Two New Acyl-Phloroglucinols from *Dryopteris atrata*[1]

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Two new acyl-phloroglucinols, called atrata-phloroglucinols A and B, were isolated from the fronds of *Dryopteris atrata*. Their structures were elucidated by two dimensional NMR spectral data to be flavaspidic acids AA and AB of which the methyl group at C-5′ is replaced with 8-aristol-9-enyl group.

Key words *Dryopteris atrata*; acyl-phloroglucinol; aristol-9-ene; flavaspidic acid; Aspidiaceae; fern

Most ferns of the genus *Dryopteris* contain acyl-phloroglucinols in their rhizomes. More than fifty acyl-phloroglucinols have been isolated[2] and used as chemical markers to examine the relationship among the taxa of *Dryopteris* and the related genera.[3] Recently, we investigated the constituents of the fronds of *Dryopteris* (*D.*) *atrata* (WALL. *ex* KUNZE) CHING (Japanese name: Iwa-hego, Aspidiaceae) and isolated two new acyl-phloroglucinols called atrata-phloroglucinols A (**1**) and B (**2**). In this paper, we describe the isolation and structure determination of these compounds.

Atrata-phloroglucinols A (**1**) and B (**2**) were isolated from the air-dried fronds of *D. atrata* collected in Arata, Fukuoka prefecture, in September.

Atrata-phloroglucinol A (**1**) was formulated as $C_{34}H_{42}O_8$ from its high-resolution (HR) FAB-MS. In the ^{1}H- and ^{13}C-NMR spectra (in $CDCl_3$), **1** showed the characteristic signals of a phloroglucinol unit (three ^{13}C signals around each of 160 and 110 ppm), a filicinic acid unit [two methyl proton signals at 1.45 (3H, s) and 1.54 (3H, s)], a methylene which links the above two units [3.55 (2H, s)] and two acetyl groups [δ_H: 2.68 (3H, s) and 2.75 (3H, s); δ_C: 29.3, 32.5, 203.4 and 204.3], indicating the presence of a diacetyl-flavaspidic acid (flavaspidic acid AA[4]) moiety in the molecule.

Considering the molecular formula and the remaining signals in the ^{13}C-NMR spectrum (two sp^2 and twelve sp^3 carbon signals), the remainder of the molecule ($C_{15}H_{23}$) was suggested to be a tricyclic sesquiterpene. As the NMR signals showed the presence of a cyclopropane ring [δ_H: 0.72 (1H, d, $J=8.8$ Hz), 0.77 (1H, d, $J=8.8$ Hz); δ_C: 18.9 (quaternary C), each signal appeared in a relatively high field], three tertiary and one secondary methyl groups, and trisubstituted double bond [δ_H: 5.26 (1H, br s)], the sesquiterpene was believed to have an aristolane skeleton.

A flavaspidic acid iBiB linking a monoterpene unit has been isolated from *Hypericum japonicum* and called sarothralen A (**3**).[5] The ^{13}C-NMR data of the flavaspidic acid moiety of **3** were in good agreement with those of **1** except that the isobutyryl groups at C-6 and C-3′ in **3** were replaced by acetyl groups in **1** (Table 1). The sesquiterpene unit was suggested to be (+)-aristol-9-ene (**4**) linked with the flavaspidic acid moiety at C-8 by comparison of the ^{13}C-NMR data with those reported.[6]

To confirm the structure deduced from the ^{13}C-NMR data, two-dimensional shift correlation spectroscopy (2D-COSY) was applied. As shown in Figs. 1 and 2, the long-range ^{13}C–^{1}H COSY and nuclear Overhauser effect correlation spectroscopy (NOESY) confirmed the plane structure and the relative stereochemistry of **1**, respectively. Thus, the structure of **1** was determined to be 5′-(aristol-9-en-8-yl)-flavaspidic acid AA. Though the absolute configuration of the aristol-9-ene moiety was not deduced, an antipodal structure of (+)-aristol-9-ene, $[\alpha]_D$ +80.9° ($CHCl_3$),[7] may be preferable, judging from the sign of the optical rotation of **1**, $[\alpha]_D$ −44° ($CHCl_3$).

Atrata-phloroglucinol B (**2**) was formulated as $C_{30}H_{46}O_8$ from the HR-FAB-MS. The ^{1}H- and ^{13}C-NMR data (Table 1 and Experimental) were in good agreement with those of **1** except that the acetyl group at C-3′ of **1** was replaced by a butyryl group in **2**. After the confirmation of the structure by 2D-COSY, **2** was determined to be 5′-(aristol-9-en-8-yl)flavaspidic acid AB.

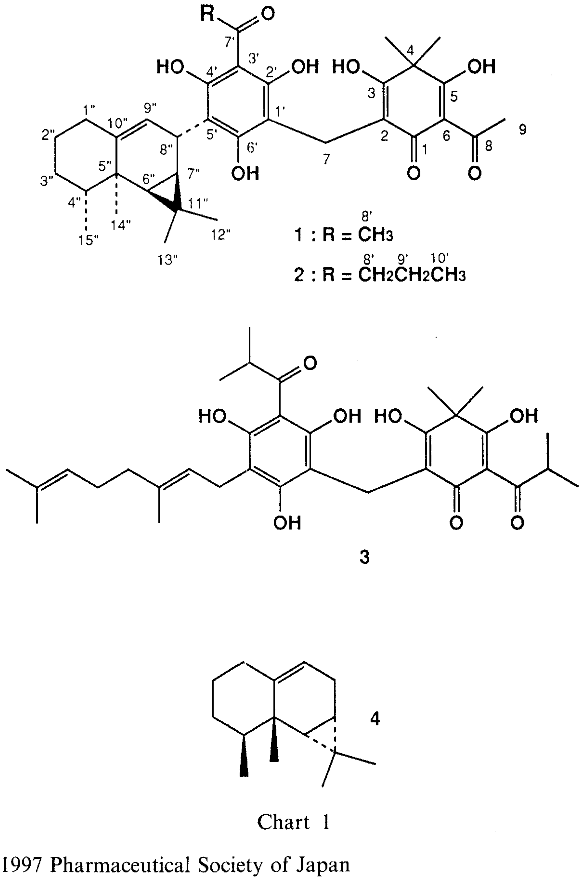

Chart 1

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Table 1. ^{13}C-NMR Data in $CDCl_3$

	1	**2**	**3**	**4**
1	198.8	198.8	199.4	
2	108.5	108.4	106.3	
3	172.0	171.9	171.1	
4	44.3	44.3	44.4	
4-Me	24.2	24.3	25.6	
4-Me	24.9	25.2	25.6	
5	187.6	187.5	187.4	
6	111.3	111.3	111.5	
7	17.1	17.1	18.8	
8	203.4	203.5	211.4	
9	29.3	29.3	36.3	
9-Me	—	—	19.1	
9-Me	—	—	19.5	
1′	105.6	105.7	105.7	
2′	160.2	160.3	160.7	
3′	104.4	104.1	108.3	
4′	158.5	158.3	158.1	
5′	110.8	110.8	107.2	
6′	161.4	161.0	161.7	
7′	204.3	207.0	210.8	
8′	32.5	45.7	37.1	
9′	—	18.0	19.3	
10′	—	14.0	—	
8′-Me	—	—	19.3	
1″	33.1	33.0		32.9
2″	27.3	27.2		27.0
3″	30.8	30.8		31.3
4″	37.9	37.8		37.7
5″	36.8	36.8		36.5
6″	32.0	31.9		32.0
7″	23.8	23.8		30.0
8″	27.5	27.4		21.6
9″	120.4	120.4		118.2
10″	146.6	146.7		141.0
11″	18.9	18.9		17.9
12″	29.5	29.5		29.2
13″	16.3	16.3		16.0
14″	21.2	21.2		21.3
15″	15.7	15.7		15.8

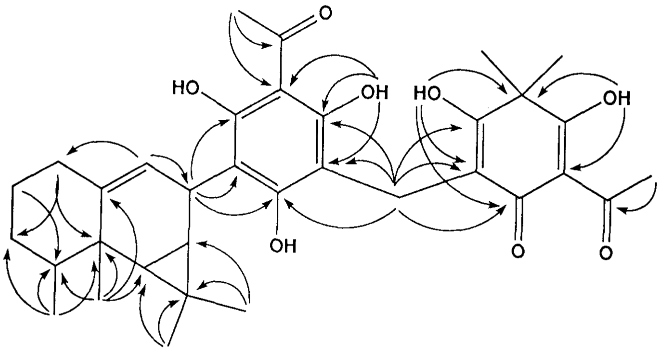

Fig. 1. Long-Range C–H Correlations for **1**

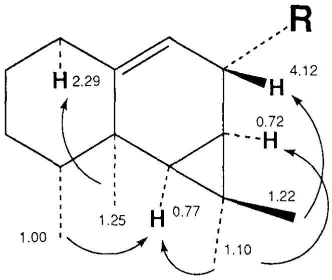

Fig. 2. NOE Correlations for **1**

Experimental

Optical rotations were taken with a JASCO DIP-360 automatic polarimeter. The ^{13}C- and ^{1}H-NMR spectra were measured with a JEOL GSX-500 spectrometer (multiplicity, s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; NC, coupling patterns were not confirmed because of overlapping signals). Ultraviolet (UV) spectra were recorded on a Hitachi 323 spectrometer. Mass spectra were measured with a JEOL SX-102 spectrometer (FAB-MS).

Isolation The air-dried fronds (640 g) of *D. atrata* collected in September in Arata, Fukuoka prefecture, were extracted twice with 3 l of MeOH under reflux for 6 h. The extracts and then 10 l of MeOH were passed over a column of activated charcoal (64 g). The resulting solution was concentrated to a syrup under reduced pressure. The syrup was chromatographed on silica gel using $CHCl_3$ and MeOH. The fractions containing **1** and **2** (2.3 g) were rechromatographed on Sephadex LH-20 (120 g) using benzene–MeOH (4 : 1) to obtain **1** (790 mg) and **2** (226 mg).

Atrata-phloroglucinol A (1) A pale yellow amorphous powder, $[\alpha]_D$ $-44°$ ($c=0.25$, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 298 (4.49), 341 (4.27). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (1H, d, $J=8.8$ Hz, H-7″), 0.77 (1H, d, $J=8.8$ Hz, H-6″), 1.00 (3H, d, $J=6.8$ Hz, H_3-15″), 1.10 (3H, s, H_3-13″), 1.22 (3H, s, H_3-12″), 1.25 (s, 3H, H_3-14″), 1.30 (1H, m, H-2″), 1.39 (1H, m, H-3″), 1.45 (3H, br s, 4-CH_3), 1.54 (3H, br s, 4-CH_3), 1.76 (2H, NC, H-2″ and H-4″), 2.06 (1H, m, H-1″), 2.29 (1H, m, H-1″), 2.68 (3H, s, H_3-8′), 2.75 (3H, s, H_3-9), 3.55 (2H, s, H_2-7), 4.12 (1H, br s, H-8″), 5.26 (1H, br s, H-9″), 10.14 (1H, s, 5-OH), 15.94 (1H, s, 2′-OH), 18.43 (1H, s, 3-OH). HR-FAB-MS (positive mode) *m*/*z*: 579.295 $[M+H]^+$. Calcd for $C_{34}H_{43}O_8$: 579.296.

Atrata-phloroglucinol B (2) A pale yellow amorphous powder, $[\alpha]_D$ $-64°$ ($c=1.0$, $CHCl_3$). UV λ_{max}^{MeOH} nm (log ε): 300 (4.40), 346 (4.23). ^{1}H-NMR ($CDCl_3$) δ: 0.72 (1H, d, $J=8.5$ Hz, H-7″), 0.77 (1H, d, $J=8.5$ Hz, H-6″), 0.99 (3H, t, $J=7.3$ Hz, H_3-10′), 1.00 (3H, d, $J=6.8$ Hz, H_3-15″), 1.10 (3H, s, H_3-13″), 1.22 (3H, s, H_3-12″), 1.26 (3H, s, H_3-14″), 1.45 (3H, br s, 4-CH_3), 1.54 (3H, br s, 4-CH_3), 1.72 (2H, m, H_2-9), 1.75 (2H, NC, H-2″ and H-4″), 2.06 (1H, m, H-1″), 2.30 (1H, m, H-1″), 2.75 (3H, s, H_3-9), 3.07 (2H, m, H_2-8′), 3.55 (2H, s, H_2-7), 4.13 (1H, br s, H-8″), 5.26 (1H, br s, H-9″), 10.19 (1H, s, 5-OH), 16.10 (1H, s, 2′-OH), 18.40 (1H, s, 3-OH). HR-FAB-MS (negative mode) *m*/*z*: 605.309 $[M-H]^-$. Calcd for $C_{36}H_{45}O_8$: 605.312.

References and Notes

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4) AA means both the acyl groups at C-6 and C-3′ are acetyl. This is the first example of flavaspidic acid AA, though dibutyryl derivative, flavaspidic acid BB, and acetyl butyryl derivative, flavaspidic acid AB, have been found in many species of *Dryopteris*.
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