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CARDENOLIDE DIGLYCOSIDES FROM *OXYSTELMA ESCULENTUM*

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Key Word Index—*Oxystelma esculentum*; Asclepiadaceae; roots; oxystelmoside; oxystelmine; steroid; cardenolide glycoside.

Abstract—Two new cardenolide diglycosides, named oxystelmoside and oxystelmine, were isolated from the dried roots of *Oxystelma esculentum*. On the basis of chemical and spectroscopic evidence their structures were established as uzarigenin 3-*O*-β-D-xylopyranosyl(1→4)-*O*-β-D-digitalopyranoside and periplogenin-3-*O*-6-deoxy-β-D-glucopyranosyl(1→4)-*O*-α-D-digitalopyranoside.

INTRODUCTION

A number of cardiac and pregnane glycosides have been isolated from the Asclepiadaceae [1-4] and it is reported that these glycosides [5, 6] are used for the treatment of heart ailments. Four glycosides, oxystine [7], oxysine [8], esculentin [9] and oxyline [10], were reported previously as constituents of *Oxystelma esculentum*. As a continuation of our studies on this plant we describe in this paper two new cardenolide diglycoside designated as oxystelmoside (**1**) and oxystelmine (**5**).

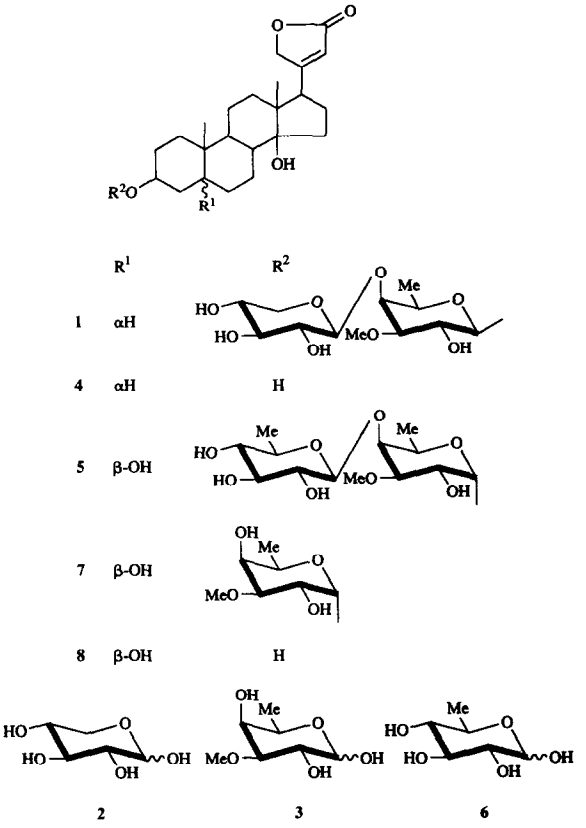
RESULTS AND DISCUSSION

Oxystelmoside (**1**) displayed positive Liebermann–Burchardt [11] and Kedde [12] tests, indicating the presence of a cardenolide moiety. The 400 MHz ^{1}H NMR spectrum displayed the presence of two doublets ($J=7.5$ and 8 Hz) for two anomeric protons at δ4.97 (1H) and 4.81 (1H), a low field one proton singlet at 5.88 of H-22 accompanied by an AB system at 4.92 (1H) and 4.86 (1H) characteristic of H-21 of a butenolide ring. This data supported the identification of **1** as cardenolide glycoside.

Kiliani hydrolysis [13] of **1** under forcing conditions, afforded a mixture of two sugars **2** and **3** identified as D-xylose and D-digitalose, respectively, by comparison with authentic samples ($[\alpha]_D$, co-chromatography). The aglycone could not be isolated as it was destroyed under the strong acidic conditions employed in the Kiliani hydrolysis.

To identify the native genin of **1** and also to determine the sequence of sugars in it, **1** was subjected to mild acid hydrolysis, with TLC and PC monitoring, using the method of Mannich and Siewert [14]. The reaction mixture contained xylose (**2**) as the first sugar unit after five days hydrolysis which indicated that xylose is the terminal sugar unit in **1**. The hydrolysis was completed in 16 days affording three chromatographically pure products, **2**–**4**. Compounds **2** and **3** were identified as xylose and digitalose, respectively.

Compound **4**, $C_{23}H_{34}O_4$, gave positive Liebermann–Burchardt and Kedde tests indicating it to be a cardenolide, which was identified as uzarigenin on comparison with the authentic sample (mp, mmp, $[\alpha]_D$ and co-chromatography). It was, therefore, concluded that in the glycoside **1**, digitalose is glycosidically linked to



uzarigenin at the C-3 hydroxyl group, this being the only available secondary hydroxyl group in the genin (**4**).

The diglycoside nature of **1** was further confirmed by the presence of a fragment ion peak at *m/z* 293 (disaccharide ion) in its EI mass spectrum. The mass spectrum did not exhibit the molecular ion but the highest mass peak recorded at *m/z* 374 was in agreement with $[M - \text{sugar}]^+$. The subsequent losses of two water molecules from this ion, giving ion peaks at *m/z* 356 and 338, were in agreement with the presence of two hydroxyl groups in its genin moiety.

In the ^{1}H NMR spectrum of **1**, the two doublets at δ4.97 (J = 7.5 Hz) and 4.81 (J = 8 Hz) were assigned to two anomeric protons and the large coupling constant typical of an axial configuration suggested the xylose and digitalose moieties were in the $^4C_1$(D) [15] conformation also suggesting that these sugars were joined through β-glycosidic linkages in **1**. The spectrum also contained a methoxy group singlet at δ3.66 and a doublet (J = 6 Hz) of a secondary methyl group at δ1.32, along with other characteristic signals of a cardenolide glycoside. The structure of oxystelmoside (**1**) was assigned as uzarigenin-3-*O*-β-D-xylopyranosyl(1→4)-*O*-β-D-digitalopyranoside.

Oxystelmine (**5**) gave positive Liebermann–Burchardt and Kedde tests. The ^{1}H NMR spectrum of **5** exhibited two doublets of one proton each at δ4.35 (J = 8 Hz) and 4.23 (J = 3 Hz) assigned to two anomeric protons. A one proton singlet of H-22 at δ5.90, along with an AB system at 5.0 (1H) and 4.81 (1H) of H-21 of a butenolide ring together with the characteristic signals of a cardenolide moiety, suggested **5** to be a cardenolide glycoside. The cardenolide diglycoside nature of **5** was confirmed from its ^{13}C NMR spectrum which contained signals for two anomeric carbons at 103.3 and 95.4, and low field signals of C-23, C-20 and C-22 of the butenolide ring at 174.5, 174.6 and 117.5, respectively.

Hydrolysis of **5** by the Kiliani method afforded two chromatographically pure sugars (**3** and **6**). Both the sugars gave the characteristic colour test for normal hexoses with Partridge reagent [16] and were identified as D-digitalose and 6-deoxy-D-glucose by comparison with an authentic sample ($[\alpha]_D$ and co-chromatography). The aglycone could not be isolated as it was destroyed under strong acidic conditions of the Kiliani hydrolysis.

In order to identify the genin and also to determine the sequence of sugars in glycoside **5**, it was subjected to mild acid hydrolysis [14]. After 6 days the hydrolysate gave 6-deoxy-glucose (**6**), unreacted **5**, and a third spot which was presumably the monoglycoside (**7**). An aliquot of this partially hydrolysed reaction mixture afforded monoglycoside (**7**) besides 6-deoxy-D-glucose (**6**) and unreacted starting material (**5**). The hydrolysis was complete in 15 days, affording three chromatographically pure products **3**, **6** and **8**. Compounds **6** and **3** were identified as 6-deoxy-D-glucose and D-digitalose, respectively.

Compound **8**, $C_{23}H_{34}O_5$, gave positive Liebermann–Burchardt and Kedde tests, indicating it to be a cardenolide, which was identified as periplogenin on comparison with the authentic sample (mp, mmp, $[\alpha]_D$ and co-chromatography). As 6-deoxy-D-glucose was the first sugar unit obtained in the mild acid hydrolysis of **5** it led to the conclusion that 6-deoxy-D-glucose was the terminal sugar and the other sugar, digitalose, was linked to periplogenin at the C-3 hydroxyl group, being the only available secondary hydroxyl group in the genin **8**.

The configuration of the glycosidic linkage in glycoside **5** was confirmed from its ^{1}HNMR spectrum. The large coupling constant (J = 8 Hz) of an anomeric proton doublet at δ4.35 was typical of an axial proton of hexopyranose suggesting that one sugar unit was in the $^4C_1$ (D) conformation [15] and linked through a β-glycosidic linkage. The small coupling constant (J = 3 Hz) of the other anomeric proton at δ4.23 was typical of an equatorial proton of a hexopyranose indicating that the second sugar was in the $^4C_1$ (D) conformation [15] and joined through an α-glycosidic linkage. To assign the glycosidic linkages in **5**, the ^{1}H NMR spectrum of monoglycoside **7** was recorded. The small coupling constant (J = 3 Hz) of the anomeric proton present at δ4.20 (1H) in **7** indicated that digitalose was linked to periplogenin through an α-glycosidic linkage. Therefore, in the glycoside **5**, 6-deoxy-D-glucose was linked to digitalose through a β-glycosidic linkage.

The EI mass spectrum of **5** did not show molecular ion but the peak recorded at *m/z* 372 corresponded to $[M - \text{disaccharide} - H_2O]^+$. The subsequent losses of the butenolide ring along with C-17, C-16 [17] and two water molecules from this ion, giving ion peaks at *m/z* 262, 244, and 226, respectively, were in agreement with the presence of three hydroxyl groups in its genin moeity. The prominent ion peaks at *m/z* 307 and 289 were attributed to the disaccharide ion and $[\text{disaccharide} - H_2O]^+$ ion, respectively. The structure of oxystelmine (**5**) was established as periplogenin 3-*O*-6-deoxy-β-D-glucopyranosyl (1→4)-*O*-α-D-digitalopyranoside.

EXPERIMENTAL

Mps: uncorr. ^{1}H and ^{13}C NMR spectra: 400 and 80 MHz spectrometers, respectively, in $CDCl_3$ with TMS as the int. standard. Sugars were made visible with 50% aq. H_2SO_4 in TLC (silica gel G BDH) and Partridge reagent on PC. PC (Whatmann no. 1) was performed using toluene–*n*-BuOH (1:2) satd with H_2O. CC was performed using silica gel (BDH 60–120 mesh).

Plant extraction. Shade-dried powdered roots (10 kg) of *O. esculentum* (Voucher No. 68528, deposited in the National Botanical Research Institute, Lucknow, India) were extracted and fractionated as reported earlier [18]. Repeated CC of the combined $CHCl_3$–EtOH (4:1 and 3:2) extract (3.5 g) over silica gel using $CHCl_3$–MeOH (47:3) as eluent afforded oxystelmoside (**1**) (40 mg) and oxystelmine (**5**) (48 mg).

Oxystelmoside (**1**) Mp 105–108°, $[\alpha]_D^{25}$ + 3.24° ($CHCl_3$; *c* 0.21) found C 63.32, H 7.96 $C_{35}H_{54}O_{12}$ required C 63.06, H 8.10%. It gave positive Liebermann–Burchardt and Kedde tests and also underwent $NaIO_4$ oxidation. ^{1}H NMR (400 MHz) δ5.88 (1H, *s*, H-22), 4.97 (1H, *d*, J = 7.5 Hz, H-1′), 4.92 (1H, *d*, J = 16 Hz, H-21), 4.86 (1H, *d*, J = 16 Hz, H-21), 4.81 (1H, *d*, J = 8 Hz, H-1′), 4.10–4.02

(1H, *m*, H-5′ of digitalose), 3.92–3.86 (1H, distorted quartet, H-5′e of xylose), 3.66 (3H *s*, –OMe), 3.46–3.38 (5H, *m*, H-5′a of xylose, 2 × H-4′, 2 × H-3′), 3.22 (1H, *q*, $J=8$ Hz, H-2′), 3.12 (1H, *q*, $J=8$ Hz, H-2′), 1.32 (3H, *d*, $J=6$ Hz, Me-6′), 1.28 (3H, *s*, Me-18), 1.22 (3H, *s*, Me-19). Ms *m/z*: $[M]^+$ not observed 374, $[M-\text{sugar}]^+$, 356 $[374-H_2O]^+$, 338 $[356-H_2O]^+$, 293 $[M-\text{genin}]^+$, 261 $[293-\text{MeOH}]^+$, 243 $[261-H_2O]^+$.

Oxystelmine (**5**) Mp 98–100°, $[\alpha]_D$ 0 ($CHCl_3$; *c* 0.19) found C 61.98, H 8.07, $C_{36}H_{56}O_{13}$ required C 62.06, H 8.04%. It gave positive Liebermann–Burchardt and Kedde tests and also underwent $NaIO_4$ oxidation. ^{1}H NMR (400 MHz) δ5.90 (1H, *s*, H-22), 5.00 (1H, *d*, $J=16$ Hz, H-21), 4.81 (1H, *d*, $J=16$ Hz, H-21), 4.35 (1H, *d*, $J=8$ Hz, H-1′), 4.23 (1H, *d*, $J=3$ Hz, H-1′), 3.94–3.84 (4H, *m*, H-3′ and H-5′), 3.66 (3H, *s*, –OMe), 3.46–3.38 (2H, *m*, H-4′), 3.28 (1H, *dd*, J = 9 and 3 Hz, H-2′), 3.19 (1H, *t*, $J=8$ Hz, H-2′), 1.31 (6H, *d*, $J=7$ Hz, 2 × Me-6′), 1.26 (3H, *s*, Me-18), 0.91 (3H, *s*, Me-19) ^{13}C NMR 27.3 (C-1), 28.1 (C-2), 66.6 (C-3), 37.2 (C-4), 74.6 (C-5), 34.7 (C-6), 26.9 (C-7), 40.4 (C-8), 39.4 (C-9), 39.6 (C-10), 22.8 (C-11), 42.2 (C-12), 50.0 (C-13), 85.4 (C-14), 31.5 (C-15), 26.6 (C-16), 50.9 (C-17), 15.9 (C-18), 15.9 (C-19), 174.6 (C-20), 73.4 (C-21), 117.5 (C-22), 174.5 (C-23), 103.2 (C-1′), 95.4 (C-1′), 18.3 (C-6′), 17.7 (C-6′), 60.6 (OMe) MS, *m/z*: $[M]^+$ not observed, 372 $[M-\text{sugar}-H_2O]^+$, 354 $[372-H_2O]^+$, 262 $[372-C_6H_6O_2]^+$, 244 $[262-H_2O]^+$, 226 $[244-H_2O]^+$, 307 [disaccharide ion]$^+$, 289 $[307-H_2O]^+$.

Kiliani hydrolysis of oxystelmoside (**1**). Compound **1**, (15 mg) was dissolved in Kiliani mixture (0.5 ml, HOAc–H_2O–HCl, 7:11:2) and heated at 100° for 1 hr and usual work-up [13] afforded mixture of two sugars, which were sepd through CC affording **2** (1.9 mg) $[\alpha]_D^{25}+19.2°$ (H_2O; *c* 0.15), **3** (2.1 mg) $[\alpha]_D^{25}+106.3°$ (H_2O; *c* 0.19). Both the sugars gave positive test with Partridge reagent. Sugars **2** and **3** were identified as D-xylose and D-digitalose by comparing with an authentic sample ($[\alpha]_D$, co-chromatography).

Kiliani hydrolysis of oxystelmine (**5**). Compound **5** (15 mg) was dissolved in Kiliani mixture (0.5 ml, HOAc–H_2O–HCl, 7:11:2) and heated at 100° for 1 hr. and usual work-up [13] afforded a mixture of two sugars, which were separated through *CC* affording **6** (3.1 mg) $[\alpha]_D^{25}+30.4°$ (H_2O; *c* 0.12), **3** (2.3 mg) $[\alpha]_D^{25}+106.6°$ (H_2O; *c* 0.14). Both the sugars gave positive test in Partridge reagent. Sugars **6** and **3** were identified as 6-deoxy-D-glucose and D-digitalose by comparing with authentic sample ($[\alpha]_D$ and co-chromatography).

Mannich and Siewert hydrolysis of compound **1**. To a soln of crystalline **1** (15 mg) in Me_2CO (2.5 ml), conc HCl (0.025 ml) was added. The soln was kept under CO_2 in the dark at room temp. After 5 days the reaction mixture exhibited two more spots (TLC) besides a spot of some unreacted starting material. The polar spot was identified as D-xylose (**2**), while the less polar spot was presumably the monoglycoside. The hydrolysis was complete in 16 days and usual workup [15] afforded three chromatographically pure compounds **2** (2.3 mg) $[\alpha]_D^{25}+19.21°$ (H_2O; *c* 0.12), **3** (2.1 mg) $[\alpha]_D^{25}+106.5°$ (H_2O; *c* 0.17) and **4** (4.1 mg) mp 244–246° $[\alpha]_D^{25}+13.6°$ (MeOH; *c* 0.12), identified as D-xylose, D-digitalose and uzarigenin, respectively.

Mannich and Siewert hydrolysis of compound **5**. To a soln of **5** (20 mg) in Me_2CO (2.5 ml) conc HCl (0.025 ml) was added. The soln was kept under CO_2 in the dark at room temp. After 6 days the reaction mixture exhibited two more spots (TLC) besides a spot of some unreacted starting material **5**. The polar spot was identified as 6-deoxy-glucose (**6**) while the less polar spot was presumably the monoglycoside (**7**). An aliquot of this partially hydrolysed reaction mixture afforded [15] chromatographically pure 6-deoxy-D-glucose (**6**), monoglycoside (**7**), $[\alpha]_D+44.4°$ (MeOH; *c* 0.11) and unreacted **5**. ^{1}H NMR (80 MHz) of **7** δ4.20 (1H, *d*, $J=3$ Hz, H-1′), 3.66 (3H, *s*, OMe), 1.25 (3H, *s*, Me-18), 0.92 (3H, *s*, Me-19). The hydrolysis was complete in 15 days. Usual work-up afforded **6** (2.7 mg) $[\alpha]_D^{25}+30.6°$ (H_2O; *c* 0.11), **3** (2.8 mg) $[\alpha]_D^{25}+106.2°$ (H_2O; *c* 0.13) and **8** (3.2 mg) mp 233–236° $[\alpha]_D^{25}+31.4°$ ($CHCl_3$; *c* 0.14) identified as 6-deoxy-D-glucose, D-digitalose and periplogenin, respectively.

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