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Aculeoside A, a Novel Steroidal Saponin Containing a Deoxyaldoketose from *Ruscus aculeatus*

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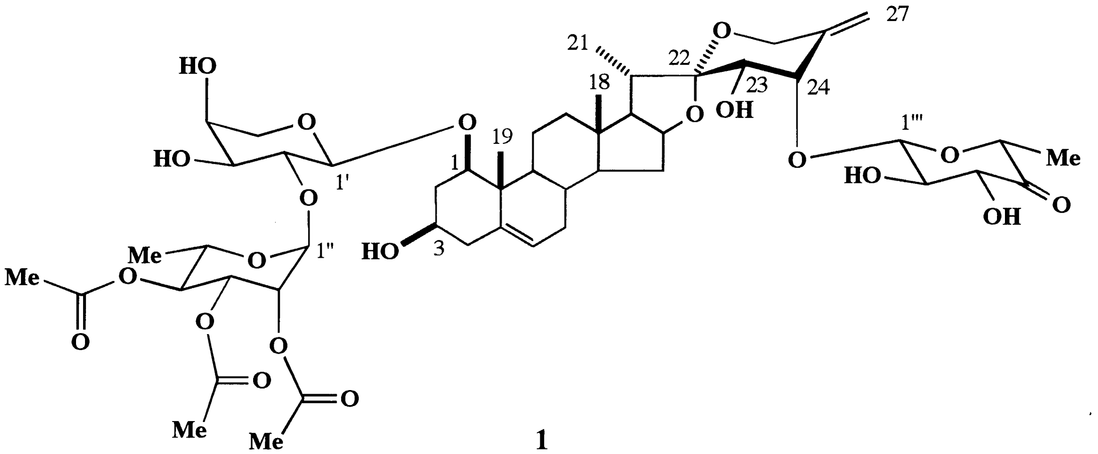
A novel steroidal saponin, named aculeoside A, embracing a deoxyaldoketose, 6-deoxy-D-glycero-L-threo-4-hexosulose, was isolated from the underground parts of *Ruscus aculeatus*. The structure was determined by extensive 2D NMR analysis and hydrolysis. Aculeoside A was active as a cyclic AMP phosphodiesterase inhibitor.

Steroidal saponins are potentially of importance as sources of steroidal hormones. Recently, several steroidal saponins have been shown to have such biological activities as inhibition of platelet aggregation,[1)] reduction of blood glucose levels[2)] and antitumor effect in association with modification of the immune system.[3)] During the course of our studies on steroidal glycosides with medicinal potential, we have examined the underground parts of *Ruscus aculeatus* (Liliaceae) and isolated a novel steroidal saponin, designated as aculeoside A (**1**), embracing a deoxyaldoketose, 6-deoxy-D-glycero-L-threo-4-hexosulose and possessing medium inhibitory activity on cAMP phosphodiesterase. This paper reports the structural elucidation of **1** based on detailed interpretation on two-dimensional (2D) NMR spectroscopies and hydrolysis.

The *n*-BuOH-soluble fraction of the MeOH extract of *R. aculeatus* yielded aculeoside A (**1**, 0.005% fresh weight) after a series of chromatographic separations.

The molecular formula of **1**[4)] was determined as $C_{50}H_{72}O_{21}$ by ^{13}C NMR data (50 carbons), negative-ion FABMS (*m/z* 1007 $[M - H]^-$), positive-ion HRFABMS (*m/z* 1031.4468 $[M + Na]^+$, Δ +0.4 mmu) and elemental analysis. The glycosidic nature of **1** was shown by the IR absorptions at 3450 and 1045 cm^{-1}.



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The presence of a carbonyl and three ester carbonyl groups in the molecule was demonstrated by the ^{13}C NMR (δ 205.2, 170.4, 170.3 and 170.2) and IR (1740 cm^{-1}) spectra. Signals for three anomeric protons at δ 6.22 (d, J = 1.3 Hz), 5.64 (d, J = 7.6 Hz) and 4.63 (d, J = 7.7 Hz), exomethylene protons at δ 5.26 and 5.12 coupled to each other (J = 1.1 Hz), an olefinic proton at δ 5.63 (br), three acetyl protons at δ 2.13, 2.00 and 1.95 (each s), two angular methyl protons at δ 1.37 and 1.00 (each s) and three methyl protons at δ 1.42 (d, J = 6.0 Hz), 1.41 (d, J = 6.4 Hz) and 1.09 (d, J = 7.0 Hz), completed the distinctive features of the ^{1}H NMR spectrum. The above ^{1}H NMR data and a quaternary carbon signal at δ 111.6 in the ^{13}C NMR spectrum[5)] suggested that **1** was a steroid of spirostanol skeleton with three acetyl groups and three monosaccharides. Attempted hydrolysis of **1** led to production of L-rhamnose and L-arabinose in a ratio of 1 : 1[6)] and unidentifed complex mixtures.

To clarify the full structure of **1**, detailed 2D NMR studies, including ^{1}H-^{1}H COSY, HMQC, HMBC and phase-sensitive NOESY experiments recorded in a mixed solvent of C_5D_5N - CD_3OD (10 : 1) to minimize signal overlap and remove exchangeable protons, were focused on it, providing useful informations for the structural assignment. The ^{1}H-^{1}H COSY spectrum led to the sequential assignments of the ^{1}H resonances of the aglycone moiety, which were correlated to the corresponding one-bond coupled ^{13}C signals. Each ^{1}H-^{1}H network was

Table 1. ^{1}H and ^{13}C NMR chemical shift assignments and HMBC correlations of the aglycone moiety of aculeoside A (**1**) in C_5D_5N - CD_3OD (10 : 1)

position	^{1}H	J (Hz)	^{13}C	HMBC (^{1}H)[a)]
1	3.79 dd	12.0, 4.0	83.6	2b, 19, 1'
2 a	2.68 ddd	11.7, 4.1, 4.0	37.6	4b
b	2.22 ddd	12.0, 11.7, 4.1		
3	3.82 m	25.0[b)]	68.0	2a, 2b, 4a, 4b
4 a	2.68		43.8	6
b	2.60 dd	12.1, 4.0		
5			139.4	4a, 4b, 19
6	5.63 br d	5.7	125.1	4a
7 a	1.83		32.1	
b	1.53			
8	1.52		33.1	6, 9
9	1.49		50.4	11b, 19
10			43.0	4b, 6, 19
11 a	2.86		24.1	
b	1.57			
12 a	1.59		40.6	11a, 17, 18
b	1.28			
13			40.9	11a, 15a, 16, 17, 18
14	1.09		56.8	12a, 18
15 a	1.85		32.6	
b	1.45			
16	4.61 q-like	6.7	83.1	
17	1.72 dd	6.7, 6.7	61.7	18, 20, 21
18	1.00 s		16.9	12b, 14, 17
19	1.36 s		15.0	1, 9
20	2.88		37.5	21
21	1.08 d	7.0	14.8	17, 20
22			111.8	20, 21, 24, 26b
23	3.96 d	4.1	70.3	24
24	4.81 d	4.1	82.8	26b, 27a, 27b, 1'''
25			143.7	
26 a	4.82 br d	11.6	61.5	27a, 27b
b	4.00 br d	11.6		
27 a	5.27 br d	1.3	114.3	24, 26b
b	5.12 br d	1.3		

[a)] Optimized for $^nJ_{C\text{-}H}$ = 8 Hz. [b)] $W_{1/2}$.

connected through quaternary carbons by the HMBC spectrum, allowing to identify the aglycone part of **1** as spirosta-5,25(27)-diene bearing oxygen atoms at C-1, -3, -23 and -24. The C-1β and C-3β orientations of the oxygen atoms were confirmed by the ^{1}H NMR parameters of the 1-H and 3-H protons ($^3J_{\text{1-H (δ 3.79) - 2ax-H (δ 2.22)}}$ = 12.0 Hz, $^3J_{\text{1-H - 2eq-H (δ 2.68)}}$ = 4.0 Hz and $W_{\text{1/2 (3-H)}}$ = 25.0 Hz). The 23-H proton clearly showed NOE correlations with 20-H and 21-Me, indicating the C-23*S* configuration. The 24-H proton was coupled to 23-H by 4.1 Hz, and showed NOEs with 23-H and 27a-H (δ 5.27), which was consistent with the C-24*S* configuration. The NOEs between 27b-H (δ 5.12) and 26b-H (δ 4.00), and 26a-H (δ 4.82) and 16-H indicated the F-ring conformation to be nearly chair-form.

The presence of α-L-arabinopyranosyl ($^1C_4$) unit and α-L-rhamnopyranosyl ($^4C_1$) uint was readily revealed by tracing out the ^{1}H-^{1}H networks, starting from the anomeric proton signals at δ 4.60 (d, *J* = 7.8 Hz) and 6.16 (1H, d, *J* = 1.5 Hz). The anomeric proton of the rhamnose showed a $^3J_{\text{C-H}}$ correlation with C-2 of arabinose and that of the arabinose with C-1 of the aglycone, leading to rhamnosyl-(1→2)-arabinose structure attached to C-1 of the aglycone. The C-2, -3 and -4 hydroxyl groups of the rhamnose were presumed to be esterified with acetic acids since the ^{1}H signals due to 2-H, 3-H and 4-H resonated at unexpected lower fields at δ 5.93 (dd, *J* = 3.4, 1.5 Hz), 5.88 (dd, *J* = 10.1, 3.4 Hz) and 5.59 (dd, *J* = 10.1, 10.1 Hz), respectively. This was confirmed by the observation of $^3J_{\text{C-H}}$ correlations from 2-H, 3-H and 4-H to the carbonyl carbons of the acetyl groups.

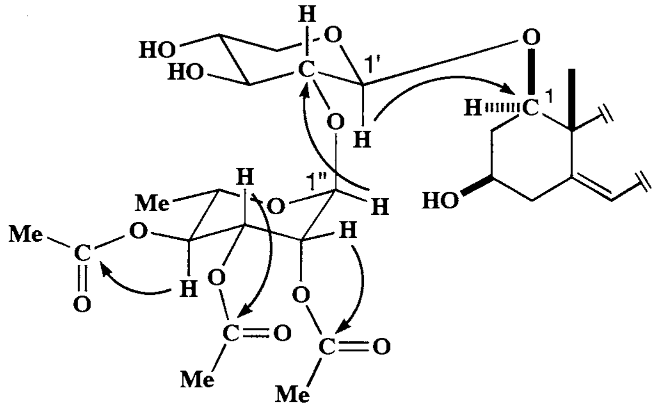

Fig. 1. Arrows indicate the HMBC correlations.

Table 2. ^{1}H and ^{13}C NMR data the saccharide moieties of **1** in C_5D_5N - CD_3OD (10 : 1)

position	^{1}H	*J* (Hz)	^{13}C
1'	4.60 d	7.8	100.0
2'	4.42 dd	9.1, 7.8	74.8
3'	4.06 dd	9.1, 3.5	75.8
4'	4.09 dd	3.5, 1.7	70.1
5'a	4.25 dd	11.6, 1.7	67.8
b	3.65 br d	11.6	
1''	6.16 d	1.5	97.8
2''	5.93 dd	3.4, 1.5	70.5
3''	5.88 dd	10.1, 3.4	70.4
4''	5.59 dd	10.1, 10.1	72.0
5''	4.96 dq	10.1, 6.2	66.6
6''	1.42 d	6.2	18.1
1'''	5.59 d	7.8	104.9
2'''	4.24 dd	9.6, 7.8	78.8
3'''	4.77 dd	9.6, 0.6 [a]	79.0
4'''			205.3
5'''	4.33 dq	6.4, 0.6 [a]	73.6
6'''	1.41 d	6.4	14.5
Ac	2.17 s		170.6 20.8
	2.02 s		170.6 20.8
	1.97 s		170.4 20.6

[a] $^4J_{\text{H-H}}$ were observed.

Finally, attention was turn out to the structure of the remaining monosaccharide, which was decomposed through acid hydrolysis. The monosaccharide was suggested to be a deoxyaldoketose from the six ^{13}C signals at δ 205.3 (C=O), 104.9 (CH), 79.0 (CH), 78.8 (CH), 73.6 (CH) and 14.5 (Me), and the ^{1}H-^{1}H COSY spectrum gave rise to two fragmnet composed of it. The carbonyl carbon exhibited $^{2 \text{ or } 3}J_{\text{C-H}}$ correlations with the ^{1}H signals at δ 4.77 (3'''-H) and 1.41 (6'''-Me), accounting for the connectivity of C-3''' and C-5''' across the carbonyl group. Compound **1** was subjected to $NaBH_4$ reduction followed by acid hydrolysis to give L-rhamnose, L-arabinose and D-fucose.[6] Thus, the deoxyaldoketose was confirmed to be 6-deoxy-D-glycero-L-threo-4-hexosulose. This sugar formed a β-glycoside linkage to the C-24 hydroxyl group of the aglycone, evidence for which was provided from $^3J_{C\text{-}H}$ correlation between the anomeric proton (δ 5.59, d, $J = 7.8$ Hz) and C-24 carbon.

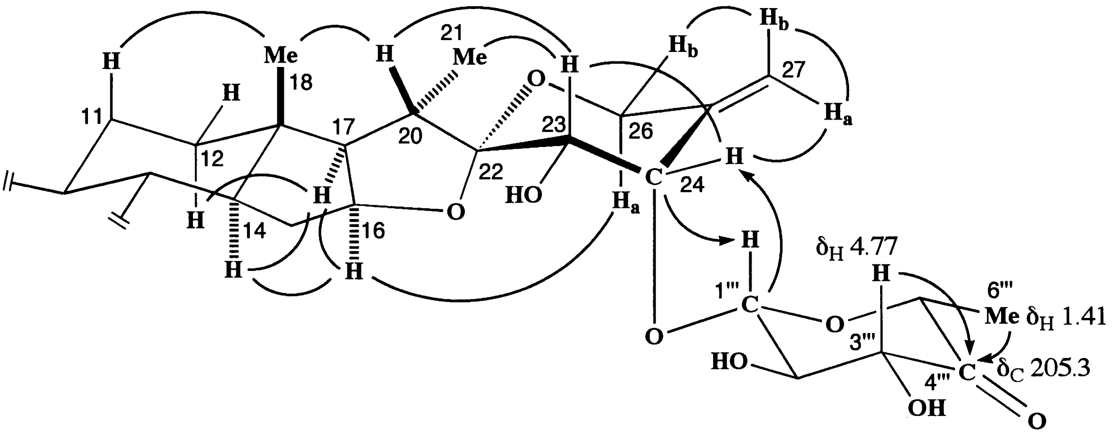

Fig. 2. Curved lines indicate the NOE and arrows the HMBC correlations.

Aculeoside A (**1**) is the first representative of a steroidal saponin bearing an aldoketose as the saccharide component, and was active as a cAMP phosphodiesterase inhibitor (IC_{50} 16.7 x 10^{-5} mol dm^{-3})[7] while inactive on Na^+/K^+ ATPase.[8]

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4) Compound **1**: amorphous powder, $[\alpha]_D$ −50.0° (*c* 0.10, MeOH); negative-ion FABMS *m/z* 1007 [M - H]⁻; positive-ion HRFABMS *m/z* 1031.4468 $[M + Na]^+$ (Calcd for $C_{50}H_{72}O_{21}$: 1031.4464); ^{13}C NMR (pyridine-d_5) δ = 83.5, 37.6, 68.0, 43.9, 139.4, 124.9, 32.0, 33.0, 50.3, 42.9, 24.0, 40.5, 40.8, 56.7, 32.5, 83.0, 61.6, 16.8, 14.9, 37.5, 14.8, 111.6, 70.3, 82.6, 143.6, 61.4, and 114.2 (C-1 - C-27), 99.9, 74.8, 75.7, 70.1, and 67.7 (C-1' - C-5'), 97.6, 70.5, 70.3, 71.9, 66.5, and 18.0 (C-1'' - C-6''), 104.9, 78.9, 79.1, 205.2, 73.5, and 14.4 (C-1''' - C-6'''), and 170.4, 170.3, 170.2, 20.7 x 2 and 20.6 (Ac x 3). Found: C, 57.46; 7.06%. Calcd for $C_{50}H_{72}O_{21}\cdot 2H_2O$: C, 57.51; H, 7.28%.
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