Calysolins V–IX, Resin Glycosides from Calystegia soldanella and Their Antiviral Activity toward Herpes

Masateru Ono, Ayako Takigawa, Yukio Kanemaru, Gen Kawakami, Kiyotaka Kabata, Masafumi Okawa, Junei Kinjo, Kazumi Yokomizo, Hitoshi Yoshimitsu, and Toshihiro Nohara

School of Agriculture, Tokai University; 5435 Minamiaso, Aso, Kumamoto 869–1404, Japan; Faculty of Pharmaceutical Sciences, Fukuoka University; 8–19–1 Nanakuma, Jonan-ku, Fukuoka 814–0180, Japan; and Faculty of Pharmaceutical Sciences, Sojo University; 4–22–2 Ikeda, Kumamoto 860–0082, Japan.

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Five new resin glycosides having macrolactone structures (jalapins), named calysolins V–IX (1–5), 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 isolated compounds could be classified into two macrolactone types—one having a 22-membered ring (1–4) and the other with a 27-membered ring (5). The sugar moieties of 1–5 were found to consist of partially acylated forms comprising 2S-methylbutyric acid and tiglic acid. Compounds 4 and 5 are the first representatives of the calysolic acid C as the component glycosidic acid. Additionally, the antiviral activity of 1–5, together with calysolic acid B, which are previously isolated jalapins from this plant, 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 activity

Calystegia soldanella Roem. et Schult. (Convolvulaceae) is widely distributed on the sandy beaches of seas and lakes in temperate regions of the world. The roots of this plant are used for the treatment of arthritis.1) In our previous investigations, 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-nic acids, upon alkaline hydrolysis of the crude resin glycoside fraction of the leaves, stems, and roots of C. soldanella.2) Further, we isolated four genuine resin glycosides named calysolins I–IV, all of which were found to possess characteristic macrolactone structures, as in already known jalapins,3) along with one known jalapin, soldanelline B.5) As part of an ongoing study of the resin glycosides from this plant, the present report deals with the isolation and structural elucidation of five new resin glycosides named calysolins V–IX. Additionally, the antiviral activity of calysolins I–IX and soldanelline B toward herpes simplex virus type 1 (HSV-1)5) is described.

The fresh leaves, stems, and roots of C. soldanella were extracted with methanol. The obtained extract was suspended in H2O and then extracted successively with ethyl acetate and n-butanol. The ethyl acetate-soluble fraction was subjected to gas chromatography (GC) revealed the presence of 2-methylbutyric and tiglic acids. The latter acid was identified as calysolic acid B (6) by comparing its 1H-NMR spectrum with that of an authentic sample.2) The 1H-NMR spectrum of 1 exhibited signals attributable to one 2-methylbutyl unit [δ 2.59 (1H, dq, J = 7.0, 7.0 Hz), 1.20 (3H, d, J = 7.0 Hz), 0.86 (3H, dd, J = 7.5, 7.5 Hz)], two tigloyl units [δ 7.42 (1H, qq, J = 1.0, 7.0 Hz), 6.86 (1H, qq, J = 1.0, 7.0 Hz), 1.99 (3H, brs), 1.73 (3H, brs), 1.71 (3H, qd, J = 1.0, 7.0 Hz), 1.51 (3H, qd, J = 1.0, 7.0 Hz)], one primary methyl group [δ 0.85 (3H, t, J = 7.5 Hz)] assignable to H3-16 of the aglycone moiety, five anomic protons [δ 5.92 (1H, d, J = 1.0 Hz), 5.53 (1H, d, J = 7.5 Hz), 5.31 (1H, d, J = 8.0 Hz), 5.00 (1H, d, J = 8.0 Hz), 4.69 (1H, d, J = 7.5 Hz)], and two secondary methyl groups [δ 1.63 (3H, d, J = 6.5 Hz), 1.58 (3H, dq, J = 6.5 Hz)] assignable to 6-deoxyhexosyl units. The 13C-NMR spectrum showed signals corresponding to four carboxyl carbon atoms (δ 176.8, 173.2, 167.7, 167.7) and five anomic carbons (δ 105.5, 103.5, 101.7, 100.4, 97.3). These 1H- and 13C-NMR signals 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). Comparison of the chemical shifts of the 1H-NMR signals of 1 and methyl ester (6a)2) of 6 indicated acylation shifts (Δδ = 0.1–0.8) of signals due to H-2 (Δδ = 0.17) and H-4 (Δδ = 0.14) of the rhamnosyl unit (Rha), H-2 (Δδ = 0.16) of the second glucosyl unit (Glc1), and H-3 (Δδ = 0.13) of the third glucosyl unit (Glc2). In addition, the 1H-NMR signals due to H-2 of the aglycone, 11S-hydroxyhexadecanoic (11S-jalapinolic) acid, moiety (Jla) of 1 were nonequivalent at δ 2.70 (1H) and δ 2.68 (1H), whereas 6a exhibited the equivalent signals due to H-2 of Jla at δ 2.32 (2H, t, J = 7.5 Hz).2) From these data, it was elucidated that 1 is composed of 1 mol each of 2-methylbutyric acid and 6 and 2 mol of tiglic acid. In addition, the ester linkages could be located at OH-3 and OH-4 of Rha, OH-2 of Glc1, and OH-3 of Glc2; further, the carbonyl group of Jla of 1 was found to be linked intramolecularly with a hydroxy group of the sugar moiety to form a macrolactone structure. The sites of each ester linkage of the organic acid units and Jla were deter-

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* To whom correspondence should be addressed. e-mail: mono@agri.u-tokai.ac.jp
mined from the HMBC spectrum of 1, with key cross-peaks observed between H-4 of Rha and C-1 of the first tigloyl unit (Tig), H-2 of Glc’ and C-1 of Jla, and H-3 of Glc’ and C-1 of the second tigloyl unit (Tig) (Fig. 1). Although no cross-peak between H-2 of Rha and C-1 of the first 2-methylbutyryl unit (Mba) was detected, the ester linkages of Mba, Tig, Tig’, and Jla could be located at the OH-2 of Rha, OH-4 of Rha, OH-3 of Glc’, and OH-2 of Glc’, respectively. These ester linkages were confirmed by the negative-ion FAB-MS of 1, which indicated fragment ion peaks at m/z 1035 [M-82 (tigloyl unit)-162 (hexosyl unit)]^-, 723 [1035-146 (6-deoxy-hexosyl unit)-84 (2-methylbutyryl unit)-82], 579 [723-144 (162-18 (H2O))], 417 [579-162], and 271 [417-146] (Fig. 2). Taking into account the J values of signals attributable to the anomic and methane protons of the sugar moiety, the conformations of the quinovopyranosyl and glucopyranosyl units were concluded to be C1′ and that of the rhamnopyranosyl unit was concluded to be C1′. The configuration of the 2-methylbutyric acid component of the crude resin glycoside fraction of this plant had been previously determined as S. Thus, the structure of 1 was assigned as 1αS-jalapinic acid 11-O-(3′-O-tigloyl)-β-D-glucopyranosyl-(1→3)-O-(2′′→2′′)-O-(2′-O-2S-methylbutyryl)-4′-O-tigloyl)-α-L-rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→2)-β-D-tiglopyranosyl(deoxyhexosyl)-1,2-ester (Fig. 3).

Compound 2, named calysolin VI, was obtained as an amorphous powder, which upon alkaline hydrolysis, furnished the same organic acids and glycosidic acid as those obtained from 1. The 1H- and 13C-NMR spectra indicated that 2 is composed of 1 mol each of tiglic acid and 6 and 2 mol of 2-methylycteric acid (Tables 1, 2). Further, the molecular formula (C16H12O12) of 2, which was obtained by HR-positive-ion FAB-MS analysis, and the nonequivalent signals due to H-2 of Jla in the 1H-NMR spectrum suggested that 2, like 1, has a macro lactone structure. The 1H-NMR spectrum of 2, when compared with that of 6a,52 showed acyl shiftings ([Δδ= δ2-Δδ6a] of signals due to H-2 (Δδ=0.95) and H-4 (Δδ=1.46) of Rha and H-2 (Δδ=ca. 1.51) and H-3 (Δδ=ca. 1.73) of Glc’. Thus, the ester linkages of 2 could be located at OH-2 and OH-4 of Rha and OH-2 and OH-3 of Glc’. In the HMBC spectrum of 2, key cross-peaks were observed between H-4 of Rha and C-1 of Tig and H-2 of Glc’ and C-1 of Jla (Fig. 1). Furthermore, the negative-ion FAB-MS of 2 indicated fragment ion peaks at m/z 1199 [M-H-82], 1035 [M-H-84-162], 807 [1035-82-146], 723 [807-84], 417 [579-162], and 271 [417-146] (Fig. 2). Therefore, Mba, Tig, Jla, and the second 2-methylbutyryl unit (Mba’) were attached to OH-2 of Rha, OH-4 of Rha, OH-2 of Glc’, and OH-3 of Glc’, respectively. Accordingly, the structure of 2 was assigned as 1αS-jalapinic acid 11-O-β-D-glucopyranosyl-(1→3)-O-(2′′→2′′)-O-(2′-O-2S-methylbutyryl)-4′-O-tigloyl)-α-L-rhamnopyranosyl-(1→2)-[O-β-D-glucopyranosyl-(1→3)]-O-β-D-glucopyranosyl-(1→2)-β-D-tiglopyranosyl(deoxyhexosyl)-1,2-ester (Fig. 3).

Compound 3, named calysolin VII, was obtained as an amorphous powder; its molecular formula was found to be C16H12O12. The 1H-NMR spectral data for 1 and 2 (in Pyridine-d5, 500 MHz)

| Position | 1          | 2          | 2          |
|----------|------------|------------|------------|
|           | 1H-NMR     | 1H-NMR     | 1H-NMR     |
|           | Spectra    | Spectra    | Spectra    |
| H-1       | 4.69 d (7.5) | 4.69 d (7.5) | 5.92 d (1)  |
| 2         | 4.15 d      | 4.13 d      | 2.32 d      |
| 3         | 4.28 dd d (9.0, 9.0) | 4.30 dd d (9.0, 9.0) | 5.04 dd (4.0, 10.0) |
| 4         | 3.55 dd d (9.0, 9.0) | 3.54 dd d (9.0, 9.0) | 5.91 dd (10.0, 10.0) |
| 5         | 3.61 d      | 3.63 d      | 5.32 q (6.5, 10.0) |
| 6         | 1.58 d (6.5) | 1.60 d (6.5) | 1.63 d (6.5) |

Table 1. Position 1 2 2 1 2
| Position | 1 | 2 | 2 | 1 | 2 |
|----------|---|---|---|---|---|
| H-1       | 4.69 d (7.5) | 4.69 d (7.5) | 5.92 d (1)  |
| 2         | 4.15 d      | 4.13 d      | 2.32 d      |
| 3         | 4.28 dd d (9.0, 9.0) | 4.30 dd d (9.0, 9.0) | 5.04 dd (4.0, 10.0) |
| 4         | 3.55 dd d (9.0, 9.0) | 3.54 dd d (9.0, 9.0) | 5.91 dd (10.0, 10.0) |
| 5         | 3.61 d      | 3.63 d      | 5.32 q (6.5, 10.0) |
| 6         | 1.58 d (6.5) | 1.60 d (6.5) | 1.63 d (6.5) |

in ppm from TMS. Coupling constants (J) in Hz are given in parentheses. a) Signals were overlapped with other signals. b) Signal due to H-2 of Glc’ of I was coupled to that [δ 7.02 (brd, J=5.0Hz)] due to OH-2 of Glc’ of I.
C₇₂H₁₁₆O₃₄ by HR-positive-ion FAB-MS. Alkaline hydrolysis of 3 furnished tiglic acid, 2-methylbutylic acid, and calysolic acid D (7). The ¹H-NMR spectrum of 3 indicated signals due to one 2-methylbutyryl unit, three tigloyl units, one non-equivalent methylene group assignable to H₂-2 of Jla, one primary methyl group, and six anomeric protons (Table 3). Thus, 3 was determined to contain 1 mol each of 2-methylbutyric acid and 7 and 3 mol of tiglic acid; the carboxyl group of Jla of 3 was determined to contain 1 mol each of 2-methylbutyryl, 4-\(^{\text{a}}\)-tigloyl)\(^{-}2\)-\(\beta\)-glucopyranosyl-(1\(\rightarrow\)4)-\(\text{O}\)-3-(\(\text{O}\)-tigloyl)\(-\beta\)-\(\text{O}\)-glucopyranosyl-(1\(\rightarrow\)3)-O-(2-O-2S-methylbutyryl, 4-O-tigloyl)\(-\alpha\)-l-rhamnopyranosyl-(1\(\rightarrow\)2)-O(\(\text{O}\)-

### Table 2. ¹³C-NMR Spectral Data for 1-5 (in Pyridine-\(d_5\), 125 MHz)

| Position | 1 | 2 | 3 | 4 | 5 |
|----------|---|---|---|---|---|
| Qui-1    | 103.5 | 103.4 | 103.4 | 103.4 | 103.6 |
| Jla-1    | 173.2 | 173.0 | 173.3 | 172.7 | 173.1 |
| 2        | 80.7 | 80.8 | 80.8 | 80.9 | 79.2 |
| Mba-1    | 162.2 | 162.1 | 162.2 | 162.2 | 162.1 |
| 3        | 79.1 | 79.1 | 79.1 | 79.1 | 79.7 |
| Tig-1    | 167.3 | 167.3 | 167.3 | 167.3 | 167.3 |
| 4        | 76.2 | 76.7 | 76.7 | 76.6 | 76.8 |
| 5        | 72.5 | 72.3 | 72.3 | 72.2 | 72.7 |
| Mba'1    | 175.3 | 175.0 | 175.8 | 175.0 | 175.8 |
| 6        | 18.4 | 18.4 | 18.4 | 18.3 | 18.5 |
| 7        | 37.1 | 37.1 | 37.1 | 37.1 | 37.1 |
| 8        | 56.3 | 56.3 | 56.3 | 56.3 | 56.3 |
| 9        | 65.3 | 65.3 | 65.3 | 65.3 | 65.3 |
| 10       | 74.3 | 74.3 | 74.3 | 74.3 | 74.3 |
| 11       | 83.3 | 83.3 | 83.3 | 83.3 | 83.3 |
| 12       | 92.3 | 92.3 | 92.3 | 92.3 | 92.3 |
| 13       | 101.3 | 101.3 | 101.3 | 101.3 | 101.3 |
| 14       | 110.3 | 110.3 | 110.3 | 110.3 | 110.3 |
| 15       | 119.3 | 119.3 | 119.3 | 119.3 | 119.3 |
| 16       | 128.3 | 128.3 | 128.3 | 128.3 | 128.3 |
| 17       | 137.3 | 137.3 | 137.3 | 137.3 | 137.3 |
| 18       | 146.3 | 146.3 | 146.3 | 146.3 | 146.3 |
| 19       | 155.3 | 155.3 | 155.3 | 155.3 | 155.3 |
| 20       | 164.3 | 164.3 | 164.3 | 164.3 | 164.3 |
| 21       | 173.3 | 173.3 | 173.3 | 173.3 | 173.3 |

Note: δ in ppm from TMS.
Compound 4, named calysolin VIII, was obtained as an amorphous powder. The molecular formula of 4 was determined to be C\textsubscript{72}H\textsubscript{120}O\textsubscript{34} by HR-positive-ion FAB-MS. Alkaline hydrolysis of 4 furnished tiglic acid, 2-methylbutyric acid, and calysolic acid C (8). The 1H- and 13C-NMR spectra indicated that 4 is composed of 1 mol each of tiglic acid and 8 and 3 mol of 2-methylbutyric acid (Tables 2, 4). Further, the molecular formula of 4 and the nonequivalent signals due to H\textsubscript{2}-2 of Jla in the 1H-NMR spectrum of 4 suggested that this compound also has a macrolactone structure. The 1H-NMR spectrum of 4 showed acylation shifts (Δδ = δ\textsubscript{methyl ester} - δ\textsubscript{tiglyl} (8a)) of the signals due to H-2 (Δδ = ca. 0.96) and H-4 (Δδ = 1.45) of Rha and H-2 (Δδ = 1.51), H-3 (Δδ = ca. 1.61), and H-4 (Δδ = ca. 1.44) of Glc', when compared with the spectrum of 8a. The HMBC spectrum of 4 showed key cross-peaks between H-4 of Rha and C-1 of Tig, H-2 of Glc' and C-1 of Jla, H-3 of Glc' and C-1 of Mba', and H-4 of Glc' and C-1 of the third 2-methylbutyryl unit (Mba") (Fig. 1). Therefore, Mba, Mba', Mba", Tig, and Jla were attached to OH-2 of Rha, OH-3 of Glc', OH-4 of Glc', OH-4 of Rha, and OH-2 of Glc', respectively. The negative-ion FAB-MS of 4, in which fragment ion peaks were observed at m/z 1283 [M-H-162-82], 1053 [1283-146-84], 891 [1053-162], 723 [891-84×2], 579 [723-144], 417 [579-162], and 271 [417-146], supported the above ester connectivities (Fig. 2). Accordingly, the structure of 4 was assigned as 11-O-β-D-glucopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2\textsuperscript{m}-ester (Fig. 3).

Compound 5, named calysolin IX, was obtained as an amorphous powder. Its negative-ion FAB-MS showed the same [M-H\textsuperscript{-}] ion peak as that of 4 at m/z 1527 together
January 2014 101

with fragment ion peaks at m/z 1445 [1527–82], 1053 [1445–162–84–146], 579 [1053–144–84×2–162], 417 [579–162], and 271 [417–146] (Fig. 2). The molecular formula of 5 was found to be the same as that of 4 by HR-positive-ion FAB-MS. Alkaline hydrolysis of 5 furnished the same organic acids and glycosidic acid as those obtained from 4. The 1H- and 13C-NMR spectra of 5, which were similar to those of 4, indicated signals corresponding to the presence of one each of tigloyl unit and 8 and three 2-methylbutyryl units (Tables 2, 4). From the preceding data, 5 was considered to be a positional isomer of 4 with respect to the ester linkage. The 1H-NMR signals corresponding to the sugar moiety of 5 were compared with those of 8a; signals due to H-2 of Rha, H-4 of Rha, H-3 of Glc', H-4 of Glc', and H-2 of the fifth glucosyl unit (Glc") showed downfield shifts of ca. 1.01, 1.46, ca. 1.34, ca. 1.63, and 1.48 ppm, respectively, owing to acylation. In addition, the HMBC spectrum of 5 showed key cross-peaks between H-4 of Rha and C-1 of Tig, H-3 of Glc' and C-1 of Mba', H-4 of Glc' and C-1 of Mba", and H-2 of Glc" and C-1 of Jla (Fig. 1). Therefore, Mba, Mba', Mba", Tig, and Jla were attached to OH-2 of Rha, OH-3 of Glc', OH-4 of Glc', OH-4 of Rha, and OH-2 of Glc", respectively. Accordingly, 5 was concluded to be a positional isomer of 4, in which the Jla of 5 was at the OH-2 of Glc" rather than at the OH-2 of Glc' (Fig. 3).

Compounds 1–5, calysolins I (9)–IV (12), and soldanelline B (13) were evaluated for their anti-HSV-1 activity, as a previous examination of the crude resin glycoside fraction of this plant indicated such activity (EC50 of anti-HSV-1

![Fig. 2. Fragment Ions Observed in the Negative-Ion FAB-MS of 1–5](image-url)
activity; 31.0 µg/mL; IC₅₀ of cytotoxic activity: 313 µg/mL). The EC₅₀ values of all compounds except 12 were ca. 8- to 22-fold of that of acyclovir, which is used for the treatment of HSV infections, and the activity of 12 was slightly weaker than that of 1–5, 9–11, and 13 (Table 5). Compounds 1–5 and 9–13 were therefore considered to be the anti-HSV-1 active components of the crude resin glycoside fraction. In addition, the results suggested that the tigloyl unit at C-4 of Rha may contribute to the anti-HSV-1 activity (see, 9 vs. 13), and that the aglycone ring size may not affect the activity (see, 4 vs. 5). However, all compounds showed relatively strong cytotoxic activity. Considering the anti-HSV-1 and cytotoxic activities of crude resin glycoside fraction as well as 1–5 and 9–13, some compounds having the strong anti-HSV-1 activity without the cytotoxic activity may be contained in the fraction. A detailed structure–activity relationship requires further sample collection, which is currently in process.

The study of the methanol extract of the fresh leaves, stems, and roots of C. soldanella resulted in the isolation and structural elucidation of five new resin glycosides. The compounds were of two macro lactone types—one having a 22-membered ring (1–4) and the other with a 27-membered ring (5). The sugar moieties of 1–5 are found to be partially acylated by some organic acids (2S-methylbutyric acid and tiglic acid). Compounds 4 and 5 are the first representatives of the calysoleic acid C as the component glycosidic acid. In addition, the anti-HSV-1 activities of 10 jalapins isolated from C. soldanella were investigated, with all compounds showing activity.

**Experimental**

**General Procedures** Optical rotations were determined with a JASCO P-1020 polarimeter. The ¹H- and ¹³C-NMR spectra were recorded by using a JEOL ECA-500 spectrometer, and chemical shifts are given on a δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. MS data were collected using a JEOL JMS-700 mass spectrometer. Analytical GC was carried out with a Shimadzu gas chromatograph GC-8A with a flame-ionization detector. Column chromatography was carried out over silica gel 60 (Merck, Art. No. 1.07734). HPLC separation was performed on a Shimadzu LC-10AS micro pump with a Shimadzu RID-10A RI detector. For HPLC column chromatography, COSMOSIL 5C18-AR-II (Nacalai Tesque, Inc., Japan, 20 mm i.d.×250 mm, column 1) and COSMOSIL π NAP (Nacalai Tesque Inc., 4.6 mm i.d.×250 mm, column 2) were used. Fetal calf serum (FCS) was purchased from Gibco BRL. Sulfonated γ-globulin (Venilon) was supplied by the Chemo-Sero Therapeutic Institute.

**Plant Material** The fresh leaves, stems, and roots of C.
The aqueous layer was desalted by Diaion HP20 (Mitsubishi Chemical Industries, Japan) column chromatography (H₂O, MeOH). Positive-ion FAB-MS m/z: 1547.7264 (Calcd for C₆₁H₁₀₀O₂₈Na⁺, 1547.7240). Negative-ion FAB-MS m/z: 1527 [M–H]⁻, 1445 [1527–82], 1283 [1445–162], 1053 [1283–144–84], 379 [84–127], 217 [84–127], 217 [147–146]. ¹H-NMR spectral data: see Table 1. ¹³C-NMR spectral data: see Table 2.

Calysolin VIII (4): Amorphous powder. [α]D²⁰ = −20.4° (c=1.6, MeOH). Positive-ion FAB-MS m/z: 1551 [M+Na]⁺. HR-positive-ion FAB-MS m/z: 1551.7572 (Calcd for C₆₁H₁₀₀O₂₈Na⁺, 1551.7553). Negative-ion FAB-MS m/z: 1527 [M–H]⁻, 1445 [1527–82], 1365 [1527–162], 1283 [1445–162], 1053 [1283–144–84], 379 [84–127], 217 [84–127], 217 [147–146]. ¹H-NMR spectral data: see Table 4. ¹³C-NMR spectral data: see Table 2.

Calysolin IX (5): Amorphous powder. [α]D²⁰ = −25.6° (c=1.0, MeOH). Positive-ion FAB-MS m/z: 1551 [M+Na]⁺. HR-positive-ion FAB-MS m/z: 1551.7572 (Calcd for C₆₁H₁₀₀O₂₈Na⁺, 1551.7553). Negative-ion FAB-MS m/z: 1527 [M–H]⁻, 1445 [1527–82], 1365 [1527–162], 1283 [1445–162], 1053 [1283–144–84], 379 [84–127], 217 [84–127], 217 [147–146]. ¹H-NMR spectral data: see Table 4. ¹³C-NMR spectral data: see Table 2.

Alkaline Hydrolysis of 1–5 Solutions of 1 (5mg), 2 (6mg), 3 (5mg), 4 (7mg), and 5 (7mg) in 1,4-dioxane–1 M KOH (1:2, 1.5mL) were each heated at 95°C for 1h. The reaction mixture was adjusted to pH 3 with 1M HCl and then diluted with H₂O (10mL) and extracted with ether (3×3mL). 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%) (GL Sciences Inc., Japan)], 3.2mm i.d.x2m glass column; carrier gas N₂, 1.0kg/cm²; column temperature, 120°C; t₀ (min)=4.50 (2-methylbutyric acid) for 1–5, 10.36 (tiglic acid) for 1–5.

The aqueous layer was desalted by Diaion HP20 (Mitsubishi Chemical Industries, Japan) column chromatography (H₂O, acetone) to give a glycolic acid as an amorphous powder (3mg from 1, 2mg from 2, 2mg from 3, 4mg from 4, 4mg from 5). The glycolic acids derived from 1–5 were each identical with 6, 7, 8, and 8, respectively, by comparison of ¹H-NMR spectra with those of authentic samples.

Anti-HSV-1 Assay The antiviral activity of test samples on HSV-1 was measured by the plaque reduction assay. Confluent monolayers of Vero cells in 6-well plates were infected with 100 TCID₅₀ of HSV-1 and treated with test samples. The samples were added to the cells 1h before virus inoculation. The cells were incubated for 2h; then, the medium was replaced with fresh medium and the culture was incubated for 48h. The plaques were stained with crystal violet and counted.

Table 3. ¹H-NMR Spectral Data for 3 (in Pyridine-d₅, 500MHz)

| Position | 3 | Position | 3 |
|----------|---|----------|---|
| Qui-1    | 4.68 d (7.5) | Glc-1 | 5.01 d (7.5) |
| 2        | 4.16 dd (7.5, 9.0) | 3 | 3.84⁹¹ |
| 3        | 4.25 dd (9.0, 9.0) | 3 | 4.21 dd (9.5, 9.5) |
| 4        | 3.54 dd (9.0, 9.0) | 4 | 5.54 dd (9.5, 9.5) |
| 5        | 3.60 dq (6.0, 9.0) | 5 | 3.82⁹¹ |
| 6        | 1.57 dd (6.0) | 6 | 3.99 dd (2.0, 12.5) |
| Glc-1    | 5.50 d (7.5) | 6 | 3.89⁹¹ |
| 2        | 4.13 dd (7.5, 9.0) | Rha-1 | 5.92 d (1.0) |
| 3        | 4.20 dd (9.0, 9.0) | 2 | 6.19 dd (1.0, 3.5) |
| 4        | 3.76 dd (9.0, 9.0) | 3 | 5.02 dd (3.5, 10.0) |
| 5        | 3.6⁴⁹² | 4 | 5.91 dd (10.0, 10.0) |
| 6        | 4.3²⁹³ | 5 | 5.30⁹¹ |
| 6        | 4.05 dd (6.5, 11.0) | 6 | 1.62 dd (6.5) |
| Glc-1    | 5.30 d (8.0) | Jia-2 | 2.71⁹¹ |
| 2        | 5.60 dd (8.0, 9.5) | 2 | 2.66⁹¹ |
| 3        | 4.58 dd (9.5, 9.5) | 3 | 11.37 mm |
| 4        | 4.15 dd (9.5, 9.5) | 16 | 0.85 s (7.0) |
| 5        | 4.1⁸⁹⁴ | Mba-2 | 2.57 dqd (7.0, 7.0, 7.0) |
| 6        | 4.51 dd (2.0, 12.0) | 3 | 1.76⁹¹ |
| 6        | 4.29 dd (5.0, 12.0) | 3 | 1.4⁸⁹⁴ |
| Glc-1    | 4.98 d (8.0) | 4 | 0.84 dd (7.5, 7.5) |
| 2        | 3.8⁷⁹ | 5 | 1.20 dd (7.0) |
| 3        | 5.65 dd (9.5, 9.5) | TIG-3 | 7.40 qq (1.0, 7.0) |
| 4        | 4.32 dd (9.5, 9.5) | 4 | 1.70 qd (1.5, 7.0) |
| 5        | 3.6⁴⁹² | 5 | 2.00 brs |
| 6        | 4.46 brs (12.0) | TIG-3 | 7.08 qq (1.0, 7.0) |
| 6        | 4.3⁴⁹² | 4 | 1.64 qd (1.5, 7.0) |
| 5        | 1.90 brs |
| TIG-3    | 6.8⁹⁴ | 4 | 1.53 qd (1.5, 7.0) |
| 5        | 1.75 brs |

δ in ppm from TMS. Coupling constants (J) in Hz are given in parentheses. a) Signals were overlapped with other signals.

soldanella were collected in Mie Prefecture, Japan, in May 2009, and identified by Dr. Hiroaki Setoguchi (Graduate School of Human and Environmental Studies, Kyoto University, Japan). A voucher specimen (CSM12009) has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Virus and Cell HSV-1 strain (KOS) and Vero cells were provided by the Chemo-Sero Therapeutic Institute.

Extraction and Isolation The cut fresh leaves, stems, and roots of C. soldanella (916.9 g) were extracted with methanol (MeOH) (10L) 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 ethyl acetate (1.6 L) and n-butanol (0.6 L) to afford a ethyl acetate soluble fraction (48.57 g) and a n-butanol soluble fraction (8.15 g). A part (45.87 g) of the ethyl acetate soluble fraction was chromatographed on silica gel column 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 1–17. HPLC (column 1) of fraction 11 (3800mg) eluted with 95% MeOH gave 5 (220mg), 2 (133mg), 4 (131mg), and fractions 11.1–11.9. Fraction 11.1 (254mg) was subjected to HPLC (column 2) eluted with 90% MeOH to afford 1 (12mg) and 3 (22mg).
1. **Table 4.** 1H-NMR Spectral Data for 4 and 5 (in Pyridine-d$_5$, 500 MHz)

| Position | 4          | 5          | Position | 4 | 5 |
|----------|------------|------------|----------|---|---|
| Qui-1    | 4.68 d (8.0)| 4.76 d (7.5)| Rha-1    | 5.77 s | 6.26 d (1.5) |
| 2        | 4.08 (a)   | 4.29 dd (7.5, 8.5)| 2        | 6.01 d (3.5) | 6.06 dd (1.5, 3.5) |
| 3        | 4.30 (a)   | 4.38 dd (8.5, 8.5)| 3        | 5.19 dd (3.5, 10.0) | 5.17 dd (3.5, 10.0) |
| 4        | 3.55 dd (9.0, 9.0)| 3.57 dd (8.5, 8.5)| 4        | 5.94 dd (10.0, 10.0) | 5.95 dd (10.0, 10.0) |
| 5        | 3.63 qd (6.5, 9.0)| 3.74 (a) | 5        | 5.29 (a) | 5.23 qd (6.0, 10.0) |
| 6        | 1.60 d (6.5) | 1.63 d (6.5) | 6        | 1.62 d (6.5) | 1.68 d (6.0) |
| Glc-1    | 5.58 d (8.0)| 5.76 d (7.5)| Jla-2    | 2.64 (a) | 2.56 (a) |
| 2        | 4.11 dd (8.0, 9.0)| 4.10 (a) | 2        | 2.61 (a) | 2.40 (a) |
| 3        | 4.48 dd (9.0, 9.0)| 3.71 dd (8.5, 8.5)| 11       | 3.72 m | 3.78 m |
| 4        | 3.85 (a)   | 3.83 (a)   | 16       | 0.86t (7.0) | 0.87t (7.0) |
| 5        | 3.87 (a)   | 3.65 ddd (2.5, 6.5, 8.5) | Mba-2    | 2.57 ddq (7.0, 7.0, 7.0) | 2.38 ddq (7.0, 7.0, 7.0) |
| 6        | 4.28 (a)   | 4.47 (a)   | 3        | 1.85 m | 1.73 (a) |
| 6        | 4.08 (a)   | 4.16 (a)   | 3        | 1.57 (a) | 1.48 (a) |
| Glc'-1   | 5.29 d (7.5)| 4.85 d (8.0) | 4        | 0.87 dd (7.5, 7.5) | 0.82 dd (7.5, 7.5) |
| 2        | 5.46 dd (7.5, 9.5)| 3.90 (a) | 5        | 1.30 d (7.0) | 1.22 d (7.0) |
| 3        | 5.87 dd (9.5, 9.5)| 5.60 dd (9.5, 9.5) | Mba'-2   | 2.36 ddq (7.0, 7.0, 7.0) | 2.44 ddq (7.0, 7.0, 7.0) |
| 4        | 5.32 dd (9.5, 9.5)| 5.51 dd (9.5, 9.5) | 3        | 1.66 (a) | 1.79 (a) |
| 5        | 4.32 (a)   | 3.95 ddd (3.0, 3.5, 9.5)| 3        | 1.42 (a) | 1.48 (a) |
| 6        | 4.28 (a)   | 4.14 (a)   | 4        | 0.85 dd (7.5, 7.5) | 0.94 dd (7.0, 7.0) |
| 6        | 4.08 (a)   | 4.05 dd (3.5, 12.0)| 5        | 1.10 d (7.0) | 1.17 d (7.0) |
| Glc''-1  | 5.13 d (7.5)| 5.13 d (7.5)| Mba''-2  | 2.24 ddq (7.0, 7.0, 7.0) | 2.52 ddq (7.0, 7.0, 7.0) |
| 2        | 3.82 (a)   | 3.95 (a)   | 3        | 1.63 (a) | 1.84 (a) |
| 3        | 3.95 (a)   | 4.00 dd (9.0, 9.0)| 3        | 1.32 (a) | 1.52 (a) |
| 4        | 3.82 (a)   | 3.86 (a)   | 4        | 0.79 dd (7.5, 7.5) | 0.96 dd (7.0, 7.0) |
| 5        | 3.94 (a)   | 4.11 (a)   | 5        | 1.05 d (7.0) | 1.22 d (7.0) |
| 6        | 4.45 brd (11.0)| 4.49 (a) | Tig-3    | 7.44 qq (1.0, 7.0) | 7.38 qq (1.5, 7.0) |
| 6        | 4.03 dd (8.0, 11.0)| 4.12 (a) | 4        | 1.74 d (7.0) | 1.74 d (7.0) |
| Glc'''-1 | 5.11 d (8.0)| 4.98 (a)   | 5        | 2.04 brs | 2.06 brs |
| 2        | 3.98 (a)   | 5.49 dd (8.0, 9.5)| 3        | 1.63 (a) | 1.84 (a) |
| 3        | 4.30 (a)   | 4.31 (a)   | 4        | 0.79 dd (7.5, 7.5) | 0.96 dd (7.0, 7.0) |
| 4        | 4.19 dd (9.0, 9.0)| 4.21 dd (9.5, 9.5)| 5        | 1.05 d (7.0) | 1.22 d (7.0) |
| 5        | 3.98 (a)   | 3.86 (a)   | 6        | 4.47 brd (11.0)| 4.48 (a) |
| 6        | 4.33 (a)   | 4.33 (a)   | 6        | 4.47 brd (11.0)| 4.48 (a) |

$\delta$ in ppm from TMS. Coupling constants (J) in Hz are given in parentheses. a) Signals were overlapped with other signals.

2. **Table 5.** Anti-herpes Activity (EC$_{50}$) and Cytotoxic Activity (IC$_{50}$) of 1-5, 9-13, and Acyclovir

| Sample | EC$_{50}$ (µg/mL) | IC$_{50}$ (µg/mL) |
|--------|------------------|------------------|
| 1      | 1.9              | 18.5             |
| 2      | 3.2              | 10.0             |
| 3      | 5.5              | 16.5             |
| 4      | 2.9              | 4.6              |
| 5      | 3.2              | 8.5              |
| 9      | 5.2              | 13.0             |
| 10     | 3.0              | 18.5             |
| 11     | 3.2              | 4.3              |
| 12     | 19.2             | 10.2             |
| 13     | 3.3              | 8.2              |
| Acyclovir | 0.25          | >100             |

Each value is the mean of duplicate assays.

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 FCS and 2% sulfonated γ-globulin including various concentrations of the test samples. The plates were incubated in the CO$_2$ 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-dimethylthiazol-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%, respectively. The optical density of each well at 570 nm was determined spectrophotometrically using a reference wavelength of 630 nm.

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