Journal of Natural Products
Vol. 48, No. 6, pp. 928-932, Nov-Dec 1985

A NOVEL PREGNANE ESTER TETRAGLYCOSIDE FROM *ORTHENTHERA VIMINEA*

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ABSTRACT.—A new pregnane ester tetraglycoside designated as orthenine (**1**) has been isolated from the dried twigs of *Orthenthera viminea*. Chemical and spectroscopic evidence is consistent with the structure 12-*O*-cinnamoyl sarcostin 3-*O*-α-L-oleandropyranosyl (1→4)-*O*-α-L-oleandropyranosyl (1→4)-*O*-α-L-oleandropyranosyl (1→4)-*O*-β-D-cymaropyranoside for orthenine.

In previous communications, we have reported the isolation and structural elucidation of four novel oligosaccharides (1-5) and pregnane ester glycosides (6,7) from the twigs of *Orthenthera viminea* W. & A. (Asclepiadaceae). As a continuation of studies on this plant, we are presenting here spectral and chemical evidence for the structure of a new oligoglycoside, designated as orthenine (**1**).

RESULTS AND DISCUSSION

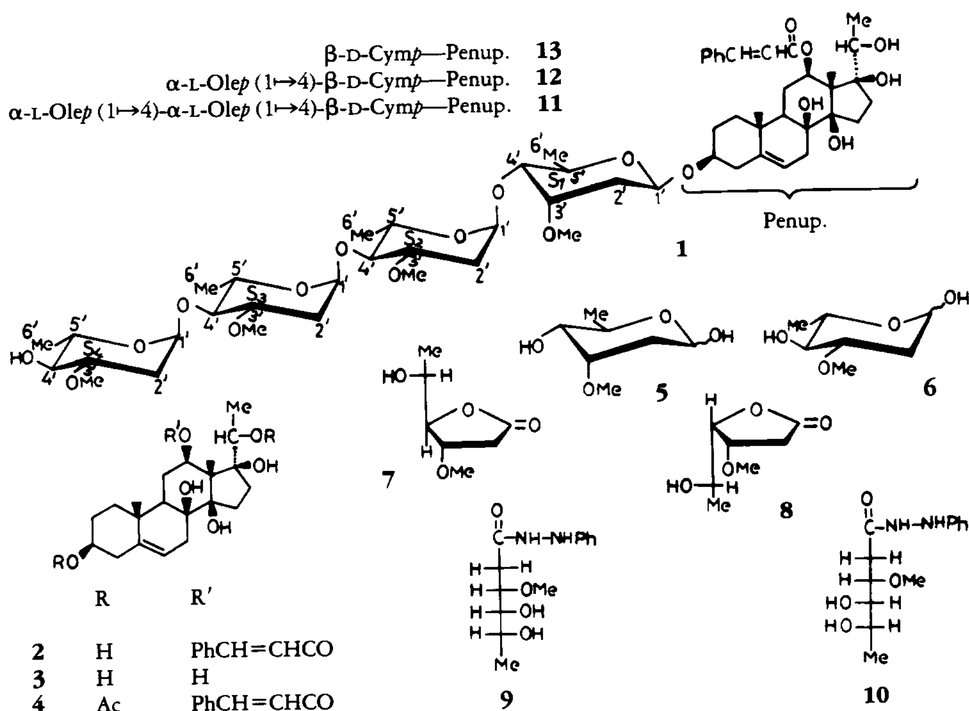
Orthenine (**1**) mp 120-124°, [α]D +115°, $C_{58}H_{88}O_{19}$, responded positively to the Liebermann Burchardt test (8), xanthydrol (9,10) and Keller-Kiliani reactions (11), indicating it to be a steroidal glycoside of a 2-deoxy sugar residue. The presence of a vicinal diol system in the molecule was indicated by its positive reaction with $NaIO_4$. In the ^{1}H-nmr spectrum of **1** at 400 MHz, the presence of characteristic methylene signals in the regions δ 2.36-2.07 (4H) and 2.02-1.80 (4H), in conjunction with four secondary methyl group doublets (J=6 Hz) at δ 1.35 (3H), 1.33 (6H), and 1.24 (3H) and four methoxy group singlets at δ 3.46, 3.45, 3.40, and 3.39, provided evidence that **1** is a tetraglycoside of 2,6-dideoxy hexoses.

Mild acid (0.025 M H_2SO_4) hydrolysis (12) of **1** afforded a crystalline genin **2** and a mixture of two sugars. The separated sugars **5** and **6** displayed characteristic color tests of 2-deoxy sugars and were identified as D-cymarose (13) (2,6-dideoxy-3-*O*-methyl-D-ribohexose) and L-oleandrose (14,15) (2,6-dideoxy-3-*O*-methyl-L-arabinohexose) (pc and [α]D). For further characterization, **5** and **6** were oxidized with bromine water to their lactones **7** and **8**, respectively, which on treatment with phenylhydrazine yielded known crystalline derivatives, i.e., D-cymaronic acid phenylhydrazide (**9**) (13) and L-oleandronic acid phenylhydrazide (**10**) (14,15). On the basis of the above results, **1** was inferred to be a tetraglycoside involving D-cymarose and L-oleandrose moieties.

The ability of the genin **2** to undergo methanolysis by the Zemplén method (16,17) indicated the presence of an ester group. The mass spectrum of the methanolysis products contained prominent ion peaks at m/z 162, 131, and 103 assigned to methyl cinnamate, confirming the presence of a cinnamoyl ester function in **2**. The hydrolyzate afforded a crystalline product **3**, mp 260-265°, [α]D +63°, identical in properties with sarcostin (18) (pregn-5-ene-3β, 8β, 12β, 14β, 17β, 20-*S*-hexol). The ease of reaction of genin **2** with sodium periodate suggested that its C-20 hydroxyl was not esterified. The genin **2** was thus identified as penupogenin (19) (12-*O*-cinnamoyl sarcostin) by mmp and comparison of its tlc with an authentic sample. Acetylation of **2** with Ac_2O in pyridine yielded the di-*O*-acetyl derivative **4**, $C_{34}H_{44}O_9$, mp 134-138°, also characterized from its ^{1}H-nmr spectrum.

More direct chemical support for **1** being a tetraglycoside of cymarose and oleandrose and determination of the sequence of the sugar units came from the results of its very mild acid (0.5 mM H_2SO_4) hydrolysis at room temperature which afforded par-

tially and completely hydrolyzed products. After 12 days, the reaction mixture exhibited the appearance of oleandrose (**6**) (pc, tlc) as the only sugar unit, with the formation of three new spots, presumably tri- (**11**), di- (**12**), and mono-glycosides (**13**), leading to the conclusion that the terminal three sugar units in **1** were oleandrose. An aliquot of this partially hydrolyzed mixture, when worked up, afforded the products **11** and **12** as a mixture and **13** as a crystalline product mp 85-90°, [α]D +19°, besides oleandrose (**6**) and some unreacted starting material **1**. After 17 days, two additional new spots (tlc) identical in mobilities with cymarose (**5**) and penupogenin (**2**) appeared, suggesting that cymarose was directly glycosidically linked to penupogenin. This was also corroborated by the mild acid hydrolysis of **13** exhibiting only two spots (tlc) identical in mobilities with cymarose (**5**) and penupogenin (**2**).



The eims of **1** did not exhibit an $[M]^+$, but the highest mass ion peak of the genin moiety, recorded at *m/z* 494.2702 was in agreement with formula $C_{30}H_{38}O_6$ corresponding to $[\text{genin fragment-}H_2O]^+$. The subsequent losses of a cinnamic acid and four water molecules from this ion giving ion peaks at *m/z* 346, 328, 310, 292, and 274 were in agreement with the presence of one cinnamoyl group and five hydroxyl groups in its genin moiety. The prominent ion peak at *m/z* 257.1402 of composition $C_{13}H_{21}O_5$ was attributed to a $[\text{disaccharide fragment ion (289)-MeOH}]^+$ ion presumably originating from the tetrasaccharide moiety of the glycoside. The low mass region contained the expected prominent cinnamic acid peak and its fragment ions at *m/z* 148, 131, and 103, as well as the common 2,6-dideoxymonomethoxy-hexose fragments (20) at *m/z* 145, 113, and 95.

The ^{1}H-nmr ($CDCl_3$) spectrum of **1** at 400 MHz not only confirmed that it was a tetraglycoside of 12-*O*-cinnamoyl sarcostin but also helped in ascertaining the configuration of the glycosidic linkages. For convenience, the one cymarose and three oleandrose units of **1** were designated, S_1, S_2, S_3, and S_4, respectively. A one proton double doublet appearing only as broad doublet at δ 4.88 (J=3 Hz) and two double doublets at

δ 4.86 (2H, J=3 and 1 Hz) and 4.51 (1H, J=9.5 and 1.5 Hz) could be assigned to four anomeric protons of the four sugars. The small coupling constants (3 Hz) of three of these anomeric protons, typical of their equatorial configuration in a 2-deoxyhexopyranose moiety in the $^{1}C_4$ (L) conformation (21), were attributed to the S_2, S_3, and S_4 L-oleandrose units linked through α-L-(1↦4) glycosidic bonds. The large coupling constant (9.5 Hz) of the fourth anomeric proton, typical of an axial configuration, suggested D-cymaropyranose moiety in a $^{4}C_1$ (D) conformation (21) joined to the aglycone through a β-D-glycosidic linkage. The ^{1}H-nmr spectrum also contained appropriate proton signals for the genin moiety consisting of 12-*O*-cinnamoyl sarcostin (see Experimental section).

In light of the foregoing evidence, the structure of orthenine (**1**) was established as 12-*O*-cinnamoyl sarcostin 3-*O*-α-L-oleandropyranosyl (1↦4)-*O*-α-L-oleandropyranosyl (1↦4)-*O*-α-L-oleandropyranosyl (1↦4)-*O*-β-D-cymaropyranoside.

EXPERIMENTAL

Mps were determined on a Boetius micromelting point apparatus and are uncorrected. All [α]D values were measured in a 1-dm tube with a Jasco-Dip 180 automatic polarimeter. The ^{1}H-nmr spectra were recorded on 400 MHz (Bruker), 90 MHz (Perkin-Elmer R 32) and 80 MHz (CFT-20, proton probe) spectrometers in $CDCl_3$, with TMS as the internal standard. Mass spectra were recorded with AEI MS-30 and JEOL High Resolution JMS-300 mass spectrometers. Sugars were made visible with 50% aqueous H_2SO_4 in tlc (silica gel G, BDH) and vanillin-$HClO_4$ reagent in pc. Pc (Whatman No. 1) was performed using $C_6H_5CH_3$-*n*-BuOH (4:1) saturated with H_2O as developing solvent. Column chromatography was performed using silica gel (BDH, 60-120 mesh).

PLANT EXTRACTION.—Shade-dried, powdered twigs (10 kg) of *O. viminea* (Voucher No. 68018, deposited in the National Botanical Research Institute, Lucknow, India) were extracted and fractionated with solvents of different polarities, as reported earlier (2). The residue from the $CHCl_3$-EtOH extract, 4:1 (9 g) and $CHCl_3$-EtOH extract, 3:2 (3 g) rich in glycosides, were combined and hydrolyzed with 25 ml H_2SO_4 in 50% MeOH, in order to obtain the genins, partially hydrolyzed glycosides, and sugars. Repeated column chromatography of the mixture of partially hydrolyzed glycosides using $CHCl_3$-MeOH (98:2) as eluent afforded orthenine (65 mg).

ORTHENINE (**1**).—Orthenine: mp 120-124° (Me_2CO-hexane), $[\alpha]^{25}D$ +115.6° (c=0.12, MeOH). It gave a pink color in xanthydrol and blue in Keller-Kiliani reactions, and underwent $NaIO_4$ oxidation. ^{1}H nmr (400 MHz) δ 7.95-7.92 (2H, m, aromatic), 7.77 (1H, d, J=16 Hz), 7.44-7.30 (3H, m, aromatic), 6.46 (1H, d, J=16 Hz), 5.41-5.37 (1H, m, H-6), 4.88 (1H, br. d, J=3 Hz, H-1′ of Ole), 4.86 (2H, dd, J=3 and 1 Hz, H-1′ of Ole), 4.76 (1H, dd, J=11.5 and 4.5 Hz, H-12), 4.51 (1H, dd, J=9.5 and 1.5 Hz, H-1′ of Cym), 3.93-3.84 (4H, m, H-5′ of S_1, S_2, S_3 and S_4), 3.64 (1H, m; D_2O shake q, J=6 Hz, H-20), 3.62-3.51 (4H, m, H-3′ of S_1, S_2, S_3 and S_4), 3.46 (3H, s, OMe), 3.45 (3H, s, OMe), 3.40 (3H, s, OMe), 3.39 (3H, s, OMe), 3.27-3.14 (4H, m, H-4′ of S_1, S_2, S_3 and S_4), 2.36-2.07 (4H, m, H-2′e of S_1, S_2, S_3 and S_4), 2.02-1.80 (4H, m, H-2′a of S_1, S_2, S_3 and S_4), 1.35 (3H, d, J=6 Hz, 6′-Me), 1.33 (6H, d, J=6 Hz, 6′-Me), 1.24 (3H, d, J=6 Hz, 6′-Me), 1.23 (3H, s, 18-Me), 1.18 (3H, s, 19-Me), 1.11 (3H, d, J=6 Hz, 21-Me); ms m/z (rel. int.) $[M]^+$ (not observed), 494.2702 (0.42) $[M\text{-sugars-}H_2O]^+$ ($C_{30}H_{38}O_6$), 476.2563 (0.92) $[494\text{-}H_2O]^+$ ($C_{30}H_{36}O_5$), 458.2472 (0.87) $[476\text{-}H_2O]^+$ ($C_{30}H_{34}O_4$), 364.2284 (0.47) $[M\text{-sugars-PhCH=CHCO}_2H]^+$ ($C_{21}H_{32}O_5$), 346.2148 (2.59) $[364\text{-}H_2O]^+$ ($C_{21}H_{30}O_4$), 328.2038 (6.48) $[346\text{-}H_2O]^+$ ($C_{21}H_{28}O_3$), 310.1936 (5.63) $[328\text{-}H_2O]^+$ ($C_{21}H_{26}O_2$), 292.1831 (4.07) $[310\text{-}H_2O]^+$ ($C_{21}H_{24}O$), 274.1722 (0.85) $[292\text{-}H_2O]^+$ ($C_{21}H_{22}$), 148.0523 (13.75) ($C_9H_8O_2$), 131.0499 (18.72) (C_9H_7O), 103.0550 (9.99) (C_8H_7); sugar fragments: 257.1402 (8.02) $[\text{tetrasaccharide ion-two sugars-MeOH}]^+$ ($C_{13}H_{21}O_5$), 239.1319 (1.59) $[257\text{-}H_2O]^+$ ($C_{13}H_{19}O_4$), 181.0895 (1.20) $[257\text{-MeCHO-MeOH}]^+$ ($C_{10}H_{13}O_3$), 145.0873 (100.00) ($C_7H_{13}O_3$), 113.0606 (40.93) ($C_6H_9O_2$) and 95.0500 (9.29) (C_6H_7O). *Anal.* calcd. for $C_{58}H_{88}O_{19}$: C, 63.97; H, 8.09. Found: C, 64.11; H, 7.95%.

MILD ACID HYDROLYSIS OF ORTHENINE (**1**).—To a solution of **1** (15 mg) in 80% aqueous dioxane (1 ml) was added 0.05M H_2SO_4 (1 ml), and the solution was warmed for 30 min at 50°; then concentrated under reduced pressure to remove dioxane. The aqueous portion was repeatedly extracted with $CHCl_3$-MeOH (99:1), and the organic layer was washed in sequence with H_2O, 2N Na_2CO_3, and again with H_2O, dried over Na_2SO_4, and evaporated to afford genin (**2**) which crystallized from Me_2CO-hexane as colorless needles (5 mg) mp 140-145°. It underwent $NaIO_4$ oxidation and was identified as penupogenin [12-*O*-cinnamoyl sarcostin, lit (19) mp 145-150°] by mmp and tlc comparison with the authentic sample;

^{1}H nmr (90 MHz) δ 7.60-7.30 (5H, m, aromatic), 7.75 (1H, d, J=16 Hz), 6.42 (1H, d, J=16 Hz), 5.42-5.22 (1H, m, H-6), 4.71 (1H, dd, J=11 and 4.5 Hz, H-12), 3.63 (1H, q, J=6.5 Hz, H-20), 1.14 (6H, s, 18-Me and 19-Me) and 1.04 (3H, d, J=6 Hz, 21-Me).

The aqueous hydrolyzate was neutralized with freshly prepared $BaCO_3$, filtered, and concentrated under reduced pressure to afford a mixture of two sugars which were isolated through column chromatography affording **5** (1.8 mg), $[\alpha]^{25}$D +49.5° (c=0.11, H_2O) and **6** (5.2 mg), $[\alpha]^{25}$D +14.1° (c=0.14, H_2O). Both gave a positive coloration in the xanthydrol and Keller-Kiliani reactions. The $[\alpha]$D, tlc, and pc comparisons of **5** and **6** showed them to be identical to D-cymarose [lit (13) $[\alpha]$D +55° (H_2O)] and L-oleandrose [lit (14, 15) $[\alpha]$D +12° (H_2O)], respectively.

DI-*O*-ACETYLPENUPOGENIN (**4**).—Crystalline **2** (2 mg) dissolved in anhydrous C_5H_5N (0.2 ml) was mixed with Ac_2O (0.2 ml), and the mixture was kept for 48 h at room temperature. After the usual work-up of the reaction mixture, it afforded the acetylated product **4** (1.7 mg) which crystallized from Me_2CO-hexane, mp 134-138°. ^{1}H nmr (80 MHz) δ 1.90 (3H, s, OAc), 1.98 (3H, s, OAc), 4.85-4.45 (3H, m, H-3, H-12 and H-20). *Anal.* calcd. for $C_{34}H_{44}O_9$: C, 68.46; H, 7.38. Found: C, 68.73; H, 7.21%.

HYDROLYSIS OF **2** BY ZEMPLÉN METHOD.—To a solution of **2** (3 mg) in absolute MeOH (1 ml) was added sodium methoxide (0.15 ml), and the mixture was kept at room temperature; when the reaction was complete (tlc), it was neutralized with IR 120 H resin and filtered. MeOH was removed under reduced pressure yielding a viscous product (2.3 mg), which gave ion peaks at m/z 162, 131, and 103 in the lower mass region of its ms. The chromatographic separation of the hydrolyzate afforded product **3** (1.5 mg) which crystallized from MeOH-Me_2CO, mp 260-265°, $[\alpha]^{25}$D +62.5° (c=0.11, MeOH). The mmp, tlc, and $[\alpha]$D comparisons with the authentic material confirmed **3** as sarcostin [lit (18) mp 150°/260-263°, $[\alpha]$D +67° (MeOH)].

OXIDATION OF CYMAROSE (**5**) WITH Br_2 WATER.—A solution of **5** (1.8 mg) in H_2O (0.4 ml) was mixed with Br_2 (6 μl) and shaken in a stoppered flask in the dark for 24 h at room temperature. The excess of Br_2 was then removed under reduced pressure, the acidic mixture was made neutral with freshly precipitated Ag_2CO_3, and the suspension was filtered. H_2S was passed through the filtrate to remove Ag^+ ions, and the suspension was again filtered. The filtrate was evaporated to dryness under reduced pressure yielding syrupy lactone **7** (1.2 mg) showing a violet spot with NH_2OH-$FeCl_3$ spray reagent.

OXIDATION OF OLEANDROSE (**6**) WITH Br_2 WATER.—A solution of **6** (3 mg) in H_2O (0.6 ml) was mixed with Br_2 (12 μl) as in the oxidation of **5** affording syrupy lactone **8** (2.2 mg) showing a violet spot with NH_2OH-$FeCl_3$ spray reagent.

D-CYMARONIC ACID PHENYLHYDRAZIDE (**9**).—A solution of **7** (1.2 mg) in absolute EtOH (0.04 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml), and the mixture was heated for 30 min at 100°. The viscous mass was cooled and repeatedly triturated with absolute Et_2O (to remove excess of phenylhydrazide), yielding a D-cymaronic acid phenylhydrazide (**9**) which crystallized from MeOH-Et_2O as colorless needles (0.6 mg) mp 150-153° [lit (13) mp 155°].

L-OLEANDRONIC ACID PHENYLHYDRAZIDE (**10**).—A solution of **8** (2 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) and heated as for **7**, affording L-oleandronic acid phenylhydrazide (**10**) which crystallized from MeOH-Et_2O as colorless needles (1.2 mg) mp 135-136° [lit (14, 15) mp 136°].

VERY MILD ACID HYDROLYSIS OF ORTHENINE (**1**).—To a solution of **1** (25 mg) in 80% aqueous dioxane (3 ml) was added 1 mM H_2SO_4 (3 ml), and the solution was kept at room temperature. After 12 days, tlc of the reaction mixture exhibited a spot due to oleandrose (**6**) (Rf 1.00, taken as reference), and three more spots of mobilities Rf 2.00, 2.08, and 2.42 presumed to be tri (**11**), di (**12**), and monoglycosides (**13**), respectively. An aliquot (3 ml) of this partially hydrolyzed reaction mixture was removed, neutralized with IRA 400 (OH), and evaporated under reduced pressure to afford a viscous mass (12 mg) which was separated on a silica gel column giving a mixture (2 mg) of **11** and **12**; **13** (4 mg), crystallized from MeOH-Et_2O, mp 85-90°, $[\alpha]^{25}$D +19.3° (c=0.11, MeOH), oleandrose (**6**) (1.0 mg) and **1** (2 mg). On the 17th day, two additional new spots identical in mobilities with cymarose (**5**) (Rf 1.5) and penupogenin (**2**) (Rf 1.53) appeared.

ACID HYDROLYSIS OF **13**.—To a solution of **13** (1 mg) in 80% aqueous dioxane (0.1 ml) was added 0.05 M H_2SO_4 (0.1 ml), and the solution was warmed for 30 min at 50°. The reaction mixture exhibited the spots of cymarose (**5**) and penupogenin (**2**) (pc, tlc).

ACKNOWLEDGMENTS

We are thankful to Prof. H. Mitsuhashi, Hokkaido University, Sapporo, Japan, for kindly supplying

an authentic sample of penupogenin and Dr. D.K. Kulshreshtha (CDRI, Lucknow) for helpful discussions. One of us (K.J.K.) thanks the UGC, New Delhi, for the award of a junior research fellowship.

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Received 28 March 1985