

New Cardenolide and Acylated Lignan Glycosides from the Aerial Parts of *Asclepias curassavica*

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Three new cardenolide glycosides and six new acylated lignan glycosides were obtained along with nineteen known compounds from the aerial parts of *Asclepias curassavica* L. (Asclepiadaceae). The structure of each compound was determined based on interpretations of NMR and MS measurements and chemical evidence.

Key words *Asclepias curassavica* L.; Asclepiadaceae; cardenolide glycoside; lignan glycoside

Asclepias curassavica L. (Asclepiadaceae), a common garden plant in Japan, contains many kinds of cardenolides and cardenolide glycosides.^{1–3} The monarch butterfly (*Danaus plexippus* L.) feeds on the *Asclepias* genus, including *A. curassavica*, for protection from vertebrate predators.^{4–6} In the course of our research on the phytochemicals in Asclepiadaceae plants, we have also investigated the constituents of the aerial parts of *A. curassavica*.

A MeOH extract from the dried aerial parts of *A. curassavica* was suspended in water. The suspension was extracted with diethyl ether and partitioned into an ether-soluble fraction and a water-soluble fraction. The residue of the water-soluble fraction, after passing through a porous polymer gel, was chromatographed on a silica gel column to give fractions of cardenolides, cardenolide glycosides, lignan and acylated lignan glycosides, from which nine new compounds were obtained.

Compounds **1**–**11**, **13**–**17** and **18** were known cardenolides and cardenolide glycosides, and identified as uzarigenin (**1**),^{1,7–9} 5,6-dehydrouzarigenin (xysmalogenin) (**2**),^{8–11} coroglaucigenin (**3**),^{1,12} gofruside (**4**),^{12,13} 6'-*O*-(*E*-4-hydroxycinnamoyl)-desglucouzararin (**5**),^{14,15} 6'-*O*-sinapinoyl-desglucouzararin (**6**),¹⁶ calactin (**7**),^{17–19} 16 α -acetoxyalactin (**8**),¹⁶ calotropin (**9**),^{17–20} 16 α -acetoxyalotropin (**10**),²¹ asclepin (**11**),¹⁹ 16 α -hydroxyasclepin (**13**),²¹ 16 α -acetoxyasclepin (**14**),²¹ uscharin (**15**),^{17–19} 16 α -hydroxyuscharin (**16**),²² uscharidin (**17**)^{17–19} and 19-nor-16 α -acetoxy-10 β -hydroxyasclepin (**18**).³ Compounds **21** and **22** were also identical to known lignans, (+)-medioresinal and (+)-syringaresinol, respectively.²³

Compound **12** was suggested to have the molecular formula C₃₁H₄₄O₁₀ based on high resolution (HR)-FAB-MS [*m/z*: 599.2806 [M+Na]⁺]. On comparison of the ¹H- and ¹³C-NMR spectra of **12** with those of **11**, hydroxy methyl proton and carbon signals were observed at δ 4.01 (1H, dd, *J*=11.5, 3.0 Hz), 3.74 (1H, dd, *J*=11.5, 3.0 Hz) and δ 59.3 instead of the aldehyde proton and carbon signals. In the heteronuclear multiple-bond connectivity (HMBC) experiment, these proton signals exhibited long-range correlations to the C-1 (δ 37.8), C-5 (δ 45.4), C-9 (δ 51.5) and C-10 (δ 41.9) signals. Thus, this hydroxy methyl group was located at the C-19 position, and compound **12** was determined as 19-dihydroasclepin.

The molecular formula of compound **19** was proposed to be C₃₀H₄₂O₁₀ based on HR-FAB-MS. The ¹H- and ¹³C-NMR

spectroscopic data of **19** were similar to those of **11** and **12** except for the exchange of a quaternary oxygenated carbon signal (δ 72.7) for a quaternary carbon and disappearance of the C-19 signal. In addition, the ¹H-NMR spectrum of **19** revealed a hydroxyl proton signal at δ 5.18. In the HMBC spectrum, long-range correlations were observed from this hydroxyl proton signal to the C-1 (δ 41.1), C-5 (δ 44.4), C-9 (δ 48.7) and above quaternary oxygenated carbon signals. Thus, this quaternary oxygenated carbon signal was assigned at the C-10 position and the hydroxy group was placed at C-10. The rotating frame nuclear Overhauser effect (ROE) difference experiment suggested that this hydroxy group retained a β orientation, owing to the observation of ROEs between this hydroxyl proton and H-1 β (δ 2.47), H-2 (δ 5.06), H-4 β (δ 1.99), and H-8 (δ 2.17). Thus, **19** was identified as be 19-nor-10 β -hydroxyasclepin. The similarity of the ¹H- and ¹³C-NMR spectroscopic data of **19** to those of **18** supported this finding (see Table 1 and Experimental).

HR-FAB-MS of compound **20** afforded a [M+Na]⁺ peak at *m/z* 531.2569, suggesting the molecular formula, C₂₉H₃₈O₆. Compound **20** was also considered to be a cardenolide glycoside, according to the appearance of typical signals due to an α,β -unsaturated- γ -lactone, one aldehyde, two secondary oxymethines, and one quaternary oxygenated carbon in the ¹H- and ¹³C-NMR spectra of the aglycone moiety, together with the anomeric proton and carbon signals at δ 5.63 (1H, s) and δ 104.6 in the sugar moiety. Based on a comparison of the ¹H- and ¹³C-NMR spectroscopic data of **20** with those of calactinic acid methyl ester,³ the sugar moiety of **20** was presumed to consist of a furanose form. This presumption was supported by the long-range correlations from H-1' (δ 5.63) to C-2' (δ 88.3), C-4' (δ 45.3) and C-5' (δ 74.1) and from H-4' (δ 2.64 and/or 2.61) to C-1', C-2' and C-5' in the HMBC experiment of **20**. Moreover, the observed HMBC correlations between H-4' and C-3' (δ 172.5), C-3' and H-2 (δ 5.56), H-1' and C-3 (δ 78.3), and H-3 (δ 3.93) and C-1' (δ 104.6) suggested that the sugar unit was attached to the C-3 position by a glycosidic linkage and the C-2 position by an esterified linkage. Because, in the ROE difference experiment, ROEs were exhibited between H-2 and H-19 (δ 10.19), H-3 and H-1 α (δ 1.23), H-5 (δ 1.40), the orientation of H-2 and H-3 was determined as β and α , respectively. In addition, observations of ROEs from H-1' to H-2 and H-5' suggested that H-1' and H-5' retained the β -orientation. The orientation of the hydroxyl group at C-2'

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Table 1. ^{13}C -NMR Data of Compounds **11**, **12**, **18**, **19** and **20**

	11	12	18	19	20
Carbon No.					
1	36.6	37.8	40.9	41.1 ^(a)	36.6
2	69.4	69.5	69.5	69.6	74.4
3	72.3	73.1	72.6 ^(a)	72.6 ^(b)	78.3
4	32.5	33.5	32.7	32.8	32.0
5	43.5	45.4	44.4	44.4	48.2
6	28.0	28.0 ^(a)	28.1	28.2	27.9 ^(a)
7	28.0	27.8 ^(a)	27.6	27.5 ^(c)	27.8 ^(a)
8	42.6	41.8 ^(b)	40.9	40.8 ^(a)	43.5
9	48.8	51.5	48.7	48.7	42.5
10	53.0	41.9 ^(b)	72.7 ^(a)	72.7 ^(b)	52.8
11	22.2	23.5	21.4	21.3	22.5
12	39.3	40.4	40.1	39.6	39.1
13	49.8	50.2	49.6	50.0	49.8
14	84.1	84.7	84.2	84.3	84.1
15	33.9	33.2	39.8	33.2	32.4
16	27.2	27.4	79.3	27.4 ^(c)	27.2
17	51.2	50.6	58.4	51.4	51.2
18	15.9	16.3	16.1	16.1	15.8
19	207.8	59.3	—	—	207.9
20	175.5	175.9	173.0	175.9	175.5
21	73.7	73.7	74.1	73.7	73.7
22	117.9	117.7	118.3	117.7	117.9
23	174.4	174.5	174.3	174.5	174.4
1'	97.0	97.3	97.3	97.3	104.6
2'	91.8	91.7	91.8	91.8	88.3
3'	75.3	75.6	75.5	75.5	172.5
4'	36.3	36.4	36.3	36.4	45.3
5'	68.1	68.0	68.1	68.1	74.1
6'	21.3	21.3	21.3	21.3	20.9
1"	170.7	170.7	170.7 ^(b)	170.7	—
2"	21.0	20.9	21.0	21.0	—
1"	—	—	170.9 ^(b)	—	—
2"	—	—	21.0	—	—

Measured in pyridine-*d*₅ at 35 °C. *a, b, c*) Signal assignments may be interchanged in each column.

was assigned as β based on the result of a X-ray analysis (Fig. 1). Thus, the structure of **20** was identified as shown in Chart 1, and the compound was named calactinolactone. It was suggested that compound **20** might be an artifact of the autoxidation of uscharidin (**17**).³⁾

HR-FAB-MS of compound **23** exhibited an $[\text{M}]^+$ ion at m/z 758.2776, revealing the molecular formula of **23** to be $\text{C}_{38}\text{H}_{46}\text{O}_{16}$. The ^{13}C -NMR spectrum of **23** showed the signals of two tetra-substituted symmetrical aromatic rings (δ 149.2 \times 2, 137.0, 134.3, 104.7 \times 2 and δ 149.2 \times 2, 136.1, 131.7, 107.3 \times 2), three methylenes (δ 68.3, 73.1, 34.3), three methines (δ 83.5, 51.3, 43.6), and four methoxy groups (δ 56.5 \times 4) in the aglycone moiety, along with the presence of β -D-glucopyranosyl and *trans*-feruloyl groups. From the ^{13}C -NMR spectroscopic data, the aglycone of **23** was expected to be a tetrahydrofuran-type lignan. The deacylated compound **23a** afforded by alkaline hydrolysis of **23** was identified as (8*R*,7'*S*,8'*R*)-5,5'-dimethoxyariciresinol 9'-*O*- β -D-glucopyranoside, the ^1H -, ^{13}C -NMR spectroscopic data and circular dichroism (CD) spectrum being consistent with those of alangilignoside C.^{24,25)} Moreover, the ^1H -NMR spectrum of **23** revealed downfield shifts of the H-6 signals of the β -D-glucopyranosyl group (δ 5.14, 5.01), and the HMBC measurement showed long-range correlations from the carbonyl carbon signal of the *trans*-feruloyl group (δ 167.6) to these H-6 signals of the β -D-glucopyranosyl group. The above re-

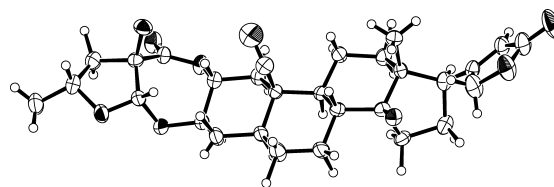
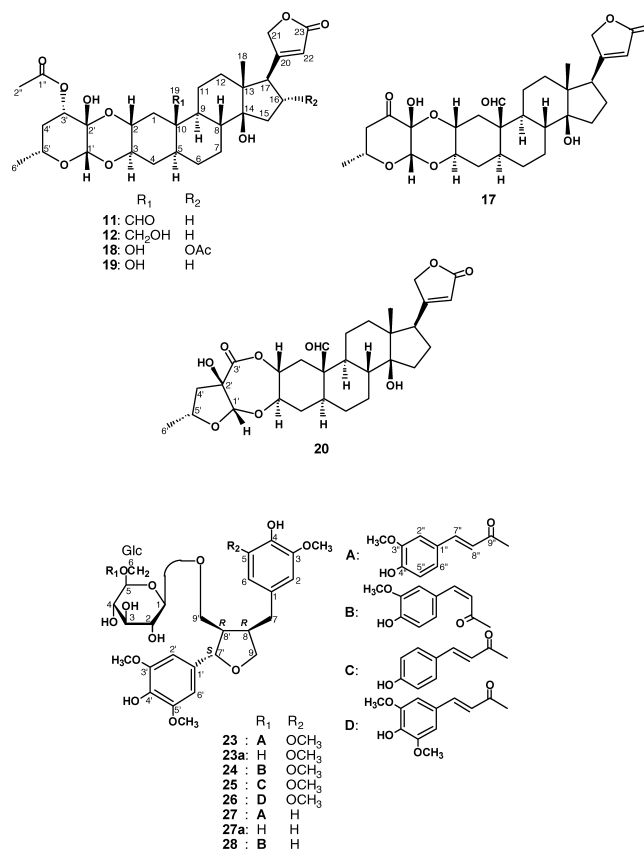
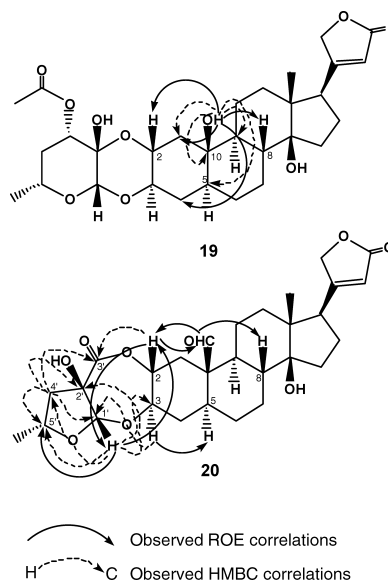
Fig. 1. ORTEP Drawing of Compound **20**Chart 1. Structures of Compounds **11**, **12**, **17**—**20**, **23**—**27** and **28**Chart 2. ROE and HMBC Correlations in Compounds **19** and **20**

Table 2. ^{13}C - and ^1H -NMR Data of Compounds **23** and **27**

	23+		23++		27++	
Carbon and Proton No.						
Aglycone moiety						
1	131.7	—	132.9	—	133.6	—
2	107.3	6.73 (s)	107.2	6.43 (s)	113.5	6.72 (d, 2.0)
3	149.2	—	149.3 ^{a)}	—	149.0	—
4	136.1	—	134.9 ^{b)}	—	145.8	—
5	149.2	—	149.3 ^{a)}	—	116.2	6.67 (d, 8.0)
6	107.3	6.73 (s)	107.2	6.43 (s)	122.2	6.56 (dd, 8.0, 2.0)
7	34.3	3.29 (dd, 13.5, 4.5)	34.5	2.90 (dd, 13.5, 5.0)	33.9	2.88 (dd, 13.5, 5.0)
		2.74 (dd, 13.5, 11.5)		2.47 (dd, 13.5, 11.0)		2.46 (dd, 13.5, 11.0)
8	43.6	3.03 (m)	44.1	2.73 (m)	44.1	2.72 (m)
9	73.1	4.27 (dd, 8.5, 6.5)	73.7	3.96 (dd, 8.5, 6.5)	73.8	3.95 (dd, 8.5, 6.5)
		4.02 (dd, 8.5, 6.5)		3.70 (dd, 8.5, 6.5)		3.67 (dd, 8.5, 7.0)
OMe	56.5×2	3.78 (s)×2	56.9 ^{c)} ×2	3.77 (s)×2	56.9	3.78 (s)
1'	134.3	—	135.0 ^{b)}	—	135.1	—
2'	104.7	7.12 (s)	104.6	6.64 (s)	104.5	6.64 (s)
3'	149.2	—	149.2 ^{a)}	—	149.2	—
4'	137.0	—	136.0	—	135.9	—
5'	149.2	—	149.2 ^{a)}	—	149.2	—
6'	104.7	7.12 (s)	104.6	6.64 (s)	104.5	6.64 (s)
7'	83.5	5.33 (d, 6.5)	84.6	4.85 (d, 6.5)	84.8	4.86 (d, 6.5)
8'	51.3	2.86 (m)	51.6	2.49 (m)	51.4	2.48 (m)
9'	68.3	4.52 (dd, 10.0, 6.5)	68.9	4.00 (dd, 10.0, 6.0)	68.9	3.99 (dd, 10.0, 6.5)
		4.15**		3.85 (dd, 10.0, 6.0)		3.85 (dd, 10.0, 6.5)
OMe'	56.5×2	3.81 (s)×2	56.8 ^{c)} ×2	3.81 (s)×2	56.9×2	3.80 (s)×2
Sugar moiety						
Glc-1	105.1	4.99 (d, 8.0)	104.6	4.33 (d, 8.0)	104.5	4.32 (d, 8.0)
-2	75.2	4.13 (t, 8.0)	75.2	3.26 (t, 8.0)	75.2	3.25 (t, 8.0)
-3	78.5	4.26 (t, 8.0)	78.0	3.39**	78.1	3.39**
-4	71.5	4.19**	71.9	3.39**	71.9	3.39**
-5	75.6	4.16**	75.5	3.54 (m)	75.5	3.55 (m)
-6	64.6	5.14 (dd, 12.0, 2.0)	64.4	4.54 (dd, 12.0, 2.0)	64.5	4.53 (dd, 12.0, 2.0)
		5.01 (dd, 12.0, 5.5)		4.40 (dd, 12.0, 6.0)		4.40 (dd, 12.0, 6.0)
Ester moiety						
1''	126.5	—	127.6	—	127.6	—
2''	111.5	7.21*	111.8	7.08 (d, 2.0)	111.8	7.09 (d, 2.0)
3''	149.0	—	149.4	—	149.4	—
4''	151.2	—	150.7	—	150.7	—
5''	116.8	7.15 (br s)	116.5	6.77 (d, 8.0)	116.5	6.78 (d, 8.0)
6''	*	7.15 (br s)	124.1	6.97 (dd, 8.0, 2.0)	124.2	6.99 (dd, 8.0, 2.0)
7''	145.8	7.93 (d, 16.0)	147.0	7.57 (d, 16.0)	147.1	7.59 (d, 16.0)
8''	115.1	6.62 (d, 16.0)	115.2	6.32 (d, 16.0)	115.3	6.34 (d, 16.0)
9''	167.6	—	169.0	—	169.0	—
OMe''	56.0	3.77 (s)	56.5	3.82 (s)	56.5	3.82 (s)

+ : Measured in pyridine- d_5 at 35 °C. ++ : Measured in MeOH- d_4 at 35 °C. a, b, c) Signal assignments may be interchanged in each column. * Overlapping with pyridine- d_5 signals. ** Overlapping with other signals.

sults suggested that the C-6 position of the β -D-glucopyranosyl group was acylated by *trans*-ferulic acid. Hence, the structure of **23** was elucidated as shown in Chart 1.

From the results of HR-FAB-MS, compounds **24**—**26** had the molecular formulae, $\text{C}_{38}\text{H}_{46}\text{O}_{16}$, $\text{C}_{37}\text{H}_{44}\text{O}_{15}$ and $\text{C}_{39}\text{H}_{48}\text{O}_{17}$, respectively. On the basis of the ^1H - and ^{13}C -NMR spectroscopic data, the aglycone and sugar moieties of **24**—**26** were identified as **23a**, their NMR spectroscopic data being consistent with those of **23** except for their ester moieties. Because alkaline hydrolysis afforded *cis*-ferulic acid, *trans-p*-coumaric acid and *trans*-sinapinic acid from **24**, **25** and **26**, respectively, together with **23a**, their structures were established as shown in Chart 1.

The molecular formula of both compounds **27** and **28** was proposed to be $\text{C}_{37}\text{H}_{44}\text{O}_{15}$. The ^1H -NMR spectra of **27** and **28** showed one set of ABX-type aromatic proton signals [**27**: δ 6.72 (1H, d, $J=2.0$ Hz), 6.67 (1H, d, $J=8.0$ Hz), 6.56 (1H,

dd, $J=8.0$, 2.0 Hz); **28**: δ 6.74 (1H, d, $J=2.0$ Hz), 6.69 (1H, d, $J=8.0$ Hz), 6.61 (1H, dd, $J=8.0$, 2.0 Hz)] with one set of tetra-substituted symmetrical aromatic proton signals. Compound **27a** produced by alkaline hydrolysis of **27** was identified as (8*R*,7'*S*,8'*R*)-5'-methoxylariciresinol 9'-*O*- β -D-glucopyranoside based on a comparison with the ^1H -, ^{13}C -NMR spectroscopic data and CD spectrum of alangilignoside D.²⁴⁾ In consideration of the production of *trans*-ferulic acid from **27** by alkaline hydrolysis, the structure of **27** was determined as shown in Chart 1. The structure of compound **28** was also established, based on a comparison of the NMR spectroscopic data with those of **24** and **27** and the result of alkaline hydrolysis of **28**.

A previous paper reported that mainly pregnane glycosides were contained in the roots of *A. curassavica* along with a few kinds of cardenolide glycosides.²⁶⁾ In this investigation, the aerial parts of this plant afforded many kinds of cardeno-

lides and cardenolide glycosides without pregnane glycosides. A marked difference in the constituents of this plant was found between the aerial parts and roots. *A. curassavica* is toxic, probably due to the cardenolides and their glycosides. We were interested in the cardiotoxicity and inhibitory effect on $\text{Na}^+ - \text{K}^+$ ATPase of these compounds.

Experimental

General Procedure The instrumental analysis was carried out as described previously.²⁷⁾ Melting points were measured on a Yanaco MP-S3.

X-Ray Analysis Suitable colorless crystals of **20** were obtained by recrystallization (CHCl_3 -MeOH). The crystal (0.40×0.40×0.40 mm) belonged to the orthorhombic system, space group $P2_12_12_1$ (#19), with $a=14.461(8)$ Å, $b=30.606(3)$ Å, $c=5.857(6)$ Å, $V=2592(4)$ Å³, $Z=4$. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated $\text{CuK}\alpha$ radiation and a rotating anode generator. The structure was solved by direct methods²⁸⁾ and expanded using Fourier techniques.²⁹⁾ The oxygen atom of the formyl group at C-19 adopted two positions, and the occupying parameters of this atom were also refined.

Plant Materials The aerial parts of *Asclepias curassavica* L. were collected from the botanical garden of the University of Shizuoka in Japan in September, 2001 and identified by Dr. T. Warashina. The dried materials were stored in a herbarium.

Extraction and Isolation The dried aerial parts of *Asclepias curassavica* L. (6.8 kg) were treated three times with MeOH under reflux. The extract was concentrated under reduced pressure and the residue was suspended in H₂O. This suspension was extracted with Et₂O. The H₂O layer of the MeOH extract was passed through a porous polymer gel (Mitsubishi Diaion HP-20) column with the absorbed material being eluted with MeOH-H₂O (1:1), MeOH-H₂O (7:3) and MeOH, respectively. The MeOH fraction from the porous polymer gel column was then evaporated dry, with the residue (13.7 g) subjected to silica gel CC with a CHCl_3 -MeOH (95:5—85:15) system and semi-preparative HPLC (Develosil-ODS, and YMC-ODS: 27—35% MeCN in water and 45—60% MeOH in water) to give compounds **1** (60 mg), **2** (5 mg), **3** (11 mg), **4** (8 mg), **5** (31 mg), **6** (6 mg), **7** (16 mg), **8** (3 mg), **9** (35 mg), **10** (21 mg), **11** (35 mg), **12** (4 mg), **13** (21 mg), **14** (42 mg), **15** (40 mg), **16** (8 mg), **17** (275 mg), **18** (16 mg), **19** (6 mg), **20** (9 mg), **21** (4 mg), **22** (28 mg), **23** (73 mg), **24** (6 mg), **25** (3 mg), **26** (4 mg), **27** (11 mg) and **28** (4 mg). However, compound **28** was not purified completely.

19-Dihydroasclepin (12): Amorphous powder. $[\alpha]_D^{25} +17^\circ$ ($c=0.37$, MeOH). FAB-MS m/z : 599 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 599.2806 (Calcd for $\text{C}_{31}\text{H}_{44}\text{O}_{10}\text{Na}$: 599.2832). ¹³C-NMR (pyridine-*d*₅ at 35 °C): shown in Table 1. ¹H-NMR (pyridine-*d*₅ at 35 °C) δ : 6.10 (1H, brt, 1.5, H-22), 5.75 (1H, brt, 3.0, C-19-OH), 5.45 (1H, dd, 12.0, 5.0, H-3'), 5.28 (1H, dd, 18.5, 1.5, H-21), 5.08 (1H, s, H-1'), 5.01 (1H, dd, 18.5, 1.5, H-21), 4.90 (1H, ddd, 12.0, 11.0, 5.0, H-2), 4.46 (1H, td, 11.0, 5.0, H-3), 4.01 (1H, dd, 11.5, 3.0, H-19), 3.79 (1H, m, H-5'), 3.74 (1H, dd, 11.5, 3.0, H-19), 2.94 (1H, dd, 12.0, 5.0, H-1), 2.76 (1H, dd, 9.0, 5.0, H-17), 1.78 (3H, s, C-3'-OCOCH₃*), 1.37 (3H, d, 6.0, H-6'), 1.11 (1H, t, 12.0, H-1), 1.00 (3H, s, H-18).

19-Nor-16 α -acetoxy-10 β -hydroxyasclepin (18): Amorphous powder. $[\alpha]_D^{25} +3.0^\circ$ ($c=0.60$, MeOH). FAB-MS m/z : 621 $[\text{M}+\text{H}]^+$, 643 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 621.2902 (Calcd for $\text{C}_{35}\text{H}_{45}\text{O}_{12}$: 621.2911). ¹³C-NMR (pyridine-*d*₅ at 35 °C): shown in Table 1. ¹H-NMR (pyridine-*d*₅ at 35 °C) δ : 6.28 (1H, brt, 1.5, H-22), 5.84 (1H, brs, C-14-OH), 5.68 (1H, td, 8.0, 4.0, H-16), 5.42 (1H, dd, 12.0, 5.0, H-3'), 5.29 (1H, s, C-10-OH), 5.29 (1H, dd, 18.0, 1.5, H-21), 5.17 (1H, dd, 18.0, 1.5, H-21), 5.07 (overlapping, H-2), 5.07 (1H, s, H-1'), 4.48 (1H, ddd, 11.5, 10.0, 4.0, H-3), 3.79 (1H, m, H-5'), 2.94 (1H, d, 4.0, H-17), 2.55 (1H, dd, 13.5, 8.0, H-15), 2.49 (1H, dd, 12.0, 4.5, H-1), 2.22 (1H, td, 11.5, 3.0, H-8), 2.11 (1H, q, 12.0, H-4'), 2.09 (3H, s, C-3'-OCOCH₃*), 1.87 (3H, s, C-16-OCOCH₃*), 1.50 (1H, t, 12.0, H-1), 1.39 (overlapping, H-5), 1.35 (3H, d, 6.5, H-6'), 1.00 (3H, s, H-18). ¹³C-NMR (CDCl₃ at 35 °C) δ : 174.2 (C-23), 172.3 (C-3'-OC*OCH₃), 171.0 (C-20), 170.7 (C-16-OC*OCH₃), 118.4 (C-22), 96.2 (C-1'), 90.9 (C-2'), 84.7 (C-14), 78.4 (C-16), 75.6 (C-3'), 73.8 (C-21), 73.0 (C-10), 71.5 (C-3), 68.9 (C-2), 67.8 (C-5'), 57.8 (C-17), 48.8 (C-13), 47.9 (C-9), 43.5 (C-5), 40.9 (C-8), 39.7, 39.6 (C-1, -12), 39.3 (C-15), 35.5 (C-4'), 31.6 (C-4), 27.3 (C-6), 26.5 (C-7), 21.1, 21.0 (C-16-OCOC*H₃, -3'-OCOC*H₃), 20.9, 20.8 (C-11, -6'), 15.6 (C-18). ¹H-NMR (CDCl₃ at 35 °C) δ : 5.94 (1H, brt, 1.5, H-22), 5.27 (1H, td, 8.0, 4.0, H-16), 4.92 (1H, dd, 18.0, 1.5, H-21), 4.85 (1H, dd, 18.0, 1.5, H-21), 4.79 (1H, dd, 12.0, 5.0, H-3'), 4.59 (1H, s, H-1'), 4.22 (1H, ddd, 12.0, 10.0, 4.5, H-2), 3.97 (1H, td, 10.0, 5.0, H-3), 3.70 (1H, m, H-5'), 2.64 (1H, d, 4.0, H-17), 2.25 (1H, dd, 13.5, 8.0, H-15), 2.15 (3H, s, C-3'-

OCOCH₃*), 2.10 (1H, dd, 12.0, 4.5, H-1), 2.07 (1H, dd, 13.5, 8.0, H-15), 2.04 (3H, s, C-16-OCOCH₃*), 1.86 (1H, ddd, 12.0, 5.0, 2.0, H-4'), 1.73 (1H, q, 12.0, H-4'), 1.31 (3H, d, 6.5, H-6'), 1.29 (1H, t, 12.0, H-1), 1.20 (1H, td, 12.0, 3.0, H-9), 0.87 (3H, s, H-18).

19-Nor-10 β -hydroxyasclepin (19): Amorphous powder. $[\alpha]_D^{22} +22^\circ$ ($c=0.60$, MeOH). FAB-MS m/z : 563 $[\text{M}+\text{H}]^+$, 585 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 563.2854, 585.2676 (Calcd for $\text{C}_{30}\text{H}_{43}\text{O}_{10}$: 563.2856 and $\text{C}_{30}\text{H}_{42}\text{O}_{10}\text{Na}$: 585.2676). ¹³C-NMR (pyridine-*d*₅ at 35 °C): shown in Table 1. ¹H-NMR (pyridine-*d*₅ at 35 °C) δ : 6.10 (1H, brt, 1.5, H-22), 5.42 (1H, dd, 12.0, 5.0, H-3'), 5.27 (1H, dd, 18.5, 1.5, H-21), 5.25 (1H, s, C-14-OH), 5.18 (1H, s, C-10-OH), 5.06 (1H, s, H-1'), 5.06 (overlapping, H-2), 5.01 (1H, dd, 18.5, 1.5, H-21), 4.46 (1H, ddd, 12.0, 10.0, 4.5, H-3), 3.79 (1H, m, H-5'), 2.74 (1H, dd, 9.0, 5.0, H-17), 2.47 (1H, dd, 12.0, 4.5, H-1), 2.17 (1H, td, 12.0, 3.0, H-8), 2.10 (overlapping, H-4'), 1.99 (1H, q, 12.0, H-4), 1.97 (overlapping, H-4'), 1.87 (3H, s, C-3'-OCOCH₃*), 1.47 (1H, t, 12.0, H-1), 0.98 (3H, s, H-18). ¹³C-NMR (CDCl₃ at 35 °C) δ : 174.3, 174.2 (C-20, -23), 172.3 (C-3'-OC*OCH₃), 117.8 (C-22), 96.1 (C-1'), 90.9 (C-2'), 85.0 (C-14), 75.7 (C-3'), 73.4 (C-21), 73.0 (C-10), 71.5 (C-3), 68.9 (C-2), 67.8 (C-5'), 50.7 (C-17), 49.4 (C-13), 48.0 (C-9), 43.4 (C-5), 41.1 (C-8), 39.6, 39.4 (C-1, -12), 35.5 (C-4'), 32.9 (C-15), 31.6 (C-4), 27.4 (C-6), 26.9, 26.5 (C-7, -16), 21.1 (C-3'-OCOC*H₃), 20.9 (C-6'), 20.7 (C-11), 15.7 (C-18). ¹H-NMR (CDCl₃ at 35 °C) δ : 5.87 (1H, brt, 1.5, H-22), 4.97 (1H, dd, 18.0, 1.5, H-21), 4.79 (1H, dd, 18.0, 1.5, H-21), 4.78 (1H, dd, 12.0, 5.0, H-3'), 4.59 (1H, s, H-1'), 4.22 (1H, ddd, 12.0, 10.5, 4.5, H-2), 3.97 (1H, td, 10.5, 6.0, H-3), 3.70 (1H, m, H-5'), 2.76 (1H, dd, 9.5, 5.5, H-17), 2.14 (3H, s, C-3'-OCOCH₃*), 2.10 (1H, dd, 12.0, 4.5, H-1), 1.86 (1H, ddd, 12.0, 5.0, 1.5, H-4'), 1.73 (1H, q, 12.0, H-4'), 1.59 (overlapping, H-8), 1.58 (overlapping, H-4), 1.38 (overlapping, H-5), 1.28 (overlapping, H-1), 0.89 (3H, s, H-18).

Calactinolactone (20): Prism from CHCl_3 -MeOH, mp 301—306 °C. $[\alpha]_D^{22} +56^\circ$ ($c=0.52$, CHCl_3 -MeOH (1:1)). FAB-MS m/z : 531 $[\text{M}+\text{H}]^+$. HR-FAB-MS m/z : 531.2569 (Calcd for $\text{C}_{29}\text{H}_{39}\text{O}_9$: 531.2594). ¹³C-NMR: shown in Table 1. ¹H-NMR (pyridine-*d*₅ at 35 °C) δ : 10.19 (1H, s, H-19), 6.11 (1H, brt, 1.5, H-22), 5.63 (1H, s, H-1'), 5.56 (1H, td, 12.0, 5.0, H-2), 5.25 (1H, dd, 18.0, 1.5, H-21), 5.00 (1H, dd, 18.0, 1.5, H-21), 4.52 (1H, dq, 9.5, 6.0, H-5'), 3.93 (1H, td, 12.0, 4.0, H-3), 2.85 (1H, dd, 12.0, 5.0, H-1), 2.74 (1H, dd, 9.0, 5.0, H-17), 2.64 (1H, dd, 13.5, 9.5, H-4'), 2.61 (1H, dd, 13.5, 6.0, H-4'), 1.91 (1H, q, 12.0, H-4), 1.91 (overlapping, H-8), 1.73 (1H, dt, 12.0, 4.0, H-4), 1.40 (overlapping, H-5), 1.36 (3H, d, 6.0, H-6'), 1.23 (1H, t, 12.0, H-1), 0.92 (3H, s, H-18).

(8*R*,7'*S*,8'*R*)-5,5'-Dimethoxyariciresinol 9'-*O*- β -D-(6-*O*-*E*-4-Hydroxy-3-methoxy-cinnamoyl)-glucopyranoside (23): Amorphous powder. $[\alpha]_D^{22} +15.6^\circ$ ($c=1.24$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 209 (4.71), 233 (4.26), 284 (sh), 299 (sh), 326 (4.12). FAB-MS m/z : 758 $[\text{M}]^+$, 781 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 758.2776 (Calcd for $\text{C}_{38}\text{H}_{46}\text{O}_{16}$: 758.2786). ¹³C- and ¹H-NMR: shown in Table 2.

(8*R*,7'*S*,8'*R*)-5,5'-Dimethoxyariciresinol 9'-*O*- β -D-(6-*O*-*Z*-4-Hydroxy-3-methoxycinnamoyl)-glucopyranoside (24): Amorphous powder. $[\alpha]_D^{22} +6.2^\circ$ ($c=0.59$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 210 (4.67), 230 (sh), 282 (3.87), 324 (4.04). FAB-MS m/z : 758 $[\text{M}]^+$, 781 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 781.2711 (Calcd for $\text{C}_{38}\text{H}_{46}\text{O}_{16}\text{Na}$: 781.2684). ¹³C-NMR (MeOH-*d*₄ at 35 °C) δ : 168.1 (C-9''), 149.6 (C-4''), 148.4 (C-3''), 145.5 (C-7''), 128.1 (C-1''), 126.7 (C-6''), 116.2 (C-8''), 115.8 (C-5''), 115.3 (C-2''), 56.5 (-OMe''). The ¹³C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **23**. ¹H-NMR (MeOH-*d*₄ at 35 °C) δ : 7.68 (1H, d, 2.0, H-2''), 7.11 (1H, dd, 8.0, 2.0, H-6''), 6.80 (1H, d, 13.0, H-7''), 6.75 (1H, d, 8.0, H-5''), 5.73 (1H, d, 13.0, H-8''), 4.49 (1H, dd, 12.0, 2.0, H_{glc}-6), 4.31 (1H, dd, 12.0, 6.5, H_{glc}-6), 4.29 (1H, d, 8.0, H_{glc}-1), 3.83 (3H, s, -OMe''), 3.50 (1H, m, H_{glc}-5), 3.38 (1H, t, 8.0, H_{glc}-3), 3.33 (1H, t, 8.0, H_{glc}-4), 3.23 (1H, t, 8.0, H_{glc}-2). The ¹H-NMR spectroscopic data of the aglycone moiety were in good agreement with those of **23**. But, the signals due to H-7' and H-9' were observed as follows: δ 4.80 (1H, d, 6.5, H-7'), 3.95 (1H, dd, 10.0, 6.0, H-9'), 3.80 (overlapping, H-9').

(8*R*,7'*S*,8'*R*)-5,5'-Dimethoxyariciresinol 9'-*O*- β -D-(6-*O*-*E*-4-Hydroxycinnamoyl)-glucopyranoside (25): Amorphous powder. $[\alpha]_D^{21} +18^\circ$ ($c=0.26$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 207 (4.90), 222 (sh), 312 (4.26). FAB-MS m/z : 751 $[\text{M}+\text{Na}]^+$. HR-FAB-MS m/z : 751.2572 (Calcd for $\text{C}_{37}\text{H}_{44}\text{O}_{15}\text{Na}$: 751.2578). ¹³C-NMR (MeOH-*d*₄ at 35 °C) δ : 169.0 (C-9''), 161.4 (C-4''), 146.8 (C-7''), 131.2×2 (C-2'', -6''), 127.0 (C-1''), 116.9×2 (C-3'', -5''), 114.9 (C-8''). ¹H-NMR (MeOH-*d*₄ at 35 °C) δ : 7.57 (1H, d, 16.0, H-7''), 7.34 (2H, d, 8.5, H-2'', -6''), 6.76 (2H, d, 8.5, H-3'', -5''), 6.28 (1H, d, 16.0, H-8''). The ¹³C- and ¹H-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **23**.

(8*R*,7'*S*,8'*R*)-5,5'-Dimethoxyariciresinol 9'-*O*- β -D-(6-*O*-*E*-4-Hydroxy-3,5-dimethoxycinnamoyl)-glucopyranoside (26): Amorphous powder. $[\alpha]_D^{21}$

+17° ($c=0.32$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 212 (5.25), 233 (sh), 283 (sh), 327 (4.26). FAB-MS m/z : 788 [M]⁺, 811 [M+Na]⁺. HR-FAB-MS m/z : 788.2900, 811.2793 (Calcd for C₃₉H₄₈O₁₇: 788.2892 and C₃₉H₄₈O₁₇Na: 811.2789). ¹³C-NMR (MeOH-*d*₄ at 35 °C) δ : 168.9 (C-9'), 149.5×2 (C-3", -5"), 147.3 (C-7"), 139.8 (C-4"), 126.6 (C-1"), 115.7 (C-8"), 107.0×2 (C-2", -6"), 56.8×2 (-OMe"). ¹H-NMR (MeOH-*d*₄ at 35 °C) δ : 7.57 (1H, d, 16.0, H-7"), 6.82 (2H, s, H-2", -6"), 6.37 (1H, d, 16.0, H-8"), 3.82 (6H, s, -OMe"). The ¹³C- and ¹H-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **23**.

(8*R*,7'*S*,8'*R*)-5'-Methoxylariciresinol 9'-*O*- β -D-(6-*O*-*E*-4-Hydroxy-3-methoxy-cinnamoyl)-glucopyranoside (**27**): Amorphous powder. $[\alpha]_{\text{D}}^{21} +15.3^\circ$ ($c=1.08$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 209 (4.55), 229 (4.28), 288 (4.02), 326 (4.15). FAB-MS m/z : 728 [M]⁺, 751 [M+Na]⁺. HR-FAB-MS m/z : 728.2666, 751.2584 (Calcd for C₃₇H₄₄O₁₅: 728.2680 and C₃₇H₄₄O₁₅Na: 751.2578). ¹³C- and ¹H-NMR: shown in Table 2.

(8*R*,7'*S*,8'*R*)-5'-Methoxylariciresinol 9'-*O*- β -D-(6-*O*-*Z*-4-Hydroxy-3-methoxy-cinnamoyl)-glucopyranoside (**28**): FAB-MS m/z : 728 [M]⁺, 751 [M+Na]⁺. HR-FAB-MS m/z : 728.2677, 751.2577 (Calcd for C₃₇H₄₄O₁₅: 728.2680 and C₃₇H₄₄O₁₅Na: 751.2578). The ¹³C- and ¹H-NMR spectra of **28** were measured in MeOH-*d*₄ solution. Its spectroscopic data of the aglycone moiety were in good agreement with those of **27**, but, the signals due to H-7' and H-9' were observed as follows: δ 4.81 (1H, d, 6.5, H-7'), 3.94 (1H, dd, 10.0, 6.0, H-9'), 3.79 (overlapping, H-9'). The data of the sugar and ester moieties were similar to those of **24**.

Alkaline Hydrolysis of Compounds 23 and 27 Compounds **23** (12 mg) and **27** (11 mg) were each dissolved in 0.25 M NaOH (1.0 ml). The solution was stirred for 1.5 h at room temperature under a N₂ atmosphere. The reaction mixture was passed through an Amberlite IR-120B column with the eluate concentrated dry. The residue was partitioned between EtOAc and H₂O. Both layers were concentrated dry, and HPLC of the residue from each EtOAc layer suggested that *trans*-ferulic acid was produced from **23** and **27**. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate 1.0 ml/min; solvent, 20% MeCN in water+0.05% trifluoroacetic acid (TFA); t_{R} 15.2 min (*trans*-ferulic acid). Purification of the residue from each H₂O layer using HPLC afforded **23a** (3 mg) and **27a** (6 mg). HPLC conditions: column, YMC-ODS 10 mm×25 cm; flow rate, 3.0 ml/min; solvent, **23a**, 35% MeOH in water, **27a**, 32.5% MeOH in water. Compounds **23a** and **27a** were identified to be (8*R*,7'*S*,8'*R*)-5,5'-dimethoxylariciresinol 9'-*O*- β -D-glucopyranoside (alangilignoside C) and (8*R*,7'*S*,8'*R*)-5'-methoxylariciresinol 9'-*O*- β -D-glucopyranoside (alangilignoside D), respectively, on the basis of the ¹H-, ¹³C-NMR spectroscopic data, optical rotation values and CD spectral data.^{24,25)}

Compound **23a**: Amorphous powder. $[\alpha]_{\text{D}}^{21} +12^\circ$ ($c=0.29$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 207 (5.06), 232 (sh), 270 (3.35). CD nm ($\Delta\epsilon$): 242 (-1.38), 278 (+0.156) ($c=0.51$ mg/ml MeOH). [lit.: $[\alpha]_{\text{D}}^{24} +16.2^\circ$ ($c=0.74$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 209 (4.80), 234 (4.12), 273 (3.45). CD nm ($\Delta\epsilon$): 211 (+11.1), 244 (-1.14); $[\alpha]_{\text{D}}^{18} +18^\circ$ ($c=0.4$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 208 (4.95), 237 (4.24), 271 (3.52). CD nm ($\Delta\epsilon$): 245 (-0.28), 278 (+0.12)].^{24,25)}

Compound **27a**: Amorphous powder. $[\alpha]_{\text{D}}^{21} +13^\circ$ ($c=0.57$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 207 (5.08), 228 (sh), 280 (3.57). CD nm ($\Delta\epsilon$): 239 (-1.27), 260 (+0.248), 288 (-0.331) ($c=0.61$ mg/ml MeOH). [lit.: $[\alpha]_{\text{D}}^{24} +10.3^\circ$ ($c=0.87$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (log ϵ): 207 (4.69), 228 (4.14), 281 (3.61). CD nm ($\Delta\epsilon$): 208 (+7.13), 239 (-0.73)].²⁴⁾

Alkaline Hydrolysis of Compounds 24—26 and 28 Solutions of compounds **24—26** and **28** (ca. 0.5 mg) in 0.25 M NaOH (1.0 ml) were stirred for 1 h at room temperature under a N₂ atmosphere. The procedures and conditions for the detection of the component ester were described above. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 20% MeCN in water+0.05% TFA; t_{R} 13.2 min (*trans-p*-coumaric acid), 14.6 min (*trans*-sinapinic acid), 17.6 min (*cis*-ferulic acid), *trans-p*-Coumaric acid and *trans*-sinapinic acid were afforded from compounds **25**

and **26**, respectively. *cis*-Ferulic acid was yielded from compounds **24** and **28**. Similarly, compounds **23a** and **27a** were detected from the H₂O layers derived from compounds **24—26** and **28** using HPLC. HPLC conditions: column, YMC-ODS 4.6 mm×25 cm; flow rate, 1.0 ml/min; solvent, 35% MeOH in water; t_{R} 16.6 min (**23a**), 18.2 min (**27a**).

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