An Androstane Bioside and 3'-Thiazolidinone Derivatives of Doubly-linked Cardenolide Glycosides from the Roots of *Asclepias tuberosa*

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Steroidal compounds in the roots of *Asclepias tuberosa* were investigated and 17 α -hydroxyandrosta-4,6,15-trien-3-one 17-*O*- α -L-arabinopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranoside, termed ascandroside, was isolated from the CHCl₃-soluble fraction. Among five doubly-linked cardenolide glycosides, two were identified as 3'-spiro-linked thiazolidinone (4) and *S*-oxythiazolidinone derivatives (5) of Δ^5 -calotropin. The stereochemistry at the C-3' in these two cardenolides is discussed. 3'-*O*- β -D-glucopyranosyl- Δ^5 -calotropin (3) was also isolated along with Δ^5 -calotropin and its 3'-acetate. Nine glycosides of uzarigenin, coroglaucigenin and corotoxigenin were identified.

Key words *Asclepias tuberosa*; androstane bioside; thiazolidinone-linked Δ^5 -calotropin; pleurisy root

While *Asclepias* is a genus known to contain cardenolides¹⁾ and the isolation of a large amount of glucofrugoside from the roots of *Asclepias tuberosa* L. has been described,²⁾ the roots have been called "pleurisy root" and used to treat pleurisy, bronchitis and other respiratory infections in the U.S.A. As part of a series of studies on the constituents of the Asclepiadaceae, we examined cardenolide glycosides from this plant material, in order to identify the type and the amount of cardiac glycosides present and investigate the biological effects of these substances on pleurisy. This paper deals with the isolation of an androstane glycoside and cardiac glycosides, including three unknown doubly-linked glycosides, from the roots of *A. tuberosa*.

An androstane glycoside termed ascandroside (1), showed a red spot on TLC following spraying with 10% H₂SO₄ and heating. The molecular formula of 1 was suggested to be C₃₀H₄₂O₁₁, based on high-resolution (HR)-FAB-MS. From the NMR spectra, 1 was suggested to be a bioside, based on two anomeric proton and carbon signals at δ 4.98 ($J=7$ Hz), 4.93 ($J=8$ Hz), and δ 105.3, 104.8, respectively. Upon acid hydrolysis, 1 afforded two sugars which were confirmed to be D-glucose and L-arabinose by GC of the thiazolidine derivatives,³⁾ although the isolation of the aglycone was unsuccessful. Since two 3H singlet signals were observed at δ 0.98 and

δ 1.16, as is usual for steroidal compounds, 1 was considered to be a bioside of a C-19-steroid.

The presence of a 4,6-dien-3-one system in 1 was suggested by the conjugated carbonyl carbon signal at δ 198.3 and the three olefinic proton signals at δ 5.86 (s), 6.12, 6.18 (each dd, $J=10$, 2 Hz) as well as the UV absorption at 283 nm. At a lower field, two disubstituted olefinic protons were observed at δ 6.06 (H-16, dt, $J=6$, 1 Hz) and 6.23 (H-15, ddd, $J=6$, 2, 1 Hz), which were coupled to the proton signal (H-17, δ 4.77, br s) attached to the oxygen-bearing carbon in the ¹H-¹H shift correlation spectroscopy (COSY) spectrum. Based on the small coupling constant ($J=6$ Hz) and connectivity from these olefin protons to three methine protons as δ 1.93 (H-14) $\rightarrow$ 2.30 (H-8) $\rightarrow$ 1.08 (H-9), the presence of a 15-en-17-ol system was suggested. The 4,6-dien-3-one and 15-en-17-ol systems in the androstane structure was further confirmed by cross peaks in the heteronuclear multiple bond connectivity (HMBC) spectrum between H-19/C-1, 5, 9, 10; H-1/C-2, 3, 10, 19; H-18/C-12, 13, 14, 17; and H-7/C-5, 8, 9, 14. The α -oriented hydroxy group at C-17 was assigned by the presence of a nuclear Overhauser effect (NOE) between H-17 and H-18. The proton signals due to H-18 and H-19 showed a response to H-8. The linkage of the sugar moiety was determined to be *via* a 17-*O*- α -L-arabi-

Chart 1

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nosyl-(1→6)- β -D-glucoside, based on cross peaks between H-1_{ara}/C-6_{glc}, C-5_{ara}; H-1_{glc}/C-17; and H-17/C-1_{glc} in HMBC. The structure of **1** was, thus, established to be 17 α -hydroxy-androsta-4,6,15-trien-3-one 17-*O*- α -L-arabinopyranosyl-(1→6)- β -D-glucopyranoside.

The usual type of cardenolide glycosides, such as uzarigenin, corotoxigenin and coroglaucigenin, were isolated along with several cardenolides. They were all identified as known cardenolides and glycosides,⁴⁾ based on their NMR and FAB-MS data, and shown to be the glucoside and gentiobioside (uzarin) of uzarigenin, the glucoside and glucosyl-(1→4)-6-deoxyalloside of corotoxigenin, and the glucoside, gentiobioside, 6-deoxyalloside (frugoside), glucosyl-(1→4)-6-deoxyalloside (glucofrugoside)²⁾ and gentiobiosyl-(1→4)-6-deoxyalloside of coroglaucigenin. Of these, glucofrugoside and frugoside were the major glycosides. Of the five doubly-linked glycosides, two were identified as the already known 5,6-didehydrocalotropin (Δ^5 -calotropin, **2**)⁵⁾ and its acetate.⁵⁾

Glycoside **3** was suggested to be a doubly-linked glycoside composed of **2** and one hexose, based on a $[M+Na]^+$ peak at m/z 715.2939 (C₃₅H₄₈O₁₄+Na). The signals due to **2** and glucose were identified by almost the same chemical shifts and multiplicities in the ¹H- and ¹³C-NMR spectra, except for the lower field shift of C-3'. The signals were also comparable with those of 3'-*O*-glucosylcalotropin^{1b)} over the range of shielding or deshielding affected by the Δ^5 -linkage in **3**. Since the anomeric proton of glucose (δ 5.24) showed an HMBC correlation to C-3' (δ 84.8), glucose was located at the 3'-hydroxy group. While the anomeric carbon of glucose was observed in the lower chemical shift (δ 108.0), and C-3' also showed remarkable deshielding (+10.9 ppm) with shielding of C-4' (-1.6 ppm) in comparison with those of **2**, the glucose was assigned to be one of the D-series.⁶⁾ Based on the coupling constant of the anomeric proton (d, $J=8$ Hz), the glycosidic linkage was assigned to be β . Glycoside **3** was, thus, determined to be Δ^5 -calotropin 3'-*O*- β -D-glucopyranoside.

Glycoside **4** afforded a $[M+Na]^+$ peak at m/z 624.2244, suggesting a molecular formula, C₃₁H₃₉NO₉S, including N and S as found in uscharin and voruscharin.^{1d)} The carbon and proton signals due to the cardenolide moiety in **4** were observed to have almost the same chemical shifts as those of **2** or **3**. In the ¹³C-NMR spectrum, two signals at δ 32.9 and 175.1 seemed to be due to the thiazoline ring and assigned as methylene (C-1'') and carbonyl (C-2'') carbons, respectively. In the HMBC spectrum, the N-H proton at δ 10.21 showed a response to the methylene carbon at δ 32.9 and to C-3' (δ 72.7), while the methylene protons at δ 3.82 (2H, s, H-1'') showed a correlation to the carbonyl carbon at δ 175.1 and C-3'. Glycoside **4** was, therefore, appeared to link a thiazolidinone ring instead of the thiazoline ring in voruscharin.

In the HR-FAB-MS of **5**, a $[M+Na]^+$ peak at m/z 640.2202 suggested the formula, C₃₁H₃₉NO₁₀S, one oxygen greater than **4**. On comparing the ¹H- and ¹³C-signals with those of **4**, the cardenolide moiety in **5** seemed to have the same structure as **4**. The ¹³C-signals of C-1'' and C-3' were deshielded as δ 55.8 and 83.5, respectively, suggesting that the extra oxygen forms an *S*-oxide. The strong absorbance at 1030 cm⁻¹ in the IR spectrum also suggested the presence of an *S*-oxide. As observed in **4**, cross peaks showed a correlation between N-H/C-1'', C-2'', C-3' and H-1''/C-2'', C-3' in

Table 1. ¹³C-NMR Spectral Data for **1**—**5** [δ ppm in Pyridine-*d*₅]

No.	1 ^{a)}	2	3 ^{b)}	4	5
1	33.9	35.5	35.6	35.6	35.1
2	34.3	68.9	68.8	70.1	69.5
3	198.3	72.0	72.0	71.3	71.5
4	124.1	33.7	33.6	33.4	33.2
5	163.1	140.6	140.7	140.7	140.6
6	128.2	120.9	120.9	120.9	120.9
7	140.3	29.6	29.5	29.5	29.4
8	35.0	44.9	44.9	44.9	44.7
9	51.2	38.8	38.9	38.8	38.7
10	36.2	52.3	52.4	52.3	52.3
11	20.4	23.7	23.7	23.7	23.7
12	35.5	38.8	38.8	38.9	38.7
13	52.4	50.7	50.7	50.8	50.7
14	54.8	84.4	84.3	84.4	84.3
15	135.5	39.5	39.5	39.6	39.5
16	130.0	27.9	27.9	28.0	27.9
17	91.4	50.7	50.6	50.6	50.6
18	13.2	16.1	16.1	16.1	16.0
19	16.1	206.6	206.7	206.4	206.3
20		175.2	175.3	175.3	175.2
21		73.6	73.6	73.6	73.6
22		117.9	117.8	117.9	117.9
23		174.3	174.3	174.3	174.3
1'		97.3	96.7	95.7	96.3
2'		92.7	92.7	93.0	92.6
3'		73.9	84.8	72.7	83.5
4'		39.9	38.3	46.8	35.4
5'		68.5	68.3	67.8	67.4
6'		21.5	21.3	21.3	21.2
1''				32.9	55.8
2''				175.1	171.9

a) 17-*O*-Glucosyl residue: 105.3 (C-1_{ara}), 104.8 (C-1_{glc}), 78.6 (C-3_{glc}), 77.2 (C-5_{glc}), 75.2 (C-2_{glc}), 74.3 (C-3_{ara}), 72.3 (C-2_{ara}), 71.8 (C-4_{glc}), 69.6 (C-6_{glc}), 69.1 (C-4_{ara}), 66.4 (C-5_{ara}). b) 3'-*O*-Glucosyl residue: δ 108.0 (C-1), 75.9 (C-2), 78.5 (C-3), 71.4 (C-4), 78.9 (C-5), 62.7 (C-6).

the HMBC spectrum.

In the difference (DIF)-NOE spectra of **4** and **5**, a response between H-1'/H-5' was observed for the two compounds. However, the H-1' signal showed a response in **4**, while the H-4' α in **5** required irradiation of the N-H proton (Chart 2). Therefore, the stereochemistry at C-3' was assigned as *S* in **4** and *R* in **5**. Although thiazoline or thiazolidine derivatives of doubly-linked cardenolide glycosides are known in *A. fruticosa*,^{1c,d)} **4** and **5** are the first the thiazolidinone derivatives. While all thiazoline or thiazolidine-linked glycosides, including uscharin and voruscharin, occur in the C-3'*S*-form, so far,^{1c,d)} the presence of the C-3'*R* isomer should be considered. Pregnane glycosides from the roots are described elsewhere.

Experimental

The melting points were determined on a hot-stage apparatus without correction. ¹H- and ¹³C-NMR spectra were recorded on a JNM-A500 spectrometer in pyridine-*d*₅. Chemical shifts are given in δ values referred to the internal standard, tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. HR-FAB-MS were recorded on a JEOL HX-110 spectrometer. Optical rotations were measured on a JASCO DIP 360 polarimeter. UV spectra were recorded on a Shimadzu 200S double beam spectrometer in MeOH and IR spectra were recorded on a JASCO FT/IR-230 in CHCl₃. GC was performed on a Shimadzu GC-17A. For silica gel column chromatography and TLC, the following solvent systems were used: CHCl₃-MeOH-H₂O (bottom layer, solvent 1), EtOAc-MeOH-H₂O (top layer, solvent 2), benzene-acetone (solvent 3). For octadecyl silica (ODS) column chromatography and HPLC, MeCN-H₂O (solvent 4) was used. Spray reagent: a) 10% H₂SO₄ b)

Table 2. ^1H -NMR Spectral Data for 1–5 [δ ppm in Pyridine- d_5 , J in Hz]

No.	1 ^{a)}	2	3 ^{b)}	4	5
1	1.48 (m) 1.73 (m)	1.18 (t, 12) 2.51 (dd, 12, 5)	1.17 (t, 12) 2.55 (dd, 12, 5)	1.21 (t, 13) 2.64 (dd, 13, 5)	1.22 (t, 12) 2.36 (dd, 12, 5)
2		4.88 (m)	4.87 (m)	4.89 (m)	4.66 (m)
3		4.30 (m)	4.30 (m)	4.32 (m)	4.33 (m)
4	5.86 (s)				
6	6.12 (dd, 10, 2)	6.39 (br d, 3)	6.39 (br d, 4)	6.39 (br d, 5)	6.39 (br s)
7	6.18 (dd, 10, 2)				
8	2.30 (br t, 12)				
9	1.08 (ddd, 12, 10, 5)				
14	1.93 (ddd, 12, 2, 1)				
15	6.23 (ddd, 6, 2, 1)				
16	6.06 (dt, 6, 1)				
17	4.77 (br s)	2.81 (dd, 9, 6)	2.81 (dd, 9, 6)	2.84 (dd, 9, 6)	2.83 (dd, 9, 6)
18	1.16 (s)	0.88 (s)	0.89 (s)	0.88 (s)	0.87 (s)
19	0.98 (s)	9.88 (s)	9.88 (s)	9.83 (d, 1)	9.79 (d, 1)
21		5.04 (dd, 18, 1) 5.27 (dd, 18, 1)	5.05 (dd, 18, 2) 5.27 (dd, 18, 1)	5.05 (dd, 18, 2) 5.27 (dd, 18, 2)	5.05 (dd, 18, 2) 5.26 (dd, 18, 1)
22		6.15 (br s)	6.16 (br s)	6.17 (br s)	6.16 (br s)
1'		5.03 (s)	4.99 (s)	5.56 (s)	5.46 (s)
3'		4.13 (dd, 12, 5)	4.17 (dd, 12, 4)		
4'		2.03 (ddd, 12, 5, 2) 2.11 (br q, 12)	2.15 (br d, 12) 2.24 (br q, 12)	2.26 (dd, 13, 2) 2.56 (dd, 13, 12)	2.69 (dd, 14, 2) 2.40 (dd, 14, 11)
5'		3.74 (m)	3.68 (m)	4.49 (m)	4.29 (m)
6'		1.36 (d, 6)	1.30 (d, 6)	1.42 (d, 6)	1.37 (d, 6)
1''				3.82 (2H, s)	3.65 (d, 16) 4.60 (d, 16)

a) 17-*O*-Glycoside residue: δ 4.98 (d, $J=7$, H-1_{ara}), 4.93 (d, $J=8$, H-1_{glc}), 4.81 (dd, $J=11$, 2, H-6a_{glc}), 4.45 (dd, $J=9$, 7, H-2_{ara}), ca. 4.30 (H-6b_{glc}, H-4_{ara}, H-5a_{ara}), 4.20 (t, $J=9$, H-3_{glc}), 4.15 (dd, $J=9$, 3, H-3_{ara}), 4.14 (t, $J=9$, H-4_{glc}), 4.01 (dd, $J=9$, 8, H-2_{glc}), 4.10 (m, H-5_{glc}), 3.76 (dd, $J=13$, 3, H-5b_{ara}). b) 3'-*O*-Glucosyl residue: δ 5.24 (d, $J=8$, H-1), 4.57 (dd, $J=12$, 2, H-6a), 4.39 (dd, $J=12.5$, H-6b), 4.24 (t, $J=9$, H-3), 4.19 (t, $J=9$, H-4), 4.13 (dd, $J=8$, 9, H-2), 4.03 (m, H-5).

Chart 2. NOEs of 4 and 5

Kedde's reagent (1 : 1 mixture of 5% 3,5-dinitrobenzoic acid in MeOH and 2 N NaOH).

Extraction and Isolation The roots of *Asclepias tuberosa* L., collected in North Carolina in March, 1994, were purchased from Wilcox Natural Products, North Carolina. Powdered dried roots (4.5 kg) were percolated with MeOH. The concentrated MeOH extract was suspended in 50% MeOH and filtered. The filtrate was partitioned with benzene (extract 34.0 g) and then with CHCl_3 (4.5 g). The H_2O layer, after partition with CHCl_3 , was passed through an MCI-gel column (Mitsubishi Kasei Co., CHP-20P) and fractionated into five fractions (eluted with H_2O , 25%, 50%, 75%, and 100% MeOH, respectively). The CHCl_3 fraction and the 75% and 100% MeOH effluents of MCI-gel column chromatography were individually chromatographed on silica gel columns with solvents 1, 2, 3 and an ODS column or HPLC (ODS) with solvent 4, finally, to afford glycoside 1, uzarigenin (50 mg), coroglaucigenin (113 mg), calotropagenin (120 mg), coroglaucigenone-3-methyl-hemiacetal-3,19-epoxide (9 mg), uzarigenin glucoside (4.6 mg), uzarigenin gentiobioside (uzarin) (230 mg), corotoxigenin glucoside (8 mg), corotoxigenin glucosyl-(1 $\rightarrow$ 4)-6-deoxyalloside (93 mg), coroglaucigenin glucoside (262 mg), coroglaucigenin 6-deoxyalloside (frugoside) (1 g), coroglaucigenin glucosyl-(1 $\rightarrow$ 4)-6-deoxyalloside (glucofrugoside) (5.2 g), coroglaucigenin gentiobioside (45 mg), coroglaucigenin gentiobiosyl-(1 $\rightarrow$ 4)-6-deoxyalloside (32 mg), Δ^5 -calotropin (2, 38 mg), Δ^5 -calotropin-3'-acetate (17 mg), glycoside 3 (14 mg), glycoside 4 (34 mg), and glycoside 5 (21 mg).

Ascardoside (1): mp 180–184 °C, $[\alpha]_D^{25} -58.7^\circ$ ($c=0.78$, MeOH), HR-FAB-MS m/z : 601.2624 (Calcd for $\text{C}_{30}\text{H}_{42}\text{O}_{11}+\text{Na}$: 601.2625), UV λ_{max} nm (log ϵ): 283 (4.04).

GC analysis: 1 (2 mg) was heated with 1 N HCl (0.5 ml) for 2 h, deacidified with IRA-410, and reacted with L-cystein methyl ester-HCl in pyridine, followed by trimethylsilylation: column Shimadzu DB-1 (0.25 mm $\times$ 30 m), temperature column 250 °C, inj. 300 °C, detector 300 °C, carrier gas: He (30 cm/s), split ratio 1/40. t_R (min): 4.06, 6.37 (authentic specimen D-arabinose: 4.31, L-arabinose: 4.07, D-glucose: 6.37, L-glucose: 6.60).

Δ^5 -Calotropin 3'-*O*- β -D-glucopyranoside (3): mp 250–260 °C (dec.), $[\alpha]_D^{23} +38.5^\circ$ ($c=0.26$, MeOH), FAB-MS m/z : 715.2939 (Calcd for $\text{C}_{35}\text{H}_{48}\text{O}_{14}+\text{Na}$: 715.2931).

Δ^5 -Calotropin (3'*S*)-3'-thiazolidinone (4): mp 260–280 °C (dec.), $[\alpha]_D^{29} -9.6^\circ$ ($c=1.87$, MeOH), FAB-MS m/z : 624.2244 (Calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_9\text{S}+\text{Na}$: 624.2243).

Δ^5 -Calotropin (3'*R*)-3'-thiazolidinone S-oxide (5): mp 240–245 °C (dec.), $[\alpha]_D^{26} +21.1^\circ$ ($c=1.07$, MeOH), FAB-MS m/z : 640.2202 (Calcd for $\text{C}_{31}\text{H}_{39}\text{NO}_{10}\text{S}+\text{Na}$: 640.2192). IR (CHCl_3) cm^{-1} : 3680, 3620, 1750, 1030.

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