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Isolation and structural determination of spilacleosides A and B having a novel 1,3-dioxolan-4-one ring

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Abstract—As the first natural product incorporating 1,3-dioxolan-4-one in spiro structure, spilacleosides A and B were isolated from *Ruscus aculeatus*, and the absolute structures determined.

In the course of our search for keratinization-controlling substances[1] in natural sources, we encountered spilacleosides A and B (**1** and **2**) having a spiro 1,3-dioxolan-4-one structures, which were composed of aculeoside A[2] and (2*R*,3*S*)-2,3-dihydroxy-3-methylpentanoic acid (Fig. 1). These are not only rare examples containing such a spiro structure but also are noteworthy in two aspects: (1) they are the first examples where both segments intermolecularly construct a 1,3-dioxolan-4-one ring with a spiro system at the C-2; (2) the naturally derived 2,3-dihydroxy-3-methylpentanoic acid has the opposite configuration at C-3 to that having (2*R*,3*R*) configuration[3] produced by a mutant strain of *Neurospora crassa*[4] and by *Heliotropium strigosum*.[5] A few natural products intramolecularly incorporating a spiro 1,3-dioxolan-4-one ring are known,[6] while its usefulness as a building block in asymmetric organic synthesis has been often reported.[7]

Herein we report their isolation and structural determination.

The 30% ethanol extracts of 5 kg of the root of butcher's-broom, *Ruscus aculeatus*, was chromatographed successively on hydrophobic resin DIAION® HP20, silica gel, Sephadex™ LH-20, and octadesylsilylated silica-gel columns, and finally on silica-gel plate (TLC) to afford **1** (90 mg) and **2** (95 mg).

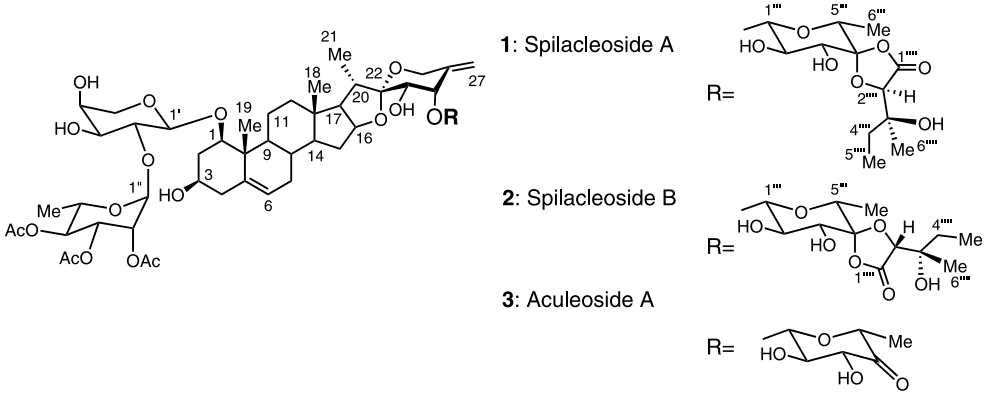

Figure 1.

Keywords: 1,3-dioxolan-4-one; butcher's-broom; *Ruscus aculeatus*.
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Table 1. Significant ^{1}H and ^{13}C spectral data, and DIS ($\Delta\delta$) for **1** and **2**

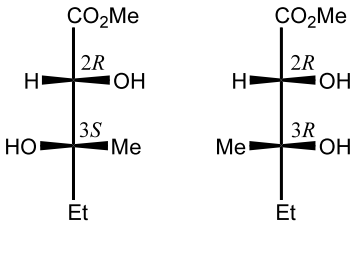
	1					**2**				
Position	$\delta^{1}H^{a}$	J (Hz)	$\delta^{13}C^{a}$	$\delta^{13}C^{b}$	$\Delta\delta$	$\delta^{1}H^{a}$	J (Hz)	$\delta^{13}C^{a}$	$\delta^{13}C^{b}$	$\Delta\delta$
1	3.75 dd	12.0, 4.0	83.40	83.38	−0.02	3.74 dd	12.0, 4.0	83.39	83.38	−0.01
3	3.78 m		67.78	67.89	0.11	3.78 m		67.79	67.90	0.11
6	5.58 br d	5.5	124.88	124.87	−0.01	5.60 br d	5.5	124.87	124.86	−0.01
18	0.90 s		16.67	16.65	−0.02	0.94 s		16.71	16.70	−0.01
19	1.31 s		14.77	14.76	−0.01	1.32 s		14.79	14.78	−0.01
21	1.01 d	7.0	14.59	14.58	−0.01	1.02 d	7.0	14.63	14.63	0.00
27a	5.19 d	1.8	113.95	113.91	−0.04	5.20 br d	1.8	113.98	113.97	−0.01
27b	5.07 d	1.8	–	–	–	5.05 br d	1.8	–	–	–
1′	4.57 d	7.5	99.80	99.79	−0.01	4.57 d	7.5	99.80	99.79	−0.01
1″	6.12 d	1.5	97.57	97.55	−0.02	6.12 d	1.5	97.57	97.57	0.00
1‴	5.23 d	7.6	105.17	105.20	0.03	5.23 d	7.5	105.54	105.59	0.05
2‴	4.16 dd	10.0, 7.6	74.23	74.35	0.12	4.32 dd	10.0, 7.5	74.26	74.39	0.13
3‴	4.25 d	10.0	75.68	75.79	0.11	4.23 d	10.0	77.56	77.71	0.15
4‴	–		108.09	108.09	0.00	–		107.01	107.02	0.01
5‴	3.88 q	6.5	72.93	72.90	−0.03	4.02 q	6.5	71.40	71.39	−0.01
6‴	1.27 d	6.5	12.48	12.45	−0.03	1.54 d	6.5	13.72	13.72	0.00
1⁗	–		171.18	171.18	0.00	–		172.34	172.34	0.00
2⁗	4.67 s		79.96	79.95	−0.01	5.10 s		82.41	82.43	0.02
3⁗	–		74.23	74.31	0.08	–		73.37	73.46	0.09
4⁗a	1.84 m		32.99	33.01	0.02	1.93 m		32.28	32.32	0.04
4⁗b	1.78 m		–	–	–	1.83 m		–	–	–
5⁗	0.95 t	7.5	8.44	8.42	−0.02	0.92 t	7.5	8.39	8.39	0.00
6⁗	1.53 s		20.17	20.20	0.03	1.53 s		21.77	21.82	0.05

[a] Spectra were measured in C_5D_5N–CD_3OD (10:1).
[b] Spectra were measured in C_5D_5N–CD_3OH (10:1).

Both their molecular formulas were determined to be $C_{56}H_{82}O_{24}$ by HRESI-MS; $[M+Na]^+$,1161.5063 and 1161.5087 (calcd 1161.5094), respectively.

Their ^{13}C NMR spectra (Table 1) were very similar to those of aculeoside A (**3**) isolated from the same plant by Sashida group,[2] except for the absence of a carbonyl signal of aldoketose at 205.3 ppm and the presence of additional signals at 171.18 (C-1⁗), 108.09 (C-4‴), 79.96 (C-2⁗), 74.23 (C-3⁗), 32.99 (C-4⁗), 20.17 (C-6⁗) and 8.44 ppm (C-5⁗) in **1**, and 172.34 (C-1⁗), 107.01 (C-4‴), 82.41 (C-2⁗), 73.37 (C-3⁗), 32.28 (C-4⁗), 21.77 (C-6⁗) and 8.39 ppm (C-5⁗) in **2**. Both **1** and **2**, on refluxing in MeOH for 1 h, cleanly gave aculeoside A (**3**: $[\alpha]_D^{21}$ −50.5°(*c* 0.10, MeOH))[2] and 2,3-dihydroxy-3-methylpentanoic acid methyl ester (**4**: $[\alpha]_D^{21}$ −28°(*c* 0.08, $CHCl_3$)). The spectral data of **3** coincided with that reported.[2] In combination with H–H COSY and HMBC spectra, those additional signals other than that at ca. 107 ppm supported the presence of 2,3-dihydroxy-3-methylpentanoic acid as a partial structure in **1** and **2**. The methyl esters **4** thus obtained from **1** and **2** were identical in all respects in their ^{1}H and ^{13}C NMR spectra,[8] showing that they were endowed with the same configuration. To determine the absolute structure of **4**, two authentic samples of 2,3-dihydroxy-3-methylpentanoic acid methyl esters were prepared in optically pure forms by the reported method:[3] *erythro* (2*R*,3*S*) isomer (**4**: $[\alpha]_D^{26}$ −29°(*c* 1.0, $CHCl_3$))[8] and *threo* (2*R*,3*R*) isomer (**5**: $[\alpha]_D^{26}$ −24° (*c* 1.2, $CHCl_3$))[8] known as strigosic acid.[4,5] The ^{1}H and ^{13}C NMR spectra of the naturally derived methyl ester were identical with those of **4**, and the coincidence of optical rotation confirmed it to be of (2*R*,3*S*) configuration (Fig. 2). Thus, it is different from the (2*R*,3*R*) isomer **5** produced by a mutant strain of *Neurospora crassa*[4] and by *Heliotropium strigosum*.[5]

The manner of combination of these two parts, aculeoside A and the dihydroxyacid, was determined as follows. The absorptions at 1811 cm^{-1} in **1** and 1805 cm^{-1} in **2** in the IR spectra suggested the presence of the γ-lactone, 1,3-dioxolan-4-one ring.[9] The corresponding absorption of the δ-lactone, 1,3-dioxan-4-one, should have appeared at ca. 1750 cm^{-1}.[10] This was confirmed by differential isotope shifts (DIS) in the ^{13}C NMR spectrum upon deuterium exchange (Table 1).[11] The C-3⁗ carbon showed 0.08 ppm and 0.09 ppm shift change in **1** and **2**, respectively, while the C-2⁗ carbon showed much smaller changes, –0.01 ppm in **1** and 0.02 ppm in **2**. The stereochemistry at the spiro carbon atom



4 (2*R*, 3*S*) **5** (2*R*, 3*R*)

Figure 2.

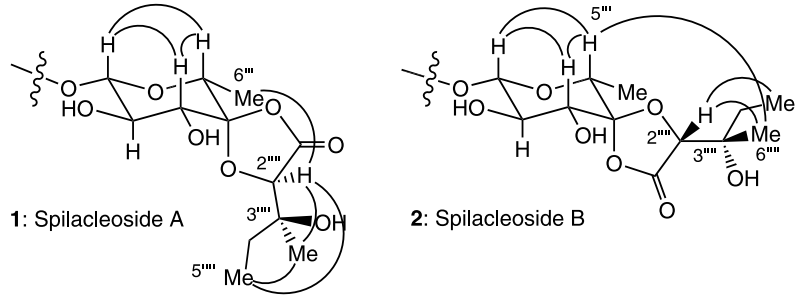


Figure 3. NOE correlation of the sugar moiety of **1** and **2**.

was determined on the basis of NOE studies (Fig. 3). The NOEs observed in the two isomers, especially between Me-6‴ and H-2‴′ in **1** and between H-5‴ and Me-6‴′ in **2**, showed the appropriateness of the stereoalignment around the spiro carbon as shown in Figure 3.

The ease of methanolysis of **1** and **2** suggested that the isolation of aculeoside A (**3**) might have been brought about by methanol extraction in reflux. Thus, the absolute structures of spilacleosides A and B were determined to be **1** and **2**. These are the first examples found in nature of intermolecularly formed 1,3-dioxolan-4-one ring with a spiro system at the C-2.

Acknowledgements

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8. The optical rotations of the corresponding carboxylic acids are the following. (2*R*,3*S*) Isomer: $[\alpha]_D^{26}$ −16° (*c* 0.66, 0.1 M aq. HCl), lit.[3] $[\alpha]_D^{26}$ −15.6° (*c* 2.3, 0.1 M aq. HCl); (2*R*,3*R*) isomer: $[\alpha]_D^{26}$ −19° (*c* 0.57, 0.1 M aq. HCl), lit.[3] $[\alpha]_D^{26}$ −23.1° (*c* 2.0, 0.1 M aq. HCl). ^{1}H NMR (600 MHz in $CDCl_3$; δ, ppm from TMS, and *J* in Hz) spectral data of the esters are the following. **4**: 0.96 (3H, t, *J*=7.0, H-5‴′), 1.16 (3H, s, H-6‴′), 1.56 (1H, dq, *J*=14.0 and 7.0, H-4‴′), 1.69 (1H, dq, *J*=14.0 and 7.0, H-4‴′), 2.38 (1H, br s, OH-3‴′), 3.10 (1H, d, *J*=7.0, OH-2‴′), 3.83 (3H, s, -CO_2Me), 4.05 (1H, d, *J*=7.0, H-2‴′). **5**: 0.97 (3H, t, *J*=7.0, H-5‴′), 1.22 (3H, s, H-6‴′), 1.43 (1H, dq, *J*=14.0 and 7.0, H-4‴′), 1.61 (1H, dq, *J*=14.0 and 7.0, H-4‴′), 2.41 (1H, s, OH-3‴′), 3.08 (1H, d, *J*=6.5, OH-2‴′), 3.84 (3H, s, -CO_2Me), 4.05 (1H, d, *J*=6.5, H-2‴′).
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