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 corotoxinogen, coroglaucigenin and 12β-hydroxyxcoroglaucigenin were characterized as cellobiosyl-allomethylglucoside.

**Keywords** *Asclepias curassavica*, seed cardenolide; 12β-hydroxyxcoroglaucigenin; 4'-O-cellobiosyl-12β-hydroxyfrugoside; 4'-O-cellobiosyl-glfruside; doubly linked cardenolide glycoside; 16α-hydroxycalotropagenin; 3'-O-gentiobiosyl-16α-hydroxy-calotropin

*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, uscharadin, calactin, calotropin and calotoxin were reported in the 1960s, and recently the biosynthesis of these cardenolides was examined. In the preceding paper, we described the isolation and structure determination of 3'-epi-19-norafroside and 12β-hydroxycalotropagenin\(^{1)}\) 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, corotoxinogen (1, 2), coroglaucigenin (3—5), 12β-hydroxyxcoroglaucigenin (6—9), calotropagenin (10) and 16α-hydroxycalotropagenin (11—14). Among these, glucosylalfruside\(^{4)}\) (1), frugoside (3)\(^{5)}\) and glucosylfrugoside (4)\(^{6)}\) have already been isolated from other genera, and 12β-hydroxyxcoroglaucigenin (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 881.784 (C\(_{42}\)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 \(\delta\) 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 \(\delta\) 208.7 in the carbon-13 nuclear magnetic resonance (\(^13\)C-NMR) spectrum. The aglycone was assignable as corotoxinogen by comparison of the NMR signals with those of 1.

Three anomic protons were observed at \(\delta\) 4.98, 5.19 and 5.33, each as an 8H 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-
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D-allomethoxyislyl) moiety was also confirmed in 2, and 2 was considered to be glucobiosyl-β-d-allomethylosyl. 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 1H- and 13C-NMR spectra. Therefore, 2 was characterized as 4β-glucobiosyl-β-glucofructose.

The 1H-NMR spectrum of 5 showed no formyl proton signal and the molecular formula was suggested to be C₄₁H₆₆O₁₉, 2H larger than 2, based on a [M + Na]⁺ signal at m/z 883.3937. Since methylene proton signals due to a primary carbonyl 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 1H- and 13C-NMR spectra, 5 was assigned as coroglucaigenin-3-O-β-cellubiosyl(1→4)-α-d-allomethylosyl (4β-glucobiosyl-frugoside).

The aglycones of 7, 8 and 9 was assigned as 12β-hydroxy-coroglucaigenin by comparison of the 1H- and 13C-NMR signals with those of 6. These compounds were considered to be a monoside, bioside and triside, 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-hydroxy-coroglucaigenin-3-O-β-glucopyranosyl(1→4)-β-d-allomethylosyl (7), 8β-glucopyranosyl(1→4)-β-d-allomethylosyl (8) and β-cellubiosyl(1→4)-β-d-allomethylosyl (9).

The molecular formula of 10 was suggested to be C₃₄H₅₅O₁₄ 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,[16] by the corresponding signals in the 1H- and 13C-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,[16] 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, C₃₄H₅₃O₁₃, and the NMR signals. Since the C-5 signal at lower field was consistent with a 5x-structure, signals at δ 3.90 (dd, J = 12, 9, 4 Hz) and 4.01 (dd, J = 12, 9, 4 Hz) were assignable to H-3x and H-2β of the 5x-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 1H- and 13C-NMR spectra with those of 11 and 12, 12 was assignable as a doubly linked monoside composed of 11 and the deoxyxugar 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
obtained. 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. oleanadrign) at δ 3.37 (d, 9 Hz). The structures of 11 and 12 were therefore characterized as 2xa,3β,14,16x-tetrahydroxy-19-oxo-5α,14β-cardenolide (16x-hydroxycalotropigen) and 16x-hydroxyxalotropin, respectively.

Based on the 1H- and 13C-NMR spectra and [M + Na]+ peaks, 13 and 14 were considered to be a bioside and a triolide 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 triolides having a gentiobiosyl moiety. Compositions 13 and 14 were assigned as the 3′-O-β-D-glucopyranoside and 3′-O-β-D-gentiobioside of 16x-hydroxyxalotropin, respectively.

Only syroside9) 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 cellulosic 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 cellosbyl moiety, while gentiobiose is attached to a doubly linked glycoside. Whereas several 16x-hydroxylated and acetylated cardenolides were reported from Asclepias vestita8) and A. subalata,10) no 16x-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, calactoxin, uscaril, uscharin, and voruscarin1,2) were obtained from the seeds.

**Experimental**

The melting points were taken on a hot stage apparatus and are recorded uncorrected. 1H- and 13C-NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine-d5. 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 = 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: CHCl₃-MeOH-H₂O (bottom layer, solvent 1), CHCl₃-MeOH-H₂O (top layer, solvent 2). Cardenolides on TLC plates were visualized by spraying Kodde’s reagent (1:1 mixture of 5% 3,5-dinitrobenzoic acid in MeOH and 2% NaOH) or 10% H₂SO₄.

Extraction and Isolation of Cardenolide Glycosides The seeds were collected from Asclepias curassavica L. cultivated in the medicinal plant garden of Fukuo University. The air-dried seeds (630 g) were ground and eluted with hexane (2.120.1g), MeOH (33.3 g) and then MeOH-H₂O (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-H₂O extract was passed through a polystryene-gel column (Mitsubishi Chemical Co. GHP-20) and the column was eluted with H₂O, 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 containing the same cardenolides from the MeOH and MeOH-H₂O (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/H₂O 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-glycoside (1, 213 mg), frugoside (3, 732 mg), 4'-O-β-D-glucopyranosyl-frugoside (4, 1.06 g), 12β-hydroxyxycycloglaucigenin (6, 15 mg).

4'-O-β-D-Cellobiosyl-glycoside (2) Fine prisms from MeOH, mp 234–235°C (dec.) [α]D° + 27.6° (c = 1, MeOH). FAB-MS m/z: 881.3784 (Calcd for C₄₆H₇₅O₂₃ + Na⁺: 881.3783).

4'-O-β-D-Cellobiosyl-frugoside (5) Fine prisms from MeOH, mp 272–283°C (dec.) (197 mg), [α]D° − 6.8° (c = 2.0, MeOH). FAB-MS m/z: 883.3997 (Calcd for C₄₆H₇₅O₂₃ + Na⁺: 883.3940).

12β-Hydroxyfrugoside (7) A solid (18 mg), [α]D° + 4.9° (c = 0.9, MeOH). FAB-MS m/z: 575.2831 (Calcd for C₃₂H₅₁O₁₅ + Na⁺: 575.2831).

4'-O-β-D-Glucopyranosyl-12β-hydroxyfrugoside (8) A solid (40 mg), [α]D° − 11.8° (c = 2.4, MeOH). FAB-MS m/z: 737.3361 (Calcd for C₅₄H₈₅O₃₁ + Na⁺: 737.3361).

4'-O-β-D-Cellobiosyl-12β-hydroxyfrugoside (9) A solid (21 mg), [α]D° − 18.1° (c = 1.0, MeOH). FAB-MS m/z: 899.3888 (Calcd for C₅₄H₇₅O₂₅ + Na⁺: 899.3889).

3'-O-β-D-Glucopyranosyl-calotropin (10) A solid (93 mg), [α]D° + 37.0° (c = 2.8, MeOH). FAB-MS m/z: 717.3097 (Calcd for C₃₀H₄₉O₁₄ + Na⁺: 717.3098).

16α-Hydroxyfrugotropogenin (11) Prisms from MeOH, mp 173–178°C (20 mg), [α]D° + 3.8° (c = 1.0, MeOH). FAB-MS m/z: 443.2044 (Calcd for C₂₉H₄₃O₁₃ + Na⁺: 443.2045).

16α-Hydroxyfrugotropogenin (12) Prisma from MeOH, mp 244–256°C (dec.) (55 mg), [α]D° + 29.9° (c = 1.5, MeOH). FAB-MS m/z: 571.2517 (Calcd for C₃₂H₅₃O₁₇ + Na⁺: 571.2519). 12 (50 mg) was acetylated with Ac₂O and pyridine at room temperature for 20 h to afford a diacetate (12a, 16 mg) and a triacetate (12b, 29 mg).


References