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A PREGNANE ESTER AND ITS GLYCOSIDE FROM *ORTHENTHERA VIMINEA*

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Key Word Index—*Orthenthera viminea*; Asclepiadaceae; ornogenin; ornine; steroid; pregnane ester and its diglycoside.

Abstract—A new pregnane ester named ornogenin and its diglycoside designated as ornine were isolated from the dried twigs of *Orthenthera viminea*. On the basis of chemical and spectroscopic evidence, their structures were established as 12,20-di-*O*-cinnamoyl-sarcostin and 12,20-di-*O*-cinnamoyl-sarcostin-3-*O*-α-L-oleandropyranosyl(1 → 4)-*O*-β-D-cymaropyranoside, respectively.

INTRODUCTION

In previous communications, we have reported the isolation and structural elucidation of four novel oligosaccharides [1–5] from the twigs of *Orthenthera viminea*. As a continuation of the studies on this plant, we are presenting here the spectral and chemical evidence for the structure of a novel pregnane ester ornogenin (**1**) and its diglycoside ornine (**4**).

RESULTS AND DISCUSSION

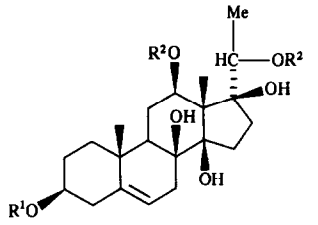
Ornogenin (**1**), $C_{39}H_{46}O_8$, was isolated by column chromatography of the partially hydrolysed glycosides and genins mixture. Alkaline hydrolysis of **1** afforded **3**, $C_{21}H_{34}O_6$, identified as sarcostin [6] (mmp, TLC), suggesting the presence of an ester function in the molecule. The mass spectrum of the methanolysis [7, 8] product of **1** contained prominent ion peaks at *m/z* 162, 131 and 103 in its lower mass region confirming the cinnamoyl ester function in **1**. The formula difference $C_{18}H_{12}O_2$ of **1** and **3** suggested **1** to be di-*O*-cinnamoyl sarcostin. This conclusion was also supported by the fact that acetylation of **1** with acetic anhydride in pyridine yielded the mono-*O*-acetyl derivative **2**, $C_{41}H_{48}O_9$, also characterized from its ^{1}H NMR spectrum. Although **1** was inert to $NaIO_4$, its deacylated product **3** reacted with this reagent. It is, therefore, evident that one of the cinnamoyl ester groups is at C-20 while the other cinnamoyl group could be at the C-12 or C-3 position in sarcostin.

The mass spectrum of **1** failed to display its molecular ion but contained the highest mass ion peak at *m/z* 494 which could be interpreted as $[M - C_6H_5CH{=}CHCOOH]^+$ which was supported by an intense peak at *m/z* 131 for the cinnamoyl group $[C_6H_5CH{=}CHCO]^+$ and the other cinnamic acid was represented by the ion peak at *m/z* 346 $[M - 2C_6H_5CH{=}CHCOOH]^+$ followed by the loss of four water molecules giving fragment ion peaks at *m/z* 328, 310, 292 and 274 which were in agreement with the two cinnamoyl groups and four hydroxyl groups of its sarcostin moiety. The fragment ion at *m/z* 468 consisting of rings C and D after the loss of 138 a.m.u. (originating by RDA fission at Δ^5), can only be explained by the presence of two cinnamoyl ester groups in rings C and D at C-12 and C-20, respectively, of the sarcostin moiety. This led to the conclusion that **1** was 12,20-di-*O*-cinnamoyl sarcostin. The 400 MHz ^{1}H NMR spectrum of **1** was also in full agreement with the derived structure (see Experimental).

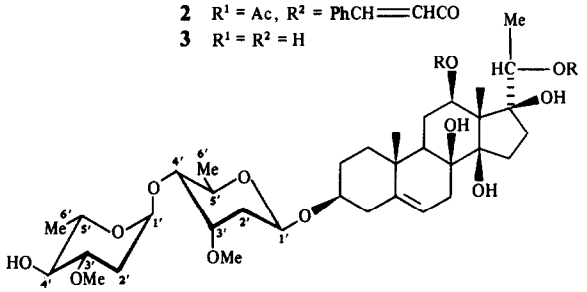
On the basis of the chemical and spectroscopic evidence, the structure of ornogenin (**1**) has been established as 12,20-di-*O*-cinnamoyl sarcostin. Ornine (**4**), $C_{53}H_{70}O_{14}$, gave characteristic tests in the xanthydrol [9, 10] and Keller–Kiliani [11] reactions indicating it to be a glycoside of 2-deoxy sugars. Mild acid (0.025 M H_2SO_4) hydrolysis [12] of **4** afforded a genin, which was found to be identical with the ornogenin (**1**) ($[\alpha]_D$, TLC, mmp), and sugars **5** and **6** displaying characteristic colour tests of 2-deoxy sugars, 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 $[\alpha]_D$). For further characterization, **5** and **6** were oxidized with bromine water to their lactones **7** and **8**, respectively, which on treatment with phenyl hydrazine 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, compound **4** was inferred to be a diglycoside of ornogenin (**1**) with D-cymarose and L-oleandrose.

A more direct chemical support for **4** being a diglycoside of cymarose and oleandrose and their sequence came from its very mild acid hydrolysis (2.5 mM H_2SO_4) and monitoring the reaction on PC. After four days, the reaction mixture exhibited the spots of oleandrose (**6**), unreacted **4** and what was presumed to be a monoglycoside. The hydrolysis was complete in seven days and working up of the hydrolysate afforded ornogenin (**1**), cymarose (**5**) and oleandrose (**6**).

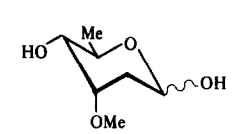
As oleandrose was the first sugar liberated in the partial hydrolysis of **4**, it was the terminal sugar and the other sugar, cymarose, was concluded to be glycosidically linked to the C-3 hydroxyl of the 12,20-di-*O*-cinnamoyl sarcostin moiety. The mass spectrum of **4** did not exhibit an $[M]^+$



1 $R^1 = H$, $R^2 = PhCH{=}CHCO$
2 $R^1 = Ac$, $R^2 = PhCH{=}CHCO$
3 $R^1 = R^2 = H$



4 $R = PhCH{=}CHCO$



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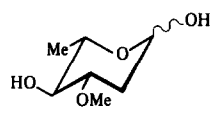

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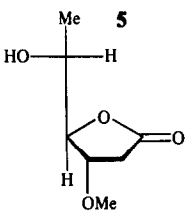

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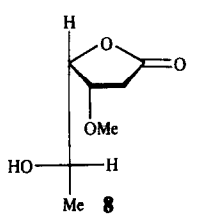

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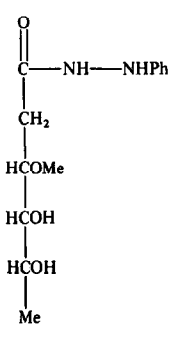

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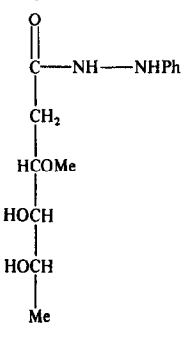

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but the highest mass ion peak recorded at m/z 494.2639 was in agreement with the formula $C_{30}H_{38}O_6$ which corresponded to [M − cinnamic acid − disaccharide unit]$^+$, supplemented with the mass ion peak at m/z 257.1396 of composition $C_{13}H_{21}O_5$ which originated by the loss of methanol from the disaccharide ion. The subsequent loss of one more cinnamic acid residue from the ion m/z 494.2639 gave a peak at m/z 346.2144 corresponding to formula $C_{21}H_{30}O_4$ which evidently represented the basic sarcostin moiety of the molecule with its four oxygens as hydroxyl functions which is in conformity with the subsequent loss of four water molecules from this ion giving peaks at m/z 328, 310, 292 and 274. The low mass region contained the expected prominent cinnamic acid and its fragment ions at m/z 148, 131 and 103 besides the common 2,6-dideoxy monomethoxy hexose fragments [16] at m/z 145, 113 and 95.

The ^{1}H NMR spectrum of the glycoside **4** at 400 MHz not only confirmed the derived structure but also established the configuration of the glycosidic linkages. The small coupling constant (3 Hz) of the anomeric proton at δ4.85 was attributed to its equatorial configuration indicating an α-glycosidic linkage commonly occurring with L-sugars in natural glycosides and assigned to the end L-oleandropyranose unit present in $^1C_4$ (L) conformation and linked through an α-L (1 → 4) glycosidic linkage [17] to cymarose. The large coupling constant (J = 9 Hz) of the other anomeric proton at δ4.50 was, therefore, attributed to its axial configuration indicating D-cymaropyranose present in $^4C_1$ (D) conformation and joined to the aglycone through a β-D-glycosidic linkage. The ^{1}H NMR spectrum also contained other appropriate proton signals present in **4** (see Experimental).

The above considerations led us to assign the structure of ornine (**4**) as 12,20-di-*O*-cinnamoyl sarcostin-3-*O*-α-L-oleandropyranosyl(1 → 4)-*O*-β-D-cymaropyranoside.

EXPERIMENTAL

Mps (Boetius micromelting point apparatus) uncorr; ^{1}H NMR: 400 MHz (Bruker), 90 MHz (Perkin–Elmer R-32) and 80 MHz (CFT-20, proton probe), $CDCl_3$, TMS as internal standard. Sugars were visualized with 50% aq. H_2SO_4 (TLC) and vanillin–$HClO_4$ reagent (PC). PC was performed using toluene–BuOH (4:1) satd with H_2O.

Plant extraction. Shade-dried powdered twigs (10 kg) of *O. viminea* were extracted and fractionated with solvents of different polarities, as reported earlier [4]. The residue from the $CHCl_3$–EtOH (4:1), 9 g, and $CHCl_3$–EtOH (3:2), 3 g, rich in glycosides (xanthydrol test positive), were combined and hydrolysed with 25 mM H_2SO_4 in 50% MeOH, in order to obtain the genins, partially hydrolysed glycosides and sugars. Repeated CC of this mixture of genins and partially hydrolysed glycosides using $CHCl_3$–MeOH (98:2) as eluent afforded ornine (50 mg) and ornogenin (40 mg).

Ornogenin (**1**). Mp 75–80° (MeOH–petrol), $[\alpha]_D^{25}$ + 111.4° (MeOH, *c* 0.12). (Found C, 73.15; H, 6.95; $C_{39}H_{46}O_8$ requires C, 72.89; H, 7.17%.) ^{1}H NMR (400 MHz): δ7.96–7.92 (4H, *m*, aromatic), 7.55 (1H, *d*, J = 16 Hz), 7.45 (1H, *d*, J = 16 Hz), 7.42–7.29 (6H, *m*, aromatic), 6.46 (1H, *d*, J = 16 Hz), 6.29 (1H, *d*, J = 16 Hz), 5.39 (1H, *m*, H-6), 4.89 (1H, *q*, J = 6 Hz, H-20), 4.82 (1H, *dd*, J = 11 and 4 Hz, H-12), 3.61–3.50 (1H, *m*, H-3), 1.36 (3H, *d*, J = 6 Hz, 21-Me), 1.26 (3H, *s*, 18-Me), 1.13 (3H, *s*, 19-Me). MS m/z (rel. int.): [M]$^+$ (not observed), 494 [M − PhCH=CHCO$_2$H]$^+$ (8), 476 [494 − H_2O]$^+$ (8), 458 [494 − 2H_2O]$^+$ (7), 440 [494 − 3H_2O]$^+$ (5), 422 [494 − 4H_2O]$^+$ (4.8), 346 [494 − PhCH=CHCO$_2$H]$^+$ (34), 328 [346 − H_2O]$^+$ (76), 310 [346 − 2H_2O]$^+$ (39), 292 [346 − 3H_2O]$^+$ (22), 274 [346 − 4H_2O]$^+$ (10), 450 [M − MeCHOCOCH=CHPh–OH]$^+$ (10), 432 [450 − H_2O]$^+$ (11), 417 [432 − Me]$^+$ (4), 468 [M − 138 − 2H_2O]$^+$ (15), 172 [468 − 2PhCH=CHCO$_2$H]$^+$ (23), 120 [138 − H_2O]$^+$ (20), 105 [120 − Me]$^+$ (62), 131 [PhCH=CHCO]$^+$ (100), 103 [C_8H_7]$^+$ (34).

Mono-O-acetyl ornogenin (**2**). A soln of **1** (10 mg) in pyridine (0.5 ml) and Ac_2O (0.4 ml) was kept for 48 hr at room temp. Usual work up yielded mono-*O*-acetyl ornogenin (**2**) which crystallized as colourless plates (5.8 mg) from Me_2CO–hexane,

mp 94–97°, $[\alpha]_D^{25}$ + 188° (MeOH, *c* 0.21). (Found C, 72.12; H, 6.89; $C_{41}H_{48}O_9$ requires C, 71.93; H, 7.02%.) ^{1}H NMR (80 MHz): δ7.95–7.23 (12H, *m*, aromatic 10H + 2PhCH=CH), 6.40 (1H, *d*, *J* = 16 Hz), 6.25 (1H, *d*, *J* = 16 Hz), 5.35 (1H, *m*, H-6), 4.50–4.45 (3H, *m*, H-3, H-12 and H-20), 1.98 (3H, *s*, OAc), 1.25–1.12 (9H, *br* signal, 19-Me, 18-Me and 21-Me). MS *m/z* (rel. int.): $[M]^+$ (not observed), 536 $[M - PhCH{=}CHCO_2H]^+$ (10), 476 $[536 - HOAc]^+$ (6), 328 $[476 - PhCH{=}CHCO_2H]^+$ (30), 310 $[328 - H_2O]^+$ (30), 292 $[310 - H_2O]^+$ (20), 274 $[292 - H_2O]^+$ (6).

Alkaline hydrolysis of compound **1**. Compound **1** (5 mg) was dissolved in 5% methanolic KOH (1 ml) and refluxed for 2 hr. After adding H_2O (0.5 ml), MeOH was removed under red. pres. The aq. concentrate was extracted with $CHCl_3$–MeOH (90:10), dried over Na_2SO_4, filtered and evapd to dryness yielding **3** (2.3 mg) which crystallized from MeOH–Me_2CO, mp 262–264°, $[\alpha]_D^{25}$ + 63.4° (MeOH, *c* 0.09). It was identified as sarcostin by comparison with an authentic sample ($[\alpha]_D$, TLC, mmp)].

Methanolysis of **1** *by the Zemplén method*. To a soln of **1** (2 mg) in absolute MeOH (0.5 ml) was added $NaOCH_3$ (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. and yielded a residue (1.5 mg). MS *m/z* (rel. int.): 162 (55), 131 (100) and 103 (60).

Ornine (**4**). Mp 124° (Me_2CO–petrol); $[\alpha]_D^{25}$ + 141.3° (MeOH, *c* 0.2). (Found C, 68.60; H, 7.40; $C_{53}H_{70}O_{14}$ requires C, 68.39; H, 7.53%.) It gave a pink colour in the xanthydrol and a blue colour in the Keller–Kiliani reactions. ^{1}H NMR (400 MHz): δ7.96–7.92 (4H, *m*, aromatic), 7.55 (1H, *d*, *J* = 16 Hz), 7.44 (1H, *d*, *J* = 16 Hz), 7.40–7.29 (6H, *m*, aromatic), 6.47 (1H, *d*, *J* = 16 Hz), 6.29 (1H, *d*, *J* = 16 Hz), 5.39 (1H, *m*, H-6), 4.88 (1H, *q*, *J* = 6 Hz, H-20), 4.85 (1H, *br d*, *J* = 3 Hz, H-1′ in Ole), 4.82 (1H, *dd*, *J* = 11 and 4.5 Hz, H-12), 4.50 (1H, *dd*, *J* = 9 and 1.5 Hz, H-1′ in Cym), 3.93–3.84 (2H, *m*, H-5′ in Cym and Ole), 3.61–3.45 (2H, *m*, H-3′ in Cym and Ole), 3.53 (3H, *s*, OMe), 3.42 (3H, *s*, OMe), 3.35–3.23 (2H, *m*, H-4′ in Cym and Ole), 2.49–2.15 (2H, *m*, H-2′e in Cym and Ole), 2.15–1.70 (2H, *m*, H-2′a in Cym and Ole), 1.36 (3H, *d*, *J* = Hz, 21-Me), 1.33 (3H, *d*, *J* = 6 Hz, sec 6′-Me), 1.27 (3H, *s*, 18-Me), 1.24 (3H, *d*, *J* = 6 Hz, sec 6′-Me), 1.22 (3H, *s*, 19-Me). MS *m/z* (rel. int.): $[M]^+$ (not observed), 494.2639 $[M - sugars - PhCH{=}CHCO_2H]^+$ (0.21) ($C_{30}H_{38}O_6$), 476.2565 $[494 - H_2O]^+$ (1.66) ($C_{30}H_{36}O_5$), 458.2466 $[476 - H_2O]^+$ (2.27) ($C_{30}H_{34}O_4$), 346.2144 $[494 - PhCH{=}CHCO_2H]^+$ (2.86) ($C_{21}H_{30}O_4$), 328.2041 $[346 - H_2O]^+$ (9.37) ($C_{21}H_{28}O_3$), 310.1938 $[328 - H_2O]^+$ (10.04) ($C_{21}H_{26}O_2$), 292.1831 $[310 - H_2O]^+$ (5.36) ($C_{21}H_{24}O$), 274.1720 $[292 - H_2O]^+$ (1.63) ($C_{21}H_{22}$); sugar fragments: 257.1396 [disaccharide ion $- MeOH]^+$ (7.44) ($C_{13}H_{21}O_5$), 145.0874 (100) ($C_7H_{13}O_3$), 113.0604 (48.75) ($C_6H_9O_2$) and 95.0499 (8.97) (C_6H_7O).

Mild hydrolysis of **4** *with acid*. To a soln of **4** (25 mg) in 80% 1,4-dioxane (1 ml) was added 0.05 M H_2SO_4 (1 ml) and the soln was warmed for 30 min at 50°. Usual work up as reported earlier [18] afforded crystalline genin **1** (15.2 mg), mp 72–78° (MeOH–petrol), $[\alpha]_D^{25}$ + 111° (MeOH, *c* 0.21) and a mixture of two sugars which were separated through CC affording **5** (3 mg), $[\alpha]_D^{25}$ + 50.7° (H_2O, *c* 0.19) and **6** (3.2 mg), $[\alpha]_D^{25}$ + 13.2° (H_2O, *c* 0.12). Both sugars gave positive colouration in the xanthydrol and Keller–Kiliani reactions. The specific rotation, TLC and PC comparison of **5** and **6** showed them to be identical to D-cymarose and L-oleandrose, respectively.

Oxidation of **5** *with bromine water*. A soln of **5** (2.5 mg) in H_2O (0.4 ml) was oxidized with Br_2 (7 μl) by the method reported earlier [3] yielding syrupy lactone **7** (2 mg). It gave a violet colouration with NH_2OH–$FeCl_3$ reagent.

Oxidation of **6** *with bromine water*. A soln of **6** (3 mg) in H_2O (0.6 ml) was mixed with Br_2 (11 μl) as in the oxidation of **5**, affording syrupy lactone **8** (2.2 mg), which also gave a violet colouration with NH_2OH–$FeCl_3$ reagent.

D-*Cymaronic acid phenylhydrazide* (**9**). A soln of lactone **7** (2 mg) in absolute EtOH (0.05 ml) on heating with freshly distilled phenylhydrazine (0.04 ml) and usual work up [4] yielded crystalline D-cymaronic acid phenylhydrazide (**9**) from MeOH–Et_2O (1 mg), mp 152–153°; lit. mp 155°.

L-*Oleandronic acid phenylhydrazide* (**10**). A soln of **8** (2 mg) in absolute EtOH (0.05 ml) was mixed with freshly distilled phenylhydrazine (0.04 ml) as in lactone **7** affording L-oleandronic acid phenylhydrazide (**10**), crystallized from MeOH–Et_2O as colourless needles (1.1 mg) mp 134–136°; lit. mp 136°.

Very mild hydrolysis of **4** *with acid*. To a soln of **4** (15 mg) in 80% aq. 1,4 dioxane (2.5 ml) was added 0.005 M H_2SO_4 (2.5 ml) and the soln was kept at room temp. After four days, it showed two more spots on PC besides a spot of some unreacted starting material **4** (R_{Ole} 1.72). The polar spot (R_{Ole} 1.00) was identified as L-oleandrose (**6**) by co-chromatography (PC) with the authentic sample, taken as the reference, while the less polar spot (R_{Ole} 2.36) was presumably the monoglycoside. The hydrolysis was complete in 7 days (PC) and working up of the hydrolysate followed by CC over silica gel afforded an amorphous product **1** (7 mg) which was found to be identical to ornogenin (**1**) ($[\alpha]_D$, TLC) and two pure viscous syrups **5** (2.0 mg) and **6** (1.5 mg) identified as D-cymarose and L-oleandrose, respectively, by comparing with authentic samples ($[\alpha]_D$, PC).

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