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Calysolins I–IV, Resin Glycosides from *Calystegia soldanella*

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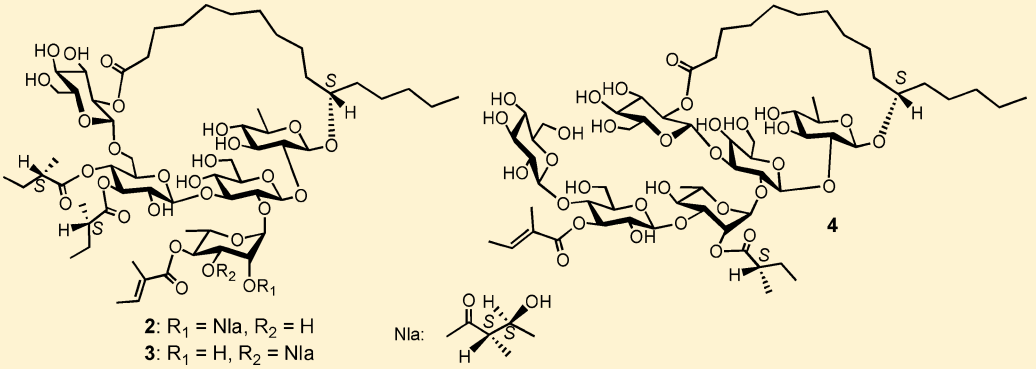
Supporting Information



ABSTRACT: Four new resin glycosides having intramolecular cyclic ester structures (jalapins), named calysolins I–IV (**1**–**4**), were isolated from the methanol extract of leaves, stems, and roots of *Calystegia soldanella*, along with one known jalapin (**5**) derivative. The structures of **1**–**4** were determined on the basis of spectroscopic data and chemical evidence. They fall into two types, one having a 22-membered ring (**1** and **4**) and the other with a 27-membered ring (**2** and **3**). The sugar moieties of **1**–**4** were partially acylated by some organic acids. Compound **4** is the first example of a hexaglycoside of jalapin.

The so-called resin glycosides are well-known as purgatives and commonly found in plants of Convolvulaceae.[1] *Calystegia soldanella* Roem. et Schult. (Convolvulaceae) is distributed widely on sandy beaches of seas and lakes in temperate regions of the world. The roots of this plant are used for the treatment of arthritis.[2] The isolation and structure elucidation were reported for two genuine resin glycosides, soldanellines A and B, as chemical constituents of the root.[3,4] In a previous paper,[5] we reported the isolation and structure elucidation of four new glycosidic acids, calysolic acids A–D, which were obtained along with a known glycosidic acid, soldanellic acid B,[4] and three organic acids, 2*S*-methylbutyric, tiglic, and 2*S*-methyl-3*S*-hydroxybutyric (2*S*,3*S*-nilic) acids, upon alkaline hydrolysis of the crude resin glycoside fraction of the leaves, stems, and roots of *C. soldanella*. As part of an ongoing study of the resin glycosides from this plant, the isolation and structure elucidation of four new genuine resin glycosides (**1**–**4**) along with a known resin glycoside (**5**) are reported herein.

The flesh leaves, stems, and roots of *C. soldanella* were extracted with methanol. This extract was suspended in H_2O and then extracted successively with ethyl acetate and *n*-butyl alcohol. The ethyl acetate–soluble fraction was subjected to silica gel and HPLC on ODS and silica gel to yield five resin glycosides (**1**–**5**).

RESULTS AND DISCUSSION

Compound **1**, named calysolin I, was obtained as an amorphous powder. The alkaline hydrolysis product of **1** was fractionated into an organic acid fraction and a glycosidic acid. GC of the organic acid fraction revealed the presence of 2-methylbutyric acid and nilic acid. The later was identified in soldanellic acid B[4] by ^{1}H NMR spectroscopic comparison with that of an authentic sample.[5] In the negative-ion FABMS, **1** exhibited a $[M - H]^-$ ion peak at *m*/*z* 1053 and fragment ion peaks at *m*/*z* 953 [1053 − 100 (niloyl unit (Nla))]$^-$, 825 [1053 − 144 (hexosyl unit − H_2O) − 84 (2-methylbutyryl unit (Mba))]$^-$, 807 [953 − 146 (methylpentosyl unit)]$^-$, 725 [825 − 100]$^-$, 579 [725 −146]$^-$, 417 [579 − 162]$^-$, and 271 [417 − 146]$^-$ (Figure S22, Supporting Information). Furthermore, the molecular formula of **1** was analyzed as $C_{50}H_{86}O_{23}$ by positive-ion HRFABMS. The ^{1}H NMR spectrum of **1** exhibited signals due to H-2 (δ 2.41 (ddq, J = 7.0, 7.0, 7.0 Hz)) of Mba, H-2 (δ 3.00 (dq, J = 7.0, 7.0 Hz)) of Nla, two primary methyl groups (δ 0.89 (dd, J = 7.0, 7.0 Hz), 0.85 (t, J = 7.5 Hz)), five secondary methyl groups (δ 1.75 (d, J = 6.5 Hz), 1.54 (d, J = 6.5 Hz), 1.52 (d, J = 7.0 Hz), 1.46 (d, J = 6.5 Hz), 1.11 (d, J = 7.0 Hz)), and four anomeric protons (δ 5.79 (s), 5.57 (d, J = 8.0 Hz), 5.27 (d, J = 8.0 Hz), 4.74 (d, J = 7.5 Hz)). The ^{13}C NMR spectrum showed signals

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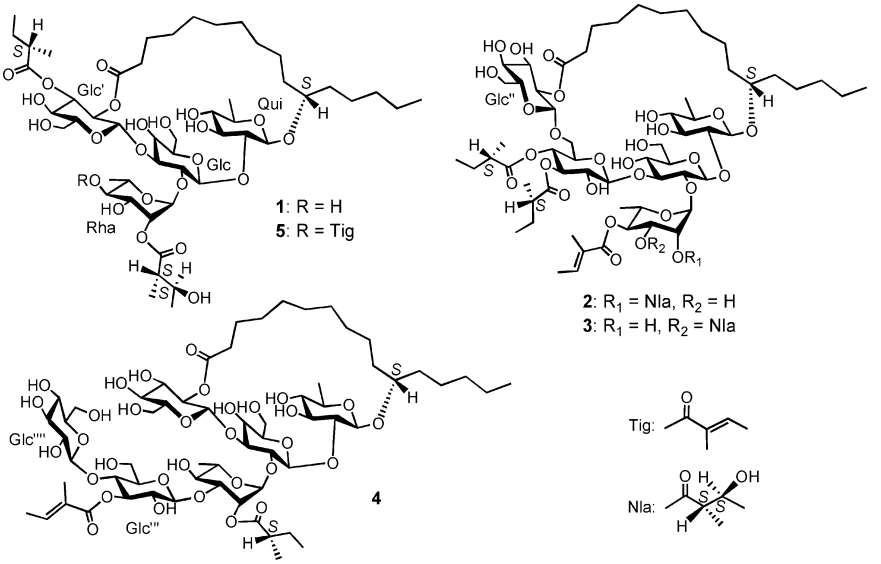

assignable to three carboxyl carbons (δ 175.4, 175.3, 172.9) and four anomeric carbons (δ 103.6, 101.7, 99.9, 98.1). These data indicated that **1** is composed of 1 mol each of 2-methylbutyric acid, nilic acid, and soldanellic acid B and that three ester groups exist in the molecule; hence, the carboxyl group of the aglycone 11*S*-hydroxyhexadecanoic (11*S*-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.[6] The presence of the macrocyclic ester structure was supported by the nonequivalent signals due to H_2-2 of the jalapinoloyl unit (Jla) of **1** observed at δ 2.71 (1H, ddd, J = 2.5, 15.5, 15.5 Hz) and 2.61 (1H, ddd, J = 4.5, 4.5, 15.5 Hz), whereas the methyl ester[5] of soldanellic acid B exhibited the equivalent signal due to H_2-2 of Jla at δ 2.32 (2H, t, J = 7.5 Hz) in the ^{1}H NMR spectrum.[7]

In order to clarify the positions of the ester linkages, the ^{1}H NMR signals due to the sugar moiety of **1** were assigned on the basis of ^{1}H–^{1}H COSY, HMQC, and HMBC spectra. On comparison of the chemical shifts of signals due to the sugar moieties between **1** and soldanellic acid B methyl ester,[5] downfield shifts ($\Delta\delta$ = δ(**1**) − δ(soldanellic acid B methyl ester)) owing to acylation were seen for signals due to H-2 ($\Delta\delta$ = 1.09) of rhamnosyl unit (Rha), H-2 ($\Delta\delta$ = ca. 1.53) and H-3 ($\Delta\delta$ = 1.73) of the second glucosyl unit (Glc′) in **1**. Thus, the ester linkages could be located at the OH-2 of Rha, the OH-2 of Glc′, and the OH-3 of Glc′. The positions of each ester linkage of Jla, Nla, and Mba were determined by negative-ion FABMS and the HMBC spectrum of **1** and the HRFABMS of the peracetate (**1a**) of **1**. The fragment ion peaks at m/z 825 and 807 in the negative-ion FABMS suggested that the ester linkage of Nla might be placed at the OH-2 of Rha (Figure S22, Supporting Information). However, the sites of the ester linkages of Mba and Jla, both of which are in the Glc′ unit, were not discernible. In the HMBC spectrum, a key cross peak was observed between H-2 of Glc′ and C-1 of Jla, while the counterparts of H-2 of Rha and H-3 of Glc′ could not be defined because the ^{13}C NMR signals due to C-1 of Mba and C-1 of Nla appeared at almost the same chemical shifts (Figure S23, Supporting Information). The HRFABMS of **1a** gave a fragment ion peak at m/z 373.1500, which was ascribable to the fragment ion of a 2-*O*-2-methyl-3-acetoxybutyryl-3,4-*O*-diacetylrhamnopyranosyl unit. Therefore, the ester linkages of Nla, Jla, and Mba could be located at the OH-2 of Rha, the OH-2 of Glc′, and the OH-3 of Glc′, respectively. Taking the J values of signals due to the anomeric and methine protons of the sugar moiety into account, the conformations of the quinovopyranosyl and glucopyranosyl units were ^{4}C$_1$ and that of the rhamnopyranosyl unit was concluded to be ^{1}C$_4$. The configurations of the component 2-methylbutyric acid and nilic acid units of the crude resin glycoside fraction of this plant have been determined previously as *S* and 2*S*,3*S*, respectively.[5] Accordingly, the structure of **1** was assigned as 11*S*-jalapinolic acid 11-*O*-(2-*O*-2*S*,3*S*-niloyl)-α-L-rhamnopyranosyl-(1→2)-[*O*-(3-*O*-2*S*-methylbutyryl)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-qinovopyranoside, intramolecular 1,2′′′′-ester.

Compound **2** (calysolin II) was obtained as an amorphous powder and furnished tiglic acid, 2-methylbutyric acid, nilic acid, and calysolic acid A on alkaline hydrolysis.[5] The ^{1}H and ^{13}C NMR spectra of **2** indicated that **2** is composed of 1 mol each of tiglic acid, nilic acid, and calysolic acid A and 2 mol of 2-methylbutyric acid. Further, a [M − H]$^-$ ion peak observed at m/z 1381 in the negative-ion FABMS of **2** and the nonequivalent signals due to H_2-2 of Jla in the ^{1}H NMR spectrum suggested that **2**, like **1**, has an intramolecular macrocyclic ester structure.

The ^{1}H NMR spectrum of **2** showed, in comparison with that of calysolic acid A methyl ester,[5] acylation shifts ($\Delta\delta$ = δ(**2**) − δ(calysolic acid A methyl ester)) of signals due to H-2 ($\Delta\delta$ = 1.19) and H-4 ($\Delta\delta$ = 1.47) of Rha, H-3 ($\Delta\delta$ = 1.47) and H-4 ($\Delta\delta$ = ca. 1.52) of Glc′, and H-2 ($\Delta\delta$ = ca. 1.45) of the third glucose unit (Glc″). Thus, the ester linkages of **2** could be located at OH-2 and OH-4 of Rha, OH-3 and OH-4 of Glc′, and OH-2 of Glc″. The sites of each ester linkage of the 2-methylbutyryl, tigloyl (Tig), Nla, and Jla units were determined using the HMBC spectrum of **2**, with key cross peaks observed between H-2 of Rha and C-1 of Nla, 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 the second 2-methylbutyryl unit (Mba′), and H-2 of Glc″ and C-1 of Jla (Figure S23, Supporting Information). Therefore, Nla, Tig, Mba, Mba′, and Jla were attached to OH-2 of Rha, OH-4 of Rha, OH-3 of Glc′, OH-4 of Glc′, and OH-2 of Glc″, respectively. These inferences were supported by the negative-ion FABMS of **2**, in which fragment ion peaks were observed at m/z 1237, 1053 [1381 − 100 − 82 (Tig) − 146]$^-$ and 907 [1237 − 162 − 84 × 2]$^-$ (Figure S22, Supporting Information). Accordingly, the structure of **2** was assigned as 11*S*-jalapinolic acid 11-*O*-(2-*O*-2*S*,

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Table 1. ^{1}H NMR Spectroscopic Data (in pyridine-d_5, 500 MHz) for 1 and 2[a]

position	1	2	position	1	2
Qui-1	4.74 d (7.5)	4.72 d(7.0)	Rha-1	5.79 s	6.34 d (1.0)
2	4.17 dd (7.5, 9.0)	ca. 4.32	2	5.91 d (3.0)	5.99 dd (1.0, 3.5)
3	ca. 4.35	ca. 4.31	3	4.62 dd (3.0, 9.5)	4.83 dd (3.5, 9.5)
4	3.51 dd (9.0, 9.0)	3.56 dd (9.0, 9.0)	4	4.12 dd (9.5, 9.5)	5.74 dd (9.5, 9.5)
5	3.65 m	3.65 dq (9.0, 6.5)	5	5.04 dq (9.5, 6.5)	5.19 dq (9.5, 6.5)
6	1.54 d (6.5)	1.60 d (6.5)	6	1.75 d (6.5)	1.61 d (6.5)
Glc-1	5.57 d (8.0)	5.83 d (7.5)	Jla-2	2.71 ddd (2.5, 15.5, 15.5)	ca. 2.54
2	4.11 dd (8.0, 9.0)	ca. 4.16	2	2.61 ddd (4.5, 4.5, 15.5)	ca. 2.42
3	4.27 dd (9.0, 9.0)	3.95 dd (9.0, 9.0)	11	ca. 3.72	ca. 3.79
4	3.81 dd (9.0, 9.0)	3.90 dd (9.0, 9.0)	16	0.85 t (7.5)	0.86 t (7.0)
5	ca. 3.70	3.71 ddd (2.5, 5.5, 9.0)	Mba-2	2.41 ddq (7.0, 7.0, 7.0)	2.42 ddq (7.0, 7.0, 7.0)
6	ca. 4.34	4.46 dd (2.5, 12.0)	3	ca. 1.77	1.77 m
6	4.06 dd (5.0, 11.5)	ca. 4.19	3	ca. 1.42	1.47 m
Glc′-1	5.27 d (8.0)	4.90 d (8.0)	4	0.89 dd (7.0, 7.0)	0.93 dd (7.5, 7.5)
2	5.54 dd (8.0, 9.5)	3.94 dd (8.0, 9.0)	5	1.11 d (7.0)	1.16 d (7.0)
3	5.87 dd (9.5, 9.5)	5.57 dd (9.0, 9.0)	Mba′-2		2.52 ddq (7.0, 7.0, 7.0)
4	4.19 dd (9.5, 9.5)	5.44 dd (9.0, 9.0)	3		1.87 m
5	4.03 m	3.88 m	3		1.54 m
6	4.42 dd (2.0, 12.0)	4.09 dd (2.5, 12.0)	4		0.96 dd (7.5, 7.5)
6	ca. 4.32	4.00 dd (4.5, 12.0)	5		1.22 d (7.0)
Glc″-1		4.94 d (8.0)	Nla-2	3.00 dq (7.0, 7.0)	2.77 dq (7.0, 7.0)
2		5.46 dd (8.0, 9.5)	3	ca. 4.36	ca. 4.28
3		ca. 4.27	4	1.46 d (6.5)	1.37 d (6.5)
4		ca. 4.18	5	1.52 d (7.0)	1.32 d (7.0)
5		3.83 m	Tig-3		7.19 dq like (1.5, 6.5)
6		4.44 dd (2.0, 12.0)	4		1.68 d (6.5)
6		ca. 4.31	5		1.85 br s

[a]Chemical shifts (δ) are in ppm relative to TMS. Coupling constants (J) in Hz are given in parentheses.

3*S*-niloyl,4-*O*-tigloyl)-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→6)-*O*-(3,4-di-*O*-2*S*-methylbutyryl)-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2‴″-ester.

Compound **3** (calysolin III) was obtained as an amorphous powder, and its molecular formula was found to be the same as that of **2**. On alkaline hydrolysis, **3** furnished the same organic acids and glycosidic acid as those obtained from **2**. The ^{1}H NMR spectrum of **3**, which was superimposable on that of **2**, indicated signals due to one unit each of Nla, Tig, and calysolic acid A, and two 2-methybutyryl units. Comparison of the ^{1}H NMR signals due to the sugar moiety between **3** and calysolic acid A methyl ester indicated acylation shifts ($\Delta\delta = \delta(\mathbf{3}) - \delta$(calysolic acid A methyl ester)) of signals due to H-3 ($\Delta\delta$ = 1.45) and H-4 ($\Delta\delta$ = 1.89) of Rha, H-3 ($\Delta\delta$ = 1.60) and H-4 ($\Delta\delta$ = ca. 1.65) of Glc′, and H-2 ($\Delta\delta$ = ca. 1.50) of Glc″. In addition, the HMBC spectrum of **3** showed cross peaks between H-3 of Rha and C-1 of Nla, 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 (Figure S23, Supporting Information). Accordingly, **3** was concluded to be a positional isomer of **2**, in which the Nla unit was at the OH-3 of Rha rather than at the OH-2 of Rha.

Compound **4** (calysolin IV) was obtained as an amorphous powder and afforded tiglic acid, 2-methylbutyric acid, and calysolic acid D[5] on alkaline hydrolysis. Negative-ion FABMS of **4** indicated a $[M - H]^-$ ion peak at m/z 1359 and fragment ion peaks at m/z 1277 $[1359 - 82]^-$, 1215 $[1359 - 144]^-$, 953 $[1277 - 162 \times 2]^-$, 723 $[953 - 84 - 146]^-$, 579 $[723 - 144]^-$, 417 $[579 - 162]^-$, and 271 $[417 - 146]^-$ (Figure S22, Supporting Information). Compound **4** showed signals due to one unit each of Mba and Tig, a nonequivalent methylene group assignable to H_2-2 of Jla, and a primary methyl group and six anomeric protons in the ^{1}H NMR spectrum and exhibited signals due to three carboxyl carbons and six anomeric carbons in the ^{13}C NMR spectrum. Thus, **4** was composed of 1 mol each of 2-methylbutyric acid, tiglic acid, and calysolic acid D, and the carboxyl group of Jla of **4** was also linked intramolecularly with a hydroxy group of the sugar moiety to form a macrocyclic ester structure.

The ^{1}H NMR signals due to the sugar moiety of **4** were compared with those of methyl ester[5] of calysolic acid D, and signals due to H-2 of Rha, H-2 of Glc′, and H-3 of Glc‴ showed downfield shifts of 1.07, ca. 1.62, and ca. 1.59 ppm, respectively, owing to acylation. In addition, the HMBC spectrum of **4** exhibited key correlations between H-2 of Glc′ and C-1 of Jla and H-3 of Glc″ and C-1 of Tig (Figure S23, Supporting Information). Accordingly, the structure of **4** was proposed as 11*S*-jalapinolic acid 11-*O*-β-D-glucopyranosyl-(1→4)-*O*-(3-*O*-tigloyl)-β-D-glucopyranosyl-(1→3)-*O*-(2-*O*-2*S*-methylbutyryl)-α-L-rhamnopyranosyl-(1→2)-[*O*-β-D-glucopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→2)-β-D-quinovopyranoside, intramolecular 1,2‴″-ester.

Compound **5** was identified as soldanelline B on the basis of its physical and spectroscopic data.[4]

The calysolins in this study have an intramolecular cyclic ester structure. Therefore, they can be classified as jalapin-type compounds.[6] Further, they fall into two types: one having a 22-membered ring with the intramolecular ester group attached at OH-2 of the second glucose unit (**1** and **4**) and the other with a 27-membered ring with an ester linkage at OH-2 of the third glucose unit (**2** and **3**). Calysolin IV (**4**) is the first example of a hexaglycoside of jalapin, and calysolines II (**2**) and III (**3**) are the first representatives of the calysolic acid A as the component glycosidic acid.

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Table 2. ^{13}C NMR Spectroscopic Data (in pyridine-d_5, 125 MHz) for 1–4[a]

position	1	2	3	4	position	1	2	3	4
Qui-1	103.6	103.6	103.2	103.4	Rha-1	98.1	97.8	101.2	97.5
2	80.4	79.5	79.1	80.9	2	73.1	73.1	69.5	72.2
3	78.8	79.0	78.9	78.7	3	70.8	68.2	73.4	79.5
4	76.9	76.7	76.8	76.9	4	74.2	75.9	72.3	73.1
5	72.2	72.6	72.5	72.4	5	69.3	66.7	67.1	69.0
6	18.4	18.4	18.5	18.3	6	18.3	18.5	18.5	18.2
Glc-1	101.7	100.8	101.4	101.9	Jla-1	172.9	173.1	173.0	173.3
2	77.0	75.3	76.7	76.4	2	33.9	34.4	34.4	33.9
3	84.7	90.3	90.4	85.3	11	81.8	82.6	82.2	81.5
4	70.1	70.7	70.6	70.2	16	14.2	14.2	14.2	14.2
5	77.0	77.4	77.3	77.0	Mba-1	175.4[b]	175.8	176.1	176.4
6	63.1	63.0	63.2	63.2	2	41.5	41.1	41.1	41.0
Glc′-1	99.9	104.1	104.6	100.4	3	26.8	26.9	26.8	27.3
2	72.9	72.5	72.5	75.0	4	11.7	11.8	11.6	11.6
3	76.1	75.9	76.3	75.0	5	16.7	16.6	16.6	16.3
4	70.0	69.0	69.1	72.6	Mba′-1		175.7	175.7	
5	78.3	73.3	73.1	78.8	2		41.2	41.1	
6	61.5	66.5	66.1	62.2	3		26.7	26.7	
Glc″-1		102.0	102.0		4		11.8	11.8	
2		75.0	75.0		5		16.3	16.4	
3		76.0	76.1		Nla-1	175.3[b]	175.4	174.8	
4		71.6	71.7		2	48.2	48.3	48.1	
5		79.0	79.1		3	70.4	69.8	68.4	
6		62.2	62.3		4	21.2	21.1	19.8	
Glc‴-1				105.4	5	13.4	13.3	11.6	
2				73.9	Tig-1		167.5	167.5	167.4
3				77.0	2		128.8	128.6	129.4
4				76.3	3		138.0	138.5	137.2
5				76.8	4		14.3	14.4	14.1
6				61.2	5		12.4	12.5	12.4
Glc⁗-1				104.6					
2				75.4					
3				78.2					
4				71.8					
5				77.9					
6				62.9					

[a]Chemical shifts (δ) are in ppm relative to TMS. [b]Assignments may be interchangeable.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were determined with a JASCO P-1020 polarimeter. The ^{1}H and ^{13}C 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 GC-8A gas chromatograph with a flame-ionization detector. Colum 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 or Shimadzu SPD-10A UV detector. For HPLC column chromatography, COSMOSIL 5C18-AR-II (Nacalai Tesque, Inc., Kyoto, Japan, 20 mm i.d. × 250 mm, column 1) and COSMOSIL SIL-06 (Nacalai Tesque Inc., 20 mm i.d. × 250 mm, column 2) were used.

Plant Material. The flesh leaves, stems, and roots of *C. 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). A voucher specimen (CSMI2009) has been deposited at the laboratory of Natural Products Chemistry, School of Agriculture, Tokai University.

Extarction and Isolation. The cut fresh leaves, stems, and roots of *C. soldanella* (916.9 g) were extracted with methanol (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). This extract was suspended in H_2O (3.5 L) and then successively extracted with ethyl acetate (1.6 L) and *n*-butyl alcohol (0.6 L) to afford an ethyl acetate soluble fraction (48.57 g) and an *n*-butyl alcohol soluble fraction (8.15 g). A part (45.87 g) of the ethyl acetate soluble fraction was chromatographed over a silica gel column using gradient mixtures of $CHCl_3$–MeOH–H_2O (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 7 (2035 mg) eluted with 95% MeOH gave **2** (18 mg), **5** (97 mg), and fractions 7.1–7.10. Fraction 7.2 (71 mg) was subjected to HPLC (column 1) with 90% MeOH as eluent to afford **1** (11 mg) and **3** (39 mg). HPLC (column 1) of fraction 16 (3044 mg) using 90% MeOH as eluent gave fractions 16.1–16.8. Fraction 16.7 (53 mg) was subjected to HPLC (column 2) with $CHCl_3$–MeOH–H_2O (10/2/0.1) as eluent to afford **4** (17 mg).

Calysolin I (1): amorphous powder; mp 148–155 °C; $[\alpha]^{25}_D$ −39.2 (*c* 0.3, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (positive mode) *m/z* 1077 $[M + Na]^+$; FABMS (negative mode) *m/z* 1053 $[M - H]^-$, 953 $[1053 - 100]^-$, 825 $[1053 - 144 - 84]^-$, 807 $[953 - 146]^-$, 725 $[825 - 100]^-$, 579 $[725 - 146]^-$, 417 $[579 - 162]^-$, 271 $[417 - 146]^-$; HRFABMS (positive mode) *m/z* 1077.5459 (calcd for $C_{50}H_{86}O_{23}Na$, 1077.5458).

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Table 3. ^{1}H NMR Spectroscopic Data (in pyridine-d_5, 500 MHz) for 3 and 4[a]

position	3	4	position	3	4
Qui-1	4.75 d (7.5)	4.73 d (7.5)	Glc‴′-1		5.04 d (8.0)
2	ca. 4.30	ca. 4.12	2		3.83 dd (8.0, 8.5)
3	ca. 4.34	4.32 dd (9.0, 9.0)	3		ca. 4.12
4	3.54 dd (9.0, 9.0)	3.51 dd (9.0 9.0)	4		ca. 4.12
5	3.65 dq (9.0, 6.5)	ca. 3.67	5		ca. 3.76
6	1.57 d (6.5)	1.53 d (6.5)	6		ca. 4.28
Glc-1	5.71 d (8.0)	5.43[b]	6		4.18 dd (4.5, 10.5)
2	4.13 dd (8.0, 9.0)	ca. 4.12	Rha-1	6.26 d (1.0)	5.84 d (1.5)
3	3.80 dd (9.0, 9.0)	ca. 4.12	2	4.96 dd (1.0, 3.0)	6.09 dd (1.5, 3.5)
4	3.89 dd (9.0, 9.0)	ca. 3.74	3	6.05 dd (3.0, 9.5)	4.86 dd(3.5, 9.5)
5	3.72 ddd (3.0, 7.0, 9.0)	ca. 3.67	4	6.16 dd (9.5, 9.5)	4.23 dd (9.5, 9.5)
6	ca. 4.47	4.32 dd (5.0, 11.5)	5	5.30 dq (9.5, 6.5)	5.09 d (9.5, 6.5)
6	ca. 4.18	4.03 dd (6.5, 11.5)	6	1.62 d (6.5)	1.73 d (6.5)
Glc′-1	4.89 d (7.5)	5.25 d (8.0)	Jla-2	2.62 ddd (7.0, 7.0, 12.5)	2.70 ddd (3.0, 11.5, 16.0)
2	ca. 4.07	5.58 dd (8.0, 9.5)	2	ca. 2.45	ca. 2.61
3	5.70 dd (9.5, 9.5)	4.59[b]	11	ca. 3.80	ca. 3.73
4	5.57 dd (9.5, 9.5)	ca. 4.15	16	0.86 t (7.0)	0.85 t (7.0)
5	4.03 ddd (3.5, 3.5, 9.5)	ca. 4.15	Mba-2	2.39 ddq (7.0, 7.0, 7.0)	ca. 2.62
6	ca. 4.18	4.50 br d (11.5)	3	ca. 1.62	1.80 m
6	ca. 4.08	ca. 4.28	3	ca. 1.32	ca.1.54
Glc″-1	5.01 d (8.0)		4	0.70 dd (7.5, 7.5)	0.94 dd (7.5, 7.5)
2	5.51 dd (8.0, 9.5)		5	1.14 d (7.0)	1.25 d (7.0)
3	ca. 4.30		Mba′-2	2.47 ddq (7.0, 7.0, 7.0)	
4	4.19 dd (9.5, 9.5)		3	1.81 m	
5	ca. 3.87		3	1.50 m	
6	ca. 4.47		4	0.95 dd (7.5, 7.5)	
6	ca. 4.30		5	1.19 d (7.0)	
Glc‴-1		5.31 d (7.5)	Nla-2	2.75 dq (7.0, 7.0)	
2		4.06 dd (7.5, 9.5)	3	ca. 4.34	
3		5.78 dd (9.5, 9.5)	4	1.21 d (7.0)	
4		4.44 dd (9.5, 9.5)	5	1.10 d (7.0)	
5		ca. 3.65	Tig-3	7.24 dq like (1.5, 7.0)	7.05 dq like (1.5, 6.5)
6		ca. 4.38	4	1.70 dd like (1.5, 7.0)	1.61 d (6.5)
6		ca. 4.37	5	1.95 br s	1.84 br s

[a]Chemical shifts (δ) are in ppm relative to TMS. Coupling constants (J) in Hz are given in parentheses. [b]Signals were deformed by virtual coupling.

Calysolin II (2): amorphous powder; mp 143−147 °C; $[\alpha]^{25}_D$ −33.0 (c 1.3, MeOH); ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS (positive mode) m/z 1405 $[M + Na]^+$; FABMS (negative mode) m/z 1381 $[M - H]^-$, 1299 $[1381 - 82]^-$, 1281 $[1381 - 100]^-$, 1237 $[1381 - 144]^-$, 1199 $[1281 - 82]^-$, 1053 $[1199 - 146]^-$, 907 $[1237 - 84 \times 2 - 162]^-$, 579 $[907 - 82 - 100 - 146]^-$, 417 $[579 - 162]^-$; HRFABMS (positive mode) m/z 1405.6981 (calcd for $C_{66}H_{110}O_{30}Na$, 1405.6979).

Calysolin III (3): amorphous powder; mp 140−145 °C; $[\alpha]^{25}_D$ −27.4 (c 1.7, MeOH); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; FABMS (positive mode) m/z 1405 $[M + Na]^+$; FABMS (negative mode) m/z 1381 $[M - H]^-$, 1299 $[1381 - 82]^-$, 1281 $[1381 - 100]^-$, 1237 $[1381 - 144]^-$, 1199 $[1281 - 82]^-$, 1053 $[1199 - 146]^-$, 907 $[1237 - 84 \times 2 - 162]^-$, 579 $[907 - 82 - 100 - 146]^-$, 417 $[579 - 162]^-$; HRFABMS (negative mode) m/z 1381.6995 (calcd for $C_{66}H_{109}O_{30}$, 1381.6704).

Calysolin IV (4): amorphous powder; mp 170−175 °C; $[\alpha]^{25}_D$ −18.2 (c 0.6, MeOH); ^{1}H NMR, see Table 3; ^{13}C NMR, see Table 2; FABMS (negative mode) m/z 1359 $[M - H]^-$, 1277 $[1381 - 82]^-$, 1215 $[1359 - 144]^-$, 953 $[1277 - 162 \times 2]^-$, 723 $[953 - 84 - 146]^-$, 579 $[723 - 144]^-$, 417 $[579 - 162]^-$, 271 $[417 - 146]^-$; HRFABMS (negative mode) m/z 1359.6423 (calcd for $C_{62}H_{103}O_{32}$, 1359.6432).

Alkaline Hydrolysis of 1−4. Solutions of **1** (5 mg), **2** (5 mg), **3** (5 mg), and **4** (5 mg) in 1,4-dioxane−1 M KOH (1/1, 2 mL) were heated to 95 °C for 1 h. The reaction mixture was adjusted to pH 3 with 1 M HCl and then diluted with H_2O (10 mL) and extracted with ether (3 × 5 mL). The ether layer was dried over $MgSO_4$ and evaporated to furnish an organic acid fraction, which was analyzed by GC (Shimadzu GC-8A gas chromatograph with flame-ionization detector; column, Unisole 30T (5%), 3.2 mm i.d. × 2 m glass column (column 1); carrier gas N_2, 1.0 kg/cm^2; column temperature, 120 °C; t_R (min) = 4.42 (2-methylbutyric acid) for **1**−**4**, 10.38 (tiglic acid) for **2**−**4**). A part of the organic acid fraction was methylated with trimethylsilyldiazomethane−ether, and then the reaction mixture was analyzed by GC (column, column 1; column temperature, 100 °C; carrier gas, N_2 1.0 kg/cm^2; t_R (min) = 8.60 (methyl nilate) for **1**−**3**).

The aqueous layer was desalted by MCI gel CHP 20 column chromatography (H_2O, acetone) to give a glycosidic acid as an amorphous powder (3 mg from **1**, 4 mg from **2**, 4 mg from **3**, 4 mg from **4**). The glycosidic acids derived from **1**−**4** were each identical with soldanellic acid B, calysolic acid A, calysolic acid A, and calysolic acid D, respectively, by comparison of ^{1}H NMR spectra with those of authentic samples.[5]

Acetylation of 1. A solution of **1** (3 mg) in Ac_2O−pyridine (1/1, 1 mL) was left to stand at room temperature overnight. The solvent was removed under an N_2 stream to give a residue. The residue was partitioned between diethyl ether (0.5 mL) and H_2O (0.5 mL). The diethyl ether layer was concentrated under a N_2 stream to afford **1a** (3 mg).

Peracetate of 1 (1a): amorphous powder; FABMS (positive mode) m/z 1455 $[M + Na]^+$, 373, 313, 83; HRFABMS (positive mode) m/z 373.1500 (calcd for $C_{17}H_{25}O_9$, 373.1499).

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■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Figures giving NMR spectra and other data for **1**−**4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ NOTE ADDED AFTER ASAP PUBLICATION

This paper was published on the Web on Oct 12, 2011, with an error in the last value in column 2 of Table 1. The corrected version was reposted on Oct 17, 2011.