Cardenolide Glycosides from the Seeds of *Asclepias curassavica*[1)]

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From the seeds of *Asclepias curassavica*, two cardenolides and twelve glycosides were obtained. Among these, four compounds were determined to be 16α-hydroxycalotropagenin, 16α-hydroxycalotropin and its 3′-*O*-glucoside and 3′-*O*-gentiobioside. Normally linked triosides of corotoxigenin, coroglaucigenin and 12β-hydroxycoroglaucigenin were characterized as cellobiosyl-allomethylosides.

Keywords *Asclepias curassavica*; seed cardenolide; 12β-hydroxycoroglaucigenin; 4′-*O*-cellobiosyl-12β-hydroxyfrugoside; 4′-*O*-cellobiosyl-gofruside; doubly linked cardenolide glycoside; 16α-hydroxycalotropagenin; 3′-*O*-gentiobiosyl-16α-hydroxycalotropin

Asclepias curassavica is well-known not only as a garden plant but also as a feeding plant for larvae of *Danaus* butterflies. From the viewpoint of cardenolide chemistry, *Asclepias* contains the unique doubly linked cardenolide glycosides as well as the glycosides of *Gomphocarpus*, *Calotropis* and *Pergularia*. From the leaves, uscharin, uscharidin, calactin, calotropin and calotoxin were reported in the 1960s,[2)] and recently the biosynthesis of these cardenolides was examined.[3)] In the preceding paper, we described the isolation and structure determination of 3′-*epi*-19-norafroside and 12β-hydroxycoroglaucigenin[1)] from the stems of *Asclepias curassavica*. This paper concerns the cardenolide glycosides from the seeds.

The usual extraction of the seeds with MeOH and fractionation with normal and reversed phase column chromatographies resulted in the isolation of 14 cardenolides and their glycosides. They were classified into five groups based on the component cardenolide, corotoxigenin (**1**, **2**), coroglaucigenin (**3**—**5**), 12β-hydroxycoroglaucigenin (**6**—**9**), calotropagenin (**10**) and 16α-hydroxycalotropagenin (**11**—**14**). Among these, glucosylgofruside[4)] (**1**), frugoside (**3**)[5)] and glucosylfrugoside (**4**)[6)] have already been isolated from other genera, and 12β-hydroxycoroglaucigenin (**6**) was isolated from the stems of this plant.[1)]

Compound **2** was isolated from the polar fraction and its fast atom bombardment mass spectrum (FAB-MS) afforded a $[M+Na]^+$ peak at *m/z* 881.3784 ($C_{41}H_{62}O_{19}$+Na), suggesting it to be a cardenolide trioside. In the proton nuclear magnetic resonance (^{1}H-NMR) spectrum, one formyl proton signal was observed at δ 10.01, along with signals due to the cardenolide framework, such as methylene protons at C-21, an olefinic proton at C-22 and a methine proton at C-17. One carbonyl carbon signal due to the formyl group was detected at δ 208.7 in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum. The aglycone was assignable as corotoxigenin by comparison of the NMR signals with those of **1**.

Three anomeric protons were observed at δ 4.98, 5.19 and 5.33, each as an 8 Hz doublet signal, suggesting the three sugars to retain β-linkages and possibly to be D-sugars. In comparison with **1**, the presence of a 4-*O*-β-D-glucosyl-6-deoxy-β-D-allopyranosyl (4-*O*-β-D-glucosyl-β-

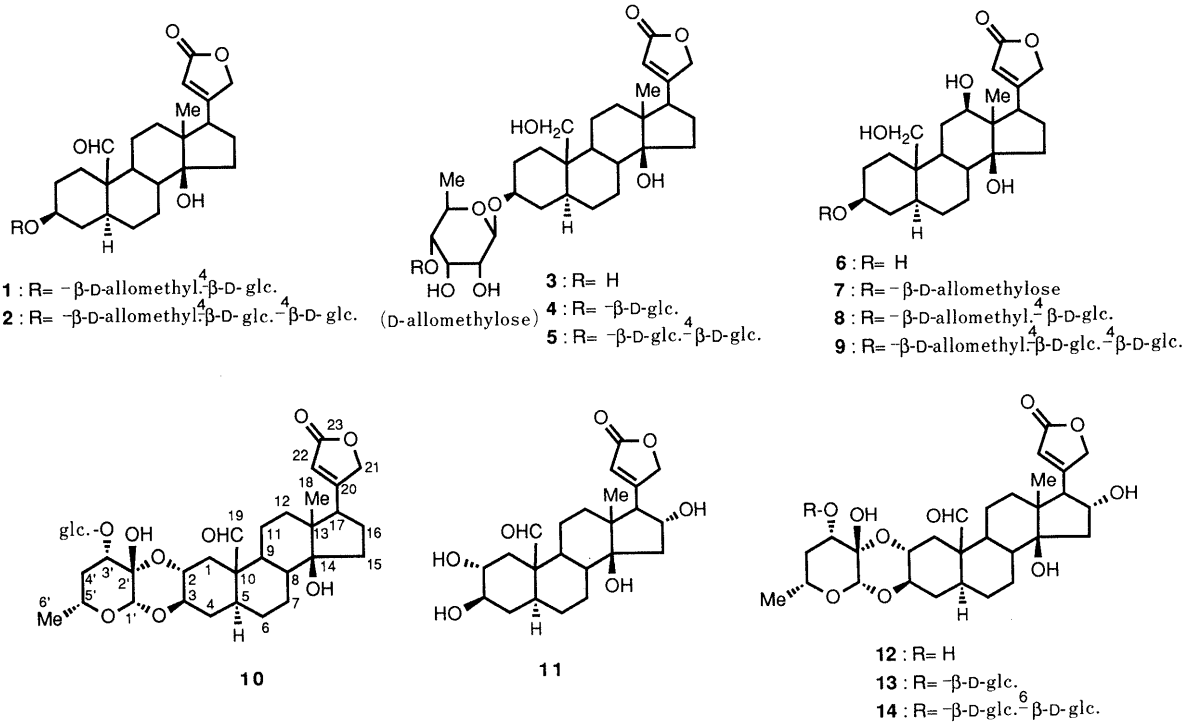

Chart 1

TABLE I. ^{1}H Chemical Shifts of **2**, **5**, **7**—**13** and **14**, δ (ppm) from Tetramethylsilane (TMS) in Pyridine-d_5 (J (Hz) in Parentheses)

H	2	5	7	8	9	10	11	12	13	14
22	6.10 br s	6.10 br s	6.23 br s	6.23 br s	6.23 br s	6.11 br s	6.22 br s	6.22 br s	6.24 br s	6.24 br s
21	4.99 dd (18, 1)	5.01 dd (18, 1)	5.09 dd (18, 2)	5.09 dd (18, 1)	5.09 dd (18, 1)	5.00 dd (18, 1)	5.03 dd (18, 2)	5.03 dd (18, 1)	5.03 dd (18, 1)	5.03 dd (18, 2)
	5.25 dd (18, 1)	5.28 dd (18, 1)	5.24 dd (18, 1)	5.24 dd (18, 2)	5.24 dd (18, 1)	5.25 dd (18, 1)	5.17 dd (18, 1)	5.17 dd (18, 2)	5.18 dd (18, 1)	5.18 dd (18, 2)
19	10.01 s	3.89 d (11)	3.95 d (11)	3.94 d (12)	3.94 d (11)	9.98 s	10.19 s	10.00 s	9.98 s	9.96 s
		4.06 d (11)	4.10 d (11)	4.09 d (12)	4.09 d (11)					
18	0.91 s	1.04 s	1.26 s	1.25 s	1.25 s	0.91 s	0.97 s	0.95 s	0.95 s	0.95 s
17	2.74 dd (9, 4)	2.77 dd (9, 5)	3.75 dd (8, 7)	3.74 dd (9, 7)	3.74 dd (8, 7)	2.74 dd (8, 5)	3.01 d (4)	3.01 d (4)	3.01 d (4)	3.01 d (4)
16β							5.04 td (8, 4)	5.05 td (8, 4)	5.05 td (8, 4)	5.04 td (8, 4)
12α			3.62 dd (11, 5)	3.61 dd (11, 5)	3.61 dd (11, 5)					
3α	3.95 m	4.00 m	4.06 m	4.01 m	3.99 m	4.31 td (10, 4)	3.90 ddd (12, 9, 4)	4.33 td (10, 4)	4.32 td (10, 4)	
2β						4.43 ddd (12, 10, 4)	4.01 ddd (12, 9, 4)	4.45 ddd (12, 10, 4)	4.43 ddd (12, 10, 4)	
1β	2.39 dt (14, 3)	2.62 dt (12, 3)	2.68 dt (13, 3)	2.67 dt (13, 3)	2.67 dt (13, 3)	2.51 dd (12, 4)	2.96 dd (12, 5)			
1α	0.94 td (14, 3)	0.84 td (12, 3)	0.87 td (13, 3)	0.85 td (13, 3)	0.85 td (13, 3)	1.15 t (12)	1.34 t (12)	1.15 t (12)	1.14 t (12)	1.14 t (12)
1′	5.33 d (8)	5.39 d (8)	5.42 d (8)	5.38 d (8)	5.39 d (7)	4.97 s		5.01 s	4.97 s	4.93 s
2′	3.86 dd (8, 3)	3.89 dd (8, 3)	3.92 dd (8, 3)	3.89 dd (8, 3)	3.89 dd (8, 3)					
3′	4.97 t (3)	4.98 t (3)	4.67 t (3)	5.02 t (3)	4.97 t (3)	4.16 dd (12, 5)		4.11 dd (12, 5)	4.17 dd (12, 5)	
4′	3.75 dd (9, 3)	3.76 dd (9, 3)	3.68 dd (9, 3)	3.80 dd (9, 3)	3.75 dd (9, 3)	2.14 ddd (12, 5, 2)		2.04 ddd (12, 5, 2)	2.16 ddd (12, 5, 2)	
						2.22 q (12)		2.13 q (12)	2.24 q (12)	2.27 q (12)
5′			4.36 m	4.48 m		3.69 m		3.76 m	3.70 m	3.78 m
6′	1.69 d (6)	1.69 d (6)	1.62 d (6)	1.69 d (6)	1.69 d (6)	1.30 d (6)		1.38 d (6)	1.32 d (6)	1.38 d (6)
1″	4.98 d (8)	4.98 d (8)		5.02 d (8)	4.98 d (8)	5.21 d (8)			5.22 d (8)	5.16 d (8)
1‴	5.19 d (8)	5.19 d (8)			5.20 d (8)					5.09 d (8)
Others				4.30 dd (12, 5 H-6″a)		4.09 t (9) (H-2″)			4.10 t (9) (H-2″)	4.49 dd (12, 3 H-6‴b)
				4.41 dd (12, 3 H-6″b)		4.16 t (9)			4.38 dd (12, 5 H-6″a)	4.84 dd (11, 1 H-6″b)
						4.21 t (9) (H-3″, 4″)			4.22 t (9) (H-3″)	
						4.01 m (H-5″)			4.56 dd (12, 2 H-6″b)	
						4.37 dd (12, 5 H-6″a)				
						4.55 dd (12, 2 H-6″b)				

D-allomethylosyl) moiety was also confirmed in **2**, and **2** was considered to be glucobiosyl-β-D-allomethyloside. In the glucobiose moiety, two sets of H-6a, b and C-6 signals were observed with almost the same chemical shifts, and one of the C-4 signals showed a glycosylation shift (+9.4 ppm) in the ^{1}H- and ^{13}C-NMR spectra. Therefore, **2** was characterized as 4′-*O*-β-cellobiosyl-gofruside.

The ^{1}H-NMR spectrum of **5** showed no formyl proton signal and the molecular formula was suggested to be $C_{41}H_{64}O_{19}$, 2H larger than **2**, based on a $[M+Na]^+$ signal at m/z 883.3937. Since methylene proton signals due to a primary carbinol were observed at δ 3.89 and 4.06 (each d, $J=11$ Hz) as in **3** and **4**, and signals due to a sugar moiety afforded a similar pattern to those of **2** in the ^{1}H- and ^{13}C-NMR spectra, **5** was assigned as coroglaucigenin-3-*O*-β-cellobiosyl(1→4)-β-D-allomethyloside (4′-*O*-β-cellobiosyl-frugoside).

The aglycones of **7**, **8** and **9** was assigned as 12β-hydroxycoroglaucigenin by comparison of the ^{1}H- and ^{13}C-NMR signals with those of **6**. These compounds were considered to be a monoside, bioside and trioside, based on $[M+Na]^+$ peaks at m/z 575.2831, 737.3361 and 899.3888, respectively. Since the sugar moieties were identical with those of **3**, **4** and **5**, respectively, the structures were determined to be 12β-hydroxycoroglaucigenin-3-*O*-β-D-allomethyloside (**7**), β-D-glucopyranosyl(1→4)-β-D-allomethyloside (**8**) and β-cellobiosyl(1→4)-β-D-allomethyloside (**9**).

The molecular formula of **10** was suggested to be $C_{35}H_{50}O_{14}$ based on FAB-MS. One formyl proton was observed at δ 9.98 as a singlet signal, suggesting **10** to be a 19-formyl cardenolide. Unlike **1**—**9**, one of the anomeric protons appeared as a singlet signal at δ 4.97, so that **10** was considered to be a doubly linked glycoside. The presence of a glucose residue was confirmed, along with the calotropagenin moiety,[1] by the corresponding signals in the ^{1}H- and ^{13}C-NMR spectra. A proton signal assignable to H-3′ was observed at δ 4.16 (dd, $J=12$, 5 Hz), and its coupling mode to the C-4′ methylene protons showed that H-3′ retained β(axial)-orientation. The NMR assignments indicated **10** to be composed of one mole each of calotropin and glucose. Since C-3′ was shifted to the lower field (+10.8 ppm) in comparison with that of calotropin,[1] glucose was linked to the 3′-hydroxyl group. Compound **10** was therefore determined to be 3′-*O*-β-D-glucopyranosyl-calotropin.

Compound **11** was considered to be a free cardenolide, having one formyl and four hydroxyl groups including 14β-hydroxyl, based on its molecular formula, $C_{23}H_{32}O_7$, and the NMR signals. Since the C-5 signal at lower field was consistent with a 5α-structure, signals at δ 3.90 (ddd, $J=12$, 9, 4 Hz) and 4.01 (ddd, $J=12$, 9, 4 Hz) were assignable to H-3α and H-2β of the 5α-cardenolide as in **10**. Another secondary hydroxyl group seemed to be located at C-16, based on the coupling mode of H-17, although the signal of H-16 duplicated that of H-21.

By comparison of the ^{1}H- and ^{13}C-NMR spectra with those of **11** and **10**, **12** was assignable as a doubly linked monoside composed of **11** and the deoxysugar moiety of **10**. In order to examine the coupling pattern of H-16 in **11** and **12**, **12** was subjected to acetylation to afford a diacetate (**12a**) and a triacetate (**12b**), in which downfield shifts of H-16 and C-16, and an upfield shift of a C-17 were

TABLE II. ^{13}C Chemical Shifts of **2**, **5**, **7**—**13** and **14**, δ (ppm) from TMS in Pyridine-d_5

C	2	5[a]	7	8[a]	9	10	11[a]	12[a]	13[a]	14
1	31.5	32.5	32.4	32.4	32.4	36.6	40.4	36.4	36.6	36.5
2	31.2	30.7	30.6	30.6	30.6	69.2	73.1	69.3	69.2	69.2
3	76.7	77.6	77.4	77.5	77.5	72.4	75.9	72.3	72.3	72.3
4	36.3	35.4	35.4	35.4	35.4	32.5	37.9	33.9	33.8	33.8
5	43.0	44.8	44.9	45.0	44.9	43.5	43.3	43.4	43.4	43.4
6	28.7[b]	28.7[b]	28.7[b]	28.7[b]	28.7[b]	27.9	28.2[b]	28.0[b]	28.2[b]	28.0[b]
7	27.9[b]	28.1[b]	28.4[b]	28.4[b]	28.4[b]	27.9	28.0[b]	27.9[b]	27.9[b]	27.9[b]
8	43.0	42.3	42.0	42.0	42.0	42.6	42.7	42.6	42.6	42.6
9	48.6	50.7	47.5	47.5	47.5	48.7	48.8	48.7	48.7	48.7
10	51.7	39.8	39.7	39.7	39.7	52.9	53.0	52.9	52.9	52.9
11	22.1	23.3	32.5	32.5	32.5	22.2	22.6	22.4	22.4	22.4
12	39.3	40.5	75.0	75.0	75.0	39.2	40.2	40.1	40.1	40.1
13	49.8	50.2	56.8	56.8	56.8	49.7	49.2	49.2	49.2	49.2
14	84.1	84.8	85.4	85.4	85.4	84.1	84.4	84.4	84.4	84.4
15	32.5	33.0	33.4	33.4	33.4	33.8	42.3	42.4	42.4	42.4
16	27.1	27.3	27.8	27.9	27.8	27.1	76.5	76.4	76.5	76.5
17	51.2	51.5	46.6	46.6	46.6	51.1	62.2	62.2	62.2	62.2
18	15.9	16.3	10.3	10.3	10.3	15.8	16.1	16.0	16.0	16.0
19	208.7	59.1	58.9	58.9	58.9	207.9	208.6	207.8	207.9	207.9
20	175.6	176.1	176.7	176.8	176.7	175.5	174.3	174.1	174.2	174.2
21	73.7	73.7	74.0	74.1	74.0	73.6	74.3	74.2	74.3	74.3
22	117.7	117.5	117.3	117.3	117.3	117.8	118.0	118.0	118.0	118.0
23	174.4	174.5	174.7	174.8	174.7	174.4	174.3	174.2	174.3	174.3
1′	99.5	99.4	99.6	99.5	99.5	96.6		97.2	96.6	96.6
2′	72.0	72.1	72.5	72.1	72.1	92.7		92.7	92.7	92.7
3′	72.3	72.4	72.9	72.4	72.4	84.6		73.8	84.5	84.2
4′	83.6	83.7	74.4	83.6	83.6	38.3		39.9	38.3	38.3
5′	68.7	68.7	70.3	68.8	68.7	68.3		68.5	68.3	68.5
6′	18.4	18.4	18.8	18.5	18.4	21.3		21.5	21.3	21.4
1″	105.9	105.9		106.2	105.9	107.8			107.8	107.5
2″	74.7	74.7		75.2	74.7	75.8			75.8	75.2[c]
3″	76.5	76.5		78.3	76.5	78.9			78.9	78.4
4″	81.0	81.0		71.6	81.0	71.5			71.5	71.5[d]
5″	76.3	76.2		78.1	76.2	78.5			78.5	77.5
6″	61.9	61.9		62.6	61.9	62.7			62.7	70.2
1‴	104.9	104.9			104.9					105.4
2‴	74.7	74.7			74.7					75.6[c]
3‴	78.4	78.4			78.4					78.4
4‴	71.5	71.5			71.5					71.6[d]
5‴	78.3	78.2			78.2					78.3
6‴	62.4	62.4			62.4					62.7

a) Signal assignments were based on two dimensional (2D) ^{13}C–^{1}H correlation spectroscopy (COSY) spectra. *b*—*d*) Signal assignments may be interchangeable in each column.

observed. The signal due to H-17 in **12a** was observed at δ 2.93 (d, 4 Hz), showing a remarkable difference from that of 16β-*O*-acetylated cardenolide (*e.g.* oleandrigenin) at δ 3.37 (d, 9 Hz).[7,8] The structures of **11** and **12** were therefore characterized as 2α,3β,14,16α-tetrahydroxy-19-oxo-5α,14β-cardenolide (16α-hydroxycalotropagenin) and 16α-hydroxycalotropin, respectively.

Based on the ^{1}H- and ^{13}C-NMR spectra and $[M+Na]^+$ peaks, **13** and **14** were considered to be a bioside and a trioside of **11**, respectively. The sugar moiety of **13** was in good agreement with that of **10**. In **14**, the presence of a gentiobiosyl unit was confirmed by comparison with other triosides having a gentiobiosyl moiety.[7] Compounds **13** and **14** were assigned as the 3′-*O*-β-D-glucopyranoside and 3′-*O*-β-gentiobioside of 16α-hydroxycalotropin, respectively.

Only syrioside[9] is known as a doubly linked glycoside with one glucose unit at the 3′-hydroxyl group, and this is the first report of a doubly linked glycoside having the gentiobiosyl residue at 3′-OH. It should be noted that cardenolide glycosides having a cellobiosyl group are not so common as those with a gentiobiosyl group. In the seeds of *Asclepias curassavica*, the normally linked glycosides, **2**, **5** and **9**, bear a cellobiosyl moiety, while gentiobiose is attached to a doubly linked glycoside. Whereas several 16α-hydroxylated and acetoxylated cardenolides were reported from *Asclepias vestita*[8] and *A. subulata*,[10] no 16α-hydroxycalotropin was found. The seeds show a different distribution of cardenolides from those of the leaves and stems, since neither 19-norafroside and its homologous glycosides nor asclepin, calactin, calotoxin,[1,2] uscharidin, uscharin and voruscharin[2,3] were obtained from the seeds.

Experimental

The melting points were taken on a hot stage apparatus and are recorded uncorrected. ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d_5. Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br s = broad singlet, dd = doublet of doublets. FAB-MS was recorded on a JEOL D-300-FD spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. For silica gel column

chromatography and thin layer chromatography (TLC), the following solvent systems were applied: $CHCl_3$–MeOH–H_2O (bottom layer, solvent 1), EtOAc–MeOH–H_2O (top layer, solvent 2). 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.

Extraction and Isolation of Cardenolide Glycosides The seeds were collected from *Asclepias curassavica* L. cultivated in the medicinal plant garden of Fukuoka University. The air-dried seeds (630 g) were ground and eluted with hexane (ext. 120.1 g), MeOH (33.3 g) and then MeOH–H_2O (1 : 1, 43.0 g) successively. The MeOH extract was chromatographed on a silica gel column with solvent 1 (7 : 1 : 1—7 : 3 : 1). The MeOH–H_2O extract was passed through a polystyrene-gel column (Mitsubishi Chemical Co. GHP-20) and the column was eluted with H_2O, 25, 50, 80 and 100% MeOH. The 80% and 100%MeOH eluates were combined and chromatographed on a silica gel column with solvent 1 (7 : 1 : 1—7 : 3 : 0.6). Fractions containig the same cardenolides from the MeOH and MeOH–H_2O (1 : 1) extracts were combined. Each fraction was chromatographed on a silica gel column with solvent 1 or 2 (4 : 1 : 5—4 : 1 : 3). A reversed phase column (YMC-gel) with MeCN/H_2O was employed when further separation was required. The following known cardenolides and glycosides were isolated along with new compounds **2**, **5**, **7**—**14**; 4′-*O*-*β*-D-glucopyranosyl-gofruside (**1**, 213 mg), frugoside (**3**, 732 mg), 4′-*O*-*β*-D-glucopyranosyl-frugoside (**4**, 1.06 g), 12*β*-hydroxycoroglaucigenin (**6**, 15 mg).

4′-*O*-*β*-Cellobiosyl-gofruside (2) Fine prisms from MeOH, mp 234—239°C (20 mg), $[\alpha]_D^{26}$ −2.7° (c=1.6, MeOH). FAB-MS m/z: 881.3784 (Calcd for $C_{41}H_{62}O_{19}$+Na: 881.3783).

4′-*O*-*β*-Cellobiosyl-frugoside (5) Fine prisms from MeOH, mp 272—283°C (dec.) (197 mg), $[\alpha]_D^{30}$ −6.8° (c=2.0, MeOH). FAB-MS m/z: 883.3937 (Calcd for $C_{41}H_{64}O_{19}$+Na: 883.3940).

12*β*-Hydroxyfrugoside (7) A solid (18 mg), $[\alpha]_D^{29}$ +4.9° (c=0.9, MeOH). FAB-MS m/z: 575.2831 (Calcd for $C_{29}H_{44}O_{10}$+Na: 575.2831).

4′-*O*-*β*-D-Glucopyranosyl-12*β*-hydroxyfrugoside (8) A solid (40 mg), $[\alpha]_D^{30}$ −11.8° (c=2.4, MeOH). FAB-MS m/z: 737.3361 (Calcd for $C_{35}H_{54}O_{15}$+Na: 737.3361).

4′-*O*-*β*-Cellobiosyl-12*β*-hydroxyfrugoside (9) A solid (21 mg), $[\alpha]_D^{26}$ −18.1° (c=1.0, MeOH). FAB-MS m/z: 899.3888 (Calcd for $C_{41}H_{64}O_{20}$+Na: 899.3889).

3′-*O*-*β*-D-Glucopyranosyl-calotropin (10) A solid (93 mg), $[\alpha]_D^{30}$ +37.0° (c=2.8, MeOH). FAB-MS m/z: 717.3097 (Calcd for $C_{35}H_{50}O_{14}$+Na: 717.3098).

16*α*-Hydroxycalotropagenin (11) Prisms from MeOH, mp 173—178°C (20 mg), $[\alpha]_D^{30}$ +3.8° (c=1.0, MeOH). FAB-MS m/z: 443.2044 (Calcd for $C_{23}H_{32}O_7$+Na: 443.2045).

16*α*-Hydroxycalotropin (12) Prisms from MeOH, mp 244—256°C (dec.) (55 mg), $[\alpha]_D^{30}$ +29.9° (c=1.5, MeOH). FAB-MS m/z: 571.2517 (Calcd for $C_{29}H_{40}O_{10}$+Na: 571.2519). **12** (50 mg) was acetylated with Ac_2O and pyridine at room temperature for 20 h to afford a diacetate (**12a**, 16 mg) and a triacetate (**12b**, 29 mg).

12a: A solid, FAB-MS m/z: 655.2729 (Calcd for $C_{33}H_{44}O_{12}$+Na: 655.2730). ^{1}H-NMR δ: 0.93 (3H, s, H-18), 1.36 (3H, d, J=6 Hz, H-6′), 1.91, 2.09 (3H each, s, –OAc), 2.32, 2.54 (1H each, dd, J=14, 8 Hz, H-15), 2.93 (1H, d, J=4 Hz, H-17), 3.79 (1H, m, H-5′), 4.36, 4.43 (1H each, td, J=11, 4 Hz, H-2, 3), 5.03 (1H, s, H-1′), 5.14, 5.26 (1H each, dd, J=18, 1 Hz, H-21), 5.42 (1H, dd, J=11, 5 Hz, H-3′), 5.64 (1H, td, J=8, 4 Hz, H-16), 6.29 (1H, br s, H-22), 9.98 (1H, s, H-19). ^{13}C-NMR δ: 15.8 (C-18), 20.9 (×2), 21.2 (C-6′, 2×–OAc), 22.3 (C-11), 27.7, 27.9 (C-6, 7), 33.8 (C-4), 36.2, 36.6 (C-1, 4′), 39.1, 39.6 (C-12, 15), 42.3, 43.4 (C-5, 8), 48.5 (C-9), 49.3 (C-13), 52.9, (C-10), 58.2 (C-17), 68.0 (C-5′), 69.3 (C-2), 72.2 (C-3), 74.3 (C-21), 75.2 (C-3′), 79.1 (C-16), 83.9 (C-14), 91.7 (C-2′), 96.9 (C-1′), 118.4 (C-22), 170.7, 170.6 (–OAc), 172.5 (C-20), 174.1 (C-23), 207.6 (C-19).

12b: A solid, FAB-MS m/z: 675.3018 (Calcd for $C_{35}H_{46}O_{13}$+H: 675.3016). ^{1}H-NMR δ: 0.95 (3H, s, H-18), 1.32 (3H, d, J=6 Hz, H-6′), 1.97, 2.09, 2.13 (3H each, s, –OAc), 2.33, 2.56 (1H each, dd, J=14, 8 Hz, H-15), 2.94 (1H, d, J=4 Hz, H-17), 2.80 (1H, m, H-5′), 4.34, 4.39 (1H each, td, J=11, 4 Hz, H-2,3), 5.14, 5.26 (1H each, dd, J=18, 1 Hz, H-21), 5.65 (1H, td, J=8, 4 Hz, H-16), 6.00 (1H, s, H-1′), 6.22 (1H, dd, J=12, 5 Hz, H-3′), 6.29 (1H, br s, H-22), 10.13 (1H, s, H-19).

3′-*O*-*β*-D-Glucopyranosyl-16*α*-hydroxycalotropin (13) A solid (24 mg), $[\alpha]_D^{29}$ +7.9° (c=1.65, MeOH). FAB-MS m/z: 733.3052 (Calcd for $C_{35}H_{50}O_{15}$+Na: 733.3047).

3′-*O*-*β*-Gentiobiosyl-16*α*-hydroxycalotropin (14) A solid (23 mg), $[\alpha]_D^{26}$ −5.1° (c=1.25, MeOH). FAB-MS m/z: 895.3574 (Calcd for $C_{41}H_{60}O_{20}$+Na: 895.3575).

Acknowledgement We thank Ms. Y. Iwase and J. Honda, and Mr. H. Hanazono of Fukuoka University for NMR and MS measurements. This work was supported in part by a grant from the Central Research Institute of Fukuoka University.

References

1) F. Abe, Y. Mōri and T. Yamauchi, *Chem. Pharm. Bull.*, **39**, 2709 (1991).
2) J. v. Euw, L. Fisherson, J. A. Parsons, T. Reichstein and M. Rothschild, *Nature* (London), **214**, 35 (1967); B. Singh and R. P. Rastogi, *Ind. J. Chem.*, **7**, 1105 (1969); *idem*, *Phytochemistry*, **11**, 757 (1972).
3) H. W. Groeneveld, A. Binnekamp and D. Seykens, *Phytochemistry*, **30**, 2577 (1991).
4) M. Keller and T. Reichstein, *Helv. Chim. Acta*, **32**, 1607 (1949); A. Hunger and T. Reichstein, *ibid.*, **35**, 1073 (1952).
5) L. Sawlewicz, E. Weiss and T. Reichstein, *Helv. Chim. Acta*, **50**, 504 (1967); K. Jaggi, H. Kaufmann, W. Stocklin and T. Reichstein, *ibid.*, **50**, 3457 (1967).
6) J. Petricic, *Arch. Pharm.*, **299**, 1007 (1966).
7) T. Yamauchi and F. Abe, *Chem. Pharm. Bull.*, **38**, 669 (1990).
8) H. T. A. Cheung and C. J. Nelson, *J. Chem. Soc., Perkin Trans. 1*, **1989**, 1563; H. T. A. Cheung, C. J. Nelson and T. R. Watson, *J. Chem. Res. (M)*, **1989**, 0145.
9) P. Brown, J. v. Euw, T. Reichstein, K. Stocken and T. R. Watson, *Helv. Chim. Acta*, **62**, 412 (1979).
10) S. D. Jolad, R. B. Bates, J. R. Cole, J. J. Hoffmann, T. J. Siahaan and B. N. Timmermann, *Phytochemistry*, **25**, 2581 (1986).