

A New Pregnane Glycoside from Fermented Leaves of *Agave americana*

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Abstract: A new minor pregnane glycoside was isolated from the fermented leaves of *Agave americana*. Its structure was elucidated as (20*S*)-5 α -pregnane-3 β , 20-diol 20-O- β -D-glucopyranoside (**1**) by spectral methods.

Keywords: *Agave americana* L., pregnane glycoside.

Pregnane glycosides are rare in *Agavaceae*. Several pregnane glycosides were discovered from *Agavaceae* plants¹⁻³. No pregnane glycoside has been isolated from a *Agave* species. Previously, we have reported two new steroidal glycosides from fermented leaves of *A. americana*⁴. In this paper, we describe the structural determination of a new pregnane glycoside from fermented leaves of *A. americana*.

The methanol extract of dried fermented residues of leaf-juices of *A. americana* (6.5 Kg) produced in Ruili County of Yunnan Province at January 2000, was subjected to repeated normal and reverse silica gel column chromatographies to give compound **1** (7 mg).

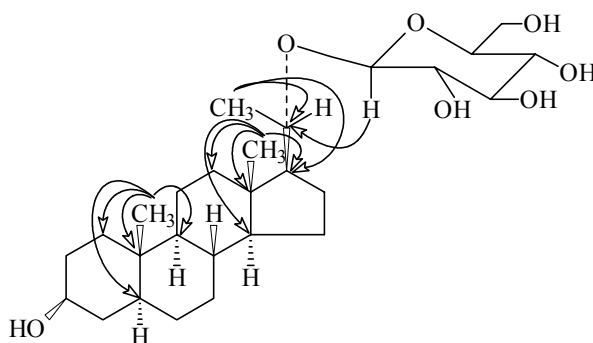
Compound **1** was obtained as a white amorphous solid, and showed positive reaction to anisaldehyde-sulphuric acid reagent on TLC. Its molecular formula was deduced as C₂₇H₄₆O₇ by DEPT and negative ion FAB-MS. A quasi molecular ion of **1** was observed at *m/z* 481.3165 ([M-H]⁻) in the high resolution mass spectrum, also indicating the molecular formula as C₂₇H₄₆O₇ (calcd for C₂₇H₄₅O₇, 481.3165). The ¹H-NMR spectrum exhibited two tertiary methyl protons at δ 0.69 (3H, s) and 0.84 (3H, s), and one secondary methyl proton at δ 1.53 (3H, d, *J*=6.0 Hz). An anomeric proton signal was also identified at δ 4.89 (1H, d, *J*=7.6 Hz). Its *J* value (> 7 Hz) of the anomer of the sugar moiety indicated the β -orientation at the anomeric center of the D-pyranose. The ¹³C NMR spectrum showed 27 carbon signals, 21 of them were to the aglycone part and 6 to a monosaccharide, suggesting that it might be a pregnane monoglycoside. It is easy to make out that there were six signals of a glucopyranosyl unit at δ 106.1, 78.5, 78.1, 75.7, 71.9 and 63.1 in ¹³C-NMR spectrum. Two carbon signals at δ 81.6 and 70.7 indicated that there were two hydroxyl groups in the molecular

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structure. Acid hydrolysis of **1** with 1 mol/L HCl only yielded D-glucose as the carbohydrate component, which was identified by GC analysis following its conversion to the trimethylsilylated derivative.

The carbon and proton signals of **1** were assigned by analysis of 1D and 2D NMR (**Table 1**). In HMBC spectrum, the tertiary methyl signal at δ_{H} 0.84 (H-19) was correlated with carbon signals at δ_{C} 37.6 (CH₂), 45.3 (CH), 54.7 (CH) and 35.9 (C), indicating that these signals were assigned to be C-1, C-5, C-9 and C-10, respectively. In the same way, from the correlations with δ_{H} 0.69 (H-18) and 1.53 (H-21), carbon signals at δ_{C} 39.6 (C-12), 42.0 (C-13), 56.6 (C-14), 58.1 (C-17) and 81.6 (C-20) were also assigned respectively. The anomeric proton signal of glucopyranosyl unit at δ_{H} 4.89 was correlated with δ_{C} 81.6, indicating that the glucopyranosyl unit was attached to the hydroxyl of the aglycone C-20 (**Figure 1**).

Figure 1 Key HMBC correlations of **1**



Comparison of the ¹³C-NMR data of **1** with those of tigogenin revealed that the structure of the ring A-C portion of the molecule (C-1 to C-14) was identical to that of tigogenin, including the orientation of oxygen atom at C-3 and hydrogen atom at C-5, as well as ring junction⁵. In the ROESY spectrum, the correlations from H-21 (δ 1.53) to H-18 (δ 0.69), and from H-20 (δ 3.87) to H-16 β (δ 1.90) were consistent with C-17 β and C-20*S* configuration. H-3 (δ 3.83) was correlated with H-1 α (δ 1.03), H-2 α (δ 2.04), H-4 α (δ 1.79) and H-5 α (δ 1.13), also indicating that the orientations of C-3 oxygen and C-5 hydrogen atoms were determined as β -orientation and α -orientation (**Figure 2**). Thus, the structure of **1** was determined as (20*S*)-5 α -pregnane-3 β , 20-diol 20-O- β -D-glucopyranoside.

Figure 2 Significant ROESY correlations of 1

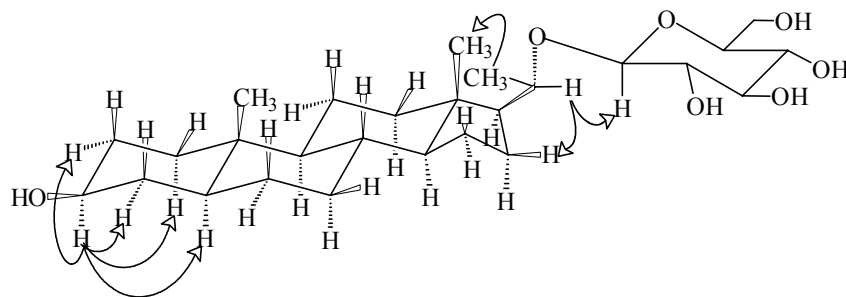


Table 1 ^1H and ^{13}C NMR data of 1 (pyridine- d_5 , δ in ppm)

Position	δ_{C}	δ_{H}
1	37.6 (t)	1.03 (1 α), 1.74 (1 β)
2	34.5 (t)	2.04 (2 α), 1.70 (2 β)
3	70.7 (d)	3.83
4	39.3 (t)	1.79 (4 α), 1.59 (4 β)
5	45.3 (d)	1.13
6	29.2 (t)	1.64 (6 α), 1.28 (6 β)
7	32.5 (t)	1.62 (7 α), 0.87 (7 β)
8	35.5 (d)	1.13
9	54.7 (d)	0.60
10	35.9 (s)	
11	21.4 (t)	1.49 (11 α), 1.08 (11 β)
12	39.6 (t)	1.12 (12 α), 1.89 (12 β)
13	42.0 (s)	
14	56.6 (d)	0.98
15	24.5 (t)	1.11 (15 α), 1.58 (15 β)
16	27.4 (t)	2.34 (16 α), 1.90 (16 β)
17	58.1 (d)	1.66
18	12.9 (q)	0.69 (s)
19	12.6 (q)	0.84 (s)
20	81.6 (d)	3.87
21	23.3 (q)	1.53 (d, $J=6.0$ Hz)
Glc 1	106.1 (d)	4.89 (d, $J=7.6$ Hz)
2	75.7 (d)	4.05
3	78.1 (d)	4.18
4	71.9 (d)	4.16
5	78.5 (d)	3.93
6	63.1 (t)	4.51 (dd, $J=2.2, 11.6$ Hz) 4.35 (dd, $J=5.3, 11.4$ Hz)

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