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A PREGNANE GLYCOSIDE FROM *STREBLUS ASPER*

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Key Word Index—*Streblus asper*; Moraceae; roots; pregnane glycoside; sioraside.

Abstract—A pregnane glycoside, named sioraside, has been isolated from *Streblus asper*. Its chemical and spectroscopic data are consistent with the structure 3β,14β-dihydroxypregn-20-one-3-*O*-β-D-(3-*O*-methyl)-glucopyranoside.

INTRODUCTION

Pregnane derivatives [1] along with cardenolides [2] have been mostly reported to be present in several species of the Asclepiadaceae. In continuation of our studies on pregnane glycosides, column chromatography of a chloroform–ethanol (2:1) extract of dried roots of *Streblus asper* gave a novel substance 'U' which was reported earlier by Reichstein *et al.* [3]. Its chemical and physical data indicated it to be a glycoside of 3-*O*-methyl-D-glucose but no other data or interpretation of its structure was reported. It was, therefore, of interest to elucidate the structure of substance 'U' now named sioraside (**1**).

RESULTS AND DISCUSSION

Sioraside (**1**), mp 214–217°, $[\alpha]_D^{25}$ −1.3°, gave the molecular formula $C_{28}H_{46}O_8$ which was in agreement with the highest mass ion peak at *m/z* 492.3092 $[M-H_2O]^+$ in the high resolution mass spectrum. A positive Liebermann–Burchardt test [4] and Feigl reaction [5] indicated **1** to be a steroidal glycoside of a normal sugar. The presence of a keto methyl group was shown by the presence of a singlet of three protons at δ2.24 in the ^{1}H NMR which could be present at the commonly reported C-17 position of the pregnane moiety. The presence of an isolated double bond in the molecule was precluded by the ^{1}H NMR data.

As **1** was a normal sugar glycoside, it was subjected to Kiliani hydrolysis [6], affording two crystalline products. The compound possessing reducing properties towards ammoniacal silver nitrate, was identified as 3-*O*-methyl-D-glucose [7] on comparison with an authentic sample (mp, mmp $[\alpha]_D$). The presence of a monomethoxy normal sugar (C_7) in **1** containing 28 carbon atoms suggested that its steroidal moiety is to be a pregnance (C_{21}) derivative. The other compound **2**, mp 163–165°, $[\alpha]_D^{25}$ −7.8°, $C_{21}H_{32}O_2$ which gave a Liebermann–Burchardt test was possibly the genin moiety.

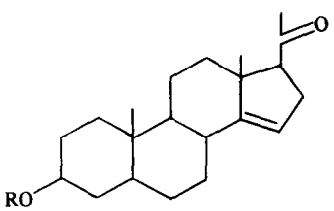
For the detailed study of **2**, it was acetylated with acetic anhydride in pyridine to yield **3**. Its ^{1}H NMR spectrum at 80 MHz contained signals for two angular methyl groups at δ0.98 and 0.96 and a six proton broad singlet at δ2.10 assigned to an acetyl group and a C-17 ketomethyl group. Interestingly it also exhibited a multiplet for an olefinic proton at δ5.35. As **1** did not contain an olefinic proton, but the genin obtained from Kiliani hydrolysis exhibited one, it was evident that this product is an anhydrogenin.

To obtain the native genin and sugar of **1**, it was subjected to mild acid (0.05 M H_2SO_4) hydrolysis [8] which afforded a crystalline genin **4**, 220–222° $[\alpha]_D^{25}$ −47.2°, $C_{21}H_{34}O_3$ and a reducing sugar. This sugar was identical to 3-*O*-methyl-D-glucose isolated earlier from the Kiliani hydrolysis of **1**. Acetylation of genin **4** with acetic anhydride in pyridine gave **5**, concluded to be a monoacetate from a singlet of six protons at δ2.09 assigned to one acetyl and a keto methyl group in the ^{1}H NMR spectrum at 80 MHz. It also contained characteristic signals for the pregnane moiety but the absence of any signal in the region δ5–6 indicated the absence of an olefinic proton in **4** which was also not observed in the ^{1}H NMR spectrum of **1**.

The anhydro product **2** was interpreted to be the dehydration product of **4** involving the C-14 hydroxyl group under the strongly acidic conditions of the Kiliani reaction leading to the conclusion that the native genin moiety is presumably 3β,14β-dihydroxy-17-keto-methyl pregnane [9] (**4**), which was confirmed by co-chromatography and undepressed mmp with an authentic sample.

The structure of **1** was largely elicited by its ^{1}H NMR spectrum. A one proton doublet ($J = 8$ Hz) at δ4.40 was evidently from the anomeric proton of the sugar moiety. The large value ($J = 8$ Hz) of the coupling constant for the anomeric proton was typical of its axial configuration in the hexopyranose in the $^4C_1$(D) conformation indicating that the sugar was joined through a β-D-glycosidic linkage. The ^{1}H NMR spectrum also contained the characteristic signals of genin and sugar moieties of **1**.

Although sioraside did not exhibit a $[M]^+$ the highest ion peak at *m/z* 492.3092 ($C_{28}H_{44}O_7$) was interpreted as $[M-H_2O]^+$. Its other prominent fragment ions in the lower mass region at *m/z* 177.1641 ($C_7H_{13}O_5$) were typical of a mono-*O*-methyl normal hexose fragment, whereas the other ion at *m/z* 317.2477 for $C_{21}H_{33}O_2$ corresponded to a genin without a hydroxyl group affording another ion at *m/z* 299.2376 after the loss of a water molecule. The other prominent ion signals for the genin moiety were recorded at *m/z* 273.2218, 255.2112, 181.1227, 141.0914, 135.1175 and 123.1136 and the significant ion peaks at *m/z* 159.1238, 144.0424, 127.1362 and 73.0301 originated from the fragmentation of the mono-*O*-methyl normal hexose moiety. Thus, **1** is 3β,14β-dihydroxy pregn-20-one-3-*O*(-3-*O*-methyl)-D-glucopyranoside.



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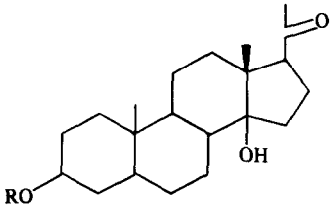

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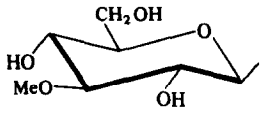

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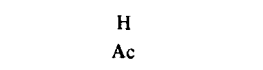

4 H
5 Ac

EXPERIMENTAL

General procedures were the same as those reported in ref. [3] except for MS which were recorded at high resolution. ^{1}H NMR were recorded at 400 and 80 MHz with TMS as int. standard unless otherwise stated.

Extraction. Shade-dried powdered roots (5.3 kg) of *S. asper* were extracted with aq. EtOH and the concentrate fractionated with different organic solvents as reported earlier [3] to afford a petrol extract (2.6 g), an Et_2O extract (1.2 g), a $CHCl_3$ extract (18 g), a $CHCl_3$–EtOH (4:1) extract (8 g) and a $CHCl_3$–EtOH (2:1) extract (8 g), respectively. The $CHCl_3$–EtOH (2:1) extract was chromatographed over silica gel to give sioraside (**1**) (72 mg).

Sioraside (**1**). Mp 214–217° (Me_2CO–Et_2O). $[\alpha]_D^{25}$ −1.3° (MeOH; *c* 0.11). (Found: C, 65.43; H, 9.00, $C_{28}H_{46}O_8$ requires C, 65.88; H, 8.98%). It gave a crimson colour with the Feigl test and a red colour with Fehling's soln. ^{1}H NMR $CDCl_3$ (400 MHz) 4.40 (1H, *d*, J = 8 Hz, H-1') 3.96–3.88 (1H, *m*, H-5'), 3.8–3.72 (1H, *m*, H-3'), 3.68 (3H, *s*, OMe), 3.44 (1H, *dd*, J = 8 and 6 Hz, H-2'), 3.40–3.34 (1H, *m*, H-4'), 2.24 (3H, *s*, Ac), 2.0–1.3 (methylenes of aglycone), 0.98 (3H, *s*, Me-18), 0.96 (*s*, 3H, Me-19). MS: *m/z* (rel. int. %): 510 ([M]$^+$) not observed 492.3092 (2.8) [M − H_2O]$^+$ [$C_{28}H_{44}O_7$], 317.2477 (67.3) [genin − OH]$^+$ [$C_{21}H_{33}O_2$], 299.2376 (100) [genin − OH − H_2O]$^+$ ($C_{21}H_{31}O$), 273.2218 (2.1) [genin − Ac − H_2O]$^+$ ($C_{19}H_{29}O$), 255.2112 (8.8) [genin − Ac − 2H_2O]$^+$ ($C_{19}H_{27}$), 249.1844 (7.8) [$C_{16}H_{25}O_2$]$^+$, 231.1733 (11.6) [$C_{16}H_{23}O$]$^+$, 213.1667 (8.26) [$C_{16}H_{21}$]$^+$, 181.1227 (15.7) [$C_{11}H_{17}O$]$^+$, 177.1641 (41.3) ($C_{13}H_{21}$]$^+$, 165.1321 (7.2) [$C_{11}H_{17}O$]$^+$, 147.1173 (12.6) [$C_{11}H_{15}$]$^+$, 135.1175 (17.1) [$C_{10}H_{15}$]$^+$, 127.1302 (2.8) [$C_6H_7O_3$]$^+$, 73.0301 (18.1) [$C_3H_6O_2$]$^+$.

Kiliani hydrolysis. A crystalline sample of sioraside **1** (10 mg) was treated in 1 ml Kiliani mixt. (3.5 parts HOAc + 5.5 parts H_2O + 1 part conc. HCl) in a small test tube. It was heated at 100° for 1 hr and usual work-up yielded genin **2** and a sugar (3 mg), which was recrystallized from Me_2CO, mp 133–135°, $[\alpha]_D^{25}$ +68° (MeOH), $C_7H_{14}O_6$. A comparison of its optical rotation, co-chromatography on PC and undepressed mmp with an authentic sample identified it as 3-*O*-methyl-D-glucose. Crystalline genin **2** (5 mg) $[\alpha]_D^{25}$ −7.8 (MeOH; *c* 0.13) Found: C, 79.76%, H, 10.08% $C_{21}H_{32}O_2$ required C, 79.74: H, 10.12%.

Acetylation of genin **2**. Compound **2** (3 mg) on acetylation with Ac_2O (0.4 ml) in pyridine (0.4 ml) at 100° for 8 hr and usual work-up afforded **3**. ^{1}H NMR (80 MHz) δ5.35 (*m*, 1H; olefinic proton), 2.05 (6H, *s*, OAc and Ac), 0.98 (3H, *s*, Me-18), 0.96 (3H, *s*, Me-19).

Mannich hydrolysis. To a soln of crystalline **1** (15 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 5 hr at 50°. Usual work-up afforded genin **4** (8 mg) and sugar (4 mg), recrystallized from dry Me_2CO, mp 133–35°, $[\alpha]_D^{25}$ +68°. Comparison of its optical rotation, PC and undepressed mmp with an authentic sample identified it as 3-*O*-methyl-D-glucose. The genin (**4**), mp 220–222°, was confirmed as 3β,14β-dihydroxy-17-keto-methylpregnane by comparison with an authentic sample (TLC and mmp). Found: C, 75.44, H, 10.18, $C_{21}H_{34}O_3$ requires 75.45, H, 10.18.

Acetylation of genin **4**. Compound **4** (5 mg) on acetylation with Ac_2O (0.4 ml) in pyridine (0.4 ml) at 100° for 8 hr and usual work-up afforded **5**. ^{1}H NMR (80 MHz), δ2.06 (6H, *s*, OAc and Ac), 0.98 (3H, *s*, Me-18), 0.96 (3H, *s*, Me-19).

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