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A PREGNANE ESTER DIGLYCOSIDE FROM *PERIPLOCA CALOPHYLLA*

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Key Word Index—*Periploca calophylla*; Asclepiadaceae; plocinine; steroid; pregnane ester diglycoside.

Abstract—A new pregnane ester diglycoside of ornogenin named as plocinine was isolated from the dried twigs of *Periploca calophylla*. On the basis of chemical and spectroscopic evidences its structure was established as 12,20-di-*O*-cinnamoyl sarcostin-3-*O*-α-L-oleandropyranosyl(1 → 4)-*O*-α-L-oleandropyranoside.

INTRODUCTION

In an earlier chemical investigation of the twigs of *Periploca calophylla* (Asclepiadaceae), the presence of cymarose [1], periplogenin [1], calocin [2], plocin [3] and triterpenoids [4] were reported. Preparative separation of the polar glycoside mixture extracted from the twigs of plant material further afforded a small quantity of a novel diglycoside, designated as plocinine (**1**).

RESULTS AND DISCUSSION

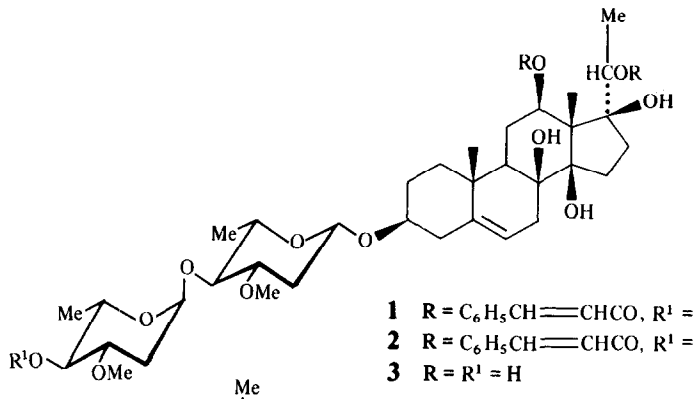
Plocinine (**1**) gave a positive Liebermann–Burchardt colour reaction [5] and characteristic colour tests for 2-deoxy sugars in xanthydrol [6, 7] and Keller–Kiliani reactions [8] which indicated it to be a steroidal glycoside of 2-deoxy sugars. Alkaline hydrolysis of **1** afforded a more polar amorphous product **3** suggesting the presence of an ester function in the molecule.

To identify the sugar and genin units of **1**, it was hydrolysed with mild acid [9] (0.025 M H_2SO_4) which afforded a genin **4**, $C_{39}H_{46}O_8$, and chromatographically pure reducing sugar **7**, $[\alpha]_D + 15°$, identified as L-oleandrose [10, 11] (2,6-dideoxy-3-*O*-methyl-L-arabino-hexose) by comparison with an authentic sample ($[\alpha]_D$, PC). Bromine water oxidation of **7** gave a lactone **8** which with phenylhydrazine afforded the known L-oleandronic acid phenylhydrazide (**9**) [10, 11] (mmp, IR).

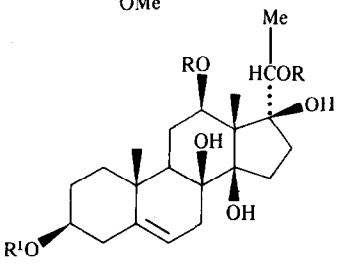
Alkaline hydrolysis of compound **4** gave **6**, $C_{21}H_{34}O_6$, identified as sarcostin [12] ($[\alpha]_D$, mmp, TLC). As the acetylation of the glycoside **1** gave a monoacetate **2**, $C_{55}H_{72}O_{15}$ (^{1}H NMR), it could be concluded that the genin moiety sarcostin in **1** was present as a diacyl derivative. This was substantiated by the very mild alkaline hydrolysis [13] of **2**. Within 2 hr it showed besides unreacted **2**, plocinine (**1**), its fully deacylated product **3**, and a fourth spot which was presumably a monoacyl derivative of **3** (TLC). In 5 hr **2** was completely deacylated into **3**. To identify the acyl groups methanolysis of **4** by the Zemplén method [14, 15] was carried out which afforded the acid component as the methyl ester. The mass spectral analysis of the reaction product in the lower mass region contained characteristic prominent mass ion peaks at *m/z* 162, 131 and 103 suggesting the presence of a cinnamate ester function in **4**. The difference, $C_{18}H_{12}O_2$, between **4** and **6** suggested **4** to be di-*O*-cinnamoyl sarcostin finally identified with the earlier reported ornogenin [16] (12,20-di-*O*-cinnamoyl sarcostin ($[\alpha]_D$, mmp, TLC) also characterized by preparing its mono-*O*-acetyl derivative **5**, $C_{41}H_{48}O_9$. The molecular formula, $C_{53}H_{70}O_{14}$, of **1** and identification of its two components as ornogenin, $C_{39}H_{46}O_8$, and oleandrose, $C_7H_{14}O_4$, suggested it to be a dioleandroside. A very mild acid hydrolysis of **1** (0.0025 M H_2SO_4) after 72 hr showed (PC) unreacted **1**, oleandrose (**7**) and a third spot which was presumed to be the monoglycoside **10**. The hydrolysis was complete in 92 hr exhibiting two spots identical with ornogenin (**4**) and oleandrose (**7**) on TLC and PC, respectively, confirming **1** to be the dioleandroside of ornogenin.

The mass spectrum of **1** did not exhibit $[M]^+$ but the highest mass ion at *m/z* 494 corresponded to $[M - \text{cinnamic acid} - \text{disaccharide unit}]^+$ which was supplemented with the mass ion peak at *m/z* 257 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 gave a peak at *m/z* 346 which evidently represented the basic sarcostin moiety of the molecule. The lower mass region contained the expected prominent cinnamic acid and its fragment ions at *m/z* 148 and 131 besides the common 2,6-dideoxymonomethoxy-hexose fragments at *m/z* 145, 113 and 95.

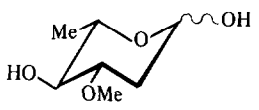
The ^{1}H NMR spectrum of **1** at 100 MHz not only substantiated the derived structure but also defined the configuration of the glycosidic linkages. It contained signals for two methoxy groups and two sets of methylene multiplets. A double doublet for anomeric protons (2H) at δ4.75 (J = 3 and 1 Hz) was in agreement with the presence of two oleandrose units in **1** showing that they are magnetically equivalent and their configurations are identical in the two oleandrose units. The small coupling constant (J = 3 Hz) of the anomeric protons was typical of an equatorial configuration indicating an α-L-(1 → 4) glycosidic linkage for L-oleandropyranose units present in $^1C_4$ (L) conformation. The low field chemical shift of one proton double doublet (J = 9 and 3 Hz) at δ4.92 and another ill resolved one proton quartet at 4.53 (J = 7 Hz) indicated that both these positions were esterified by the cinnamoyl groups and were assigned to the C-12 and C-20 methine protons of ornogenin, respectively. The spectrum



1 R = $C_6H_5CH{=}CHCO$, R^1 = H
2 R = $C_6H_5CH{=}CHCO$, R^1 = Ac
3 R = R^1 = H



4 R = $C_6H_5CH{=}CHCO$, R^1 = H
5 R = $C_6H_5CH{=}CHCO$, R^1 = Ac
6 R = R^1 = H



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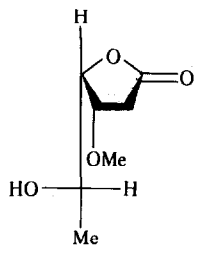

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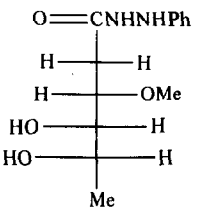

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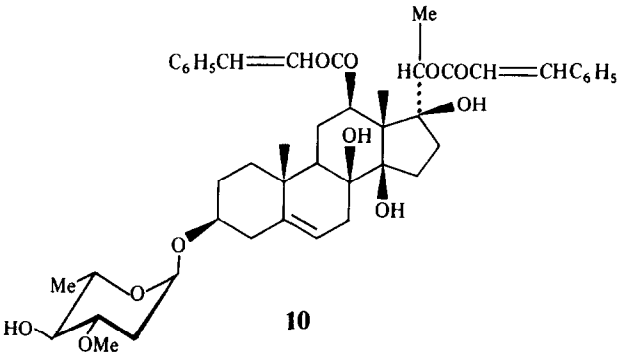

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also accounted for other appropriate proton signals (see Experimental).

In the light of the foregoing evidence, the structure of plocinine (**1**) was thus established as 12,20-di-*O*-cinnamoyl sarcostin-3-*O*-α-L-oleandropyranosyl-(1 → 4)-*O*-α-L-oleandropyranoside.

EXPERIMENTAL

The general procedures were the same as those reported recently [3], except ^{1}H NMR ($CDCl_3$): 100 MHz (JEOL JNM FX-100FT).

Plant extraction. Shade dried twigs of *P. calophylla* (5 kg) were extracted as reported earlier [3, 17] yielding with $CHCl_3$–EtOH. (4:1) 7 g and with $CHCl_3$–EtOH (3:2) 5.2 g. These combined extracts by column chromatography on silica gel yielded plocinine (**1**) (70 mg).

Plocinine (**1**). Mp 144–148° (Me_2CO–Et_2O), $[\alpha]_D^{25}$ + 37° (MeOH, *c* 0.14). (Found C, 68.21; H, 7.42; $C_{53}H_{70}O_{14}$ requires C, 68.38; H, 7.52%.) It gave a pink colour in the xanthydrol and a blue colour in the Keller–Kiliani reactions. It also gave a positive colour in the Liebermann–Burchardt test. ^{1}H NMR (100 MHz): δ7.96 (1H, *d*, *J* = 16 Hz), 7.94–7.62 (10H, *m*, Ar) 7.48 (1H, *d*, *J* = 16 Hz), 6.50 (1H, *d*, *J* = 16 Hz), 6.14 (1H, *d*, *J* = 16 Hz), 5.42 (1H, *m*, H-6), 4.92 (1H, *dd*, *J* = 9 and 3 Hz), 4.75 (2H, *dd*, *J* = 3 and 1 Hz, H-1 of sugars), 4.53 (1H, *q*, H-20), 4.16–3.82 (4H, *m*, H-3′ and H-5′ of sugars), 3.42 (6H, *s*, 2 × OMe), 3.48–3.24 (2H, *m*, H-4′ of sugars), 2.40–2.24 (4H, *m*, H-2′a, H-2′e of sugars), 2.00–1.80 (–CH_2–aglycone), 1.38 (3H, *d*, *J* = 6 Hz, 21-Me), 1.34 (6H, *d*, *J* = 6 Hz, 6′-Me of sugars), 1.12 (3H, *s*, 18-Me),

0.97 (3H, *s*, 19-Me). MS *m/z* (rel. int.): $[M]^+$ not observed, 494 $[M - \text{sugars} - PhCH{=}CHCOOH]^+$ (2), 476 $[494 - H_2O]^+$ (3), 458 $[476 - H_2O]^+$ (3), 346 $[494 - PhCH{=}CHCOOH]^+$ (44.6), 328 $[346 - H_2O]^+$ (89.1), 310 $[328 - H_2O]^+$ (82.8), 292 $[310 - H_2O]^+$ (28), 274 $[292 - H_2O]^+$ (22), 148 $[PhCHCHCOOH]^+$, 131 $(PhCHCHCO)^+$; sugar fragments 257 [disaccharide ion − MeOH] (34), 145 (100), 113 (8), 95 (35).

Alkaline hydrolysis of **1**. Compound **1** (5 mg) was dissolved in 5% methanolic KOH (2 ml) and kept at 50° for 1 hr. The hydrolysate yielded amorphous **3** (2.5 mg) of lower mobility than **1** (TLC). It gave a pink colour in the xanthydrol reaction, a blue colour in the Keller–Kiliani reaction and it gave a positive colour in the Liebermann–Burchardt test.

Mono-O-acetyl plocinine (**2**). Substance **1** (8 mg) in pyridine (0.5 ml) was acetylated with Ac_2O (0.4 ml) at room temp. Usual work-up yielded mono-*O*-acetyl plocinine **2** as colourless crystals (5 mg), mp 105–110° (Et_2O), $[\alpha]_D + 159°$ (MeOH, *c* 0.18). (Found: C, 67.37; H, 7.31. $C_{55}H_{72}O_{15}$ requires C, 67.90; H, 7.40%.) ^1H NMR: δ2.05 (3H, *s*, OAc), 4.15 (1H, *m*, H-4').

Mild hydrolysis of **2** *with alkali*. A soln of **2** (5 mg) in 0.5% methanolic KOH (1.2 ml) was kept at room temp., after 2 hr the hydrolysate showed four spots on TLC. The spot with R_f 0.85 was identical in mobility with the triacyl starting material **2**; spot with R_f 0.65 had the same mobility as that of plocinine (**1**); the spot with R_f 0.40 was presumably the monoacyl derivative of **3** and the last spot (R_f 0.25) was the deacylated plocinine **3** alternatively obtained by the alkaline hydrolysis of **1**. In 5 hr the reaction was complete, showing only one spot having the same mobility as that of **3**.

Mild hydrolysis of **1** *with acid*. To a soln of crystalline **1** (20 mg) in 80% aq. 1,4-dioxane (1.3 ml) was added 0.025 M H_2SO_4 (1.3 ml) and the soln was warmed for 30 min at 50° which after usual work-up afforded **4** (7.5 mg), mp 72–78° (MeOH–petrol), $[\alpha]_D^{25} + 108°$ (MeOH, *c* 0.20). (Found: C, 72.78; H, 7.1%; $C_{39}H_{46}O_8$ requires C, 72.89; H, 7.17%) and syrupy sugar **7** (6 mg), $[\alpha]_D^{25} + 13.8°$ (H_2O, *c* 0.12), which gave a pink colouration in the xanthydrol and a blue colouration in the Keller–Kiliani reactions and it was found to be L-oleandrose ($[\alpha]_D$, PC).

Oxidation of **7** *with bromine water*. A soln of **7** (5 mg) in H_2O (0.7 ml) was oxidized with Br_2 (12 μl) [10, 11], made neutral with Ag_2CO_3 and yielding syrupy lactone **8** (3.5 mg). It gave a violet colouration with NH_2OH–$FeCl_3$ reagent.

L-*Oleandronic acid phenyl hydrazide* (**9**). A soln of **8** (3 mg) in absolute EtOH (0.05 μl) was heated with phenylhydrazine (0.06 ml) and the usual work up gave the crystalline phenylhydrazide (**9**), mp 133–135° (MeOH–Et_2O) (2.3 mg). (Found: C, 58.12; H, 7.39; N, 10.41; $C_{13}H_{20}N_2O_4$ requires C, 58.20; H, 7.46; N, 10.44%.) It was found identical with authentic L-oleandronic acid phenylhydrazide (mmp, IR).

Very mild hydrolysis of **1** *with acid*. To a soln of **1** (16 mg) in 80% aq. 1,3-dioxane (2.6 ml) was added 0.0025 M H_2SO_4 (2.6 ml) and the soln was kept at room temp. After 72 hr the reaction mixture showed four spots on TLC. By co-chromatography the slowest spot was identified as oleandrose (**7**) and taken as reference (R_{Ole} 1.0). The other spot (R_{Ole} 5.4) was genin **4**, spot (R_{Ole} 3.4) was unchanged plocinine **1** and the new spot (R_{Ole} 4.1) was presumably the monoglycoside **10**. The hydrolysis was complete in 95 hr and working up of hydrolysate followed by a column chromatography over silica gel afforded a viscous syrupy **7** (5 mg), $[\alpha]_D^{25} + 15°$ and a crystalline compound **4** (8 mg), mp 72–78°, $[\alpha]_D^{25} + 108°$. The crystalline compound **4** was found to be identical with ornogenin (**4**) (TLC and $[\alpha]_D$) and the viscous syrup **7** was identical with authentic L-oleandrose ($[\alpha]_D$, PC).

Alkaline hydrolysis of **4**. Compound **4** (5 mg) in 5% methanolic KOH (1.5 ml) was heated to yield **6** (2.3 mg), mp 261–263° (MeOH–Et_2O), $[\alpha]_D^{25} + 63.6°$ (MeOH, *c* 0.09) which was found to be sarcostin (TLC, mmp and $[\alpha]_D$).

Hydrolysis of **4** *by the Zemplén method*. To a soln of **4** (2 mg) in absolute MeOH (0.5 ml) was added NaOMe (0.04 ml) and the mixture was kept at room temp. After 15 min it was neutralized with IR 120H resin and filtered; MeOH was removed under red. pres. and yielded a residue (1.5 mg) containing the acyl group as methyl ester. MS in lower mass region: *m/z* 162, 131 and 103.

Acetylation of **4**. Compound **4** (5 mg) on acetylation with Ac_2O (0.5 ml) in pyridine (0.5 ml) at 100° for 9 hr and usual work-up afforded a crystalline monoacetate **5** (5 mg). (Found: C, 71.06; H, 7.09; $C_{41}H_{48}O_9$ requires C, 71.92; H, 7.01%.) Mp 93–95°, $[\alpha]_D^{25} + 192°$ (MeOH, *c* 0.18).

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