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Two New Steroidal Glycosides from Fermented Leaves of *Agave americana*

Jian Ming JIN, Xi Kui LIU, Rong Wei TENG, Chong Ren YANG*

Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650204

Abstract: Two new spirostanol glycosides named agamenoside A and B, were isolated from the fermented leaves of *Agave americana*. Their structures were elucidated as (23*S*, 25*R*)-5α-spirostan-3β, 6α, 23-triol 3-O-α-L-rhamnopyranosyl-(1→3)- β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside (**1**) and (25*R*)-5α-spirostan-3β, 6α-diol 3-O-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]- β-D-glucopyranosyl-(1→4)- β-D-galactopyranoside (**2**) by a combination of chemical and spectral methods.

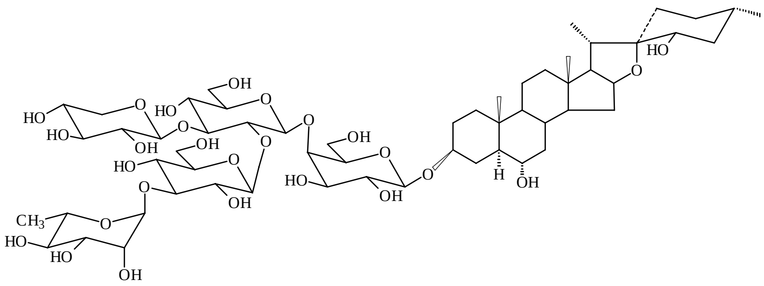
Keywords: *Agave americana* L., steroidal glycosides, agamenosides.

Agave americana L., an xerophilous succulent plant is original native in Mexico and widely cultivated in tropical and subtropical area of the world. In the southern part of China, the leaves of this plant are used as a fiber and folk medicinal herb. It is also used to produce steroidal sapogenins such as hecogenin[1]. It is well know that several species of *Agave* are rich in steroidal saponins and could be use as an important resource in steroidal industry[2-10]. Usually the utilization of the leaves of *Agave* plants needs natural fermentative process. Previously the isolation and structural elucidation of several steroidal saponins from fermented leaf-juice residues of a cultivated of *A. sisalana* have been reported[11-12]. In this paper, we describe the isolation and structural determination of two new steroidal glycosides from fermented leaves of *A. americana*.

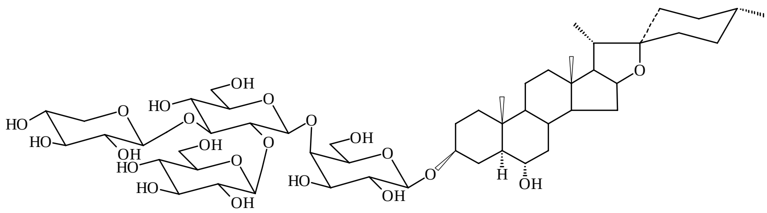
The methanol extracts of dried residues of fermented leaves of *A. americana* L. produced in Ruili County of Yunnan Province at January 2000, were subjected to repeated column chromatography of normal and reverse silica gel to afford compound **1** and **2**.

Compound **1** was obtained as a white amorphous solid, $[\alpha]_D^{15.8}$=-47.62 (*c* 0.0504, methanol). Its molecular formula ($C_{56}H_{92}O_{28}$) was determined by ^{13}C DEPT NMR and negative-ion FABMS, which showed at *m/z* 1211 $[M-H]^-$, and *m/z* 1079. The ^1H NMR spectrum showed two singlets at δ 0.64 and 1.00 ppm (each 3H), indicating the presence of two angular methyl groups, as well as two doublets at δ 0.70 and 1.19 ppm (each 3H) assignable to secondary methyl groups. In addition, the presence of five sugar units in **1** was indicated by five anomeric proton signals [δ 4.77 (d, J = 7.8 Hz), 5.07 (d, J= 7.4 Hz), 5.11 (d, J =7.5 Hz), 5.42 (d, J = 7.5 Hz) and 6.06 (s)] and five anomeric carbon signals at δ 100.5, 102.7, 104.4, 104.8 and 104.9 ppm in ^1H and ^{13}C NMR spectra respectively. Broad singlet peak of δ 6.06 indicated the α- orientation at the anomeric center of L- rhamnose. The J values of the other four anomers of the sugar moieties indicated the β-orientation at the anomeric center of the D-pyranoses. Acid hydrolysis of **1** with 1 mol/L HCl gave a steroidal sapogenin, which was identified as (23*S*, 25*R*)-5α-spirostan-3β, 6α, 23-triol (hongguanggenin) (**1a, Table 1**)[13].

Figure 1 structures of compound **1** and **2**



1



2

The sequence of the sugar linkage and its binding site at the aglycone were determined by 2D NMR experiments. ^{13}C chemical shifts due to sugar moieties were assigned and one sugar was easily identified as D-galactose (From δ 4.77, only four correlation sites can be observed) by HMQC-TOCSY spectrum (**Table 1**). In the HMBC spectrum, the following correlation peaks were observed: δ 4.77 [anomer of galactose] to 77.6 [C-3 of the aglycone], δ 5.11 [anomer of glucose I] to 79.7 [C-4 of galactose], δ 5.07 [anomer of xylose] to 87.3 [C-3 of glucose I], δ 5.42 [anomer of glucose II] to 81.0 [C-2 of glucose I], δ 6.06 [anomer of rhamnose] to 83.3 [C-3 of glucose II], which confirmed the sugar sequence and its linkage position to the aglycone. Thus, the structure of **1** was determined as (23*S*, 25*R*)-5α-spirostan-3β, 6α, 23-triol 3-O-α-L-rhamnopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-[β-D-xylopyranosyl-(1→3)]- β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, which was named agamenoside A.

Table 1 ^{13}C NMR data of compounds **1, 1a, 2** and **2a**

position	**1**	**2**	**1a**	**2a**	position	**1**	**2**
1	37.8 (t)	37.9 (t)	38.1 (t)	38.2 (t)	Gal 1	102.5 (d)	102.5 (d)
2	29.9 (t)	30.0 (t)	32.4 (t)	32.3 (t)	2	73.2 (d)	73.3 (d)
3	77.6 (d)	78.0 (d)	71.1 (d)	71.1 (d)	3	75.3 (d)	75.6 (d)
4	29.6 (t)	29.6 (t)	33.8 (t)	33.8 (t)	4	79.7 (d)	80.0 (d)
5	52.3 (d)	52.3 (d)	52.8 (d)	52.9 (d)	5	75.6 (d)	75.3 (d)
6	68.6 (d)	68.7 (d)	68.7 (d)	68.7 (d)	6	60.7 (t)	60.7 (t)
7	42.7 (t)	42.7 (t)	42.9 (t)	43.0 (t)	Glc(I) 1	104.8 (d)	105.1(d)
8	34.3 (d)	34.4 (d)	34.4 (d)	34.5 (d)	2	81.0 (d)	81.3 (d)
9	54.3 (d)	54.3 (d)	54.5 (d)	54.4 (d)	3	87.3 (d)	87.1 (d)
10	36.7 (s)	36.7 (s)	36.7 (s)	36.7 (s)	4	70.4 (d)	70.5 (d)
11	21.4 (t)	21.4 (t)	21.5 (t)	21.5 (t)	5	78.0 (d)	77.6 (d)
12	40.5 (t)	40.2 (t)	40.6 (t)	40.3 (t)	6	63.0 (t)	63.1 (t)
13	41.5 (s)	41.0 (s)	41.5 (s)	41.0 (s)	Glc(II) 1	104.4 (d)	104.9 (d)
14	56.5 (d)	56.5 (d)	56.6 (d)	56.6 (d)	2	76.4 (d)	76.2 (d)
15	32.2 (t)	32.3 (t)	32.2 (t)	32.5 (t)	3	83.3 (d)	77.8 (d)
16	81.8 (d)	81.4 (d)	81.7 (d)	81.2 (d)	4	69.4 (d)	71.1 (d)
17	62.8 (d)	63.1 (d)	62.7 (d)	63.2 (d)	5	78.5 (d)	78.7 (d)
18	17.0 (q)	16.8 (q)	17.0 (q)	16.7 (q)	6	62.3 (t)	62.5 (t)
19	13.6 (q)	13.7 (q)	13.8 (q)	13.7 (q)	Xyl 1	104.9 (d)	105.0 (d)
20	35.9 (d)	42.2 (d)	35.9 (d)	42.1 (d)	2	75.3 (d)	75.2 (d)
21	14.9 (q)	15.2 (q)	14.8 (q)	15.1 (q)	3	78.5 (d)	78.7 (d)
22	111.8 (s)	109.4 (s)	111.7 (s)	109.3(s)	4	70.7 (d)	70.9 (d)
23	67.5 (d)	32.0 (t)	67.5 (d)	32.0 (t)	5	67.3 (t)	67.4 (t)
24	38.8 (t)	29.4 (t)	38.9 (t)	29.4 (t)	Rha 1	102.7 (d)	
25	31.8 (d)	30.8 (d)	31.8 (d)	30.7 (d)	2	72.4 (d)	
26	66.1 (t)	67.1 (t)	66.1 (t)	67.0 (t)	3	72.6 (d)	
27	17.0 (q)	17.5 (q)	17.0 (q)	17.4 (q)	4	74.2 (d)	
					5	69.8 (d)	
					6	18.7 (q)	

Compound **2** was isolated as a white amorphous solid, $[\alpha]_D^{16.1}$ =-53.04 (*c* 0.0542, methanol), with a molecular formula $C_{50}H_{82}O_{23}$, determined by negative ion FABMS and ^{13}C DEPT NMR data. The negative-ion FABMS spectrum of **2** exhibited a molecular ion peak at *m/z* 1049 $[M-H]^-$, and the fragment ions at *m/z* 917, 887, 755, 593. The ^1H NMR spectrum of **2** showed two tertiary methyl proton signals at δ 0.68 and 0.82 ppm, as well as two secondary methyl protons at δ 0.86 and 1.14 ppm. In addition, four anomeric proton signals were observed at δ 4.87, 5.12, 5.18 and 5.51 ppm. These ^1H NMR spectral features and a diagnostic acetal carbon signal at δ 109.4 ppm in ^{13}C NMR indicated that **2** should be a spirostanol saponin with a sugar chain containing four monosaccharides. Acid hydrolysis of **2** gave a steroidal sapogenin (**2a**), which was identified as (25*R*)-5α-spirostan-3β, 6α-diol (Chlorogenin) by ^1H and ^{13}C NMR data [14]. The sugar linkage position was determined by 2D NMR experiments. ^{13}C chemical shifts due to each sugar moieties were assigned by HMQC-TOCSY spectrum (**Table 1**). In the HMBC spectrum, correlation peaks were observed from δ 4.87 [anomer of galactose] to 78.0 [C-3 of the aglycone], δ 5.12 [anomer of glucose I] to 80.0 [C-4 of galactose], δ 5.18 [anomer of xylose] to 87.1 [C-3 of glucose I], δ 5.51 [anomer of glucose II] to 81.2 [C-2 of glucose I]. Thus, the structure of **2** was determined as (25*R*)-5α-spirostan-3β, 6α-diol 3-O-β-D-glucopyranosyl-(1→2)-β-D-xylopyranosyl- (1 →3)]-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside, which was named agamenoside B.

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References

1. Jiangsu New Medical College, *Zhong-yao-da-ci-dian*, Shanghai People's Publisher, **1977**, 1414.
2. A. Yokosuka, Y. Mimaki, M. Kuroda, Y. Sashida, *Planta Med*., **2000**, *6*, 393.
3. A. T. Alessandra, D. L. M. Mario, M. Vincenza, D. Guiseppe, P. Proto, *Planta Med*., **1997**, *63*, 199.
4. P. K. Kintya, V. A., Bobeiko, *Tezisy Dokl.-Vses. Simp. Bioorg. Khim.*, **1975**, 20.
5. V. A. Bobeiko, T. A. Pkheidza, P. K. Kintya, *Soobshch Akad. Nauk. Gruz.*, **1975**, *80*, 621.
6. B. Wildomirski, V. A. Bobeko, P. K. Kintya, *Phytochemistry*, **1975**, *14*, 2657.
7. G. V. Lazur'evskii, V. A. Bobeiko, P. K. Kintya, *Dokl. Akad. Nauk SSSR*, **1975**, *224*, 1442.
8. P. K. Kintya, V. A. Bobeiko, V. V. Krokhmalyuk, V. Y. Chirva, *Pharmazie*, **1975**, *30*, 396.
9. P. K. Kintya, V. A. Bobriko, A. P. Gulya, *Khim. Prir. Soedin*., 1**975**, *11*, 104.
10. V. A. Bobeiko, *Tezisy Dokl. Soobshch.-konf. Molodykh. Mold.* **1974**, *9*, 100.
11. Y. Ding, Y. Y. Chen, D. Z. Wang, C. R. Yang, *Phytochemistry*, **1989**, *28*, 2787.
12. Y. Ding, R. H. Tian, C. R. Yang, *Chem. Pharm. Bull.*, **1993**, *41*, 557.
13. O. P. Sati, G. Pant, *J. Nat. Prod.*, **1985**, *48*, 395.
14. Y. Miaki, Y. Sashida, K. Kawashima, *Phytochemistry*, **1991**, *30*, 3721.

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