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

Tsutomu WARASHINA,*[,a] Kimiko SHIKATA,[b] Toshio MIYASE,[b] Satoshi FUJII,[b] and Tadataka NORO[a]

[a] *Institute for Environmental Sciences, University of Shizuoka; and* [b] *School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.*

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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 Asclepiadaceaus 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)-desglucouzarin (**5**),[14,15] 6′-*O*-sinapinoyl-desglucouzarin (**6**),[16] calactin (**7**),[17—19] 16α-acetoxycalactin (**8**),[16] calotropin (**9**),[17—20] 16α-acetoxycalotropin (**10**),[21] asclepin (**11**),[19] 16α-hydroxyasclepin (**13**),[21] 16α-acetoxyasclepin (**14**),[21] uscharin (**15**),[17—19] 16α-hydroxyuscharin (**16**),[22] uscharidin (**17**)[17—19] and 19-nor-16α-acetoxy-10β-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 $C_{31}H_{44}O_{10}$ based on high resolution (HR)-FAB-MS [*m*/*z*: 599.2806 $[M+Na]^+$]. On comparison of the ^{1}H- and ^{13}C-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 $C_{30}H_{42}O_{10}$ based on HR-FAB-MS. The ^{1}H- and ^{13}C-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 ^{1}H-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-1β (δ 2.47), H-2 (δ 5.06), H-4β (δ 1.99), and H-8 (δ 2.17). Thus, **19** was identified as be 19-nor-10β-hydroxyasclepin. The similarity of the ^{1}H- and ^{13}C-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, $C_{29}H_{38}O_9$. 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 ^{1}H- and ^{13}C-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 ^{1}H- and ^{13}C-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-1α (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′

* To whom correspondence should be addressed. e-mail: warashin@sea.u-shizuoka-ken.ac.jp

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 $[M]^+$ ion at *m*/*z* 758.2776, revealing the molecular formula of **23** to be $C_{38}H_{46}O_{16}$. The ^{13}C-NMR spectrum of **23** showed the signals of two tetra-substituted symmetrical aromatic rings (δ 149.2×2, 137.0, 134.3, 104.7×2 and δ 149.2×2, 136.1, 131.7, 107.3×2), three methylenes (δ 68.3, 73.1, 34.3), three methines (δ 83.5, 51.3, 43.6), and four methoxy groups (δ 56.5×4) 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′-dimethoxylariciresinol 9′-*O*-β-D-glucopyranoside, the ^{1}H-, ^{13}C-NMR spectroscopic data and circular dichroism (CD) spectrum being consistent with those of alangilignoside C.[24,25)] Moreover, the ^{1}H-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-

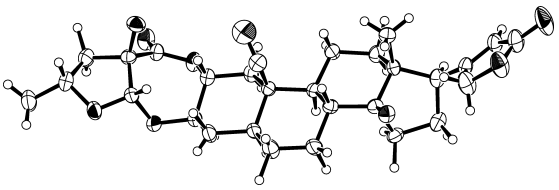

Fig. 1. ORTEP Drawing of Compound **20**

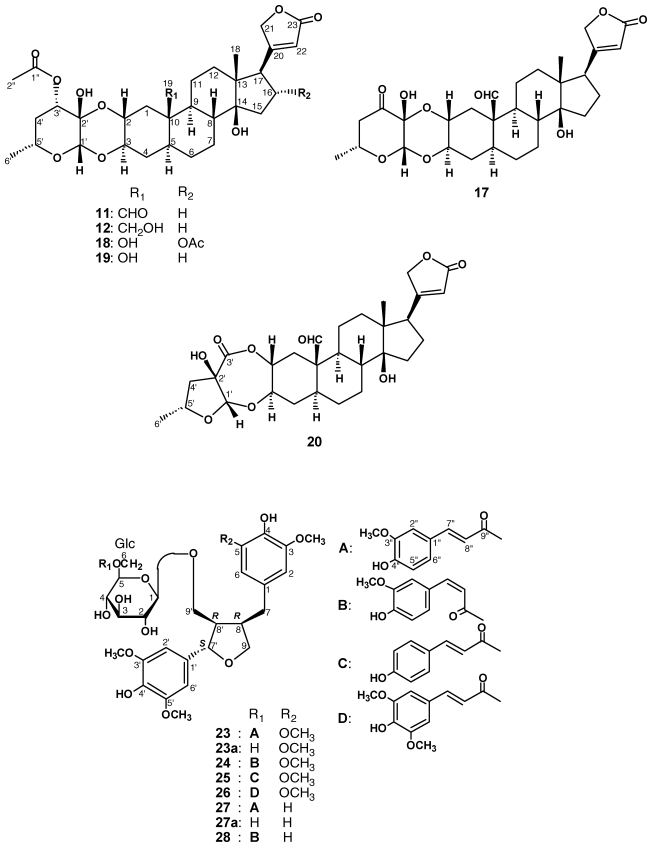

Chart 1. Structures of Compounds **11**, **12**, **17**—**20**, **23**—**27** and **28**

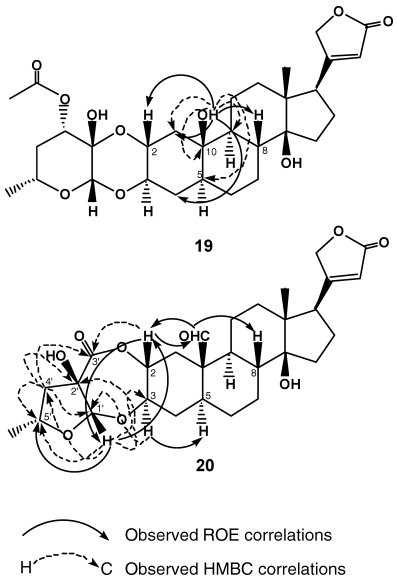

Chart 2. ROE and HMBC Correlations in Compounds **19** and **20**

Table 2. ^{13}C- and ^{1}H-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) 2.74 (dd, 13.5, 11.5)	34.5	2.90 (dd, 13.5, 5.0) 2.47 (dd, 13.5, 11.0)	33.9	2.88 (dd, 13.5, 5.0) 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) 4.02 (dd, 8.5, 6.5)	73.7	3.96 (dd, 8.5, 6.5) 3.70 (dd, 8.5, 6.5)	73.8	3.95 (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) 4.15**	68.9	4.00 (dd, 10.0, 6.0) 3.85 (dd, 10.0, 6.0)	68.9	3.99 (dd, 10.0, 6.5) 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) 5.01 (dd, 12.0, 5.5)	64.4	4.54 (dd, 12.0, 2.0) 4.40 (dd, 12.0, 6.0)	64.5	4.53 (dd, 12.0, 2.0) 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, $C_{38}H_{46}O_{16}$, $C_{37}H_{44}O_{15}$ and $C_{39}H_{48}O_{17}$, respectively. On the basis of the ^{1}H- 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 $C_{37}H_{44}O_{15}$. The ^{1}H-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 ^{1}H-, ^{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 Na^+–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) Å^3, Z=4. All measurements were made on a Rigaku AFC7R diffractometer with graphite monochromated Cu$K\alpha$ radiation and a rotating anode generator. The structure was solved by direct methods[28] 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 H_2O. This suspension was extracted with Et_2O. The H_2O 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–H_2O (1 : 1), MeOH–H_2O (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. $[\alpha]_D^{22}$ +17° (c=0.37, MeOH). FAB-MS m/z: 599 $[M+Na]^+$. HR-FAB-MS m/z: 599.2806 (Calcd for $C_{31}H_{44}O_{10}Na$: 599.2832). ^{13}C-NMR (pyridine-d_5 at 35 °C): shown in Table 1. ^{1}H-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′-OCOCH_3*), 1.37 (3H, d, 6.0, H-6′), 1.11 (1H, t, 12.0, H-1), 1.00 (3H, s, H-18).

19-Nor-16α-acetoxy-10β-hydroxyasclepin (**18**): Amorphous powder. $[\alpha]_D^{22}$ +3.0° (c=0.60, MeOH). FAB-MS m/z: 621 $[M+H]^+$, 643 $[M+Na]^+$. HR-FAB-MS m/z: 621.2902 (Calcd for $C_{32}H_{45}O_{12}$: 621.2911). ^{13}C-NMR (pyridine-d_5 at 35 °C): shown in Table 1. ^{1}H-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′-OCOCH_3*), 1.87 (3H, s, C-16-OCOCH_3*), 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). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 174.2 (C-23), 172.3 (C-3′-OC*OCH$_3$), 171.0 (C-20), 170.7 (C-16-OC*OCH$_3$), 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*H$_3$, -3′-OCOC*H$_3$), 20.9, 20.8 (C-11, -6′), 15.6 (C-18). ^{1}H-NMR ($CDCl_3$ 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′-OCOCH_3*), 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-OCOCH_3*), 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-10β-hydroxyasclepin (**19**): Amorphous powder. $[\alpha]_D^{22}$ +22° (c=0.60, MeOH). FAB-MS m/z: 563 $[M+H]^+$, 585 $[M+Na]^+$. HR-FAB-MS m/z: 563.2854, 585.2676 (Calcd for $C_{30}H_{43}O_{10}$: 563.2856 and $C_{30}H_{42}O_{10}Na$: 585.2676). ^{13}C-NMR (pyridine-d_5 at 35 °C): shown in Table 1. ^{1}H-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′-OCOCH_3*), 1.47 (1H, t, 12.0, H-1), 0.98 (3H, s, H-18). ^{13}C-NMR ($CDCl_3$ at 35 °C) δ: 174.3, 174.2 (C-20, -23), 172.3 (C-3′-OC*OCH$_3$), 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*H$_3$), 20.9 (C-6′), 20.7 (C-11), 15.7 (C-18). ^{1}H-NMR ($CDCl_3$ 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′-OCOCH_3*), 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° (c=0.52, $CHCl_3$–MeOH (1 : 1)). FAB-MS m/z: 531 $[M+H]^+$. HR-FAB-MS m/z: 531.2569 (Calcd for $C_{29}H_{39}O_9$: 531.2594). ^{13}C-NMR: shown in Table 1. ^{1}H-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, dquint, 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′-Dimethoxylariciresinol 9′-*O*-β-D-(6-*O*-*E*-4-Hydroxy-3-methoxy-cinnamoyl)-glucopyranoside (**23**): Amorphous powder. $[\alpha]_D^{22}$ +15.6° (c=1.24, MeOH). UV λ_{max}^{MeOH} nm (log ε): 209 (4.71), 233 (4.26), 284 (sh), 299 (sh), 326 (4.12). FAB-MS m/z: 758 $[M]^+$, 781 $[M+Na]^+$. HR-FAB-MS m/z: 758.2776 (Calcd for $C_{38}H_{46}O_{16}$: 758.2786). ^{13}C- and ^{1}H-NMR: shown in Table 2.

(8*R*,7′*S*,8′*R*)-5,5′-Dimethoxylariciresinol 9′-*O*-β-D-(6-*O*-*Z*-4-Hydroxy-3-methoxycinnamoyl)-glucopyranoside (**24**): Amorphous powder. $[\alpha]_D^{22}$ +6.2° (c=0.59, MeOH). UV λ_{max}^{MeOH} nm (log ε): 210 (4.67), 230 (sh), 282 (3.87), 324 (4.04). FAB-MS m/z: 758 $[M]^+$, 781 $[M+Na]^+$. HR-FAB-MS m/z: 781.2711 (Calcd for $C_{38}H_{46}O_{16}Na$: 781.2684). ^{13}C-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 ^{13}C-NMR spectroscopic data of the aglycone and sugar moieties were in good agreement with those of **23**. ^{1}H-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 ^{1}H-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′-Dimethoxylariciresinol 9′-*O*-β-D-(6-*O*-*E*-4-Hydroxycinnamoyl)-glucopyranoside (**25**): Amorphous powder. $[\alpha]_D^{21}$ +18° (c=0.26, MeOH). UV λ_{max}^{MeOH} nm (log ε): 207 (4.90), 222 (sh), 312 (4.26). FAB-MS m/z: 751 $[M+Na]^+$. HR-FAB-MS m/z: 751.2572 (Calcd for $C_{37}H_{44}O_{15}Na$: 751.2578). ^{13}C-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″). ^{1}H-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 ^{13}C- and ^{1}H-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′-Dimethoxylariciresinol 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 λ_{max}^{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 $C_{39}H_{48}O_{17}$: 788.2892 and $C_{39}H_{48}O_{17}Na$: 811.2789). ^{13}C-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″). ^{1}H-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 ^{13}C- and ^{1}H-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. $[\alpha]_D^{21}$ +15.3° (c=1.08, MeOH). UV λ_{max}^{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 $C_{37}H_{44}O_{15}$: 728.2680 and $C_{37}H_{44}O_{15}Na$: 751.2578). ^{13}C- and ^{1}H-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 $C_{37}H_{44}O_{15}$: 728.2680 and $C_{37}H_{44}O_{15}Na$: 751.2578). The ^{13}C- and ^{1}H-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 N_2 atmosphere. The reaction mixture was passed through an Amberlite IR-120B column with the eluate concentrated dry. The residue was partitioned between EtOAc and H_2O. 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 H_2O 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 ^{1}H-, ^{13}C-NMR spectroscopic data, optical rotation values and CD spectral data.[24,25]

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

Compound **27a**: Amorphous powder. $[\alpha]_D^{21}$ +13° (c=0.57, MeOH). UV λ_{max}^{MeOH} nm (log ε): 207 (5.08), 228 (sh), 280 (3.57). CD nm ($\Delta\varepsilon$): 239 (−1.27), 260 (+0.248), 288 (−0.331) (c=0.61 mg/ml MeOH). [lit.: $[\alpha]_D^{24}$ +10.3° (c=0.87, MeOH). UV λ_{max}^{MeOH} nm (log ε): 207 (4.69), 228 (4.14), 281 (3.61). CD nm ($\Delta\varepsilon$): 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 N_2 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 H_2O 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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