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A PREGNANE ESTER OLIGOGLYCOSIDE FROM *OXYSTELMA ESCULENTUM*

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Key Word Index—*Oxystelma esculentum*, Asclepiadaceae, oxysine, steroid, pregnane ester tetraglycoside

Abstract—A novel pregnane ester oligoglycoside named, oxysine has been isolated from *Oxystelma esculentum* and characterized as calogenin-3-*O*-*β*-D-oleandropyranosyl (1→4)-*O*-*β*-D-thevetopyranosyl (1→4)-*O*-*β*-D-cymaropyranosyl (1→4)-*O*-*β*-D-digitoxopyranoside.

INTRODUCTION

Plants of the Asclepiadaceae family are excellent sources of pregnane derivatives [1] In an earlier chemical investigation of the roots of *Oxystelma esculentum* the presence of a pregnane oligoglycoside oxystine [2] was reported. As a continuation of studies on this plant, we present here spectral and chemical evidence for the structure of the newly isolated oligoglycoside designated as oxysine (**1**)

RESULTS AND DISCUSSION

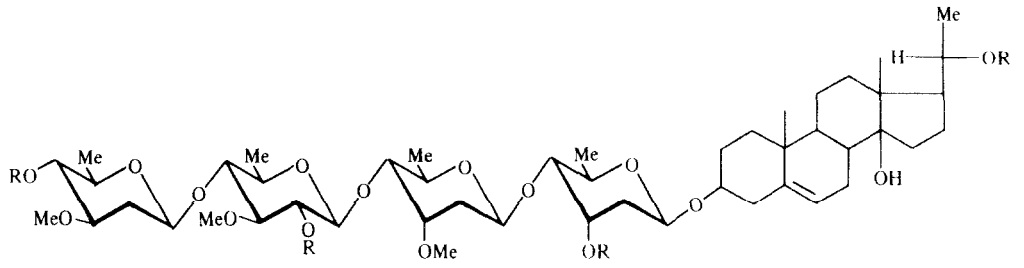
Oxysine (**1**), mp 120–122°, $[\alpha]_D$ −17.5°, $C_{48}H_{80}O_{16}$, responded positively to the Liebermann–Burchard [3], xanthydrol [4, 5] and Keller–Kiliani [6] reactions, indicating it to be a steroidal glycoside of a 2-deoxy sugar [5]

Mild acid hydrolysis [7] of **1** with 0.025 M sulphuric acid afforded a genin **3** and a mixture of three sugars **5**–**7**, which were isolated as viscous syrups All three sugars displayed characteristic colour reactions of 2-deoxy sugars. The sugars **5** and **6** were identified as D-oleandrose [8] (2,6-dideoxy-3-*O*-methyl-D-arabinohexose) and D-digitoxose [9] (2,6-dideoxy-D-ribohexose), respectively, by comparison with authentic samples (PC, $[\alpha]_D$). For further characterization, **5** and **6** were oxidised with bromine water to their lactones **8** and **9**, respectively, which on treatment with phenylhydrazine in turn afforded crystalline D-oleandronic acid phenylhydrazide (**10**) [8] and D-digitoxonic acid phenylhydrazide (**11**) [9]. The sugar **7** had an intermediate mobility between **5** and **6** on PC, suggesting it was a disaccharide

To characterize the disaccharide **7**, it was subjected to Kiliani hydrolysis [10] which afforded a sugar **12**, identical with D-thevetose (3-*O*-methyl 6-deoxy D-glucose) [11] (PC, $[\alpha]_D$). The fact that 2-deoxy sugars are decomposed under these hydrolysis conditions, suggested that disaccharide **7** contained a normal 6-deoxy hexose at the non-reducing end and a 2,6-dideoxy hexose at the reducing end. The mobility of **7**, relative to that of oleandrose, suggested it to be lilacinabiose [11] which was further supported by comparison of the optical rotation of **7** with that reported for lilacinabiose

The genin **3**, mp 195–197°, $C_{21}H_{34}O_3$ from the hydrolysate, was identified as calogenin [12] by comparison with an authentic sample (mp, mmp, TLC) Further characterization of **3** came from its acetylation with acetic anhydride in pyridine which afforded a di-*O*-acetyl derivative **4**, mp 167° identical with an authentic sample of calogenin diacetate [12] (mp, mmp, TLC) Chemical support for **1** being a tetraglycoside composed of oleandrose, digitoxose and the disaccharide lilacinabiose, and determination of the sequence of the three sugar units came from TLC and PC monitoring of the products of its very mild acid (0.5 mM H_2SO_4) hydrolysis at room temperature, which afforded partially and completely hydrolysed products After four days under these conditions, the reaction mixture contained oleandrose (**5**) (PC, TLC) as the only monosaccharide unit, together with another new component, presumably triglycoside **14**, leading to the conclusion that the terminal sugar unit of **1** was oleandrose. After six days, two additional components, one having a mobility comparable with that of lilacinabiose (**7**) and the other presumably monoglycoside **13**, appeared indicating that lilacinabiose was next in sequence after oleandrose in compound **1** Finally, after 10 days, the appearance of two new additional components with TLC mobilities identical with digitoxose (**6**) and calogenin (**3**), suggested that digitoxose was glycosidically linked to calogenin either at the C-3 or C-20 hydroxy group.

A close analysis of the 400 MHz ^{1}H NMR spectrum of compound **1** not only confirmed the derived structure, but also permitted determination of the configuration of the four glycosidic linkages Its spectrum contained three methoxy group singlets at δ3 63, 3 46, 3.42 and four secondary methyl group doublets (J = 6 Hz) at 1 33, 1.31, 1.27, 1 26 The six C-2 methylene protons of three 2-deoxy sugar units appeared as two sets of three-proton multiplets in the regions 2.45–2 03 and 2.01–1.51 for the equatorial and axial protons, respectively, accompanied by a triplet (J = 7 Hz) at 3.51 attributable to the C-2 proton of the normal sugar. The three anomeric protons of 2-deoxy hexopyranoses appeared as double doublets (J = 10 and 2 Hz) at 4 83 (2H) and 4.75 (1H); a fourth anomeric proton appeared as a doublet at 4 35 (1H, J = 8 Hz) and was assigned to the normal hexopyranose unit The large values of the coupling constants (J = 10 and 8 Hz) of these anomeric protons were typical of the axial configuration of the hexopyranoses in the $^4C_1$ (D) conformation [13], indicating that these sugars were



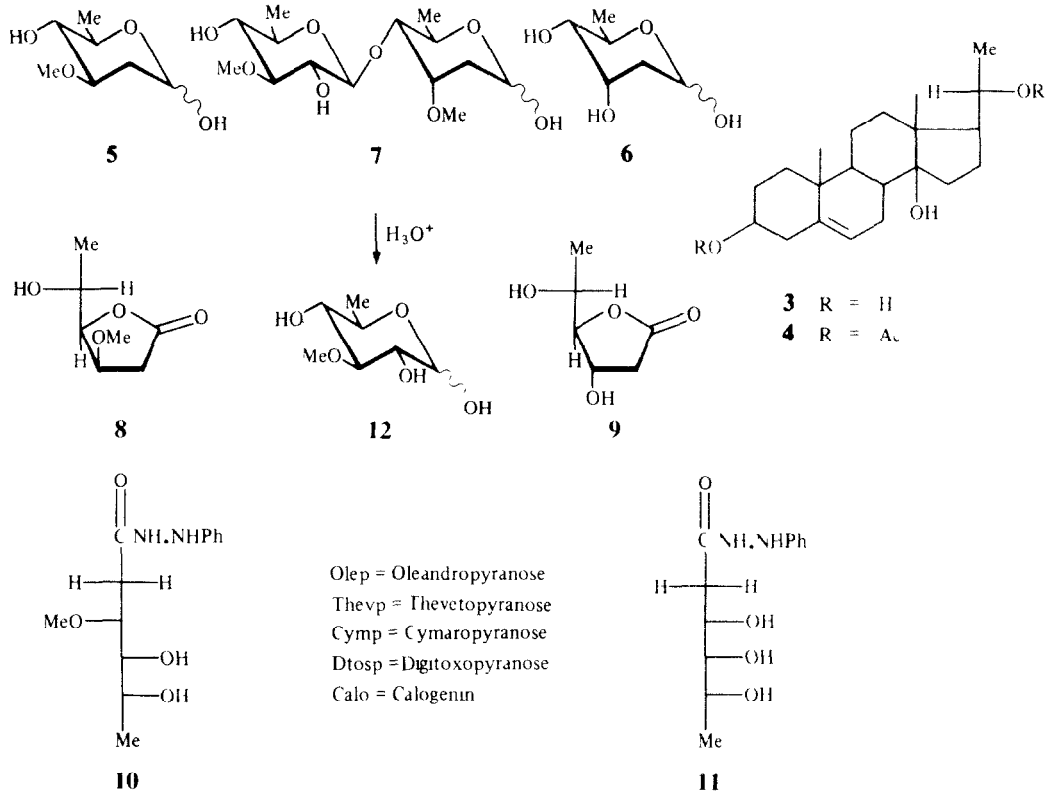
1 R = H, β-D-Olep(1→4)-β-D-Thevp(1→4)-β-D-Cymp(1→4)-β-D-Dtosp-Calo
2 R = Ac

β-D-Thevp(1→4)-β-D-Cymp(1→4)-β-D-Dtosp-Calo

14

β-D-Dtosp-Calo

13



Olep = Oleandropyranose
Thevp = Thevetopyranose
Cymp = Cymaropyranose
Dtosp = Digitoxopyranose
Calo = Calogenin

joined through β-D-glycosidic linkages The characteristic one proton quartet ($J = 6$ Hz) centred at δ 3 12 could be assigned to the C-20 methine proton of the genin moiety in glycoside **1** suggesting that digitoxose (**6**) was glycosidically linked to calogenin (**3**) at the C-3 hydroxyl group and not at the C-20 hydroxyl group This was confirmed by the downfield shifting of the methine proton quartet of C-20 carbinol in the ^{1}H NMR spectrum of the *O*-acetyl derivative (**2**) of **1**.

In light of the above evidence, the structure of oxysine (**1**) was established as calogenin-3-*O*-β-D-oleandropyranosyl (1→4)-*O*-β-D thevetopyranosyl (1→4)-*O*-β-D cymaropyranosyl (1→4)-*O*-β-D digitoxopyranoside

EXPERIMENTAL

The general procedures were as previously reported [14]

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 with solvents of different polarities, as reported earlier [15] Repeated CC of the CHCl$_3$ extract (2 8 g) over silica

gel using $CHCl_3$–MeOH (24 1) as eluent afforded oxysine (52 mg)

Oxysine (**1**) Mp 120–122° (MeOH), $[\alpha]_D^{25}$ −17 5° (MeOH, *c* 0 10) Compound **1** gave a pink colour in the xanthydrol test and a blue colour in the Keller–Kiliani reaction ^{1}H NMR (400 MHz), δ 5 39 (1H, *m*, H-6), 4 83 (2H, *dd*, *J* = 10 and 2 Hz, H-1'), 4.75 (1H, *dd*, *J* = 10 and 2 Hz, H-1'), 4 35 (1H, *d*, *J* = 8 Hz, H-1'), 4 27–4 13 (4H, *m*, H-5'), 3 99–3 76 (4H, *m*, H-3'), 3 63 (3H, *s*, OMe), 3 51 (1H, *t*, *J* = 7 Hz, H-2' of normal sugar), 3 46 (3H, *s*, OMe), 3 42 (3H, *s*, OMe), 3 42–3 16 (4H, *m*, H-4'), 3 12 (1H, *q*, *J* = 6 Hz, H-20), 2 45–2 03 (3H, *m*, H-2' eq), 2 01–1 51 (3H, *m*, H-2' *ax*), 1 33 (3H, *d*, *J* = 6 Hz, 6'-Me), 1 31 (3H, *d*, *J* = 6 Hz, 6'-Me), 1.27 (3H, *d*, *J* = 6 Hz, 6'-Me), 1 26 (3H, *d*, *J* = 6 Hz, 6'-Me), 1 25 (3H, *s*, 18-Me), 1 24 (3H, *s*, 19-Me), 1 23 (3H, *d*, *J* = 6 Hz, 21-Me) (Anal Calcd for $C_{48}H_{80}O_{16}$ C, 63 15, H, 8 77 Found C, 63 48, H, 8 44%).

Mild acid hydrolysis of oxysine (**1**) To a soln of **1** (25 mg) in 80% aq dioxane (1 ml) was added 0 05 M H_2SO_4 (1 ml), and the solution warmed for 30 min at 50° Usual work-up as reported earlier [15] afforded genin **3** which crystallized from MeOH as colourless needles (5 mg), mp 195–197°, and a mixture of three sugars which were separated by CC on silica gel affording **5** (3 10 mg), $[\alpha]_D^{25}$ −14 1° (H_2O, *c* 0 14), **7** (5 7 mg), $[\alpha]_D^{25}$ −26 8° (H_2O, *c* 0 18) and **6** (3 5 mg) $[\alpha]_D^{25}$ +42 5° (MeOH; *c* 0 16) All sugars gave positive coloration in the xanthydrol and Keller–Kiliani reactions The $[\alpha]_D$, TLC and PC comparison of **5** and **6** showed them to be identical to D-oleandrose and D-digitoxose, respectively, and **7** was identified as lilacinabiose from its mobility relative to that of oleandrose and by comparison of its $[\alpha]_D$ with that of reported for lilacinabiose [11]

Oxidation of oleandrose (**5**) *with* Br_2 *water* A soln of **5** (3 mg) in H_2O (0 5 ml) was mixed with Br_2 (8 μl) by the method reported earlier [15] yielding the syrupy lactone **8** (2 5 mg) It gave a violet coloration with NH_2OH–$FeCl_3$ spray reagent

Oxidation of digitoxose (**6**) *with* Br_2 *water* A soln of **6** (3 3 mg) in H_2O (0 5 ml) was mixed with Br_2 (8 μl) as in the oxidation of **5** affording the syrupy lactone **9** (2 8 mg) showing a violet spot with NH_2OH–$FeCl_3$ spray reagent

D-*Oleandronic acid phenylhydrazide* (**10**) A soln of **8** (2 5 mg) in absolute EtOH (0 05 ml) was heated with freshly dist phenylhydrazine (0 05 ml), and usual work-up [15] yielded crystalline D-oleandronic acid phenylhydrazide (**10**) which crystallized from MeOH–Et_2O as colourless needles (1 9 mg) mp 134–136° [lit mp 136°]

D-*Digitoxonic acid phenylhydrazide* (**11**) A soln of **9** (2 8 mg) in absolute EtOH (0 05 ml) was mixed with freshly dist phenylhydrazine (0 05 ml) as for lactone **8**, affording D-digitoxonic acid phenylhydrazide (**11**) which crystallized from MeOH–Et_2O as colourless needles (2 mg) mp 120–121° [lit mp 123°]

Kiliani hydrolysis of (**7**) Compound **7** (4 5 mg) was dissolved in Kiliani mixture (0 25 ml, AcOH–H_2O–HCl, 3 5 5 5 1), and heated at 100° for 1 hr Usual work-up [10] yielded 3-*O*-methyl-6-deoxy D-glucose (**12**) which crystallized from MeOH–Et_2O as colourless needles (1 9 mg) mp 118–120°, $[\alpha]_D^{25}$ + 39 4° (H_2O, *c* 0 11).

Very mild acid hydrolysis of oxysine (**1**) To a soln of **1** (15 mg) in 80% aq dioxane (3 ml) was added 1 mM H_2SO_4 (3 ml), and the soln kept at room temp After 4 days, TLC of the reaction mixture exhibited a spot due to oleandrose (**5**) (R_{Ole} 1 00, taken as ref), and two spots of mobilities (R_{Ole} 1 90) and (R_{Ole} 1 35) presumed to be triglycoside **14** and the unhydrolysed starting material **1**, respectively After 6 days two additional new spots appeared, one comparable with the calculated mobility of lilacinabiose (**7**) (R_{Ole} 0 63) and the other presumed to be monoglycoside **13** (R_{Ole} 2 10) The hydrolysis was complete in 10 days when two additional new spots identical in mobilities with calogenin (**3**) (R_{Ole} 1 2) and digitoxose (**6**) (R_{Ole} 0.2) appeared. The reaction mixture was then worked-up, followed by CC, affording calogenin (**3**) which crystallized from MeOH as colourless needles (2.8 mg) mp 195–197°, and three chromatographically pure reducing sugars as viscous syrups, *viz* **5** (2 3 mg), **7** (4.0 mg) and **6** (2 0 mg) identified as D-oleandrose, lilacinabiose and D-digitoxose, respectively

Di-O-acetyl calogenin (**4**) Crystalline **3** (2 mg) dissolved in dry pyridine (0 2 ml) was mixed with Ac_2O (0 2 ml), and the mixture kept for 48 hr at room temp After usual work-up [12] of the reaction mixture it afforded the acetylated product **4** (1 8 mg) which crystallized from MeOH, mp 167°

Tetra-O-acetyl oxysine (**2**) Crystalline **1** (3 mg) dissolved in dry pyridine (0 2 ml) was mixed with Ac_2O (0 2 ml) and the mixture kept for 48 hr at room temp to afford the acetylated product **2** (2 7 mg) $[\alpha]_D^{25}$ −53 2° (MeOH; *c* 0 11) ^{1}H NMR (300 MHz) δ 3 83 (1H, *m*, H-20), 3.44 (3H, *s*, OMe), 3 43 (3H, *s*, OMe), 3 37 (3H, *s*, OMe), 2 10 (6H, *s*, 2 × OAc), 2 07 (6H, *s*, 2 × OAc) (Anal Calcd for $C_{56}H_{88}O_{20}$ C, 62 22, H, 8 14 Found C, 62 66, H, 8 48%

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