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A PREGNANE ESTER TRIGLYCOSIDE FROM *SARCOSTEMMA BREVISTIGMA*

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

Abstract—A new pregnane ester glycoside, brevine, was isolated from the dried twigs of *Sarcostemma brevistigma*. Its chemical and spectroscopic properties were consistent with the structure 11-*O*-benzoyl-sarcogenin-3-*O*-α-L-diginopyranosyl(1 → 4)-*O*-α-L-diginopyranosyl(1 → 4)-*O*-α-L-diginopyranoside.

INTRODUCTION

In the chemical investigation of the aerial part of the plant *Sarcostemma brevistigma* W. & A., a mixture of chloroform soluble 2-deoxy glycosides was obtained which after very mild acid hydrolysis afforded three novel disaccharides brevobiose [1], sarcobiose [2] and tigmobiose [3]. In a reinvestigation of its chemical constituents the title plant was extracted using the earlier method [4, 5] to give a novel ester triglycoside which we have named brevine (**1**). This paper describes the structure elucidation of this glycoside.

RESULTS AND DISCUSSION

Brevine (**1**) obtained as colourless rhombs from acetone–petrol was isolated as the unhydrolysed glycoside from the very mild acid hydrolysis product of the chloroform extract. Elemental analysis of this non reducing substance gave the formula $C_{49}H_{72}O_{17}$. It displayed positive xanthydrol [6, 7] and Keller–Kiliani [8] colour reactions characteristic of 2-deoxy sugar derivatives. Its IR spectrum contained absorption bands for hydroxyl groups (3540–3250 cm^{-1}), a methyl keto chain (1688 cm^{-1} accompanied by 1368 cm^{-1}) and a benzoate ester group (1710 cm^{-1} and phenyl C–H bending at 710 cm^{-1}) indicating it to be a 2-deoxy sugar ester glycoside. The ease with which compound **1** undergoes alkaline hydrolysis further suggested the presence of an ester function in the molecule. Although **1** was inert to sodium periodate, its deacylated product reacted with this reagent. It is, therefore, evident that the vicinal diol grouping present in the latter is involved in the ester function in **1**.

Mild acid hydrolysis of **1** using the earlier reported method of Mannich and Siewert [9] yielded an amorphous genin **2** and diginose **4** as a reducing viscous product ($[\alpha]_D$, PC, TLC) [10] confirmed through its crystalline phenyl hydrazide **6** (mp, mmp) obtained from its oxidation product lactone. Alkaline hydrolysis of genin **2** yielded sarcogenin **3** [11] (mp, mmp, $[\alpha]_D$ and IR).

The monobenzoate nature of the ester function in **1** was supported by its IR, UV (λ_{max} 282, log ε2.92) and ^{1}H NMR spectral data. However, inertness of **2** to sodium periodate in contrast to its debenzoylated product **3** suggested **2** to be 11- or 12-mono-*O*-benzoyl-sarcogenin of formula $C_{28}H_{36}O_8$.

The difference of $C_{21}H_{36}O_9$ between the formulae of glycoside **1**, and its aglycone **2**, indicated that **1** was a tridiginoside. The same conclusions could be drawn from the mass spectrum of **1**, which recorded fragment ions for a trisaccharide unit (*m/z* 450) and the genin moiety (*m/z* 500) besides the prominent fragment ions of sarcogenin monobenzoate giving ions for benzoic acid at *m/z* 122 and the expected other ions of the sarcogenin moiety including the fragment ions due to the losses of its five molecules of water in sequence at *m/z* 482, 465, 447, 430 and 412.

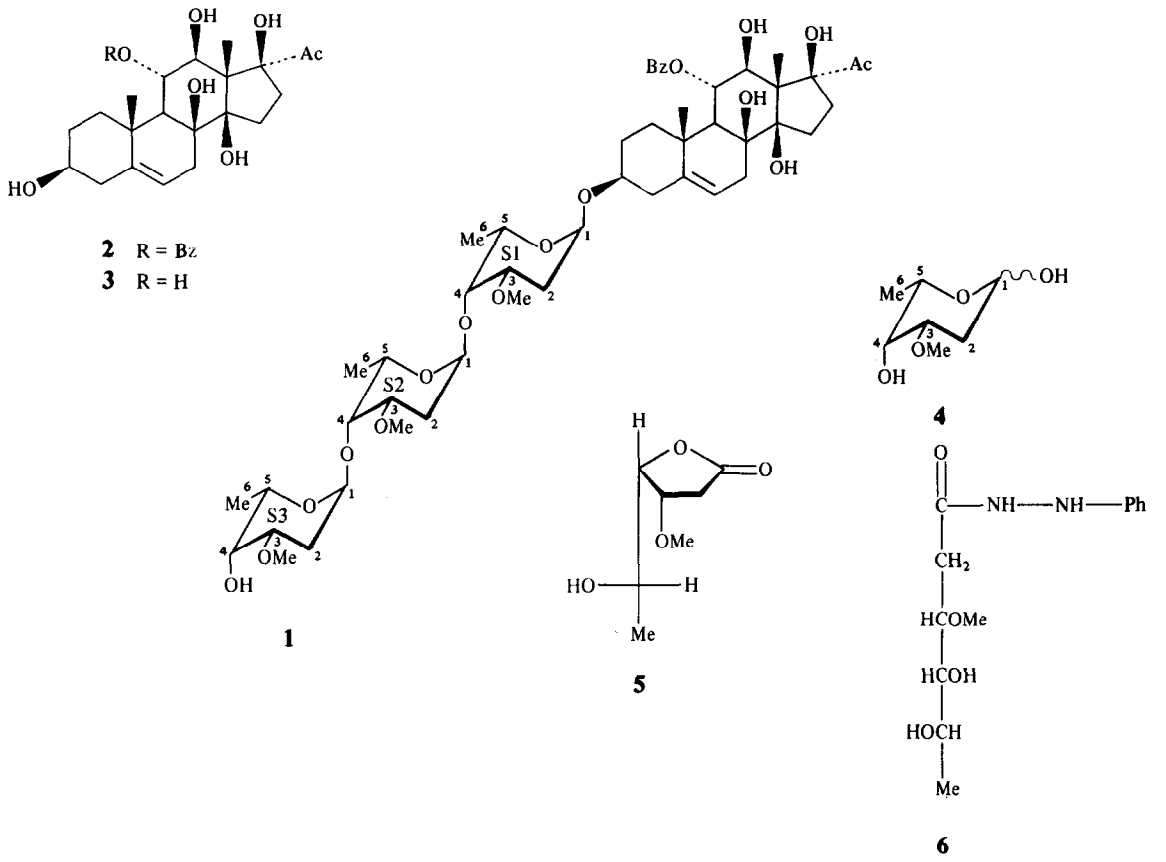
Confirmation of the triglycoside structure of compound **1** and the position of its monobenzoate ester group in the genin moiety was provided by its ^{1}H NMR spectrum which contained signals for three methoxy groups and doublets for secondary methyl groups of three diginose units. The three double doublets of one proton each were assigned to the three anomeric protons with a small coupling constant, typical of an equatorial anomeric proton in the $^1C_4$ (L) [12] conformation of three diginose units. A low field triplet was assigned to the proton adjacent to the benzoate ester group at C-11.

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

EXPERIMENTAL

Mps (uncorr.) PC: toluene–BuOH (4:1) satd with H_2O. Sugars were visualised with 50% aq. H_2SO_4 (TLC) or vanillin–$HClO_4$ reagent (PC). ^{1}H NMR: 400 MHz or 80 MHz, $CDCl_3$, TMS as internal standard.

Plant extraction. The shade dried, powdered twigs of *Sarcostemma brevistigma* (5.4 kg) were extracted by the method used for pregnane glycosides [1], using 50–95% aq. EtOH and the aq. concentrate fractionated with different organic solvents to afford a petrol extract (2.2 g), a $CHCl_3$ extract (12.35 g) and a $CHCl_3$–EtOH (4:1) extract (4.35 g). The $CHCl_3$-soluble (xanthydrol positive) extract admixed with phenolic substances was freed from it by treatment with cold 2 M NaOH. It was mildly



hydrolysed with very dilute acid to obtain a mixture of partially hydrolysed glycosides.

Very mild acid hydrolysis of $CHCl_3$ *extract.* The phenol free $CHCl_3$ extract (10.00 g) dissolved in MeOH (360 ml) mixed with 0.05 M H_2SO_4 (360 ml) was refluxed for 1 hr and MeOH removed under red. pres. The aq. concentrate was further heated for 1 hr at 60°. It was exhaustively extracted with $CHCl_3$–MeOH (4:1) and the organic layer was washed in turn with H_2O, 10% $KHCO_3$ and H_2O, dried over Na_2SO_4, and evapd to afford partially hydrolysed glycosides (7 g) which on repeated CC over silica gel gave crystalline brevine (**1**, 30 mg).

Brevine (**1**). Mp 100–105° (Me_2CO–petrol), $[\alpha]_D^{25}$ + 21.2° (MeOH, *c* 0.17). (Found C, 63.36; H, 7.65; $C_{49}H_{72}O_{17}$ requires C, 63.09; H, 7.73%.) It exhibited a pink colour in the xanthydrol and a blue colour in the Keller–Kiliani reactions. It exhibited negative $NaIO_4$ reaction and underwent $NaBH_4$ reduction (TLC). UV λ_{max}^{EtOH} nm (log ε): 282 (2.92). IR ν_{max} cm^{-1}: 3540–3250 (ass. OH groups), 1710 (C=O of benzoate ester), 1688 (C=O of Ac), 1368 (Me def.), 710 (C–H def. arom.). ^1H NMR (400 MHz): δ 7.98–7.90 (2H, *m*, aromatic), 7.64–7.38 (3H, *m*, arom), 5.38 (1H, *m*, H-6), 5.02 (1H, *dd*, *J* = 2.5 and 1 Hz, H-1 in S1 or S2 or S3), 4.61 (1H, *dd*, *J* = 2.5 and 1 Hz, H-1 in S1 or S2 or S3), 4.82 (1H, *t*, *J* = 8 Hz, H-11), 4.57 (1H, *dd*, *J* = 2.5 and 1 Hz, H-1 in S1 or S2 or S3), 4.42–4.37 (3H, *m*, H-5 in S1, S2 and S3), 4.3–4.2 (3H, *m*, H-3 in S1, S2 and S3), 3.64 (3H, *s*, OMe), 3.6–3.42 (3H, *m*, H-4 in S1, S2 and S3), 3.38 (3H, *s*, OMe), 3.31 (3H, *s*, OMe), 2.16–2.08 (3H, *s*, H-2e in S1, S2 and S3), 2.04 (3H, *s*, 17-Ac), 1.8–1.64 (3H, *m*, H-2a in S1, S2 and S3), 1.32 (6H, *d*, *J* = 6 Hz, sec. Me), 1.28 (3H, *d*, *J* = 6 Hz, sec. Me), 1.24 (3H, *s*, 18-Me), 1.14 (3H, *s*, 19-Me); MS *m/z* (rel. int.): $[M]^+$ (not observed), 500 $[M - \text{trisaccharide}]^+$ (3), 482 $[500 - H_2O]^+$ (5), 465 $[500 - H_2O - OH]^+$ (2), 447 $[500 - 2H_2O - OH]^+$ (1), 430 $[500 - 2H_2O - 2OH]^+$ (1), 412 $[500 - 3H_2O - 2OH]^+$ (3), 343 $[500 - H_2O - OH - BzOH - Ac]^+$ (3), 290 $[500 - 3H_2O - 2OH - BzOH]^+$ (100); sugar fragments: 450 [diginotriose]$^+$ (2), 354 $[450 - 3MeOH]^+$ (25), 290 [rearranged disaccharide fragment]$^+$ (100), 162 [diginose]$^+$ (22), 130 $[162 - MeOH]^+$ (22), 86 (21).

Mild hydrolysis of **1** *with acid.* A soln of **1** (20 mg) in 80% aq. 1,4-dioxane (1.2 ml) was mixed with 0.05 M H_2SO_4 (1.2 ml) and warmed for 30 min at 50°. Dioxane was then removed under red. pres. The aq. concentrate was repeatedly extracted with $CHCl_3$ and the organic layer washed in turn with H_2O, 1 M Na_2CO_3 and H_2O, dried over Na_2SO_4 and evapd to afford an amorphous genin **2** (12 mg), $[\alpha]_D^{25}$ + 65° (MeOH, *c* 0.21). ^1H NMR (80 MHz) of **2**: δ 7.90–7.45 (5H, *m*, aromatic), 5.32 (1H, *m*, H-6), 2.02 (3H, *s*, 17-Ac), 1.27 (6H, *s*, 18-Me and 19-Me).

The aq. hydrolysate was neutralized with freshly precipitated $BaCO_3$, filtered and concd under red. pres. to yield the syrupy sugar **4** (4 mg), $[\alpha]_D^{25}$ − 60° (H_2O, *c* 0.10), that reduced Fehling's soln and gave a pink colouration in the xanthydrol and a blue colouration in the Keller–Kiliani reactions. A comparison of the mobility of **4** on TLC and PC with the authentic sample and its optical rotation identified it as L-diginose (lit. [10], $[\alpha]_D^{25}$ − 65.2° (H_2O)).

Oxidation of **4** *with bromine water.* A soln of **4** (3 mg) in H_2O (0.5 ml) was oxidized with Br_2 (8.5 μl) by the method reported earlier [13] yielding a syrupy lactone **5** (2.2 mg) which gave a violet colour in the spot test with NH_2OH–$FeCl_3$ reagent [14].

L-Diginonic acid phenylhydrazide (**6**). A soln of **5** (2.2 mg) in absolute EtOH (0.6 ml) on heating with freshly distilled phenylhydrazine (0.05 ml), and usual work up as reported earlier [13]

yielded the crystalline phenylhydrazide **6** from MeOH–Et_2O (1.2 mg), mp 132–135°, identical with L-diginonic acid phenylhydrazide (mp, mmp).

Alkaline hydrolysis of compound **2**. Compound **2** (6 mg) was dissolved in 5% methanolic KOH (1.2 ml) and refluxed for 2 hr. After adding H_2O (0.6 ml) MeOH was removed under red. pres. The aq. concentrate was extracted with $CHCl_3$–MeOH (90:10), dried over Na_2SO_4, filtered and evapd to dryness yielding **3** (2.5 mg) which crystallized from Me_2CO–petrol, mp 100–104°, $[\alpha]_D^{25}$ + 47° (MeOH, *c* 0.1). It was identified as sarcogenin (**3**) [11] by comparison with an authentic sample ($[\alpha]_D$, TLC, mmp).

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