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Two New Spirostanol Steroidal Sapogenins from Fermented Leaves of *Agave americana*

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Abstract: Two new spirostanol sapogenins named agavegenin A and B were isolated from the fermented leaves of *Agave americana* L. Their structures were elucidated as (23*S*, 25*R*)-5α-spirostan-3β, 6α, 11α, 23-tetraol (**1**) and (23*S*, 25*S*)-5α-spirostan-3β, 23, 27-triol (**2**) by spectral methods.

Keywords: *Agave americana* L., spirostanol sapogenins, agavegenin A, agavegenin B.

The genus *Agave* is well known as rich sources of steroidal saponins and sapogenins [1]. More than ten steroidal sapogenins have been isolated from *Agave americana* L.[2-4] In this paper, we describe the structural determination of two new steroidal sapogenins from fermented leaves of *A. americana* L.

The methanolic 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 phase silica gel to afford compounds **1** and **2**.

Compound **1** was isolated as a white amorphous solid, $[\alpha]_D^{20.6}$ –34.79(*c* 0.194, pyridine), with a molecular formula $C_{27}H_{44}O_6$, determined by EI-MS and ^{13}C DEPT NMR data. Its molecular formula was also in accordance with HR EI-MS at *m*/*z* 464.3140 (calcd. for $C_{27}H_{44}O_6$, 464.3179). It was determined as 25*R* configuration according to the characteristic absorb band at 962，945，920，899 and 864 cm^{-1} (intensity: 899>920) in its IR spectrum. The ^1H NMR spectrum of **1** showed two tertiary methyl proton signals at δ 1.09 and 1.11, as well as two secondary methyl protons at δ_H 1.15 (d_H, 3H, *J*=7.0 Hz) and 0.71 (d, 3H, *J*=6.5 Hz). These ^1H NMR spectral features and a diagnostic acetal carbon signal at δc 111.7 in ^{13}C NMR spectrum indicated **1** was a spirostanol sapogenin[1].

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Table 1 ^{13}C and ^{1}H data of compounds **1** and **2**

Position	**1** δ_C	**1** δ_H	**2** δ_C	**2** δ_H
1	40.1 (t)	1.65 (1α), 3.04 (1 β)	37.5 (t)	0.91 (1α), 2.64 (1 β)
2	32.9 (t)	2.15 (2α), 1.85 (2 β)	32.2 (t)	2.05 (2α), 1.58 (2 β)
3	71.0 (d)	3.95	70.6 (d)	3.95
4	34.1 (t)	3.06 (4α), 1.68 (4 β)	39.3 (t)	1.76 (4α), 1.52 (4 β)
5	53.4 (d)	1.50	45.2 (d)	1.06
6	68.8 (d)	3.62	29.1 (t)	1.17 (2H)
7	43.0 (t)	1.30 (7α), 2.28 (7 β)	32.5 (t)	1.66 (7α), 0.81 (7 β)
8	33.6 (d)	1.65	35.3 (d)	1.64
9	60.6 (d)	1.14	54.6 (d)	0.56
10	38.8 (s)		35.9 (s)	
11	68.2 (d)	4.17	21.4 (t)	1.24 (11α), 1.48 (11 β)
12	52.7 (t)	1.65 (12α), 2.37 (12 β)	40.6 (t)	1.12 (12α), 1.76 (12 β)
13	41.7 (s)		41.4 (s)	
14	56.0 (d)	1.40	56.0 (d)	1.10
15	32.3 (t)	2.13 (15α), 1.58 (15 β)	32.6 (t)	2.09 (15α), 1.54 (15 β)
16	81.8 (d)	4.65 (1H, q-like, 7.2 Hz)	81.8 (d)	4.68 (1H, q-like, 8.6 Hz)
17	62.5 (d)	1.98 (dd, 6.8, 8.0Hz)	62.7 (d)	1.93 (dd, 7.1, 8.6Hz)
18	18.0 (q)	1.09 (s)	16.9 (q)	1.03 (s)
19	14.3 (q)	1.11 (s)	12.5 (q)	0.73 (s)
20	35.9 (d)	3.02	35.9 (d)	3.08
21	14.7 (q)	1.15 (d, *J*=7.0 Hz)	14.8 (q)	1.21 (d, *J*=6.8 Hz)
22	111.7 (s)		112.2 (s)	
23	67.5 (d)	3.82 (dd, *J*=8.4, 7.0 Hz)	67.7 (d)	3.96 (dd, *J*=8.4, 4.1 Hz)
24	38.8 (t)	1.74 (24α), 2.11 (24 β)	33.6 (t)	2.02 (24α), 2.29 (24 β)
25	31.7 (d)	1.80	40.6 (d)	2.28
26	66.0 (t)	3.46 (26α), 3.51 (26 β)	63.3 (t)	3.87 (26α), 4.10 (26 β)
27	17.0 (q)	0.71 (d, *J*=6.3 Hz)	64.0 (t)	3.70

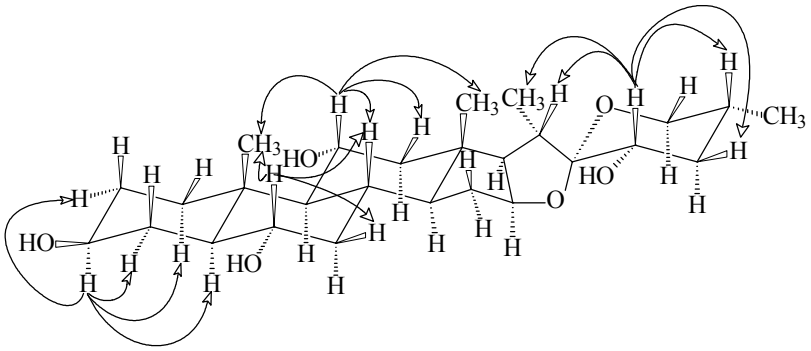
*Spectra were measured at 125 MHz for ^{13}C and 500 MHz for ^{1}H in pyridine-d_5.

The full assignments of proton and carbon signals were based on analysis of ^{13}C DEPT NMR, ^{1}H-^{1}H COSY, ROESY, HMQC and HMBC spectra (**Table 1**). In HMBC spectrum, proton signal at δ_H 1.09 (3H, s) was correlated with carbon signals at δc 52.7 (CH_2), 41.7 (C), 56.0 (CH) and 62.5 (CH), which were assigned as C-12, C-13, C-14 and C-17, respectively. In the same way, the carbon signals at δ 38.8 (C-10), 40.1 (C-1), 53.4 (C-5) and 60.6 (C-9) correlated with H-19 (δ_H 1.11) were determined. From the correlations with H-21 (δ 1.15) and H-27 (δ_H 0.71), carbon signals at δc 35.9 (C-20), 62.5 (C-17), 111.7 (C-22), 31.7 (C-25), 38.8 (C-24) and 66.0 (C-26) were also assigned. In addition, proton signal at δ_H 4.17 was correlated with carbon signal at δ 60.6 (C-9), indicating carbon signal at δc 68.2 was assigned to C-11. Analysis of the ^{1}H-^{1}H COSY spectrum allowed the assignments of the protons from H-11 (δ_H 4.17) to H-9 and H-12, from H-3 (δ_H 3.95) to H-2 and H-4, from H-23 (δ_H 3.82) to H-24, as well as from H-6 (δ_H 3.62) to H-5 and H-7. The above analysis revealed the four hydroxyls were attached to C-3, C-6, C-11 and C-23, respectively.

The relative stereochemistry of **1** was determined by ROESY spectrum. In ROESY spectrum, the NOE correlations were observed from H-11 (δ_H 4.17) to H-18 (δ_H

1.09), H-19 (δ_H 1.11) and H-12β (δ_H 2.37), which indicated H-11 was oriented in a β fashion. On base of the NOE correlations from H-3 (δ_H 3.95) to H-1α (δ_H 1.65), H-2α (δ_H 2.15), H-4α (δ_H 3.06) and H-5α (δ_H 1.50), from H-6 (δ_H 1.50) to H-19 (δ_H 1.50), H-7β (δ_H 1.50) and H-8 (δ_H 1.50), as well as from H-23 (δ_H 3.82) to H-20 (δ_H 3.02), H-25 (δ_H 1.80) and H-24β (δ_H 2.11), H-3, H-5, H-6 and H-23 were suggested to be in α, α, β and β orientation, respectively (**Figure 1**). Therefore, the structure of **1** was elucidated as (23*S*, 25*R*)-5α-spirostan-3β, 6α, 11α, 23-tetraol, which was named agavegenin A. As our well known, 11-hydroxyspirostanol sapogenins are very rare in nature.

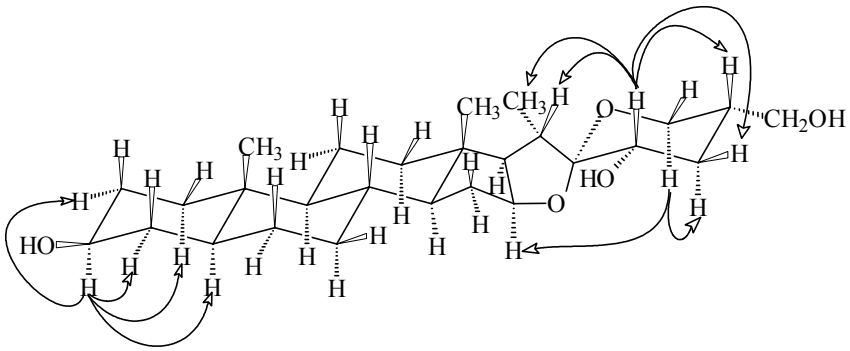
Figure 1 The significant NOE correlations of **1** in the ROESY spectrum



The molecular formula of **2** was deduced as $C_{27}H_{44}O_5$ by DEPT and HR EI-MS at *m*/*z* 448.3172 $[M-H]^-$ (calcd for $C_{27}H_{44}O_5$, 448.3189). Its IR spectrum did not show the characteristic absorb bands at 980, 920, 900 and 860 cm^{-1}. The ^1H NMR spectrum showed three methyl proton signals at δ 0.73 (3H, s)，1.03 (3H, s) and 1.21 (d, 3H, *J*=6.8 Hz). In ^{13}C NMR spectrum, the ketal carbon resonating at δ 112.2 was assigned to a spiroketal carbon with two oxygens attached.

The proton and carbon signals of **2** were assigned by analysis of 1D and 2D NMR experiments (**Table 1**). Comparing the NMR data of **2** with those of tigogenin[5], the structure of the two compounds was identical to each other, except for those belonging to ring F. The ^1H-^1H COSY and ROESY spectra were carefully inspected to assign the structure of ring F portion of **2**. In ROESY spectrum, proton signal at δ_H 3.87 (H-26α) was correlated with proton signals at δ 2.02 (H-24α) and 4.68 (H-16). In ^1H-^1H COSY spectrum, proton signal at δ_H 3.97 (H-23) was coupled with proton signals at δ 2.02 and 2.31, which were assigned as H-24. Proton signal at δ_H 2.28 (H-25) was coupled with proton signals at δ_H 2.02 (H-24α), 3.70 (H-27), 3.87 (H-26α) and 4.10 (H-26β). The above analysis revealed that two hydroxyls were attached to C-23 and C-27, respectively. In addition, those NOE correlations from H-23 (δ_H 3.97) to H-21 (δ_H 1.21), H-24 (δ_H 2.31), H-25 (δ_H 2.28) and H-20 (δ_H 3.08) in ROESY spectrum were consistent with the C-22α, C-23α, and C-25*S* configuration (**Figure 2**). Thus, the structure of **2** was determined as (23*S*, 25*S*)-5α-spirostan-3β, 23, 27-triol, which was named agavegenin B.

Figure 2 The significant NOE correlations of **2** in the ROESY spectrum



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References

1. Jiangsu New Medical College, *Zhong-yao-da-ci-dian* (The Dictionary of Chinese Medicines), Shanghai People's Publisher, **1977**, 1414.
2. J. Zhou, D. G. Wu, W. G. Huang, *Acta Pharm. Sinica*, **1965**, *12*, 392.
3. Y. Y. Chen, P. Z. Cong, L. Huang, *Acta Chemica Sinica*, **1975**, *33*, 149.
4. Y. Y. Chen, L. Huang, *Acta Botanica Sinica*, **1976**, *18*, 156.
5. P. K. Agrawal, D. C. Jain, R. K. Gupta, *Phytochemistry*, **1985**, *24*, 2479.

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