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PREGNANE GLYCOSIDES FROM *HEMIDESMUS INDICUS*

RUCHI CHANDRA, DESH DEEPAK and ANAKSHI KHARE

Khare's School of Natural Products, Department of Chemistry, Lucknow University, Lucknow, 226007, India

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Key Word Index—*Hemidesmus indicus*; Asclepiadaceae; emidine; hemidescine; calogenin; pregnane glycoside.

Abstract—Two novel pregnane glycosides, hemidescine and emidine, have been isolated from the dried stem of *Hemidesmus indicus*. Chemical and spectroscopic evidence is consistent with the structures 20-*O*-acetyl calogenin 3-*O*-*β*-D-digitoxopyranosyl(1→4)-*O*-*β*-D-oleandropyranoside and calogenin-3-*O*-*β*-D-digitoxopyranosyl(1→4)-*O*-*β*-D-digitoxopyranosyl(1→4)-*O*-*β*-D-digitoxopyranoside, respectively.

INTRODUCTION

Pregnane [1] derivatives along with cardenolides [2] have been reported from the plants of the Asclepiadaceae family. In previous studies on *Hemidesmus indicus* [3] various compounds along with pregnane glycosides [4] have been isolated. The chloroform–alcohol (4:1) extract of dried stem of *H. indicus* was investigated, which on repeated column chromatography afforded two novel pregnane glycosides named hemidescine (**1**) and emidine (**5**).

RESULTS AND DISCUSSION

Hemidescine (**1**) gave positive Liebermann–Burchardt [5], xanthydrol [6] and Keller Kiliani [7] reactions, along with three secondary methyl group doublets in the region δ1.32–1.03 (9H), and two double doublets at δ4.72 (1H) and 4.24 (1H) in the ^{1}H NMR spectrum of **1** showed that **1** was a steroidal glycoside of 2,6-dideoxy hexoses.

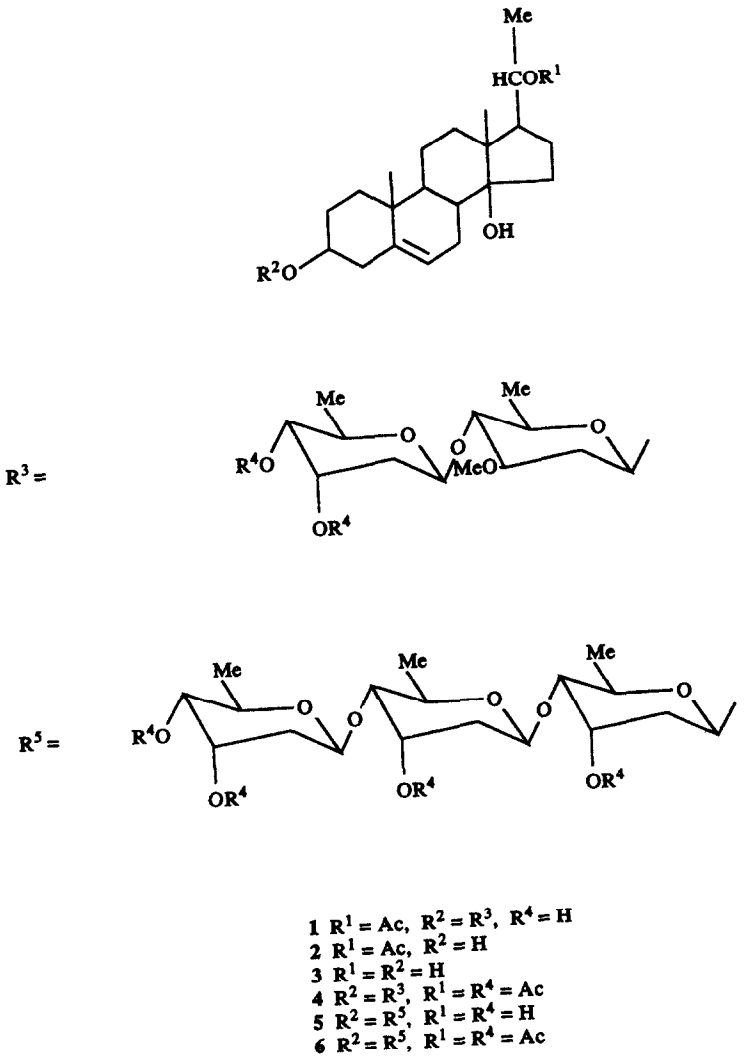
To identify the sugar(s) and genin of **1**, it was hydrolysed with 0.05 M H_2SO_4 [8] which afforded a genin **2** and two chromatographically pure sugars. The genin, **2**, on methanolysis by the Zemplen method [9] afforded a crystalline product **3** which was identified as calogenin (**3**) [4] by comparison with the authentic sample ($[\alpha]_D$, mp, TLC). The sugars were identified as D-digitoxose [10] and D-oleandrose [11] by direct comparison with the authentic samples (PC, TLC, $[\alpha]_D$). The further characterization of the sugars was done by their bromine water oxidation, resulting in the formation of their respective lactones, which on treatment with phenyl hydrazine afforded known D-digitoxonic acid phenyl hydrazide [10] and D-oleandronic acid phenyl hydrazide [11].

The ^{1}H NMR spectrum of glycoside **1** at 400 MHz showed a singlet of three protons at δ2.00, along with a multiplet in the region δ5.04–5.02 which could be assigned to the presence of one acetyl group and H-20 methine proton, which was found shifted down-field in comparison to the H-20 methine proton of calogenin, suggesting that the aglycone of **1** was 20-*O*-acetyl calogenin, which was finally confirmed by comparison with an authentic sample.

The difference of $C_{13}H_{22}O_8$ between the molecular formulae of the glycoside **1** and 20-*O*-acetyl calogenin **2** further supported the fact that **1** was a diglycoside. The diglycoside nature and sequence of sugars in **1** were confirmed by its very mild acid hydrolysis (0.005 M H_2SO_4) at room temperature which afforded partially and completely hydrolysed products. After 24 hr, TLC monitoring of the hydrolysate showed two new spots besides the unreacted starting compound. The most polar spot was identified as D-digitoxose, while the spot with the intermediate mobility was presumably the monoglycoside leading to the conclusion that D-digitoxose was the terminal sugar and D-oleandrose was glycosidically linked to 20-*O*-acetylcalogenin at the C-3 hydroxyl group, as it was the only available free secondary hydroxyl group in the genin moiety of **1**. The hydrolysis was complete in 96 hr showing three spots identical to the genin **2** and the sugars which were earlier isolated from mild acid hydrolysis of **1**.

The configuration of the glycosidic linkages was ascertained by the 400 MHz ^{1}H NMR spectrum of **1**. The two double doublets of one proton each at δ4.24 (J = 8 and 1.5 Hz) and δ4.72 (J = 8 and 1.5 Hz) were incorporated for two anomeric protons of the two monosaccharides. The large coupling constants (8 Hz) of the two anomeric protons were typical of their axial configuration in a $^4C_1$ (D) conformation, suggesting that the two sugars were linked to each other and to the aglycone through *β*-glycosidic linkages.

The EI mass spectrum of **1** did not exhibit the molecular ion peak $[M]^+$, but the highest mass ion peak recorded at m/z 340 could be assigned for $[M-\text{disaccharide}-2H_2O]^+$. The other important peaks at m/z 289



1 R^1 = Ac, R^2 = R^3, R^4 = H
2 R^1 = Ac, R^2 = H
3 R^1 = R^2 = H
4 R^2 = R^3, R^1 = R^4 = Ac
5 R^2 = R^5, R^1 = R^4 = H
6 R^2 = R^5, R^1 = R^4 = Ac

and 274 were due to [genin $-C_{17}$ side chain]$^+$ and [disaccharide $-H_2O$]$^+$.

The acetylation of **1** with acetic anhydride and pyridine confirmed the derived structure of **1** by affording di-*O*-acetyl hemidescine (**4**). The ^{1}H NMR spectrum of **4** contained three singlets of three acetyl groups which accounted for two acetylable hydroxyl groups present in the sugar part and one acetyl group present in 20-*O*-acetyl calogenin.

In the light of foregoing evidence the structure of hemidescine was established as 20-*O*-acetyl calogenin 3-*O*-*β*-D-digitoxopyranosyl(1→4)-*O*-*β*-D-oleandropyranoside.

Emidine (**5**) responded positively to the Liebermann–Burchardt, xanthydrol and Keller Kiliani reactions. In the ^{1}H NMR spectrum of **5** at 400 MHz the presence of three double doublets at δ4.62 (1H), 4.50 (1H) and 4.43 (1H), along with four secondary methyl group doublets at δ1.34, 1.26, 1.20 and 1.18 provided evidence that **5** is a steroidal triglycoside of 2,6-dideoxy hexoses.

To identify the sugar(s) and genin of **5** it was hydrolysed with 0.05 M H_2SO_4 which afforded a crystalline genin **3** and a chromatographically pure syrupy sugar. The genin **3**, was identified as calogenin by comparison with the authentic sample [10]. The sugar was identified as D-digitoxose [11] by direct comparison of its rotation and mobility on PC with the authentic sample. Further characterization was achieved by its oxidation with bromine water to a lactone, which on treatment with phenylhydrazine yielded a known crystalline D-digitoxonic acid phenyl hydrazide. The difference of $C_{18}H_{30}O_8$ between the molecular formulae of **5** and calogenin, and identification of D-digitoxose as the only sugar in the acid hydrolysate clearly indicated that three digitoxose units were present in **5**.

To confirm the triglycoside nature of **5**, it was subjected to very mild acid hydrolysis (0.005 M H_2SO_4). A TLC monitoring of the hydrolysate after 24 hr showed two new spots, besides the spot of unreacted starting material. The most polar compound was identified as D-digitoxose and the second spot was presumably the diglycoside. After 96 hr TLC showed five spots. The most and least polar compounds were identified as D-digitoxose and calogenin (**3**), respectively. The remaining three spots of

intermediate mobility were identified as unreacted **5**, diglycoside and monoglycoside in increasing order of their mobility. The monoglycoside nature of the spot was confirmed by its direct comparison (TLC) with the authentic sample of indicine [4]. The hydrolysis was complete in 140 hr, showing only two spots identical to genin **3** and D-digitoxose, earlier isolated from the mild acid hydrolysis of **5**.

The configuration of the glycosidic linkages of the sugars was ascertained by the ^{1}H NMR spectrum of **5**. Three double doublets of one proton each at δ4.62 (J=9 and 2 Hz), 4.50 (J=9 and 2 Hz) and 4.43 (J=9 and 2 Hz) could be assigned to the three anomeric protons of the three sugars. The large coupling constant (9 Hz each) of the three anomeric protons was typical of their axial configuration in $^4C_1$ (D) conformation, suggesting that all the glycosidic linkages in **5** were β.

The chemical shift assignments of these anomeric protons was further confirmed by double resonance experiments. Irradiation of the double doublets at δ4.43, 4.50 and 4.62 caused the collapse of the methylene signals for the equatorial and axial protons in the regions δ2.33–2.22 and δ2.02–1.92, 2.12–2.0 and 1.98–1.86, and 2.18–2.02 and 1.92–1.18, respectively, which again confirmed the presence of three 2-deoxy sugars in the glycoside.

To confirm the triglycosidic nature of **5** it was acetylated to afford a penta-*O*-acetyl-emidine (**6**) which was characterized by its ^{1}H NMR spectrum. The spectrum contained five singlets of three protons each for acetyl groups at δ2.16, 2.08, 2.07, 2.05 and 2.00 which indicated the presence of five acetylable hydroxyl groups in the glycoside **5**. Besides this, the low field shifting of the C-20 methine proton multiplet at δ5.12–5.02 suggested that the C-20 hydroxyl group had not participated in the glycosidation, and therefore it is the C-3 hydroxyl group which was glycosidically linked to the sugar moiety.

The EI mass spectrum of **5** did not exhibit the $[M]^+$ but the highest mass ion peak recorded at m/z 464 was due to monoglycoside. The other mass ion peaks recorded at m/z 408 and 334 were in agreement with the trisaccharide and genin, respectively. The mass ion peak at m/z 402 (monoglycoside $-$ CHOHMe $-$ H_2O)$^+$ further confirmed that the sugar chain is linked at the C-3 hydroxyl group of the aglycone.

In the light of foregoing evidence, the structure of emidine was established as calogenin 3-*O*-β-D-digitoxopyranosyl-(1→4)-*O*-β-D-digitoxopyranosyl(1→4)-*O*-β-D-digitoxopyranoside.

EXPERIMENTAL

The general procedures and extraction method were the same as those reported earlier [12], except that the NMR spectra were recorded on a 400 MHz (Brucker) spectrometer. Repeated CC on silica gel 60–120 ($CHCl_3$–MeOH 95:5) of the $CHCl_3$–EtOH (4:1, 2.4 g) extract afforded 2 glycosides: hemidescine and emidine.

Hemidescine (**1**, 42 *mg*). Mp 158°, $[\alpha]_D$ +13.33° (MeOH; *c* 0.11). (Found: C, 66.48; H, 8.91, $C_{36}H_{58}O_{10}$ requires: C, 66.46; H, 8.92%). ^{1}H NMR ($CDCl_3$) (400 MHz): δ5.34–5.26 (1H, *m*, H-6), 5.04–5.02 (1H, *m*, H-20), 4.72 (1H, *dd*, J=8 and 1.5 Hz, H-1′), 4.24 (1H, *dd*, J=8 and 1.5 Hz, H-1″), 4.14–4.10 (1H, *m*, H-3′), 4.04–3.92 (2H, *m*, H-5′, H-5″), 3.86–3.80 (1H, *m*, H-3″), 3.76–3.72 (1H, *m*, H-4′), 3.48–3.44 (1H, *m*, H-4″), 3.38 (3H, *s*, OMe), 2.51–2.46 (2H, *m*, H-2′ eq, H-2″ eq), 2.28–2.24 (2H, *m*, H-2′ ax, H-2″ ax), 2.00 (3H, *s*, OAc), 1.32–1.03 (9H, 6′-Me, 6″-Me, 21-Me), 0.86 (3H, *s*, 18-Me), 0.82 (3H, *s*, 19-Me). MS *m/z*: 340 [M − disaccharide − $2H_2O$]$^+$, 289 [M − disaccharide − Me − CHOCOMe]$^+$, 274 [disaccharide(292) − H_2O]$^+$, 271 [289 − H_2O]$^+$, 253 [340 − MeCHOCOMe]$^+$, 198 [274 − MeOH − MeCHO]$^+$, 163 [aglycone (376) − 213]$^+$, 162 [monosaccharide]$^+$, 151 [aglycone (376) − 225]$^+$, 148 [monosaccharide]$^+$, 145 [162 − OH]$^+$, 145 [163 − H_2O]$^+$, 137 [aglycone (376) − 239]$^+$, 133 [151 − H_2O]$^+$, 131 [148 − OH]$^+$, 113 [145 − MeOH]$^+$, [131 − H_2O]$^+$, 95 [113 − H_2O]$^+$.

Emidine (**5**, 35 *mg*). Mp 192–196°, $[\alpha]_D$ +10.3° (MeOH; *c* 0.15). (Found: C, 64.63; H, 8.86, $C_{39}H_{64}O_{12}$ requires: C, 64.64; H, 8.83%). ^{1}H NMR (400 MHz) ($CDCl_3$): δ5.38–5.32 (1H, *m*, H-6), 4.62 (1H, *dd*, J=9 and 2 Hz, H-1′), 4.50 (1H, *dd*, J=9 and 2 Hz, H-1″), 4.43 (1H, *dd*, J=9 and 2 Hz, H-1‴), 3.90–3.82 (1H, *m*, H-20), 3.76–3.72 (2H, *m*, H-4′, H-4″), 3.68–3.64 (3H, *m*, H-3′, H-3″, H-3‴), 3.48–3.44 (3H, *m*, H-5′, H-5″, H-5‴), 3.40–3.36 (1H, *m*, H-4‴), 2.33–2.02 (3H, *m*, H-2′ eq, H-2″ eq, H-2‴ eq), 2.02–1.78 (3H, *m*, H-2′ ax, H-2″ ax, H-2‴ ax), 1.34 (3H, *d*, J=6 Hz, 6′-Me), 1.26 (3H, *d*, J=6 Hz, 21-Me), 1.20 (3H, *d*, J=6 Hz, 6″-Me), 1.18 (3H, *d*, J=6 Hz, 6‴-Me), 1.02 (3H, *s*, 18-Me), 0.89 (3H, *s*, 19-Me). MS *m/z*: 464 [M − disaccharide]$^+$, 408 [trisaccharide]$^+$, 401 [464 − MeCHOH − H_2O]$^+$, 334 [M − trisaccharide]$^+$, 316 [334 − H_2O]$^+$, 289 [334 − MeCHOH]$^+$, 271 [316 − MeCHCHOH]$^+$, 260 [disaccharide − H_2O]$^+$, 253 [271 − H_2O]$^+$, 196 [334 − $C_9H_{14}O$]$^+$, 151 [196 − MeCHOH]$^+$, 148 [monosaccharide]$^+$, 133 [151 − H_2O]$^+$, 131 [148 − OH]$^+$, 113 [131 − H_2O]$^+$, 95 [113 − H_2O]$^+$.

Mild hydrolysis of **1**. To a soln of crystalline **1** (15 mg) in 80% aq. dioxane (1 ml) was added 0.1 M H_2SO_4 (1 ml) and the mixt. was warmed for 90 min at 50°. Usual work-up [4] followed by CC on silica gel using $CHCl_3$–MeOH as eluent afforded genin (**2**) and 2 pure sugars, $[\alpha]_D$ +41.8° (MeOH; *c* 0.10) and $[\alpha]_D$ +13.8° (H_2O; *c* 0.13). The specific rotation, TLC and PC comparison of these sugars with the authentic sample showed them to be identical to D-digitoxose and D-oleandrose.

Mild hydrolysis of **5**. A soln of **5** (10 mg) in 80% dioxane (1 ml) was hydrolysed with 0.1 M H_2SO_4 (1 ml) at 50° for 90 min. The usual work-up as above afforded genin **3** (3.1 mg), mp 202–204°, $[\alpha]_D$ −49.82° (MeOH; *c* 0.11) and the syrupy sugar (1.4 mg), $[\alpha]_D$ +40.5° (MeOH; *c* 0.14) identified as D-digitoxose by comparison with the authentic sample.

Hydrolysis of **2** *by the Zemplen method*. To a soln of **2** (2 mg) in absolute MeOH (0.5 ml) was added NaOMe (0.05 ml) and the mixt. was kept at room temp. After 15 min it was neutralized with IR 120 $[H]^+$ resin and filtered. MeOH was removed under red. pres. yielding **3** (1.4 mg) mp 200–202°, $[\alpha]_D$ −50° (MeOH; *c* 0.14).

(Found: C, 75.41; H, 10.13 $C_{21}H_{34}O_4$ requires: C, 75.44; H, 10.17%).

Very mild hydrolysis of **1**. To a soln of **1** (15 mg) in 80% aq. dioxane (1 ml) was added 0.01 M H_2SO_4 and the soln was kept at room temp. After 2 days the reaction mixt. exhibited 3 spots on TLC ($CHCl_3$–MeOH) identical in mobility with digitoxose, unreacted starting **1** and a new spot presumably monoglycoside. The hydrolysis was complete in 4 days showing 3 spots identical in mobilities with digitoxose, oleandrose and 20-*O*-acetyl calogenin. The reaction mixt. was then worked-up, followed by CC affording 20-*O*-acetyl calogenin, digitoxose and oleandrose.

Very mild hydrolysis of **5**. To a soln of **5** (10 mg) in 80% aq. dioxane (1 ml) was added 0.01 M H_2SO_4 (1 ml) and the soln was kept at room temp. After 2 days, it showed 2 spots besides a spot of unreacted **5**. The polar spot was identified as digitoxose, while the less polar spot was presumed to be the diglycoside. After 4 days on TLC the hydrolysate showed 1 more new spot presumed to be the monoglycoside besides the spot of digitoxose, diglycoside, calogenin and unreacted **5**. The hydrolysis was complete in 140 hr. Usual work-up afforded chromatographically pure calogenin (2.5 mg) and digitoxose (1.7 mg).

D-*Digitoxonic acid phenyl hydrazide*. A soln of D-digitoxose (2 mg) in H_2O (0.51 ml) was oxidized with Br_2 yielding syrupy lactone. This lactone on treatment with phenylhydrazine yielded known D-digitoxonic acid phenyl hydrazide (1.1 mg) mp 120–122°.

D-*Oleandronic acid phenyl hydrazide*. A soln of D-oleandrose (1.5 mg) in H_2O (0.5 ml) was oxidized with Br_2 yielding syrupy lactone, which on treatment with phenylhydrazine in EtOH yielded known D-oleandronic acid phenyl hydrazide (0.4 mg) mp 131–133°.

Acetylation of **1**. Compound **1** (2.5 mg) on acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temp. for 24 hr afforded **4** (1.4 mg). ^{1}H NMR: δ5.16–5.06 (1H, *m*, H-20), 2.04 (3H, *s*, OAc), 1.96 (3H, *s*, OAc), 1.90 (3H, *s*, OAc).

Acetylation of **5**. Compound **5** (2 mg) on acetylation with Ac_2O (0.3 ml) in pyridine (0.3 ml) at room temp. for 24 hr afforded **6** (1.5 mg). ^{1}H NMR: δ5.12–5.02 (1H, *m*, H-20), 2.16 (3H, *s*, OAc), 2.08 (3H, *s*, OAc), 2.07 (3H, *s*, OAc), 2.05 (3H, *s*, OAc), 2.00 (3H, *s*, OAc).

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