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A PREGNANE ESTER DIGLYCOSIDE FROM *SARCOSTEMMA BREVISTIGMA*

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Key Word Index—*Sarcostemma brevistigma*; Asclepiadaceae; brevinine; steroid; pregnane ester glycoside.

Abstract—A pregnane ester diglycoside, brevinine, has been isolated from the dried twigs of *Sarcostemma brevistigma*. Its chemical and spectroscopic data are consistent with the structure 11-*O*-benzoyl-sarcogenin-3-*O*-α-L-diginopyranosyl-(1 → 4)-α-L-diginopyranoside.

INTRODUCTION

In the chemical investigation of the aerial part of *Sarcostemma brevistigma* W. & A., a mixture of chloroform-soluble pregnane glycosides of 2-deoxy sugars was extracted. This mixture could not be resolved satisfactorily on TLC or PC. It was, therefore, subjected to very mild acid hydrolysis which yielded a novel pregnane ester diglycoside, named as brevinine (**1**), besides a triglycoside, brevine [1], and three new disaccharides, brevobiose [2], sarcobiose [3], and tigmobiose [4].

RESULTS AND DISCUSSION

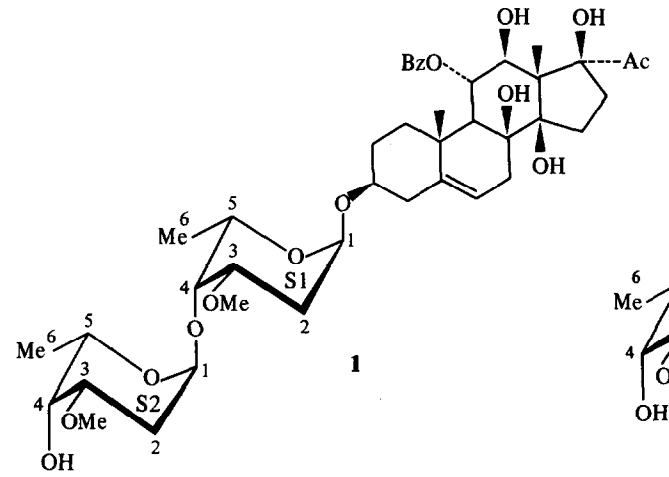
Brevinine (**1**), $C_{42}H_{60}O_{14}$, mp 260–262°, $[\alpha]_D$ +27, was obtained by repeated column chromatography of the partially hydrolysed glycoside mixture over silica gel. Positive xanthydrol [5, 6] and Keller–Kiliani [7] reactions indicated that it contained 2-deoxy sugar(s). Its IR spectrum suggested the presence of hydroxyl groups (3380–3520 cm^{-1}), a methyl keto chain (1680 cm^{-1} accompanied by 1375 cm^{-1}) and a benzoate ester group (1705 cm^{-1} supplemented by strong phenyl group C–H bending at 705 cm^{-1}). The presence of the carbonyl group was also indicated by its reduction with sodium borohydride and reaction with dinitrophenylhydrazine. The keto methyl nature of **1** was supported by a characteristic colour reaction with sodium nitroprusside [8]. The ease with which **1** underwent alkaline hydrolysis, its ^1H NMR data and its UV absorption maximum at 282 nm (log ε2.90) [9] suggested the presence of a monobenzoate ester group in the molecule.

Mild acid hydrolysis of **1** using 0.025 M sulphuric acid in aqueous dioxane [10] afforded the amorphous genin **3**, $[\alpha]_D$ +64, as a non-reducing component and a viscous reducing product as the only sugar. The sugar ($[\alpha]_D$ −60) was characterised as L-diginose (2,6-dideoxy-3-*O*-methyl-L-lyxohexose, **5**) [11] by its co-chromatography with authentic **5** on PC and TLC. Bromine water oxidation of **5** gave the amorphous lactone **6** which with phenyl hydrazine afforded a crystalline product **7**, identified as L-diginonic acid phenyl hydrazide [12] by mmp. This finding confirmed that the sugar **5** was L-diginose.

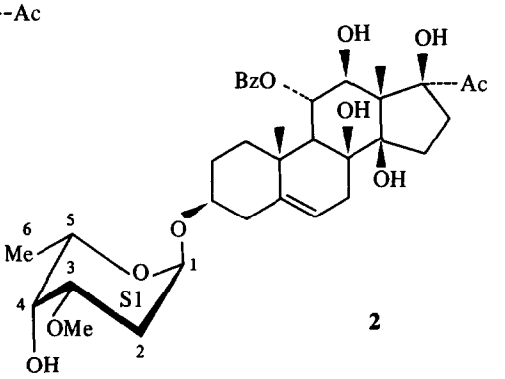
The crystalline product **4**, $C_{21}H_{32}O_7$, mp 103–104°, $[\alpha]_D$ +47, obtained by alkaline hydrolysis of the amorphous genin **3** was identified as sarcogenin [13] (3β,8β,11α, 12β,14β,17β-hexahydroxy-Δ^5-pregnen-20-one) by direct comparison with the authentic material isolated earlier from this plant. However, the inertness of **3** to sodium periodate in contrast to its debenzoylated product **4** suggested **3**, $[\alpha]_D$ +64, was 11 or 12-mono-*O*-benzoyl-sarcogenin ($C_{28}H_{36}O_8$). It was shown to be identical to 11-*O*-benzoyl-sarcogenin [1] by $[\alpha]_D$, ^1H NMR and TLC.

The difference of $C_{14}H_{24}O_6$ between the formulae of **1** and **3** indicated that **1** was a didiginoside. The mass spectrum of **1** was also consistent with a didiginoside structure. For convenience the two sugar units of **1** were labelled as S1 and S2. The mass spectrum of **1** did not exhibit an $[M]^+$ (*m/z* 788) but the highest mass ion peaks at *m/z* 506 and 493 were interpreted as $[M-145-122-15]^+$ and $[M-130-122-43]^+$ or $[M-122-43-113-17]^+$, respectively, representing the losses of monosaccharide fragment ions [14], benzoic acid, a methyl group, a hydroxyl group and the keto methyl side chain from the molecular ion. **1** also exhibited ion peaks at *m/z* 482, 464, 446, 428 and 410 corresponding to the loss of one, two, three, four and five water molecules from the genin ion (*m/z* 500). The loss of 122 amu for benzoic acid giving the ion peak at *m/z* 301 [500 (genin) − 2 × OH − COMe − BzOH] suggested the presence of a sixth benzoylated hydroxyl group. The lower mass region contained the common 2,6-dideoxy monomethoxy hexose fragments [14] at *m/z* 145, 113 and 95.

Direct chemical support that **1** was 11-*O*-benzoyl-sarcogenin-didiginoside was provided by products of its very mild hydrolysis with acid (0.001 N) for six days [15]. Under these conditions **1** gave diginose (**5**), genin **3**, starting material (**1**) and what was presumed to be the monoglycoside **2**. Further chemical support that brevinine (**1**) and brevine [1] were related to each other as the didiginoside and tridiginoside of the same genin 11-*O*-benzoyl-sarcogenin (**3**) came from the TLC comparison of their very mild acid hydrolysis products. The triglycoside brevine under similar hydrolysis conditions afforded partially hydrolysed products with identical mobilities to the didiginoside brevinine **1** and the monoglycoside **2** from brevinine. The hydrolysis of both these glycosides was complete in 12 days, when the hydrolysates gave two spots on TLC identical in mobility to the diginose and 11-*O*-benzoyl-sarcogenin confirming that both brevinine and brevine were composed of the same genin and sugar units



1



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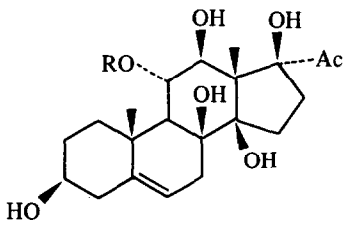
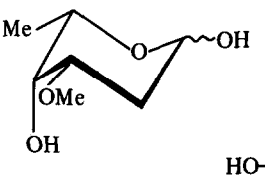


3 R = Bz
4 R = H



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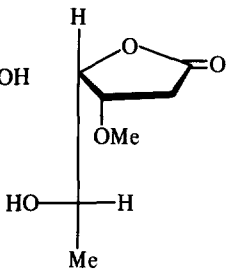

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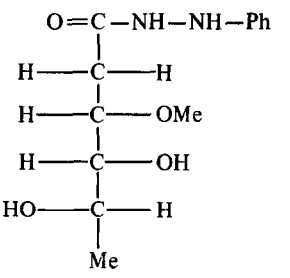

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and that brevinine was a didiginoside and brevine was a tridiginoside.

Confirmation of the diglycoside structure of **1** was provided by its ^{1}H NMR (400 MHz) spectrum which contained two methoxy group singlets at δ3.7 and 3.42 and two secondary methyl group doublets (J = 6 Hz) at 1.34 and 1.30. The four C-2 methylene protons of the two 2-deoxy sugar units S1 and S2 appeared as two sets of two proton multiplets in the region 2.18–2.30 and 1.86–1.96 for the equatorial and axial protons respectively. A very prominent double doublet of two protons (J = 3 and 1 Hz) centred at δ5.04 was assigned to the two anomeric protons of the S1 and S2 sugar units. The small coupling constant (J = 3 Hz) was typical of an equatorial anomeric proton in $^1C_4$ (L) conformation, indicating the sugars were involved in α-L-glycosidic linkages. A low field triplet (J = 8 Hz) centred at δ4.85 was attributed to the C-11 methine proton carrying the benzoyloxy function. The C-17 methyl keto signal appeared as a three proton singlet at δ2.06 and the C-18 and C-19 tertiary methyl groups gave rise to singlets at 1.28 and 1.14 besides the other signals of the sarcogenin moiety and sugar residues.

In the light of this evidence the structure of brevinine (**1**) was established as 11-*O*-benzoyl-sarcogenin-3-*O*-α-L-diginopyranosyl-(1 → 4)-α-L-diginopyranoside.

EXPERIMENTAL

Mps (Boetius micromelting point apparatus): uncorr; ^{1}H NMR: 400 MHz, $CDCl_3$, 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 (5.4 kg) of *Sarcostemma brevistigma* were extracted with aq. EtOH and the concentrate fractioned with different organic solvents. The $CHCl_3$ soluble strongly xanthydrol positive extract was mildly hydrolysed with very dil. acid by the method reported earlier [1] to afford a mixture of partially hydrolysed glycosides. Repeated CC of this glycoside mixture over silica gel afforded crystalline brevinine (**1**) (35 mg).

Brevinine (**1**). Mp 260–262° (Me_2CO–petrol), $[\alpha]_D^{25}$ + 27.5° ± 2 (MeOH, *c* 0.08), (Found C, 63.50; H, 7.40. $C_{42}H_{60}O_{14}$ requires C, 63.90; H, 7.51%). Pink colour in the xanthydrol and blue colour in Keller–Kiliani reactions. **1** did not react with $NaIO_4$ but underwent reduction with $NaBH_4$ (TLC). UV λ_{max}^{EtOH} nm (log ε): 282 (2.90); IR ν_{max}^{KBr} cm^{-1}: 3380–3520 (ass. OH), 1705 (>C=O of benzoate ester), 1680 (>C=O of Ac), 1375 (CH_3 def.) and 705 (C–H def. aromatic); ^{1}H NMR (400 MHz): δ7.94–8.0 (2H, *m*, aromatic), 7.42–7.64 (3H, *m*, aromatic), 5.42 (1H, *m*, H-6) 5.04 (2H, *dd*, J = 3 and 1 Hz, H-1 in S1 and S2), 4.85 (1H, *t*, J = 8 Hz, H-11), 4.34–4.44 (2H, *m*, H-5 in S1 and S2), 4.22–4.3 (2H, *m*, H-3 in S1 and S2), 3.7 (3H, *s*, OMe), 3.54–3.6 (2H, *m*, H-4 in S1 and S2), 3.42 (3H, *s*, OMe), 2.18–2.30 (2H, *m*, H-2e in S1 and S2), 2.06 (3H, *s*, 17-COMe), 1.86–1.96 (2H, *m*, H-2a in S1 and S2), 1.34 (3H, *d*, J = 6 Hz, sec. Me), 1.28 (3H, *s*, 18-Me), 1.24 (3H, *d*, J = 6 Hz, sec. Me) and 1.14 (3H, *s*, 19-Me); MS *m/z* (rel. int.): 788 $[M]^+$ not observed, 506 (6) $[M - BzOH - Me - monosaccharide\ ion\ (145)]^+$, 493 (8) $[M - BzOH - COMe - monosaccharide\ ion\ (130)]^+$, 482 (9) $[M - disaccharide\ (306)]^+$ or [500 (genin) $- H_2O$], 466 (77) [500 − 2 × OH], 464 (39) [482 − H_2O], 449 (46) [466 − OH], 446 (34) [464 − H_2O], 441 (100) $[M - BzOH - COMe - monosaccharide\ ion\ (130) - H_2O - 2 \times OH]^+$, 431 (17) [449 − H_2O], 428 (8) [446 − H_2O], 423 (54), [466 − COMe], 413 (10) [431 − H_2O], 410 (7) [428 − H_2O], 405 (14) [423 − H_2O], 301 (15) [423 − BzOH], 283 (17) [301 − H_2O], 265 (5)

$[283-H_2O]$, 161 (21) $[C_{11}H_{13}O$ (genin fragment)$]^+$, 139 (6) $[C_8H_{11}O_2]^+$, 122 (20) [BzOH], 113 (38) $[C_6H_9O_2]^+$, 105 (99) $[C_7H_5O]^+$ and 43 (55) [COMe]. Sugar fragments: 145 (47) [monosaccharide (162) – OH], 113 (38) [145 – MeOH] and 95 (32) $[113-H_2O]$.

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 warmed for 30 min at 50°. Dioxane was then removed under reduced pressure. The aq. portion was repeatedly extracted with $CHCl_3$ and the organic layer washed in turn with H_2O, 1 M Na_2CO_3, H_2O, dried over Na_2SO_4 and evaporated to afford genin **3** (8 mg), $[\alpha]_D^{25}+64°$ (MeOH, *c* 0.12). The aq. hydrolysate was neutralised with freshly prepared $BaCO_3$, filtered and concentrated under reduced pressure to afford the syrupy sugar **5** (6 mg), $[\alpha]_D^{25}-60.3°$ (MeOH, *c* 0.18), that reduced Fehling's soln gave a pink colouration in the xanthydrol and a blue colouration in the Keller–Kiliani reactions. A comparison of the mobility of **5** on PC and its optical rotation identified it as L-diginose. [lit. [11] $[\alpha]_D^{25}-65.2$ (H_2O)].

Oxidation of **5** *with* Br_2*–water.* A soln of **5** (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 of Br_2 was then removed under reduced pressure. The acidic mixture was neutralized with freshly prepared Ag_2CO_3 and the suspension filtered, H_2S was passed through the filtrate to remove Ag^+ ions and filtered. The filtrate was evaporated to dryness under reduced pressure yielding syrupy lactone **6** (3.0 mg) which gave a violet colour in the spot test with NH_2OH–$FeCl_3$ reagent [16].

L-*Diginonic acid phenyl hyrazide* (**7**). A soln of **6** (2.5 mg) in absolute EtOH (0.5 ml) was mixed with freshly distilled phenylhydrazine (0.4 ml), and the mixture heated for 30 min at 50°. The viscous mass was cooled and repeatedly triturated with Et_2O (to remove the excess of phenyl hydrazine) yielding the phenyl hydrazide **7** as a brown powder which crystallized from MeOH–Et_2O as colourless needles (1 mg), mp 133–134° (lit. [13] L-diginonic acid phenyl hydrazide mp 135°).

Alkaline hydrolysis of **3**. Compound **3** (7 mg) was dissolved in 5% methanolic KOH (1.5 ml) and refluxed for 2 hr. After adding H_2O (0.8 ml), MeOH was removed under reduced pressure. The aq. concentrate was extracted with $CHCl_3$, dried over Na_2SO_4, filtered and evaporated to dryness yielding **4** which crystallised from Me_2CO-petrol (4.5 mg), mp 103–104°, $[\alpha]_D^{25}+47.5$ (MeOH, *c* 0.2) (Found C, 62.87; H, 8.19. $C_{21}H_{32}O_7$ requires C, 63.18; H, 8.08%). The identity of **4** was confirmed by comparison (TLC, $[\alpha]_D$, mmp) with an authentic sample of sarcogenin (lit. [13] mp 102–105°, $[\alpha]_D^{25}+49°$).

Very mild hydrolysis of **1** *with acid.* To a soln of **1** (3 mg) in aq. 1,4-dioxane (1:1) (0.2 ml) was added 0.001 M H_2SO_4 (0.2 ml). After 6 days at room temp., the reaction mixture showed four spots on TLC. By co-chromatography the slowest spot was identified as diginose (**5**) and taken as reference (R_{Dig} 1.0). The other spots were genin **3** (R_{Dig} 5.2), unchanged brevinine (**1**) (R_{Dig} 3.2) and what was presumed to be the monoglycoside **2** (R_{Dig} 4.0). Hydrolysis was complete in 12 days when the reaction mixture exhibited spots of only the genin **3** and the sugar **5**. Brevine under similar hydrolysis conditions exhibited five spots on TLC identical in mobility with the genin **3** (R_{Dig} 5.2), brevinine **1** (R_{Dig} 3.2), monoglycoside **2** (R_{Dig} 4.0), diginose (R_{Dig} 1.0) and the unchanged starting material (R_{Dig} 2.5).

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