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A PREGNANE ESTER DIGLYCOSIDE FROM *HEMIDESMUS INDICUS*

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Key Word Index—*Hemidesmus indicus*; Asclepiadaceae; desinine; steroid; pregnane ester diglycoside.

Abstract—A new pregnane ester diglycoside named desinine has been isolated from the dried twigs of *Hemidesmus indicus*. On the basis of chemical and spectroscopic evidence, its structure has been established as drevogenin B-3-*O*-β-D-oleandropyranosyl-(1 → 4)-β-D-oleandropyranoside.

INTRODUCTION

In the roots of *Hemidesmus indicus* R. Br. (family: Asclepiadaceae), the presence has been reported of resin acid, fatty acids, tannins, saponins, an oil containing *p*-methoxysalisaldehyde [1, 2], lupeol, sitosterol and amyrins [3]. In a reinvestigation of the chemical constituents, shade-dried twigs of the plant were extracted which, on repeated CC, afforded a novel diglycoside which we have named desinine. This paper describes the structure elucidation of this glycoside.

RESULTS AND DISCUSSION

Desinine (**1**) mp 115–118°, $[\alpha]_D$ 0°, $C_{37}H_{58}O_{12}$, was obtained from the chloroform-soluble glycoside mixture by CC over silica gel. It gave a positive Liebermann–Burchardt [4] colour reaction and characteristic colour tests for 2-deoxy sugars in the xanthydrol [5, 6] and Keller–Kiliani [7] reactions, indicating it to be a steroidal glycoside of a 2-deoxy sugar.

The ^{1}H NMR spectrum of **1** at 400 MHz showed two methoxy group singlets at δ3.45 and 3.35, two secondary methyl group doublets (J = 6 Hz) at δ1.23 and 1.20 and the equatorial and axial protons of four C-2 methylene protons of two 2-deoxy sugar units as two sets of multiplets in the regions δ2.28–2.46 and 1.72–1.88. This suggested it to be a diglycoside of monomethoxy-2,6-dideoxyhexoses for which the anomeric protons could be assigned to the double doublet for two protons at δ4.88 (J = 9, 2.5 Hz) presumably in the axial conformation. This glycoside also contained the signal of one acetyl group at δ2.10, possibly present in the genin moiety.

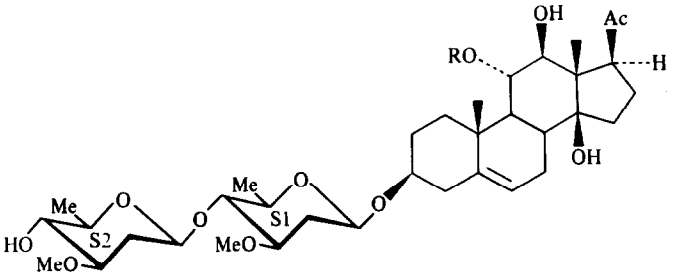
The mass spectrum of **1** was also consistent with its diglycoside structure. Although the mass spectrum of **1** did not exhibit the $[M]^+$ expected at m/z 694, there were peaks at m/z 270, 244, 238 and 213 which originated from the disaccharide fragment after the loss of acetaldehyde, methanol and a water molecule(s). The presence of an acetyl group and a keto methyl chain was substantiated by the peaks corresponding to the loss of acetic acid and a keto methyl chain from different fragments.

The inertness of **1** to sodium periodate, in contrast to its deacetylated product **2**, suggested that one of the hydroxyl groups, presumably present in the basic genin moiety of **1** in a vicinal diol arrangement, was acetylated. Mild acid hydrolysis of **1** with 0.025 M sulphuric acid afforded an acetyl genin, **4**, mp 238–240°, $[\alpha]_D$ +56°, $C_{23}H_{34}O_6$, which, on deacetylation with alkali, gave a mixture of two products having very similar mobility. One was obtained from the mixture as a crystalline product, **5**, mp 185–188°, $[\alpha]_D$ +32°, $C_{21}H_{32}O_5$, identified as drevogenin P [8, 9], thus leading to the conclusion that **4** is 11- or 12-mono-*O*-acetyldrevogenin P. A survey of the mps and ORDs of mono-*O*-acetyldrevogenin P indicated **4** to be drevogenin B [10, 11] (11α-*O*-acetyl,3β,12β,14β-trihydroxy-Δ^5-pregnen-20-one) which was finally confirmed by its comparison with the authentic material (TLC and undepressed mmp). The 11-*O*-acetyl position in the genin moiety in **1** conforms with its ^{1}H NMR spectrum consisting of a lower field one proton triplet at δ5.14 (J = 8 Hz) attributable to a C-11 methine proton and the higher field one-proton doublet at δ4.44 (J = 8 Hz) assigned to a methine proton at C-12 bearing a free hydroxyl group.

The syrupy sugar **6**, $[\alpha]_D$ −14°, was identified as D-oleandrose [12, 13] (2,6-dideoxy-3-*O*-methyl-D-arabinohexose) by comparison of ORD and mobility on PC. Bromine water oxidation of **6** gave a lactone **7** which, with phenylhydrazine, afforded the known D-oleandronic acid phenylhydrazide **8**, mp 132–134° which was characterized by comparison with the authentic material (mmp and IR spectrum) [12, 13].

The difference, $C_{14}H_{24}O_6$, between the molecular formulae of **1** and **4** and the identification of D-oleandrose as the only sugar in the acid hydrolysate clearly indicated that two oleandrose units are present in desinine, suggesting it to be a di-oleandroside. For convenience, the two sugar units in **1** are designated as S1 and S2. A double-resonance experiment was helpful in confirming the assignment of the anomeric protons at δ4.88. Irradiation of the signal at 1952 Hz resulted in the collapse of the C-2 methylene multiplets, confirming that this signal represented the anomeric protons of 2-deoxy sugar units. The configuration of the anomeric protons in S1 and S2 could be derived easily from the large coupling constant (J = 9 Hz) of their signal at δ4.88, attributed to their axial configuration indicating the sugar units to be present in a $^4C_1$ (D) conformation and linked through β-D-(1 → 4) glycosidic linkage [14].

More direct chemical support that **1** is drevogenin B-dioleandroside was provided by the results of its very mild



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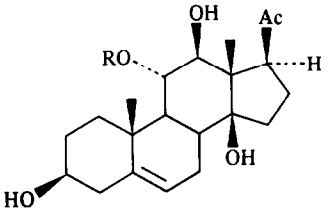
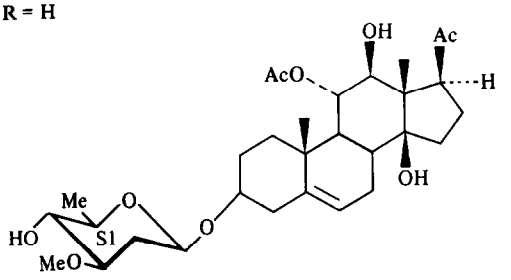
2 R = H

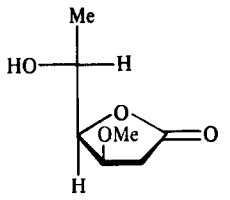




4 R = Ac
5 R = H

3

6



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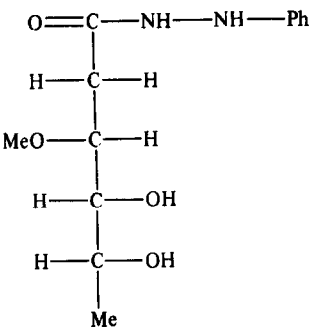

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acid (0.001 M) hydrolysis [15]. Under these conditions **1** afforded, after 6 days, four spots on TLC. The most polar spot (R_f 0.18) had the same mobility as the starting material **1**. The least polar spot (R_f 0.59) was identical in mobility to the genin **4**. The spot at R_f 0.31 was found to have identical mobility with oleandrose (**6**) and the other vanillin perchloric acid positive spot (R_f 0.51), still containing the 2-deoxy sugar, was presumed to be the monoglycoside **3**. The hydrolysis was complete in ten days when the hydrolysate exhibited only two spots identical in mobility to sugar **6** and genin **4**.

In the light of the foregoing evidence, the structure of desinine (**1**) was established as drevogenin B-3-*O*-*β*-D-oleandropyranosyl-(1 → 4)-*β*-D-oleandropyranoside.

EXPERIMENTAL

Mps were determined on a Boetius micro mp apparatus and are uncorr. MS were recorded with an AEI MS-30 instrument. ORs were measured in a 0.1 m long tube with a Jasco-Dip 180 automatic polarimeter. The ^{1}H NMR spectrum ($CDCl_3$) was recorded on a 400 MHz (Brüker) spectrometer with TMS as int. standard. Sugars were made visible with 50% aq. H_2SO_4 for TLC and vanillin–$HClO_4$ reagent for PC. PC was performed using C_6H_5Me–BuOH (4:1) satd with H_2O.

Plant extraction. The twigs of *H. indicus* were collected from the National Botanical Garden, Lucknow, India. The identity of the plant was confirmed by Dr. S. L. Kapoor, Systemic Botanist, National Botanical Research Institute, Lucknow, India where a voucher specimen was deposited (voucher No. 67957). Shade-dried powdered twigs (5 kg) of *H. indicus* were extracted by the method employed for pregnane glycosides [16] using 50–95% aq. EtOH. The EtOH extracts were combined and concd under red. pres. and without treatment with $Pb(OH)_2$, the concentrate was exhaustively extracted successively with petrol, Et_2O, $CHCl_3$, $CHCl_3$–EtOH (4:1) and $CHCl_3$–EtOH (3:2). Detailed examination of the $CHCl_3$ extract was undertaken. Although it did not resolve satisfactorily on TLC, it afforded one pure compound, given the name of desinine (**1**), by repeated CC over silica gel.

Desinine (**1**). Mp 115–118°, $[\alpha]_D^{25}$ 0°, $C_{37}H_{58}O_{12}$. (Found: C, 63.75; H, 7.20. $C_{37}H_{58}O_{12}$ requires: C, 63.97; H, 7.35%.) It gave a

violet colour in the Liebermann–Burchardt test, a pink colour in the xanthydrol and a blue colour in the Keller–Kiliani reactions. ^{1}H NMR (400 MHz): δ5.38 (1H, *m*, H-6), 5.14 (1H, *t*, J = 8 Hz, H-11), 4.88 (2H, *dd*, J = 9, 2.5 Hz, H-1 in S1 and S2), 4.44 (1H, *d*, J = 8 Hz, H-12), 3.76–3.88 (2H, *m*, H-5 in S1 and S2), 3.66–3.74 (2H, *m*, H-3 in S1 and S2), 3.45 (3H, *s*, OMe), 3.35 (3H, *s*, OMe), 3.16–3.24 (2H, *m*, H-4 in S1 and S2), 2.28–2.46 (2H, *m*, H-2e in S1 and S2), 2.18 (3H, *s*, COMe), 2.10 (3H, *s*, OAc), 1.72–1.88 (2H, *m*, H-2a in S1 and S2), 1.28 (3H, *s*, Me-18), 1.23 (3H, *d*, J = 6 Hz, sec. Me), 1.20 (3H, *d*, J = 6 Hz, sec. Me), 1.02 (3H, *s*, Me-19). MS *m/z* (rel. int.) glycoside fragments: 414 [M – S2 – HOAc – COMe – Me]$^+$ (16), 397 [M – S2 – HOAc – COMe – MeOH]$^+$ (34), 396 [M – S2 – HOAc – MeCHO – MeOH]$^+$ (56), 381 [M – S2 – HOAc – COMe – MeCHO – Me]$^+$ (12), 357 [M – disaccharide fragment (288) – 2OH – Me]$^+$ (5); genin fragments: 406 (genin) not observed, 329 [406 – HOAc – OH]$^+$ (9.8), 314 [406 – HOAc – OH – Me]$^+$ (75), 296 [329 – H_2O – Me]$^+$ (55), 281 [296 – Me]$^+$ (17), 244 [329 – C_5H_9O]$^+$ (92), 238 [281 – COMe]$^+$ (5); sugar fragments: 306 (disaccharide) not observed, 244 [306 – H_2O – MeCHO]$^+$ (92), 270 [306 – 2H_2O]$^+$ (4), 238 [270 – MeOH]$^+$ (5), 213 [306 – MeCHO – MeOH – OH]$^+$ (2).

Mild hydrolysis of **1** *with acid.* To a soln of crystalline **1** (18 mg) in 80% aq. 1,4-dioxane (1.2 ml) was added 0.05 M H_2SO_4 (1.2 ml) and the soln was warmed for 30 min at 50°. Dioxane was then removed under red. pres. The aq. portion was repeatedly extracted with $CHCl_3$ and the organic layer was washed in turn with H_2O, 1 M Na_2CO_3, again with H_2O, dried over Na_2SO_4 and evaporated to dryness. Crystallization with MeOH–Me_2CO afforded genin **4** (9 mg), mp 238–240°, $[\alpha]_D^{25}$ +56.2° (MeOH; *c* 0.18). (Found: C, 67.84; H, 8.20. $C_{23}H_{34}O_6$ requires: C, 67.98; H, 8.37%.) The aq. hydrolysate was neutralized with freshly prepared $BaCO_3$, filtered and concd under red. pres. to afford the syrupy sugar **6** (6 mg), $[\alpha]_D^{25}$ −14.2° (MeOH; *c* 0.12). It gave a pink colouration in the xanthdrol and a blue colouration in the Keller–Kiliani reactions. It had identical mobility on PC as oleandrose and its ORD was comparable to D-oleandrose.

Oxidation of compound **6** *with bromie water.* A soln of **6** (4 mg) in H_2O (0.6 ml) was mixed with Br_2 (10 μl) and shaken in the dark for 24 hr at room temp. The excess Br_2 was then removed under red. pres. The acidic mixture was neutralized with freshly prepared Ag_2CO_3 and filtered. H_2S was passed through the filtrate to remove Ag^+ ions followed by filtration. The filtrate was evaporated to dryness under red. pres. yielding the syrupy lactone **7** (3.2 mg) which gave a violet colour in the spot test with NH_2OH–$FeCl_3$ reagent [17].

D-*Oleandronic acid phenyl hydrazide* (**8**). A soln of lactone **7** (3 mg) in absolute EtOH (0.5 ml) was mixed with freshly distilled phenylhydrazine (0.5 ml) and heated for 30 min at 100°. The viscous mass was cooled and repeatedly triturated with absolute Et_2O (to remove excess of phenylhydrazine) to yield D-oleandronic acid phenylhydrazide which crystallized from MeOH–Et_2O as colourless needles (1.2 mg) mp 132–134°. It had a superimposable IR spectrum and gave no depression in mmp when compared with the authentic material (lit. [12, 13] mp 135°).

Very mild hydrolysis of **1** *with acid.* To a soln of **1** (2 mg) in aq. 1,4-dioxane (1:1) (0.2 ml) was added 0.001 M H_2SO_4 (0.2 ml) and the soln was kept at room temp. After 6 days the reaction mixture showed four spots on TLC. By co-chromatography the most polar spot (R_f 0.18) was identified as the starting material (**1**). The other spot (R_f 0.31) was sugar **6** and the least polar spot (R_f 0.59) the genin **4**. The last vanillin–$HClO_4$ positive spot (R_f 0.51) which still contained the 2-deoxy sugar was, presumably, the monoglycoside **3**.

Alkaline hydrolysis of **4**. Compound **4** (7 mg) was dissolved in 5% methanolic KOH (1.5 ml) and heated for 30 min. After adding H_2O (0.8 ml), MeOH was removed under red. pres. The aq. concentrate was extracted with $CHCl_3$, dried over Na_2SO_4, filtered and evaporated to dryness to yield **5** which crystallized from MeOH–Et_2O as colourless prisms (5 mg), mp 185–188°, $[\alpha]_D^{25}$ +32.2°. (Found: C, 69.15; H, 8.70. $C_{21}H_{32}O_5$ requires: C, 69.23; H, 8.79%.) It was identified as drevogenin P (lit. mp 185–190°, $[\alpha]_D^{25}$ +34.4°) by comparison with the authentic sample on TLC, ORD and undepressed mmp.

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