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A PREGNANE ESTER GLYCOSIDE FROM *PERIPLOCA CALOPHYLLA*

DESH DEEPAK, MAHESHWARI P KHARE and ANAKSHI KHARE

Department of Chemistry, Lucknow University, Lucknow, India

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Key Word Index—*Periploca calophylla*, Asclepiadaceae, plocigenin, plocin, steroid, pregnane ester diglycoside

Abstract—A new pregnane ester genin, plocigenin, and a new pregnane ester diglycoside, plocin, have been isolated from the dried twigs of *Periploca calophylla* The chemical and spectroscopic properties are consistent with the structures 12,20-di-*O*-benzoyl drevogenin-D and 12,20-di-*O*-benzoyl drevogenin-D-3-*O*-*β*-D-oleandropyranosyl (1 → 4)-*O*-*β*-D-oleandropyranoside, respectively

INTRODUCTION

The members of the Asclepiadaceae are known to be rich in cardenolide and pregnane derivatives [1, 2] The twigs of *Periploca calophylla* L contain a 2-deoxyhexose, cymarose, a cardenolide, periplogenin [3], and a pregnane glycoside, calocin [4] In a re-investigation of their chemical constituents, the shade-dried twigs of the plant were extracted using an earlier method [5, 6] to give a novel glycoside which we have named plocin (**1**) This paper describes the structure elucidation of this glycoside

RESULTS AND DISCUSSION

Plocin (**1**), $C_{49}H_{66}O_{13}$, mp 148–150°, $[\alpha]_D$ +40° A positive Liebermann–Burchardt [7] colour reaction and characteristic colour tests for 2-deoxy sugars in the xanthydrol [8, 9] and Keller–Kiliani [10] reactions indicated it to be a steroidal 2-deoxyglycoside

Mild acid hydrolysis of **1** with 0 025 M sulphuric acid afforded a new genin, plocigenin (**2**) and a chromatographically pure reducing sugar (**5**) Comparison of the optical rotation value and co-chromatography (PC) with an authentic sample identified the sugar as D-oleandrose [11, 12] (2,6-dideoxy-3-*O*-methyl-D-arabino-hexose) Bromine–water oxidation of **5** gave a lactone (**6**), which with phenyl hydrazine afforded the known D-oleandronic acid phenyl hydrazide [11, 12]

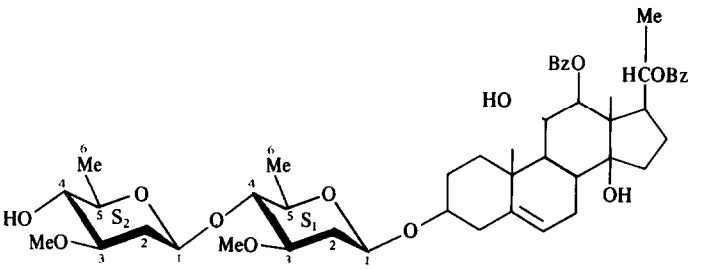
The difference, $C_{14}H_{24}O_6$, between the molecular formulae of plocin (**1**) ($C_{49}H_{66}O_{13}$) and plocigenin (**2**) ($C_{35}H_{42}O_7$) clearly indicated that plocin contained two oleandrose units and was in fact a di-oleandroside For convenience, the two D-oleandrose units of **1** were designated as S_1 and S_2

Although the genin **2** was inert to sodium periodate, its deacylated product (**3**), $C_{21}H_{34}O_5$, reacted with this reagent which suggested a vicinal diol arrangement in the molecule This pregnane genin (C_{21}) was characterized as drevogenin-D [13] (3*β*,11*α*,12*β*,14*β*,20*R*-pentahydroxy-Δ^5-pregnane) by co-chromatography (TLC) and mmp with an authentic sample of **2** The ability of plocin (**1**) and its aglycone (**2**) to undergo alkaline hydrolysis indicated the presence of ester functions The difference ($C_{14}H_8O_2$) between the formulae of aglycone **2** and its deacylated product **3**, supplemented by the UV absorption maximum at 282 nm (log *ε* 3 25), indicated the presence of two benzoyl groups, suggesting that the aglycone was a dibenzoyl drevogenin-D As both glycoside **1** and its aglycone, di-*O*-benzoyl drevogenin-D (**2**), were inert to sodium periodate, it was inferred that one of the vicinal hydroxyl groups was benzoylated at C-11 or C-12 whereas the other benzoyl group could be at any of the remaining three secondary carbinol groups (C-3, C-12 or C-11, C-20) of drevogenin-D

In the ^{1}H NMR ($CDCl_3$) spectrum of **1**, the chemical shift of a one-proton sharp doublet at *δ*4 96 (J = 8 Hz) and another one-proton multiplet at *δ*4 8–4 6 in the lower field indicated these methine protons to carry ester functions, which were assigned to C-12 and C-20 methine protons, respectively Plocigenin was thus characterized as 12,20-di-*O*-benzoyl drevogenin-D and is reported for the first time from a natural source Confirmation of this conclusion was forthcoming from the acetylation of **2** with acetic anhydride in pyridine which afforded in amorphous product, **4**, $[\alpha]_D$ −21°, which contained the expected two acetyl groups as three-proton singlets at *δ*1 97 and 1 93 in its 80 MHz ^{1}H NMR spectrum

For further confirmation of **1** being a diglycoside, it was subjected to very mild acid hydrolysis (2 5 mM sulphuric acid) After 3 days, the partially hydrolysed reaction mixture showed two new spots besides some unreacted starting material (PC) The more polar spot had the same mobility as oleandrose (**5**) whereas the other more mobile vanillin–perchloric acid positive spot (R_{Ole} 2 3) was presumed to be the monoglycoside **8** The hydrolysis was complete in 85 hr and gave two new spots which were identical with the genin **2** and oleandrose (**5**) on TLC and PC, confirming that **1** was the dioleandroside of 12,20-di-*O*-benzoyl drevogenin-D

The 250 MHz ^{1}H NMR ($CDCl_3$) spectrum of **1** was in full agreement with the derived structure It contained ten proton signals in the aromatic region in two sets of multiplets at *δ*7 98–7 9 (4H) and 7 58–7 20 (6H) for the two phenyl groups of the benzoates It also contained two methoxyl group singlets at *δ*3 42 and 3 40, two secondary methyl doublets at *δ*1 32 (J = 6 Hz) and 1 26 (J = 6 Hz), and four protons of 2-deoxymethylene of both the sugar units S_1 and S_2 as two sets of multiplets in the region *δ*2 52–2 22 and 1 98–1 88 for equatorial and axial protons,



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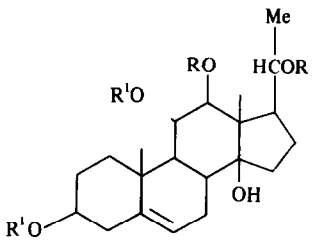
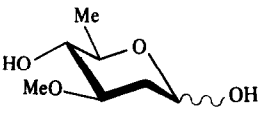


	R	R^1
2	Bz	H
3	H	H
4	Bz	Ac



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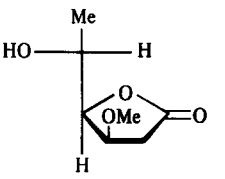

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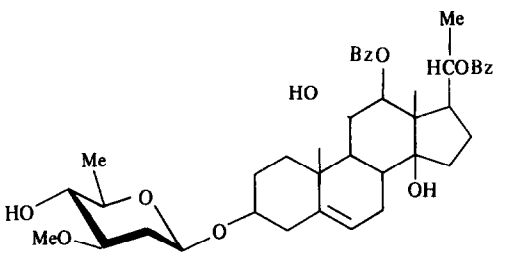

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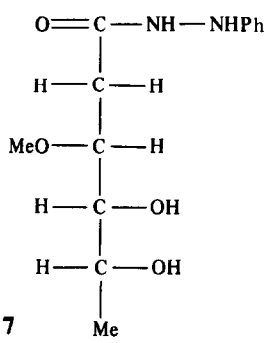

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respectively Two double-doublets (J = 8 and 3 Hz) of one proton each at δ4 84 and 4 52 were attributed to the anomeric protons of two oleandrose units in the molecule The large coupling constant (8 Hz) of both these anomeric protons, typical of their axial configuration in a 2-deoxyhexopyranose moiety in $^4C_1$ (D) conformation, suggested that both sugars were linked in a β-D-glycosidic linkage [14] The low-field chemical shift of a one-proton doublet (J = 8 Hz) at δ4 96 and a one-proton multiplet centred at δ4 7 was evidently due to the methine protons of esterified carbon atoms respectively in the pregnane moiety The C-11 methine proton carrying the hydroxyl group appeared at a high field as a triplet (J = 8 Hz) centred at δ3 34 In addition, the spectrum also contained other appropriate proton signals of drevogenin-D and sugar residues

In the light of all this evidence, the structure of plocin (1) is established as 12,20-di-*O*-benzoyl drevogenin-D-3-*O*-β-D-oleandropyranosyl (1 → 4)-*O*-β-D-oleandropyranoside

EXPERIMENTAL

Mps uncorr PC toluene–BuOH (4 1) saturated with H_2O Sugars were visualized with 50% aq H_2SO_4 (TLC) or vanillin–$HClO_4$ reagent (PC) ^{1}H NMR $CDCl_3$, TMS as internal standard

Extraction Shade-dried twigs (5 kg) of *P calophylla* were extracted and fractionated with solvents of different polarities as reported earlier [4] to afford a petrol extract (2 5 g), an Et_2O extract (1 g), a $CHCl_3$ extract (20 g), a $CHCl_3$–EtOH (4 1) extract (7 g) and a $CHCl_3$–EtOH (3 2) extract (5 2 g), respectively The $CHCl_3$–EtOH (3 2) extract was chromatographed over silica gel to give plocin (1) (64 mg).

Plocin (1) Mp 148–150° (MeOH–Et_2O), $[\alpha]_D^{25}$ +40 2° (MeOH, c 0 11) (Found C, 71 38, H, 6 82 $C_{49}H_{66}O_{13}$ requires C, 71 45, H, 6 87). It gave a pink colour in the xanthydrol and a blue colour in Keller–Kiliani reactions UV λ_{max}^{EtOH} nm (log ε) 282 (3 25) ^{1}H NMR (250 MHz): δ7 98–7 9 (m, 4H, aromatic),

7 58–7 20 (*m*, 6H, aromatic), 5 36 (*m*, 1H, H-6), 4 96 (*d*, 1H, J = 8 Hz, H-12), 4 84 (*dd*, 1H, J = 8 and 3 Hz, H-1 in S_1 or S_2), 4 80–4 64 (*m*, 1H, H-20), 4 52 (*dd*, 1H, J = 8 and 3 Hz, H-1 in S_1 or S_2), 3 84–3 74 (*m*, 2H, H-5 in S_1 and S_2), 3 66–3 50 (*m*, 2H, H-3 in S_1 and S_2), 3 42 (*s*, 3H, OMe), 3 40 (*s*, 3H, OMe), 3 34 (*t*, 1H, J = 8 Hz, H-11), 3 24–3 14 (*m*, 2H, H-4 in S_1 and S_2), 2 52–2 22 (*m*, 2H, H-2_{eq} in S_1 and S_2), 1 99–1 88 (*m*, 2H, H-2_{ax} in S_1 and S_2), 1 66–1 5 (methylenes of aglycone), 1 32 (*d*, 3H, J = 6 Hz, 6-Me in S_1 or S_2), 1 29 (*d*, 3H, J = 6 Hz, 21-Me), 1 26 (*d*, 3H, J = 6 Hz, 6-Me in S_1 or S_2), 1 24 (*s*, 3H, 18-Me), 1 12 (*s*, 3H, 19-Me)

Mild acid hydrolysis of **1** To a soln of crystalline **1** (18 mg) in 80% aq 1,4-dioxane (1 3 ml) was added 0 05 M H_2SO_4 (1 2 ml) and the solution warmed for 30 min at 50° Dioxane was then removed under reduced pressure The aq portion was repeatedly extracted with $CHCl_3$ (97 3) and the organic layer was washed successively with H_2O, Na_2CO_3 and H_2O, dried over Na_2SO_4 and evapd to afford genin **2** (8 mg), crystallized from MeOH–petrol, mp 187–189°, $[\alpha]_D^{25}$ −18 6° (MeOH, *c* 0 18) ($C_{35}H_{42}O_7$) The aq hydrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concd under reduced pressure to afford the syrupy sugar **5** (6 mg), $[\alpha]_D^{25}$ −14 2° (H_2O, *c* 0 12), which gave a pink coloration in the xanthydrol and a blue coloration in the Keller–Kiliani reactions Comparison of its optical rotation and co-chromatography (PC) identified **5** as D-oleandrose

Oxidation of **5** *with* Br_2–H_2O A soln of **5** (4 mg) in H_2O (0 6 ml) was mixed with Br_2 (10 μl) and shaken in a stoppered flask in the dark for 24 hr at room temp Excess Br_2 was then removed under reduced pressure The acidic mixture was made neutral with freshly precipitated Ag_2CO_3 and the suspension filtered, H_2S was passed through the filtrate to remove Ag^+ ions, and the suspension filtered The filtrate was evapd to dryness under reduced pressure yielding syrupy lactone **6** (3 2 mg)

D-Oleandronic acid phenyl hydrazide (**7**) A soln of lactone **6** (3 mg) in EtOH (0 05 μl) was mixed with freshly distilled phenyl hydrazine (0 05 ml) and the mixture heated for 30 min at 100° The viscous mass was cooled and repeatedly triturated with Et_2O (to remove excess phenyl hydrazine) yielding D-oleandronic acid phenyl hydrazide (2 2 mg), which crystallized from MeOH–Et_2O as colourless needles (1 2 mg), mp 132–134° It had superimposable IR and gave no depression in mp when mixed with authentic material (lit mp 135°)

Very mild acid hydrolysis of **1** To a soln of **1** (15 mg) in 80% aq 1,4-dioxane (2 5 ml) was added 5 mM H_2SO_4 (2 5 ml) and the soln was kept at room temp After 3 days, the reaction mixture showed two new vanillin–perchloric acid positive spots on PC besides a spot of some unreacted starting material The more polar spot (R_{Ole} 1 0) was identified as oleandrose (**5**) by co-chromatography with an authentic sample, while the less polar spot (R_{Ole} 2 3) was presumably the monoglycoside **8** The hydrolysis was complete in 85 hr (TLC) and work-up of the hydrolysate followed by CC over silica gel afforded a viscous syrup (4 5 mg), $[\alpha]_D^{25}$ −14 6°, and a crystalline compound (6 8 mg), mp 186–188° The crystalline compound was identical with the known 12,20-di-*O*-benzoyl drevogenin-D (**2**) (mmp, $[\alpha]_D$) and the viscous syrup was identified as D-oleandrose by comparison with an authentic sample ($[\alpha]_D$ and PC)

Alkaline hydrolysis of **2** Compound **2** (4 mg) was dissolved in 5% methanolic KOH (1 ml) and refluxed for 2 hr After addition of H_2O (0 5 ml), the MeOH was removed under reduced pressure The aq concentrate was extracted with $CHCl_3$–MeOH (9 1), dried over Na_2SO_4, filtered and evapd to dryness yielding **3** (1 8 mg), which was crystallized from Me_2CO–Et_2O, mp 225–228°, $[\alpha]_D^{25}$ −10 2° (MeOH, *c* 0 12) ($C_{21}H_{34}O_5$) No depression in mmp, comparison of TLC, and optical rotation with authentic sample confirmed **3** as drevogenin-D

Acetylation of **2** Compound **2** (6 mg) on acetylation with Ac_2O (0 4 ml) in pyridine at 100° for 8 hr afforded **4** as an amorphous residue (6 mg), $[\alpha]_D^{25}$ −21 5° (MeOH, *c* 0 11) It showed ^{1}H NMR (80 MHz) signals at δ1 97 (*s*, 3H, OAc), 1 93 (*s*, 3H, OAc)

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