# A PREGNANE ESTER TETRAGLYCOSIDE FROM *OXYSTELMA ESCULENTUM*

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**Abstract**—A new pregnane ester tetraglycoside designated as oxystine has been isolated from the dried roots of *Oxystelma esculentum*. Chemical and spectroscopic evidence is consistent with the structure 12-*O*-cinnamoyl desacylmetaplexigenin-3-*O*-β-D-cymarophyranosyl $(1 \rightarrow 4)$-*O*-β-D-thevetopyranosyl $(1 \rightarrow 4)$-*O*-β-D-cymaropyranosyl $(1 \rightarrow 4)$-*O*-β-D-digitoxopyranoside.

## INTRODUCTION

Cardenolides along with pregnane derivatives have been reported to be present in several species of Asclepiadaceae plants [1]. In the course of chemical investigation on Asclepiadaceae plants our interest was drawn to an uninvestigated twining shurb *Oxystelma esculentum* fairly widespread in marshy places of U.P. India. Its roots, which were found to be specially rich in 2-deoxy sugar glycosides, were collected in April 1986 from the suburbs of Lucknow. Shade dried powdered roots were extracted for its glycosides by an earlier reported method [2]. It afforded glycoside mixture of different polarities extracted by different solvents. Repeated chromatography of the ether and chloroform soluble glycosides over silica gel afforded a novel pregnane ester glycoside, named oxystine (1). This paper describes the structure elucidation of this glycoside.

## RESULTS AND DISCUSSION

Oxystine (1), $C_{57}H_{84}O_{20}$, gave a positive Liebermann–Burchardt colour reaction for steroids [3] and also exhibited positive tests characteristic of 2-deoxy sugars in xanthydrol [4, 5] and Keller–Kiliani [6] reactions suggesting it to be a steroidal glycoside of 2-deoxy sugars. In the $^1$H NMR spectrum of 1 at 400 MHz the presence of four anomeric protons at $\delta 4.84$ (2H), 4.76 (1H) and 4.30 (1H), along with four secondary methyl group doublets ($J = 6$ Hz) at $\delta 1.30$, 1.29, 1.27, 1.20 besides the characteristic methylene signals in the regions $\delta 2.19$–1.86 (3H) and 1.70–1.52 (3H) for the equatorial and axial protons, provided evidence that 1 is a tetraglycoside further indicating that three of these sugar units were 2,6 dideoxy hexoses and the fourth sugar unit was a 6-deoxy hexose. The tetraglycoside nature of 1 was further substantiated from its $^{13}$C NMR spectrum which contained signals for four anomeric carbons at $\delta 104.2$ (1C), 99.5 (1C) and 96.0 (2C).

To identify the sugar and genin units of 1, it was hydrolysed with mild acid (0.025 M $H_2SO_4$) using the method of ref. [7] which afforded genin 2 and a mixture of three sugars, 3–5 (TLC) which were preparatively isolated. All the three sugars exhibited individually also the characteristic colour reactions of 2-deoxy sugars. A comparison of rotation and mobility on paper chromatography of 3 and 5 with those of authentic specimens indicated them to be D-cymarose [8] (2,6-dideoxy-3-*O*-methyl-D-ribohexose) and D-digitoxose [9] (2,6-dideoxy-D-ribohexose), respectively. For further characterization, they were oxidized with bromine water to their lactones 6, 7 which in turn were converted to crystalline phenylhydrazides by reaction with phenyl hydrazine affording D-cymaronic acid phanylhydrazide (8) [8] and D-digitoxonic acid phenylhydrazide (9) [9] found identical with authentic samples (mp, mmp). The third sugar, 4, which had an intermediate mobility on paper chromatography was presumed to be a disaccharide.

To ascertain the disaccharide nature of 4 it was hydrolysed with acid under forcing conditions using the method of ref. [10] which afforded sugar 10 exhibiting the same mobility on paper chromatography as 3-*O*-methyl-6-deoxy glucose, the 2-deoxy component being decomposed under this condition, suggesting that this disaccharide belongs to the class consisting of a normal 6-deoxy hexose at the nonreducing end and another 2,6-dideoxy hexose at the reducing end, reported earlier [11] from various Asclepiadaceae plants. Optical rotation measurement of 10 confirmed it as thevetose (3-*O*-methyl-6 deoxy D-glucose) [11]. A comparison of relative mobility on paper chromatography of 4, calculated taking cymarose as the reference, indicated it to be lilacinabiose [11]. The identity of 4 was therefore concluded from its comparable mobility and specific rotation with the reported values.

The genin 2, $C_{30}H_{38}O_7$, from the hydrolysate, contained a carbonyl group which was indicated by its ability to undergo facile reduction [12] with sodium borohydride as well as its reaction with 2,4-dinitrophenyl hydrazine. The nature of this carbonyl as a methyl ketone was shown by a positive sodium nitroprusside [13] test and it was supported by the presence of a three proton

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

**1**

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

**13**

β-D-Dtosp-Kidjo

**14**

**3**   **4**   **5**

**2** R¹ = PhCH=CHCO, R² =

**11** R¹ = H, R² = O

**6**   **10**   **7**

**12** R¹ = PhCH=CHCO, R² =

**8**   **9**

Cymp = Cymaropyranose

Thevp = Thevetopyranose

Dtosp = Digitoxopyranose
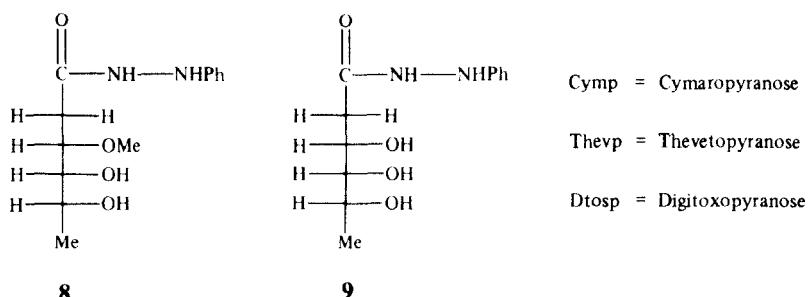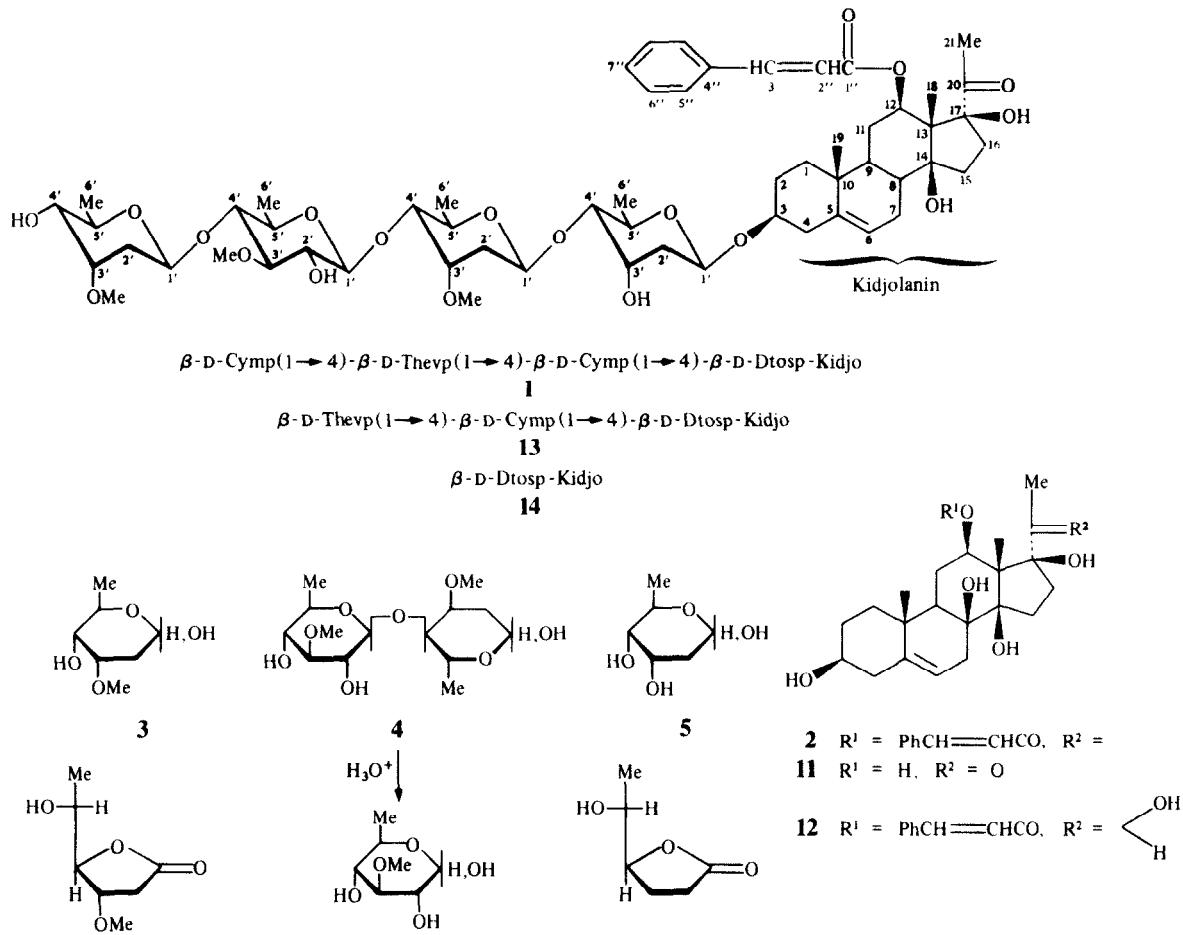
singlet at $\delta$2.15 in the $^1$H NMR spectrum. The ability of **2** to undergo hydrolysis with methanolic potassium hydroxide [14] affording **11** idennified as desacylmetaplexigenin (mmp and cochrom), indicated the presence of an ester function in the molecule. Diagnostic signals at $\delta$7.60 (d, J = 16 Hz, 1H), 6.29 (d, J = 16 Hz, 1H), 7.98–7.95 (2H, arom), 7.55–7.35 (3H, arom) in the $^1$H NMR spectrum of **1** suggested it to be cinnamoyl ester, which was in conformity to the prominent ion peaks in the lower mass region at m/z 162, 131 and 103 in the mass spectrum of the hydrolysis product of **2** [15, 16] atrributable to methyl cinnamate formed in the hydrolysate. A survey of the reported mp and rotation values of cinnamoyl pregnanes containing a C-17 methyl keto side chain indicated

**2** to be kidjolanin [17]. As the authentic sample of kidjolanin was not available, an indirect identification of **2** could be achieved through its sodium borohydride reduced product **12** with the authentic sample of pneupogenin [18] (12-O-cinnamoyl sarcostin (mmp and cochrom) which was available in our laboratory.

More direct chemical support of **1** being the tetraglycoside of cymarose, digitoxose and the disaccharide lilacinabiose and determination of the sequence of the sugar units came from the results of its very mild acid hydrolysis (2.5 mM $H_2SO_4$) at room temp. which afforded partially and completely hydrolysed products. After four days the reaction mixture exhibited the appearance of cymarose (**3**) (PC, TLC) as the only sugar unit with the

formation of another new spot, presumably triglycoside (13) leading to the conclusion that the terminal sugar unit of 1 was cymarose. After six days, two additional new spots (TLC) one comparable with the calculated mobility of lilacinabiose (4) and the other presumably for monoglycoside (14) appeared indicating that lilacinabiose was next in sequence after cymarose in compound 1. Finally after 10 days the appearance of two additional new spots (TLC) identical in mobilities with digitoxose (5) and kidjolanin (2) suggested that digitoxose was glycosidically linked to kidjolanin possibly at its C-3 hydroxyl group.

The $^1$H NMR spectrum of 1 at 400 MHz not only confirmed the derived structure but also ascertained the configuration of the glycosidic linkages. The configuration of the four anomeric protons in the four sugar units could be easily derived from the coupling constants of their signals at $\delta 4.84$ (2H, $dd$, $J = 10$ and 2Hz), 4.76 (1H, $dd$, $J = 10$ and 2 Hz) and 4.30 (1H, $d$, $J = 8$ Hz). The large coupling constants of all the four anomeric protons of these sugars were typical of axial configurations of hexopyranoses in $^4C_1$ (D) conformations indicating $\beta$-glycosidic linkages [19].

In the light of the foregoing evidence the structure of oxystine (1) was proposed as 12-O-cinnamoyl desacylmetaplexigenin-3-O-$\beta$-D-cymaropy.anosyl $(1 \rightarrow 4)$-O-$\beta$-D-thevetopyranosyl $(1 \rightarrow 4)$-O-$\beta$-D-cymaropyranosyl $(1 \rightarrow 4)$-O-$\beta$D-digitoxopyranoside.

## EXPERIMENTAL

The general procedures were the same as those reported earlier [20] except $^{13}$C NMR: 400 MHz, CDCl$_3$.

*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 [2]. Repeated CC of the Et$_2$O extract (2.5 g) over silica gel using CHCl$_3$–MeOH (96:4) as eluent afforded oxystine (62 mg).

*Oxystine* (1). Mp 145–150° (MeOH), $[\alpha]_D^{25} - 10.90°$ (MeOH, $c$ 0.10). (Found C, 62.86; H, 7.80; C$_{57}$H$_{84}$O$_{20}$ requires C, 63.16; H, 7.85%). It gave a pink colour in the xanthydrol and a blue colour in the Keller–Kiliani reactions. $^1$H NMR (400 MHz): $\delta 7.98–7.95$ (2H, $m$, aromatic), 7.60 (1H, $d$, $J = 16$ Hz), 7.55–7.35 (3H, $m$, aromatic), 6.29 (1H, $d$, $J = 16$ Hz), 5.37 (1H, $m$, H-6), 4.84 (2H, $dd$, $J = 10$ and 2 Hz, H-1′), 4.76 (1H, $dd$, $J = 10$ and 2 Hz, H-1′), 4.65 (1H, $dd$., $J = 6$ and 11 Hz, H-12), 4.30 (1H, $d$, $J = 8$ Hz, H-1′), 3.93–3.74 (4H, $m$, H-5′), 3.65 (3H, $s$, OMe), 3.53–3.48 (1H, $t$, H-2′ in normal sugar), 3.45 (3H, $s$, OMe), 3.43 (3H, $s$, OMe), 3.40–3.20 (4H, $m$, H-3′), 3.25–3.15 (4H, $m$, H-4′), 2.22 (3H, $s$, $-$COMe), 2.19–1.86 (3H, $m$, H-2′e), 1.70–1.52 (3H, $m$, H-2′a), 1.38 (3H, $s$, 18-Me), 1.30 (3H, $d$, $J = 6$ Hz, sec 6′-Me), 1.29 (3H, $d$, $J = 6$ Hz, sec 6′-Me), 1.27 (3H, $d$, $J = 6$ Hz, sec 6′-Me), 1.20 (3H, $d$, $J = 6$ Hz, sec 6′-Me), 1.13 (3H, $s$, 19-Me).

$^{13}$C NMR (400 MHz): $\delta 38.7$ ($t$, C-1), 29.3 ($t$, C-2), 77.3 ($d$, C-3), 38.7 ($t$, C-4), 139.7 ($s$, C-5), 117.7 ($d$, C-6), 34.1 ($t$, C-7), 74.2 ($s$, C-8), 43.7 ($d$, C-9), 35.3 ($s$, C-10), 27.1 ($t$, C-11), 74.6 ($d$, C-12), 57.9 ($s$, C-13), 88.0 ($s$, C-14), 35.7 ($t$, C-15), 19.2 ($t$, C-16), 91.4 ($s$, C-17), 18.0 ($q$, C-18), 18.2 ($q$, C-19), 208.8 ($s$, C-20), 31.9 ($q$, C-21), 165.5 ($s$, C-1′′), 117.4 ($d$, C-2′′), 145.1 ($d$, C-3′′), 134.3 ($s$, C-4′′), 139.4 ($d$, C-5′′), 128.7 ($d$, C-6′′), 130.2 ($d$, C-7′′), 104.2 ($d$, C-1′), 96.0 ($d$, 2C-1′), 99.5 ($d$, C-1′), 57.9 ($q$, 2x OMe), 60.4 ($q$,-OMe).

*Mild hydrolysis of* 1 *with acid.* To a soln of 1 (25 mg) in 80% 1,4-dioxane (1 ml) was added 0.05 M H$_2$SO$_4$ (1 ml) and the soln was warmed for 30 min at 50°. Usual work-up as reported earlier [3] afforded crystalline genin 2 (8.5 mg), mp 143–145° (Et$_2$O), $[\alpha]_D^{25} + 66.0°$ (CHCl$_3$, $c$ 0.11) and a mixture of three sugars

which were separated through CC affording 3 (2.1 mg), $[\alpha]_D^{25}$ +49.41° (H$_2$O, $c$ 0.18), 4 (6.5 mg), $[\alpha]_D^{25} + 26.2°$ (H$_2$O, $c$ 0.16) and 5 (2.3 mg), $[\alpha]_D^{25} + 43.3°$ (MeOH, $c$ 0.12). All sugars gave positive colouration in the xanthydrol and Keller–Kiliani reactions. The specific rotation, TLC and PC comparison of 3 and 5 showed them to be identical to D-cymarose and D-digitoxose, respectively and 4 was identified as lilacinabiose by comparing its relative mobility calculated with the mobility of cymarose as reference which was further supported by comparison of $[\alpha]_D$ with that reported for lilacinabiose.

*Oxidation of* 3 *with bromine water.* A soln of 3 (2 mg) in H$_2$O (0.4 ml) was oxidized with Br$_2$ (7 $\mu$l) by the method reported earlier [2] yielding syrupy lactone 6 (1.8 mg). It gave a violet colouration with NH$_2$OH–FeCl$_3$ reagent.

*Oxidation of* 5 *with bromine water.* A soln of 5 (2 mg) in H$_2$O(0.4 ml) was mixed with Br$_2$ (7 $\mu$l) as in the oxidation of 3, affording syrupy lactone 7 (1.8 mg) which also gave a violet colouration with NH$_2$OH–FeCl$_3$ reagent.

D-*Cymaronic acid phenylhydrazide* (8). A soln of lactone 6 (1.8 mg) in absolute EtOH (0.05 ml) on heating with freshly distilled phenylhydrazine (0.04 ml) and usual work-up [2] yielded crystalline D-cymaronic acid phenylhydrazide (8) from MeOH–Et$_2$O (0.9 mg), mp 151–153°; lit. mp 155° [8].

D-*Digitoxonic acid phenylhydrazide* (9). A soln of lactone 7 (1.5 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) as in lactone 6, affording D-digitoxonic acid phenylhydrazide (9), crystallized from MeOH–Et$_2$O as colourless needles (1.0 mg), mp 120–123°; lit. mp 123° [9].

*Kiliani hydrolysis of* 4. Compound 4 (4.3 mg) was dissolved in Kiliani mixture (0.25 ml; AcOH–H$_2$O–HCl; 7:11:2), and heated at 100° for 1 hr and usual work-up [10] yielded 3-O-methyl-6-deoxy D-glucose (10) (1.9 mg), mp 115–120° (MeOH–petrol), $[\alpha]_D^{25} + 39.4°$ (H$_2$O, $c$ 0.11).

*Alkaline hydrolysis of* 2. Compound 2 (4 mg) was dissolved in 5% methanolic KOH (1 ml) and refluxed for 2 hr. Usual work-up as reported earlier [20] afforded 11 (3.0 mg) crystallized from MeOH–Me$_2$CO, mp 220–225°, $[\alpha]_D^{25} + 44.3°$ (MeOH, $c$ 0.11), which was identified as desacylmetaplexigenin by comparison with an authentic sample ($[\alpha]_D$, TLC, mmp).

*Methanolysis of* 2 *by the Zemplén method.* To a soln of 2 (2 mg) in absolute MeOH (0.5 ml) was added NaOMe (0.05 ml) and the mixture was kept at room temp. After 15 min it was neutralized with IR 120 H resin and filtered, MeOH was removed under red. pres. which yielded a residue (1.5 mg). MS: prominent ions in lower mass region at $m/z$ (rel. int.): 162 (55), 131 (100) and 103 (60).

NaBH$_4$ *reduction of* 2. Compound 2 (2 mg) was dissolved in MeOH (0.42 ml) and NaBH$_4$ (2 mg) was added to it. After keeping it at room temp. for 2 hr, the usual work-up [21] yielding 12 crystallized from Me$_2$CO–hexane (1.5 mg), mp 142–145°. It was identical with pneupogenin (mmp and cochrom on TLC).

*Very mild hydrolysis of* 1 *with acid.* To a soln of 1 (20 mg) in 80% aq. 1,4-dioxane (2.5 ml) was added 0.005 M H$_2$SO$_4$ (2.5 ml) and the soln was kept at room temp. After 4 days, TLC of the reaction mixture exhibited a spot due to cymarose (3) ($R_{Cym}$ 1.00, taken as reference) and two spots of mobilities ($R_{Cym}$ 1.95) and ($R_{Cym}$ 1.35) presumed to be triglycoside (13) and the unhydrolysed starting material 1, respectively. After six days two additional new spots, one comparable with the calculated mobility of lilacinabiose (4) ($R_{Cym}$ 0.65) and the other presumed to be monoglycoside (14) ($R_{Cym}$ 2.20). The hydrolysis was complete in 10 days when two additional new spots identical in mobilities with kidjolanin (2) ($R_{Cym}$ 1.5) and digitoxose (5) ($R_{Cym}$ 0.2) appeared. The reaction mixture was then worked-up followed by

CC affording a crystalline kidjolanin (2), mp 142–146° (Et$_2$O), $[\alpha]_D^{25}$ + 66.2° (CHCl$_3$, *c* 0.21) and three chromatographically pure reducing sugars as viscous syrups viz. 3 (2.0 mg), 4 (3.5 mg) and 5 (1.5 mg) identified as D-cymarose, lilacinabiose and D-digitoxose, respectively.

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