*₅, 500 MHz) δ : 2.54 (3H, s), 2.25 (3H, s), 2.17 (3H, s), 2.17 (6H, s), 2.12 (3H, s), 2.10 (3H, s), 2.07 (3H, s), 2.06 (3H, s), 2.01 (3H, s), 2.01 (6H, s), 2.00 (3H, s), 1.93 (3H, s), 1.90 (3H, s).

Anti-HSV-1 Assay The antiviral activity of test samples on HSV-1 was measured by the plaque reduction assay.⁶⁾ Confluent monolayers of Vero cells in 6-well plates were infected with HSV-1 at 100 plaque-forming units per well. After a 1 h adsorption period, the cultures were overlaid with Dulbecco's modified Eagle's minimum essential medium (DMEM) containing 2% heat-inactivated fetal calf serum and 2% sulfonated γ -globulin including various concentrations of the test samples. The plates were incubated in the CO₂ incubator for 3 d, then fixed with formalin and stained with crystal violet

in MeOH. Infections HSV-1 production was quantified by observing the virus-induced cytopathic effect.

Cytotoxic Assay The anticellular activity was measured by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Vero cells were seeded in 96-well plates at 1×10^4 cells per well. After 1 d incubation, the cells were refed with DMEM containing 5% fetal bovine serum and various concentrations of the test samples. After 3 d incubation, cells were washed with phosphate buffered saline and incubated for 4 h with MTT solution at a final concentration of 0.5 mg/mL. Isopropanol and hydrochloric acid were added to the culture medium at final concentrations of 50% and 20 mM, respectively. The optical density of each well at 570 nm was determined spectrophotometrically using a reference wavelength of 630 nm.

References

- 1) "Useful Plants of the World," ed. by Hotta M., Ogata K., Nitta A., Hosikawa K., Yanagi M., Yamazaki K., Heibonsha Ltd., Tokyo, 1989, p. 197.
- 2) Takigawa A., Setoguchi H., Okawa M., Kinjo J., Miyashita H., Yokomizo K., Yoshimitsu H., Nohara T., Ono M., *Chem. Pharm. Bull.*, **59**, 1163–1168 (2011).
- 3) Ono M., Nakagawa K., Kawasaki T., Miyahara K., *Chem. Pharm. Bull.*, **41**, 1925–1932 (1993).
- 4) Takigawa A., Muto H., Kabata K., Okawa M., Kinjo J., Yoshimitsu H., Nohara T., Ono M., *J. Nat. Prod.*, **74**, 2414–2419 (2011).
- 5) Ono M., Takigawa A., Kanemaru Y., Kawakami G., Kabata K., Okawa M., Kinjo J., Yokomizo K., Yoshimitsu H., Nohara T., *Chem. Pharm. Bull.*, **62**, 97–105 (2014).
- 6) Schinazi R. F., Peters J., Williams C. C., Chance D., Nahmias A. J., *Antimicrob. Agents Chemother.*, **22**, 499–507 (1982).