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# Steroidal Constituents from the Roots and Stems of *Asclepias fruticosa*

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**Steroidal constituents from the roots and stems of *Asclepias fruticosa* L. were investigated separately. From the roots, twelve pregnane pentaosides and uzarigenin β-sophoroside were isolated together with three known coroglaucigenin and corotoxigenin glycosides. Pregnane glycosides were composed of ikemagenin or kidjolanin as an aglycone, and D-digitoxose, D-cymarose, D-oleandrose and terminal D-glucose as component sugars. Among the constituents from the stems, cardenolides show a similar pattern to those from the leaves. 17α-Hydroxycalactin and 17α-hydroxyafroside were newly obtained along with known doubly linked and normally linked glycosides. Two pregnane glycosides and uzarigenin β-sophoroside obtained from the roots were also isolated.**

**Keywords** *Asclepias fruticosa*; ikemagenin pentaoside; 17α-hydroxyafroside; kidjolanin pentaoside; uzarigenin β-sophoroside; Asclepiadaceae

It is well known that larvae of *Danaus* butterflies feed on the leaves of *Asclepias* plants, and the cardenolides from the leaves of these plants have been investigated intensively.[1] Previously, we have studied on the cardenolide glycosides from the stems[2a] and seeds[2b] of *Asclepias curassavica* in order to compare them with those from the leaves. The present paper deals with glycosides of pregnanes and cardenolides from the roots and stems of *Asclepias fruticosa* L. (*Gomphocarpus fruticosus* R.BR.)

**Roots** When the powdered roots were percolated with MeOH and the MeOH-soluble substances were processed in the usual manner, pregnane glycosides were obtained from the $CHCl_3$-soluble fraction, showing one broad spot on TLC due to a mixture of glycosides. In order to determine the component pregnanes and sugars, the mixture of the glycosides was subjected to acid hydrolysis. Two aglycones obtained were identified as 12-*O*-cinnamoyl-3β,8β,12β,14β-tetrahydroxy-17α-pregn-5-en-20-one (ikemagenin, A-1)[3] and 12-*O*-cinnamoyl-3β,8β,12β,14β,17β-pentahydroxypregn-5-en-20-one (kidjolanin, A-2)[4] based on a consideration of the $^1$H- and $^{13}$C-NMR spectra, FAB-MS and UV spectra ($\lambda_{max}$ 217, 223, 277 nm in MeOH). The component sugars were fractionated by silica gel column chromatography, and D-digitoxose, D-cymarose, D-oleandrose, β-D-glucosyl-D-cymarose (strophanthobiose)[5] and β-D-glucosyl-D-oleandrose (cynanchobiose)[5] were identified based on comparison with authentic sugars (TLC and optical rotation values). The mixture of glycosides was subjected to silica gel and then octadecyl silica (ODS) column chromatography. Each fraction containing glycosides was further purified by HPLC to afford 12 glycosides (**1**—**12**).

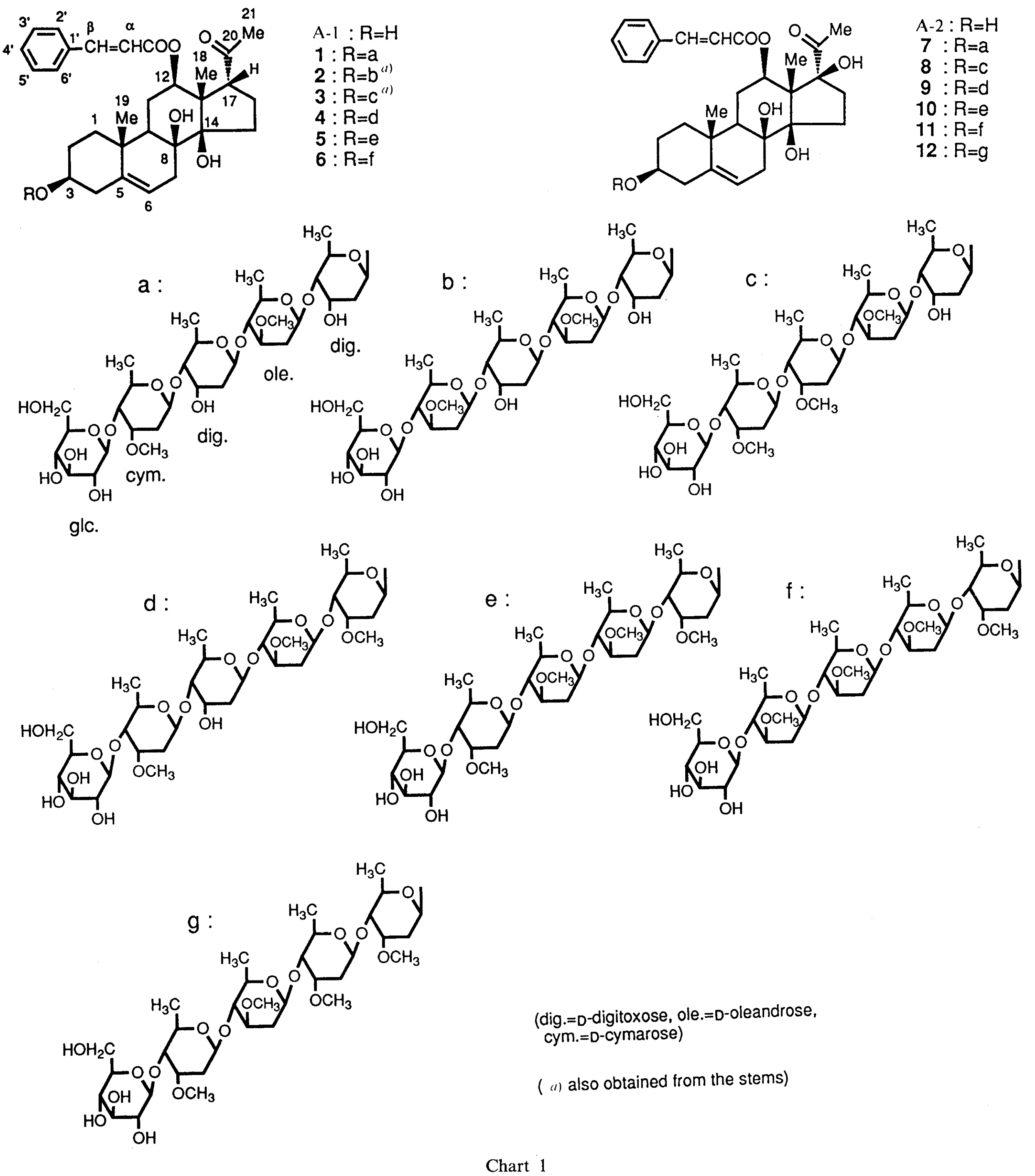
Compound **1** was suggested to have the molecular formula $C_{62}H_{92}O_{23}$, based on FAB-MS. In the $^{13}$C-NMR spectrum, signals due to the aglycone moiety were in good agreement with those of A-1 within the range of glycosylation shifts at C-3 (+6.2 ppm), C-2 (−2.2 ppm) and C-4 (−4.1 ppm), and **1** was considered to be ikemagenin 3-*O*-pentaoside.

On hydrolysis of **1** with 0.05 N $H_2SO_4$–50% dioxane, A-1, digitoxose, oleandrose and strophanthobiose were detected. The presence of only two methoxyl signals (δ 3.52, 3.53) in the $^1$H-NMR spectrum and an $[M+Na]^+$ ion peak in FAB-MS suggested that the sugar moiety includes two digitoxose, one oleandrose, and one cymarose, on which glucose was attached. The signals of each sugar were assigned completely based on the $^1$H–$^1$H shift correlation spectroscopy (COSY) spectrum. The location of glucose at the terminal position of the sugar sequence was confirmed by the $^{13}$C-NMR signals due to the glucose unit and the presence of the $[M-1-\text{glucose}]^-$ ion peak at *m*/*z* 1041 in the negative FAB-MS.

In order to determine the sequence, the nuclear Overhauser effect (NOE) difference spectra were examined. The irradiation of the anomeric proton signal of glucose at δ 4.91 (d, $J=8$ Hz) caused enhancement of the signal intensity at δ 3.60 (dd, $J=10$, 2 Hz, H-4 of cymarose), and similarly, correlations between 5.12 (dd, $J=9$, 2 Hz, H-1 of cymarose)/3.42 (dd, $J=9$, 2 Hz, H-4 of digitoxose); 5.45 (dd, $J=9$, 2Hz, H-1 of digitoxose)/3.44 (t, $J=9$ Hz, H-4 of oleandrose); 4.73 (dd, $J=10$, 2 Hz, H-1 of oleandrose)/3.51 (dd, $J=9$, 2 Hz, H-4 of digitoxose); 5.48 (dd, $J=9$, 2 Hz, H-1 of digitoxose)/3.89 (m, H-3 of aglycone) were observed. Consequently, the sequence of sugars was established to be as presented in Table III. The linkage of cymarose (or oleandrose) to digitoxose was determined to be 1→4 by the occurrence of NOE between H-1 of cymarose (or oleandrose) and H-4 of digitoxose, and by comparison of the $^{13}$C-NMR data with those in the literature.[5] Since the component sugars were all determined to be in the D-series, the sugar sequence was established as D-glucose-D-cymarose-(1→4)-D-digitoxose-D-oleandrose-(1→4)-D-digitoxose-ikemagenin. Thus, the structure of **1** was determined to be as presented in Chart 1.

Compound **2** had the same molecular formula as **1** and two methoxyl signals were observed at δ 3.52 and 3.55. On hydrolysis, A-1, digitoxose, oleandrose and cynanchobiose were obtained. The sequence of the sugar moiety was determined in the same manner as that of **1**.

Compounds **3** and **4** both showed the molecular formula, $C_{63}H_{94}O_{23}$, suggesting an increment of $CH_2$ in comparison with that of **1** or **2**. In the $^1$H-NMR spectra, three methoxyl signals were observed (**3**: δ 3.52, 3.53, 3.61; **4**: δ 3.52, 3.55, 3.58), and hydrolysis of **3** and **4** both afforded digitoxose, oleandrose, cymarose and strophanthobiose, along with A-1. The sugar sequences were



(dig.=D-digitoxose, ole.=D-oleandrose, cym.=D-cymarose)

( a) also obtained from the stems)

Chart 1

assigned on the basis of the $^1$H–$^1$H COSY and NOE difference spectra as shown in Chart 1.

Since the molecular formulae of **5** and **6** were both suggested to be $C_{64}H_{96}O_{23}$ by FAB-MS and the aglycone was identified as A-1, the sugar chains seem to be composed of four 2,6-dideoxy-3-*O*-methylhexose and one glucose. Compound **5** was hydrolyzed to A-1 and oleandrose, cymarose and strophanthobiose, and **6** to A-1, oleandrose, cymarose and cynanchobiose, respectively. Based on the characteristic proton signal of H-3 in D-cymarose ($\delta$ 4.05—4.07, br s) and the $^{13}$C-chemical shifts of methoxyl groups ($\delta$ 57.3 ± 0.1 ppm in D-oleandrose; $\delta$ 58.7 ± 0.2 ppm in D-cymarose), **5** seemed to have two cymarose and two oleandrose, and **6**, one cymarose and three oleandrose. By using the same procedure as for **1**—**4**, the structures were elucidated to be as presented in Chart 1.

Compounds **7**—**12** afforded A-2 on acid hydrolysis. In the $^{13}$C-NMR spectra, glycosylation shifts were observed in C-3 of all aglycone moieties (*e.g.* in **7**: C-3 (+6.2 ppm), C-2 (−2.2 ppm), C-4 (−4.1 ppm)) and other carbon signals in the pregnane retained the same chemical shifts as those of A-2. Therefore, the sugar moiety in each glycoside was located at the 3-hydroxyl group. The molecular formula of **7** was considered to be $C_{62}H_{92}O_{24}$ from the FAB-MS. The component sugars were the same as those of **1**, digitoxose, oleandrose and strophanthobiose and

Table I. $^{13}$C- and $^{1}$H-NMR Data for the Aglycone Moieties of **1** and **7** (δ ppm from TMS in $C_5D_5N$)

| | **1** | | **7** | |
|---|---|---|---|---|
| | C$^{a)}$ | H$^{b)}$ | C$^{a)}$ | H$^{b)}$ |
| 1 | 38.8 | | 38.8 | |
| 2 | 29.8 (−2.2) | | 29.8 (−2.2) | |
| 3 | 77.7 (+6.2) | 3.89 (m) | 77.7 (+6.2) | 3.89 (m) |
| 4 | 39.2 (−4.1) | | 39.2 (−4.1) | |
| 5 | 139.4 | | 139.4 | |
| 6 | 119.1 | 5.30 (br s) | 119.1 | 5.30 (br s) |
| 7 | 34.2$^{c)}$ | | 33.8$^{c)}$ | |
| 8 | 74.5 | | 74.3 | |
| 9 | 44.8 | | 44.5 | |
| 10 | 37.4 | | 37.4 | |
| 11 | 25.0 | | 25.0 | |
| 12 | 73.3 | 5.28 (dd, 11, 6) | 73.3 | 5.19 (dd, 12, 4) |
| 13 | 55.8 | | 55.8 | |
| 14 | 87.5 | | 89.4 | |
| 15 | 35.1$^{c)}$ | | 34.7$^{c)}$ | |
| 16 | 21.9 | | 33.0 | |
| 17 | 60.5 | | 92.4 | |
| 18 | 15.8 | 2.01 (s) | 10.6 | 2.03 (s) |
| 19 | 18.2 | 1.35 (s) | 18.2 | 1.35 (s) |
| 20 | 209.7 | | 209.7 | |
| 21 | 32.1 | 2.28 (s) | 27.6 | 2.50 (s) |
| Cinnamoyl | | | | |
| CO | 165.9 | | 165.8 | |
| α | 119.3 | 6.78 (d, 16) | 119.2 | 6.81 (d, 16) |
| β | 144.8 | 7.98 (d, 16) | 144.8 | 8.00 (d, 16) |
| 1′ | 135.0 | | 135.0 | |
| 2′ | 128.5 | 7.61—7.63 | 128.5 | 7.61—7.63 |
| 3′ | 129.2 | 7.34—7.36 | 130.5 | 7.33—7.36 |
| 4′ | 130.5 | 7.34—7.36 | 130.5 | 7.33—7.36 |
| 5′ | 129.2 | 7.34—7.36 | 129.2 | 7.33—7.36 |
| 6′ | 128.5 | 7.61—7.63 | 128.5 | 7.61—7.63 |

*a*) Δ(1−A-1) or Δ(7−A-2) ppm in parentheses. *b*) *J* Hz in parentheses. *c*) Signal assignments may be interchangeable.

Table II. $^{13}$C-NMR Data for the Sugar Moieties of **1**—**6** and **12** (δ ppm from TMS in $C_5D_5N$)

| | **1**$^{a)}$ | **2** | **3** | **4** | **5** | **6**$^{a)}$ | **12**$^{a)}$ |
|---|---|---|---|---|---|---|---|
| Sugar-1 | dig. | dig. | dig. | cym. | cym. | cym. | cym. |
| 1 | 96.4 | 96.3 | 96.3 | 96.3 | 96.3 | 96.4 | 96.4 |
| 2 | 39.1 | 39.1 | 39.1 | 37.2 | 37.2 | 37.2 | 37.2 |
| 3 | 67.5 | 67.5 | 67.5 | 77.8 | 77.8 | 77.8 | 77.8 |
| 4 | 83.6 | 83.6 | 83.6 | 82.8$^{b)}$ | 82.7$^{b)}$ | 82.7 | 82.6 |
| 5 | 68.4 | 68.4 | 68.4 | 68.9 | 68.9 | 68.9 | 68.9 |
| 6 | 18.6 | 18.6 | 18.6 | 18.6$^{c)}$ | 18.6 | 18.6$^{b)}$ | 18.2 |
| OMe | | | | 58.8 | 58.7 | 58.8 | 58.8 |
| Sugar-2 | ole. | ole. | ole. | ole. | ole. | ole. | cym. |
| 1 | 101.4 | 101.4$^{b)}$ | 101.3 | 101.9 | 101.9 | 101.9 | 100.3 |
| 2 | 37.5 | 37.5 | 37.5 | 37.5 | 37.6$^{c)}$ | 37.5$^{c)}$ | 37.0 |
| 3 | 78.8 | 78.8$^{c)}$ | 78.7 | 78.8 | 79.0 | 79.0$^{d)}$ | 77.9 |
| 4 | 82.8 | 82.7 | 82.6 | 82.9$^{b)}$ | 82.8$^{b)}$ | 82.9$^{e)}$ | 83.1 |
| 5 | 71.7 | 71.7$^{d)}$ | 71.7 | 71.7 | 71.5$^{d)}$ | 71.5$^{f)}$ | 69.2 |
| 6 | 18.5 | 18.5 | 18.5$^{b)}$ | 18.4$^{c)}$ | 18.6 | 18.7$^{b)}$ | 18.6 |
| OMe | 57.4 | 57.4 | 57.3 | 57.4 | 57.4$^{e)}$ | 57.3$^{g)}$ | 58.7 |
| Sugar-3 | dig. | dig. | cym. | dig. | ole. | ole. | ole. |
| 1 | 98.4 | 98.4 | 98.3$^{c)}$ | 98.4 | 100.0 | 100.0 | 101.9 |
| 2 | 38.9 | 38.9 | 37.1$^{d)}$ | 38.9 | 37.8$^{c)}$ | 37.6$^{c)}$ | 37.4 |
| 3 | 67.6 | 67.6 | 78.0$^{e)}$ | 67.5 | 79.0 | 79.6$^{d)}$ | 78.8 |
| 4 | 83.0 | 83.1$^{e)}$ | 83.1 | 83.4$^{b)}$ | 83.4$^{b)}$ | 83.5$^{e)}$ | 83.4 |
| 5 | 68.8 | 68.7 | 69.2 | 68.7 | 71.8$^{d)}$ | 71.6$^{f)}$ | 71.7 |
| 6 | 18.6 | 18.6 | 18.6$^{b)}$ | 18.6$^{c)}$ | 18.6 | 18.9$^{b)}$ | 18.6 |
| OMe | | | 58.8$^{f)}$ | | 57.3$^{e)}$ | 57.2$^{g)}$ | 57.4 |
| Sugar-4 | cym. | ole. | cym. | cym. | cym. | ole. | cym. |
| 1 | 99.7 | 101.3$^{b)}$ | 100.3$^{c)}$ | 99.6 | 98.3 | 100.0 | 98.4 |
| 2 | 36.4 | 37.5 | 36.7$^{d)}$ | 36.4 | 36.7 | 37.7$^{c)}$ | 36.7 |
| 3 | 77.9 | 79.2$^{c)}$ | 77.9$^{e)}$ | 77.9 | 78.1 | 79.6$^{d)}$ | 78.0 |
| 4 | 82.9 | 83.2$^{e)}$ | 83.0 | 83.0$^{b)}$ | 83.2$^{b)}$ | 83.4$^{e)}$ | 83.0 |
| 5 | 69.4 | 71.7$^{d)}$ | 69.3 | 69.4 | 69.6 | 71.7$^{f)}$ | 69.4 |
| 6 | 18.5 | 18.7 | 18.4$^{b)}$ | 18.5$^{c)}$ | 18.6 | 18.9$^{b)}$ | 18.6 |
| OMe | 58.6 | 57.2 | 58.6$^{f)}$ | 58.6 | 58.5 | 57.3$^{g)}$ | 58.9 |
| Sugar-5 | glc. | glc. | glc. | glc. | glc. | glc. | glc. |
| 1 | 106.4 | 104.4 | 106.5 | 106.4 | 106.5 | 104.4 | 106.5 |
| 2 | 75.3 | 75.7 | 75.3 | 75.3 | 75.3 | 75.7 | 75.4 |
| 3 | 78.3 | 78.6 | 78.3 | 78.3 | 78.3 | 78.6 | 78.3 |
| 4 | 71.8 | 72.0$^{d)}$ | 71.8 | 71.8 | 71.8 | 72.0 | 71.9 |
| 5 | 78.4 | 78.1 | 78.3 | 78.4 | 78.3 | 78.0 | 78.3 |
| 6 | 63.0 | 63.1 | 63.0 | 63.0 | 63.0 | 63.1 | 63.0 |

*a*) Signal assignments were based on $^{13}$C–$^{1}$H COSY spectra. *b*—*g*) Signal assignments may be interchangeable.

the $^{1}$H- and $^{13}$C-NMR signals of the sugar moiety were in good agreement with those of **1**. The structure of **7** was thus characterized to be a kidjolanin glycoside having the same sugar sequence as **1**.

Compounds **8** and **9** gave the molecular formula, $C_{63}H_{94}O_{24}$, and afforded the same component sugars, digitoxose, oleandrose, cymarose and strophanthobiose. By comparison of the $^{1}$H- and $^{13}$C-NMR spectra of the sugar moieties with those of **3** and **4**, the structures were determined to be kidjolanin glycosides with the sugar moieties corresponding to those of **3** and **4**, respectively. By the similar considerations, **10** and **11** were determined to have the same sugar moieties as **5** and **6**, respectively.

Compound **12**, $C_{64}H_{96}O_{24}$, afforded cymarose, oleandrose and strophanthobiose on acid hydrolysis. Three H-3 signals of cymarose and oleandrose (δ 4.05 × 2, 4.11, each br s) and the $^{13}$C-chemical shifts of the methoxyl groups suggested that **12** has three cymarose (δ 58.7, 58.8, 58.9) and one oleandrose (δ 57.4). Based on a consideration of the $^{1}$H–$^{1}$H COSY and NOE difference spectra, the structure was elucidated to be as shown in Chart 1.

Four cardenolide glycosides were isolated from the water layer after extraction of the pregnane glycosides with $CHCl_3$. Three of them were known glycosides, identified as coroglaucigenin 3-*O*-β-D-allomethyloside (frugoside) (**13**),[2b)] coroglaucigenin 3-*O*-β-D-glucosyl-(1→4)-β-D-allomethyloside (glucosylfrugoside) (**14**)[2b)] and corotoxigenin 3-*O*-β-D-glucosyl-(1→4)-β-D-allomethyloside (glucosylgofruside)(**15**).[2b)]

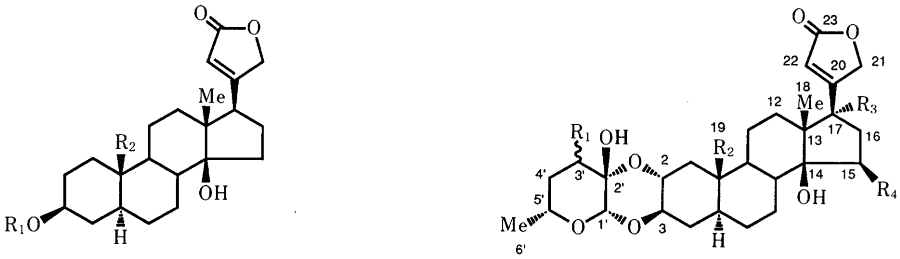
The aglycone of **16** was considered to be uzarigenin based on the C-19 signal observed at δ 12.3[6)] as well as other evidence from the NMR spectra. FAB-MS of **16** afforded an $[M+Na]^+$ peak at *m*/*z* 721.3422, suggesting **16** to be a bioside. Two anomeric protons were observed at δ 5.07 ($J=8$ Hz) and 5.26 ($J=8$ Hz), and the coupling patterns of all carbinyl protons in the sugar moiety identified axial orientations, suggesting the component sugar to be glucose. In fact, **16** was hydrolyzed with β-glucosidase to uzarigenin and uzarigenin β-D-glucoside.[2a)] Based on the signal pattern of the sugar moiety in the $^{13}$C-NMR spectrum, **16** was determined to be uzarigenin β-sophoroside.

**Stems** From the benzene- and $CHCl_3$-soluble fractions of MeOH percolate, seven known cardenolide and cardenolide glycosides, uzarigenin (**17**), calactin (**18**),[2a,7)]

TABLE III. $^1$H-NMR Data for the Sugar Moieties of **1**—**6** and **12** (δ ppm from TMS in $C_5D_5N$)[a]

| H | **1** | **2** | **3** | **4** | **5** | **6** | **12** |
|---|---|---|---|---|---|---|---|
| Sugar-1 | dig. | dig. | dig. | cym. | cym. | cym. | cym. |
| 1 | 5.48 (dd, 9, 2) | 5.49 (dd, 9, 1) | 5.48 (dd, 9, 1) | 5.27 (br d, 10) | 5.27 (br d, 10) | 5.29 (dd, 9, 2) | 5.27 (br d, 10) |
| 3 | 4.61 (br s) | 4.62 (br s) | 4.62 (br s) | 4.05 (br s) | 4.06 (br s) | 4.07 (br s) | 4.05 (br s) |
| 4 | 3.51 (dd, 9, 2) | 3.51 (dd, 9, 2) | 3.51 (dd, 9, 2) | 3.50 (dd, 9, 3) | 3.52 (dd, 9, 3) | 3.52 (dd, 9, 3) | 3.44 (dd, 9, 3) |
| 5 | 4.30 (m) | 4.31 (m) | 4.30 (m) | [c] | [c] | 4.22 (m) | 4.13 (m) |
| 6 | 1.47 (d, 6) | 1.48 (d, 6) | 1.47 (d, 6) | 1.44 (d, 6) | 1.43 (d, 6)[d] | 1.45 (d, 6) | 1.34 (d, 6) |
| Sugar-2 | ole. | ole. | ole. | ole. | ole. | ole. | cym. |
| 1 | 4.73 (dd, 10, 2) | 4.74 (dd, 10, 2) | 4.73 (dd, 9, 1) | 4.69 (br d, 10) | 4.70 (br d, 10) | 4.71 (dd, 10, 2) | 5.08 (br d, 10) |
| 3 | [b] | [b] | [b] | [b] | [b] | [b] | 4.05 (br s) |
| 4 | 3.44 (t, 9) | 3.46 (t, 9) | 3.42 (t, 9) | 3.51 (t, 9) | 3.49 (t, 9) | 3.50 (t, 9) | 3.50 (dd, 9, 2) |
| 5 | [b] | [b] | [b] | [b] | [b] | [b] | 4.22 (m) |
| 6 | 1.37 (d, 6) | 1.36 (d, 6) | 1.36 (d, 6) | 1.44 (d, 6) | 1.44 (d, 6)[d] | 1.45 (d, 6) | 1.42 (d, 6) |
| Sugar-3 | dig. | dig. | cym. | dig. | ole. | ole. | ole. |
| 1 | 5.45 (dd, 9, 2) | 5.48 (br d, 10) | 5.26 (br d, 10) | 5.46 (br d, 10) | 4.88 (br d, 10) | 4.89 (dd, 10, 1) | 4.69 (dd, 9, 2) |
| 3 | 4.59 (br s) | 4.60 (br s) | 4.05 (br s) | 4.59 (br s) | [b] | [b] | [b] |
| 4 | 3.42 (dd, 9, 2) | 3.45 (dd, 9, 2) | 3.43 (dd, 9, 3) | 3.43 (dd, 9, 2) | 3.50 (t, 9) | 3.50 (t, 9) | 3.50 (t, 9) |
| 5 | 4.24 (m) | 4.30 (m) | 4.20 (m) | [c] | [b] | [b] | [b] |
| 6 | 1.39 (d, 6) | 1.43 (d, 6) | 1.34 (d, 6) | 1.38 (d, 6) | 1.43 (d, 6) | 1.45 (d, 6) | 1.42 (d, 6) |
| Sugar-4 | cym. | ole. | cym. | cym. | cym. | ole. | cym. |
| 1 | 5.12 (dd, 9, 2) | 4.73 (dd, 9, 2) | 5.08 (dd, 10, 1) | 5.12 (br d, 10) | 5.27 (br d, 10) | 4.89 (dd, 10, 1) | 5.27 (br d, 10) |
| 3 | 4.08 (br s) | [b] | 4.11 (br s) | 4.09 (br s) | 4.15 (br s) | [b] | 4.11 (br s) |
| 4 | 3.60 (dd, 10, 2) | 3.56 (t, 9) | 3.66 (dd, 10, 2) | 3.60 (dd, 9, 2) | 3.68 (dd, 9, 2) | [b] | 3.65 (dd, 9, 2) |
| 5 | 4.26 (m) | [b] | 4.24 (m) | [c] | [c] | [b] | 4.25 (m) |
| 6 | 1.55 (d, 6) | 1.65 (d, 6) | 1.61 (d, 6) | 1.55 (d, 6) | 1.62 (d, 6) | 1.73 (d, 6) | 1.61 (d, 6) |
| Sugar-5 | glc. | glc. | glc. | glc. | glc. | glc. | glc. |
| 1 | 4.91 (d, 8) | 5.10 (d, 8) | 4.93 (d, 8) | 4.91 (d, 8) | 4.93 (d, 8) | 5.12 (d, 8) | 4.93 (d, 8) |
| 2 | 3.99 (dd, 8, 9) | 3.99 (dd, 8, 9) | 4.00 (dd, 8, 9) | 3.99 (dd, 8, 9) | 3.99 (dd, 8, 9) | 3.99 (dd, 8, 9) | 4.00 (dd, 8, 9) |
| 3 | 4.22 (t, 9) | 4.21 (t, 9) | 4.23 (t, 9) | 4.22 (t, 9) | 4.22 (t, 9) | 4.20 (t, 9) | 4.23 (t, 9) |
| 4 | 4.16 (t, 9) | 4.17 (t, 9) | 4.17 (t, 9) | 4.15 (t, 9) | 4.17 (t, 9) | 4.18 (t, 9) | 4.18 (t, 9) |
| 5 | 3.97 (m) | 3.94 (m) | 3.98 (m) | 3.95 (m) | 3.96 (m) | 3.94 (m) | 3.98 (m) |
| 6 | 4.36 (dd, 12, 5)<br>4.56 (br d, 12) | 4.34 (dd, 12, 5)<br>4.51 (dd, 12, 1) | 4.38 (dd, 12, 6)<br>4.57 (br d, 12) | 4.36 (dd, 12, 6)<br>4.56 (dd, 12, 2) | 4.38 (dd, 12, 5)<br>4.57 (br d, 12) | 4.34 (dd, 12, 5)<br>4.50 (dd, 12, 2) | 4.38 (dd, 12, 5)<br>4.57 (br d, 12) |
| OMe | 3.52<br>3.53 | 3.52<br>3.55 | 3.52<br>3.53<br>3.61 | 3.52<br>3.55<br>3.58 | 3.50<br>3.54 (×2)<br>3.59 | 3.51 (×2)<br>3.55<br>3.59 | 3.53 (×2)<br>3.58<br>3.61 |

a) Signal assignments were based on $^1$H–$^1$H COSY and NOE difference spectra. b) Signals overlapped with each other within δ 3.50—3.70 ppm. c) Signals overlapped with each other within δ 4.20—4.30 ppm. d) Signal assignments may be interchangeable.



**13** : $R_1$=β-D-allomethylose, $R_2$=$CH_2OH$ (r)
**14** : $R_1$=β-D-glucosyl$\xrightarrow{4}$allomethylose, $R_2$=$CH_2OH$ (r)
**15** : $R_1$=β-D-glucosyl$\xrightarrow{4}$allomethylose, $R_2$=CHO (r)
**16** : $R_1$=β-D-glucosyl$\xrightarrow{2}$β-D-glucose, $R_2$=$CH_3$ (s, r)
**17** : $R_1$=H, $R_2$=$CH_3$ (s)
**26** : $R_1$=H, $R_2$=$CH_2OH$ (s)
**27** : $R_1$=β-D-glucose, $R_2$=$CH_2OH$ (s)
**28** : $R_1$=β-cellobiose, $R_2$=$CH_3$ (s)

**18** : $R_1$=β-OH, $R_2$=CHO, $R_3$=$R_4$=H (s)
**19** : $R_1$=β-OH, $R_2$=$CH_3$, $R_3$=H, $R_4$=OH (s)
**20** : $R_1$=α-OH, $R_2$=$CH_3$, $R_3$=H, $R_4$=OH(s)
**21** : $R_1$=α-OAc, $R_2$=$CH_3$, $R_3$=H, $R_4$=OH (s)
**22** : $R_1$=α-OH, $R_2$=CHO, $R_3$=$R_4$=H (s)
**23** : $R_1$=β-OH, $R_2$=$CH_3$, $R_3$=$R_4$=H (s)
**24** : $R_1$=β-OH, $R_2$=CHO, $R_3$=OH, $R_4$=H (s)
**25** : $R_1$=β-OH, $R_2$=$CH_3$, $R_3$=OH, $R_4$=OH (s)

(s : obtained from the stems, r : obtained from the roots)

Chart 2

afroside (**19**),[7] 3′-epiafroside (**20**),[7] 3′-epiafroside-3′-acetate (**21**),[7] calotropin (**22**)[2a,7] and gomphoside (**23**),[7] were obtained along with two pregnane glycosides, **2** and **3**. The water layer was chromatographed on an MCI gel column. From the 100% MeOH eluate, two new cardenolide glycosides (**24**, **25**) were isolated along with coroglaucigenin (**26**), coroglaucigenin glucoside (**27**), uzarigenin β-cellobioside (**28**) and **16**.

Table IV. $^{13}$C-NMR Data for **24**, **25**, and **16** ($\delta$ ppm from TMS in $C_5D_5N$)

| C | **24**$^{a)}$ | **25**$^{b)}$ | **16** |
|---|---|---|---|
| 1 | 36.5 | 42.8 | 37.3 |
| 2 | 69.7 | 69.5 | 29.9 |
| 3 | 72.2 | 72.9 | 77.8 |
| 4 | 32.6 | 32.6 | 34.7 |
| 5 | 43.4 | 44.9 | 44.4 |
| 6 | 27.1$^{c)}$ | 28.3$^{c)}$ | 29.0 |
| 7 | 27.9$^{c)}$ | 26.9$^{c)}$ | 27.9 |
| 8 | 43.4 | 41.7 | 41.6 |
| 9 | 48.8 | 49.6 | 50.0 |
| 10 | 52.9 | 37.9 | 36.0 |
| 11 | 22.2 | 21.5 | 21.5 |
| 12 | 34.0 ($-$5.2) | 32.9 ($-$5.6) | 39.3 |
| 13 | 53.3 (+3.2) | 53.2 (+4.3) | 49.9 |
| 14 | 84.0 | 83.0$^{c)}$ | 84.5 |
| 15 | 31.1 ($-$2.8) | 70.4 ($-$2.5) | 33.1 |
| 16 | 38.5 (+11.4) | 49.4 (+11.2) | 27.2 |
| 17 | 85.7 (+34.6) | 83.6$^{c)}$ (+34.2) | 51.4 |
| 18 | 15.2 | 15.6 | 16.1 |
| 19 | 208.0 | 13.9 | 12.3 |
| 20 | 180.8 (+5.4) | 180.2 (+5.2) | 175.9 |
| 21 | 73.9 | 74.0 | 73.3 |
| 22 | 117.5 | 117.8 | 117.6 |
| 23 | 174.5 | 174.5 | 174.5 |
| 1′ | 95.4 | 95.5 | 101.5 |
| 2′ | 91.8 | 91.8 | 84.5 |
| 3′ | 71.6 | 71.7 | 77.9 |
| 4′ | 38.2 | 38.2 | 71.5 |
| 5′ | 66.5 | 66.4 | 78.2$^{c)}$ |
| 6′ | 21.5 | 21.5 | 62.7 |
| 1″ | | | 106.4 |
| 2″ | | | 76.9 |
| 3″ | | | 78.6$^{c)}$ |
| 4″ | | | 71.5 |
| 5″ | | | 78.5$^{c)}$ |
| 6″ | | | 62.7 |

*a)* $\Delta$(**24**$-$**18**) ppm in parentheses. *b)* $\Delta$(**25**$-$**19**) ppm in parentheses. *c)* The assignments in each column may be interchangeable.

Compound **24** (mp 215—220 °C) was suggested to have the molecular formula, $C_{29}H_{40}O_{10}$. The fact that an anomeric proton was observed as a singlet signal, H-3′ as a broad singlet signal at $\delta$ 4.27 and a formyl proton at $\delta$ 10.08, strongly suggested **24** to be a calactin-type doubly linked glycoside. In the $^{13}$C-NMR spectrum, signals due to rings A, B, C of the steroidal framework and a sugar moiety were in good agreement with those of calactin. Proton signals due to the butenolide ring showed lower-field shifts in comparison with those of **18** (H-22: +0.52 ppm, H-21a: +0.19 ppm, H-21b: +0.38 ppm). The tertiary carbon signal for C-17, was no longer observed in **24**, but instead, a quaternary carbinol carbon signal was seen at $\delta$ 85.7 ppm, besides C-14 signal at $\delta$ 84.0. C-13, C-16 and C-20 were shifted to the lower field (+3.2 ppm, +11.4 ppm and +5.4 ppm, respectively) and C-12 to upper field ($-$5.2 ppm). From the NMR spectrum and the molecular formula, **24** was characterized to be 17-hydroxycalactin. The configuration at C-17 was determined to be a 17$\alpha$-hydroxy based on the NOE between H-22 and H-18.

Table V. $^1$H-NMR Data for **24**, **25**, and **16** ($\delta$ ppm from TMS in $C_5D_5N$, $J$= Hz in Parentheses)

| H | **24** | **25** | **16** |
|---|---|---|---|
| 2 | 4.48 (m) | 4.50 (m) | |
| 3 | 4.38 (m) | 4.31 (m) | 3.95 (m) |
| 15α | | 4.98 (dd, 11, 5) | |
| 16α | | 3.00 (dd, 15, 11) | |
| β | | 2.59 (dd, 15, 5) | |
| 17 | | | 2.76 (dd, 9, 5) |
| 18 | 1.09 (s)<br>(+0.18)$^{a)}$ | 1.23 (s)<br>(+0.23)$^{b)}$ | 0.99 (s) |
| 19 | 10.08 (s) | 0.72 (s) | 0.75 (s) |
| 21 | 5.18 (dd, 18, 1)<br>(+0.19)$^{a)}$ | 5.24 (dd, 18, 2)<br>(+0.24)$^{b)}$ | 5.01 (dd, 18, 1) |
| | 5.62 (dd, 18, 1)<br>(+0.38)$^{a)}$ | 5.63 (dd, 18, 2)<br>(+0.34)$^{b)}$ | 5.29 (dd, 18, 1) |
| 22 | 6.66 (br s)<br>(+0.52)$^{a)}$ | 6.72 (br s)<br>(+0.62)$^{b)}$ | 6.11 (br s) |
| 1′ | 5.44 (s) | 5.44 (s) | 5.07 (d, 8) |
| 2′ | | | 4.14 (dd, 8, 9) |
| 3′ | 4.27 (br s) | 4.26 (br s) | 4.36 (t, 9) |
| 4′ | | | 4.28 (t, 9) |
| 5′ | 4.55 (m) | 4.55 (m) | 3.90—4.00 |
| 6′ | 1.39 (d, 6) | 1.38 (d, 6) | 4.35 (dd, 12, 4) |
| | | | 4.54 (dd, 12, 2) |
| 1″ | | | 5.26 (d, 8) |
| 2″ | | | 4.11 (t, 8) |
| 3″ | | | 4.22 (dd, 8, 9) |
| 4″ | | | 4.20 (t, 9) |
| 5″ | | | 3.90—4.00 |
| 6″ | | | 4.42 (dd, 12, 4) |
| | | | 4.51 (dd, 12, 2) |

*a)* $\Delta$(**24**$-$**18**) ppm in parentheses. *b)* $\Delta$(**25**$-$**19**) ppm in parentheses.

Compound **25**[8] (mp 248—252 °C) showed the molecular formula, $C_{29}H_{42}O_{10}$, one oxygen larger than **19**, and the presence of a quaternary carbinol was suggested by a signal at $\delta$ 83.6 besides that of C-14 at $\delta$ 83.0. Proton signals due to the butenolide ring were shifted to lower field (H-22: +0.62 ppm, H-21a: 0.24 ppm, H-21b: 0.34 ppm) as observed in **24**. Lower-field shifts of the neighboring carbons (C-13: +4.3 ppm, C-16: +11.2 ppm, C-20: +5.2 ppm) and an upper-field shift of C-12 ($-$5.6 ppm) indicated the structure of **14** to be 17-hydroxyafroside. Although H-15$\alpha$ in afroside (**19**) was observed as a triplet signal ($J$=8 Hz), a carbinyl proton assignable to H-15$\alpha$ in **25** appeared as a doublet of doublets. In order to confirm the configuration at C-15, NOE measurements were examined. Since NOE was observed between H-22 and H-18, the cardenolide-side chain was established to have $\beta$-orientation. The NOE connection from H-22 was also observed at one of the C-16 methylene protons at $\delta$ 2.59 (dd, $J$=15, 5 Hz), which was assignable to H-16$\beta$. Accordingly, a signal at $\delta$ 3.00 (dd, $J$=15, 11 Hz) showing geminal coupling with H-16$\beta$, was determined to be H-16$\alpha$. The $\beta$-orientation of 15-OH in **25** was confirmed by the presence of NOE between H-16$\alpha$ and H-15$\alpha$ ($\delta$ 4.98, dd, $J$=11, 5 Hz).

While cardenolides from the stems show a similar pattern to those from the leaves as regards the distribution of normally and doubly linked glycosides,[1] the roots contain pregnane glycosides having a variety of sugar sequences along with a few normally linked cardenolide glycosides. Although thiazolidine or thiazoline derivatives of doubly linked 19-formyl cardenolides occur commonly in *Asclepias* and *Calotropis*,[1] such derivatives were not detected in this study.

## Experimental

The melting points were taken on a hot stage apparatus without correction. $^{1}$H- and $^{13}$C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-$d_5$. Chemical shifts are given in $\delta$ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s = singlet, d = doublet, t = triplet, m = multiplet, br s = broad singlet, dd = doublet of doublets. FAB-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter and UV spectra on a Shimadzu UV-200S in MeOH. For silica gel column chromatography and TLC, the following solvent systems were applied: $CHCl_3$–MeOH–$H_2O$ (bottom layer, solvent 1), EtOAc–MeOH–$H_2O$ (top layer, solvent 2). For ODS column chromatography, HPLC and reversed-phase TLC (RP-18 $WF_{254}$), solvent 3 ($CH_3CN$–$H_2O$) was used. Cardenolides on TLC plates were visualized by spraying Kedde's reagent (1 : 1 mixture of 5% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH) or 10% $H_2SO_4$. The latter reagent was applied for pregnane glycosides.

**Plant Materials** *Asclepias fruticosa* L. was cultivated in the medicinal plant garden of Fukuoka University in 1991 and harvested in November. The roots and the stems were separated, air-dried and powdered.

**Extraction and Isolation of Pregnane Glycosides and Cardenolide Glycosides** From Roots: Powdered roots (0.9 kg) were percolated with MeOH, the MeOH solution was concentrated to 0.9 l *in vacuo*, and 0.9 l of $H_2O$ was added to the concentrate. The mixture was filtered and the filtrate was first extracted with benzene and then $CHCl_3$. The $CHCl_3$ extract (4.5 g) was chromatographed on a silica gel column with solvents 1 (7 : 2 : 1) and 2 (5 : 1 : 4) to give a mixture of pregnane glycosides (2.8 g), showing one broad spot on TLC. The mixture (500 mg) was subjected to hydrolysis and the rest of the mixture was fractionated on an ODS column (YMC-gel) with solvent 3 (35—55%) and finally by HPLC (column: Capcell Pak AG-120/40—50%$CH_3CN$). Twelve glycosides, **1**—**12**, were obtained (**1**; 15 mg, **2**; 15 mg, **3**; 24 mg, **4**; 8 mg, **5**; 6 mg, **6**; 15 mg, **7**; 24 mg, **8**; 12 mg, **9**; 6 mg, **10**; 8 mg, **11**; 24 mg, **12**; 7 mg). The $H_2O$ layer was concentrated *in vacuo* and passed through an MCI-gel (Mitsubishi Chem. Ind. CHP-20) column. The eluate with 50—75% MeOH was further chromatographed repeatedly on a silica gel column with solvents 1 (7 : 2 : 1—7 : 3 : 1) and 2 (5 : 1 : 4—4 : 1 : 3), and four cardenolide glycosides, frugoside (**13**, 8 mg), glucosylfrugoside (**14**, 15 mg), glucosylgofruside (**15**, 23 mg), and **16** (22 mg) were obtained.

From Stems: Powdered stems (3.2 kg) were treated according to the same procedure as described above. The 50% MeOH fraction was extracted with benzene and then with $CHCl_3$. The $H_2O$ layer was fractionated in the same manner as that of the roots. The benzene and $CHCl_3$ fractions were combined and chromatographed on a silica gel column with solvents 1 (10 : 1 : 2—7 : 2 : 1) and 2 (10 : 1 : 2) to afford uzarigenin (**17**, 20 mg), calactin (**18**, 40 mg), afroside (**19**, 8 mg), 3′-epiafroside (**20**, 15 mg), 3′-epiafroside-3′-acetate (**21**, 14 mg), calotropin (**22**, 6 mg), gomphoside (**23**, 38 mg), **2** (4 mg) and **3** (3 mg). After extraction with $CHCl_3$, the $H_2O$ layer was concentrated *in vacuo* and passed through an MCI-gel column. The column was eluted with $H_2O$–MeOH containing increasing concentrations of MeOH. The eluate with 100% MeOH was then chromatographed on a silica gel column with solvents 1 (7 : 2 : 1—7 : 3 : 1) and 2 (5 : 1 : 5—4 : 1 : 3), and on an ODS column (YMC-gel) with $CH_3CN$–$H_2O$ to give two new cardenolide glycosides, **24** (3 mg) and **25** (4 mg), together with **16** (12 mg), coroglaucigenin (**26**, 10 mg), coroglaucigenin $\beta$-D-glucoside (**27**, 55 mg) and uzarigenin $\beta$-cellobioside (**28**, 55 mg).

**Hydrolysis of a Mixture of Pregnane Glycosides** A mixture (500 mg) was heated (85—90 °C) with 0.05 N $H_2SO_4$–50% dioxane (5.0 ml) for 40 min. The mixture was diluted with $H_2O$, extracted with $CHCl_3$ and then the $H_2O$ layer was deacidified with Amberlite IRA-410. The $CHCl_3$ fraction was chromatographed on a silica gel column with solvent 2 (20 : 1 : 0.1) to give ikemagenin (A-1, 20 mg) and kidjolanin (A-2, 17 mg). A-1: solid, $[\alpha]_D^{28}$ −14.7° ($c$ = 0.55, MeOH). UV $_{\max}$ nm (log $\varepsilon$): 217 (4.25), 223 (4.18), 277 (4.40). $^{1}$H-NMR $\delta$: 1.42 (3H, s, H-19), 2.02 (3H, s, H-18), 2.29 (3H, s, H-21), 3.53 (1H, t, $J$ = 9 Hz, H-17), 3.89 (1H, m, H-3), 5.05 (1H, s, –OH), 5.29 (1H, dd, $J$ = 12, 4 Hz, H-12), 5.34 (1H, br d, $J$ = 3 Hz, H-6), 5.56 (1H, s, –OH), 6.06 (1H, br s, –OH), 6.79 (1H, d, $J$ = 16 Hz, H-$\alpha$), 7.34—7.37 (3H, H-3′, 4′, 5′), 7.61—7.64 (2H, H-2′, 6′), 7.98 (1H, d, $J$ = 16 Hz, H-$\beta$). A-2: mp 150—153 °C, $[\alpha]_D^{26}$ +28.6° ($c$ = 0.21, MeOH). UV $_{\max}$ nm (log $\varepsilon$): 216 (4.32), 222 (4.25), 276 (4.46). $^{1}$H-NMR $\delta$: 1.42 (3H, s, H-19), 2.02 (3H, s, H-18), 2.51 (3H, s, H-21), 3.88 (1H, m, H-3), 5.02 (1H, s, –OH), 5.21 (1H, dd, $J$ = 12, 4 Hz, H-12), 5.35 (1H, br d, $J$ = 2 Hz, H-6), 6.14 (1H, br s, –OH), 6.45 (1H, s, –OH), 6.81 (1H, d, $J$ = 16 Hz, H-$\alpha$), 7.34—7.37 (3H, H-3′, 4′, 5′), 7.62—7.65 (2H, H-2′, 6′), 8.00 (1H, d, $J$ = 16 Hz, H-$\beta$). The $H_2O$ layer was concentrated *in vacuo* and chromatographed on a silica gel column with solvent 1 (7 : 1 : 1.2—7 : 3 : 1.5) to give D-cymarose (9 mg, $[\alpha]_D^{24}$ +55.7° ($c$ = 0.21, 24 h after dissolution in $H_2O$)), D-oleandrose (14 mg, $[\alpha]_D^{24}$ −15.6° ($c$ = 0.55, 24 h after dissolution in $H_2O$)), D-digitoxose (18 mg ($[\alpha]_D^{24}$ +48.4° ($c$ = 0.90, 24 h after dissolution in $H_2O$)), strophanthobiose (15 mg, $[\alpha]_D^{26}$ +30.9° ($c$ = 0.33, 24 h after dissolution in $H_2O$)) and cynanchobiose (8 mg, $[\alpha]_D^{28}$ +7.0° ($c$ = 0.20, 24 h after dissolution in $H_2O$)). All sugars were identified by comparison with authentic samples on TLC with solvents 1 (7 : 2 : 1), 2 (9 : 1 : 0.1) and solvent 3 (1 : 9) (RP-18 $WF_{254}$). Hydrolysis of each pregnane glycoside (5 mg each) was conducted with 0.05 N $H_2SO_4$–50% dioxane (0.25 ml) for 40 min at 90 °C followed by dilution with MeOH. After deacidification with Amberlite IRA-410, the mixture was concentrated *in vacuo* and the residue was examined by TLC.

Compound **1**: Solid, $[\alpha]_D^{26}$ +16.1° ($c$ = 0.23, MeOH). FAB-MS $m/z$: 1227.5924 (Calcd for $C_{62}H_{92}O_{23}$ + Na: 1227.5927). FAB-MS(negative) $m/z$: 1203 $[M-1]^-$, 1073 $[M-1-\text{cinnamoyl}]^-$, 1041 $[M-1-\text{glucose}]^-$. $^{1}$H- and $^{13}$C-NMR: see Tables I, II, and III. On acid hydrolysis, **1** gave A-1, D-digitoxose, D-oleandrose and strophanthobiose.

Compound **2**: Solid, $[\alpha]_D^{28}$ +9.6° ($c$ = 0.25, MeOH). FAB-MS $m/z$: 1227.5925 (Calcd for $C_{62}H_{92}O_{23}$ + Na: 1227.5927). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **2** gave A-1, D-digitoxose, D-oleandrose and cynanchobiose.

Compound **3**: Solid, $[\alpha]_D^{27}$ +20.8° ($c$ = 0.38, MeOH). FAB-MS $m/z$: 1241.6089 (Calcd for $C_{63}H_{94}O_{23}$ + Na: 1241.6084). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **3** gave A-1, D-digitoxose, D-oleandrose, D-cymarose and strophanthobiose.

Compound **4**: Solid, $[\alpha]_D^{28}$ +24.08° ($c$ = 0.23, MeOH). FAB-MS $m/z$: 1241.6077 (Calcd for $C_{63}H_{94}O_{23}$ + Na: 1241.6084). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **4** gave A-1, D-cymarose, D-oleandrose, D-digitoxose and strophanthobiose.

Compound **5**: Solid, $[\alpha]_D^{28}$ +20.0° ($c$ = 0.15, MeOH). FAB-MS $m/z$: 1255.6232 (Calcd for $C_{64}H_{96}O_{23}$ + Na: 1255.6240). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **5** gave A-1, D-cymarose, D-oleandrose and strophanthobiose.

Compound **6**: Solid, $[\alpha]_D^{27}$ +9.0° ($c$ = 0.29, MeOH). FAB-MS $m/z$: 1255.6259 (Calcd for $C_{64}H_{96}O_{23}$ + Na: 1255.6240). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **6** gave A-1, D-cymarose, D-oleandrose and cynanchobiose.

Compound **7**: Solid, $[\alpha]_D^{26}$ +24.3° ($c$ = 0.38, MeOH). FAB-MS $m/z$: 1243.5874 (Calcd for $C_{62}H_{92}O_{24}$ + Na: 1243.5876). $^{1}$H- and $^{13}$C-NMR: see Tables I, II and III. On acid hydrolysis, **7** gave A-2, D-digitoxose, D-oleandrose and strophanthobiose.

Compound **8**: Solid, $[\alpha]_D^{27}$ +26.0° ($c$ = 0.24, MeOH). FAB-MS $m/z$: 1257.6034 (Calcd for $C_{63}H_{94}O_{24}$ + Na: 1257.6033). The $^{1}$H- and $^{13}$C-NMR spectra of the aglycone and sugar moieties were in good agreement with those of **7** and **3**, respectively. On acid hydrolysis, **8** gave A-2, D-digitoxose, D-cymarose, D-oleandrose and strophanthobiose.

Compound **9**: Solid, $[\alpha]_D^{28}$ +33.5° ($c$ = 0.20, MeOH). FAB-MS $m/z$: 1257.6050 (Calcd for $C_{63}H_{94}O_{24}$ + Na: 1257.6033). The $^{1}$H- and $^{13}$C-NMR spectra of the aglycone and sugar moieties were in good agreement with those of **7** and **4**, respectively. On acid hydrolysis, **9** gave A-2, D-cymarose, D-oleandrose, D-digitoxose and strophanthobiose.

Compound **10**: Solid, $[\alpha]_D^{28}$ +25.2° ($c$ = 0.23, MeOH). FAB-MS $m/z$: 1271.6190 (Calcd for $C_{64}H_{96}O_{24}$ + Na: 1271.6189). The $^{1}$H- and $^{13}$C-NMR spectra of the aglycone and sugar moieties were in good agreement with those of **7** and **5**, respectively. On acid hydrolysis, **10** gave A-2, D-cymarose, D-oleandrose and strophanthobiose.

Compound **11**: Solid, $[\alpha]_D^{27}$ +12.7° ($c$ = 0.37, MeOH). FAB-MS $m/z$: 1271.6177 (Calcd for $C_{64}H_{96}O_{24}$ + Na: 1271.6189). The $^{1}$H- and $^{13}$C-NMR spectra of the aglycone and sugar moieties were in good agreement with those of **7** and **6**, respectively. On acid hydrolysis, **11** gave A-2, D-cymarose, D-oleandrose and cynanchobiose.

Compound **12**: Solid, $[\alpha]_D^{28}$ +32.0° ($c$ = 0.20, MeOH). FAB-MS $m/z$: 1271.6187 (Calcd for $C_{64}H_{96}O_{24}$ + Na: 1271.6189). $^{1}$H- and $^{13}$C-NMR: see Tables II and III. On acid hydrolysis, **12** gave A-2, D-cymarose, D-oleandrose and strophanthobiose.

Compound **16**: Solid, $[\alpha]_D^{23}$ −9.8° ($c$ = 1.52, MeOH). FAB-MS $m/z$: 721.3422 (Calcd for $C_{35}H_{54}O_{14}$ + Na: 721.3411). $^{1}$H- and $^{13}$C-NMR: see Tables IV and V. Compound **16** (3 mg) was dissolved in 20% EtOH–$H_2O$ (1 ml) and shaken with $\beta$-glucosidase (P-L Biochemicals Inc.) (10 mg) at 37 °C for 8 h. The mixture was diluted with $H_2O$ and extracted with BuOH. The BuOH layer was evaporated and the residue was examined by TLC. The presence of uzarigenin and uzarigenin β-D-glucoside was confirmed by comparison with authentic samples.

Compound **24**: Colorless prisms, mp 215—220 °C, $[\alpha]_D^{24}$ +57.5° ($c=0.12$, MeOH). FAB-MS $m/z$: 571.2520 (Calcd for $C_{29}H_{40}O_{10}+Na$: 571.2519). $^1$H- and $^{13}$C-NMR: see Tables IV and V.

Compound **25**: Colorless prisms, mp 248—252 °C, $[\alpha]_D^{24}$ +12.1° ($c=0.36$, MeOH). FAB-MS $m/z$: 573.2677 (Calcd for $C_{29}H_{42}O_{10}+Na$: 573.2676). $^1$H- and $^{13}$C-NMR: see Tables IV and V.

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