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 flavaspicic acids AA and AB of which the methyl group at C-5' is replaced with 8-aristol-9-etyl group.

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

Most ferns of the genus *Dryopteris* contain acyl-phloroglucinols in their rhizomes. More than fifty acyl-phloroglucinols have been isolated² 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 C₃₄H₄₂O₉ from its high-resolution (HR) FAB-MS. In the ¹H- and ¹³C-NMR spectra (in CDCl₃), 1 showed the characteristic signals of a phloroglucinol unit (three ¹³C 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 [2.85 (2H, m)] and two acetyl groups [δₐ: 2.68 (3H, s) and 2.75 (3H, s); δₓ: 32.5, 203.4 and 204.3], indicating the presence of a diacetyl-flavaspicic acid (flavaspicic acid AA⁴) moiety in the molecule.

Considering the molecular formula and the remaining signals in the ¹³C-NMR spectrum (two sp² and twelve sp³ carbon signals), the remainder of the molecule (C₁₃H₁₃) was suggested to be a tricyclic sesquiterpene. As the NMR signals showed the presence of a cyclopropane ring [δₚ: 0.72 (1H, d, J = 8.8 Hz), 0.77 (1H, d, J = 8.8 Hz); δₓ: 18.9 (quaternary C), each signal appeared in a relatively high field], three tertiary and one secondary methyl groups, and trisubstituted double bond [δₚ: 5.26 (1H, br s)], the sesquiterpene was believed to have an aristolane skeleton.

A flavaspicic acid iBIB linking a monoterpene unit has been isolated from *Hypericum japonicum* and called sarothralen A (3).⁵ The ¹³C-NMR data of the flavaspicic acid moiety of 3 were in good agreement with those of 1 except that the isobutyl 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 flavaspicic acid moiety at C-8 by comparison of the ¹³C-NMR data with those reported.⁶

To confirm the structure deduced from the ¹³C-NMR data, two-dimensional shift correlation spectroscopy (2D-COSY) was applied. As shown in Figs. 1 and 2, the long-range ¹H-¹H 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-flavaspicic acid AA. Though the absolute configuration of the aristol-9-ene moiety was not deduced, an antipodal structure of (+)-aristol-9-ene, [α]₂₀⁺80.9° (CHCl₃),⁷ may be preferable, judging from the sign of the optical rotation of 1, [α]₂₀⁻44° (CHCl₃).

*Atrata-phloroglucinol* B (2) was formulated as C₃₀H₄₄O₇ from the HR-FAB-MS. The ¹H- and ¹³C-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 butyl group in 2. After the confirmation of the structure by 2D-COSY, 2 was determined to be 5'-aristol-9-en-8-yl-flavaspicic acid AB.

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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 332 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 101 of MeOH were passed over a column of activated charcoal (64 g). The resulting solution was concentrated 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).

Atracta-vaginata A (1) A pale yellow amorphous powder, [α]$_D$ -44° ($c = 0.25$, CHCl$_3$) UV $A_{max}$ (nm (log e)): 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$_2$-15), 1.10 (3H, s, H$_2$-13), 1.22 (3H, s, H$_2$-12), 1.25 (s, 3H, H$_2$-14*), 1.30 (1H, m, H-2*), 1.39 (1H, m, H-3'), 1.45 (3H, brs, 4-CH$_3$), 1.54 (3H, brs, 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, brs, H-8*), 5.26 (1H, brs, H-9').

Atracta-vaginata B (2) A pale yellow amorphous powder, [α]$_D$ -64° ($c = 1.0$, CHCl$_3$). UV $A_{max}$ (nm (log e)): 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$_2$-15'), 1.10 (3H, s, H$_2$-13'), 1.22 (3H, s, H$_2$-12'), 1.26 (3H, s, H$_2$-14'), 1.45 (3H, brs, 4-CH$_3$), 1.54 (3H, brs, 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 (3H, m, H-1*), 2.75 (3H, s, H$_3$-9), 3.07 (2H, m, H-8*), 3.55 (2H, s, H$_2$-7'), 4.13 (1H, brs, H-8*), 5.26 (1H, brs, H-9'), 10.19 (1H, s, O-H), 16.10 (1H, s, 2-OH), 18.40 (1H, s, 3-OH). HR-FAB-MS (negative mode) $m/z$: 605.309 [M - H]$^+$. Calculated for C$_{36}$H$_{54}$O$_{16}$: 605.312.}

References and Notes
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 dibuteryl derivative, flavaspidic acid BB, and acetyl butyryl derivative, flavaspidic acid AB, have been found in many species of Dryopteris.
7. Wu C.-L., Asakawa Y., Phytochemistry, 27, 940-945 (1988); (--)Aristol-9-ene has been isolated from such plants as Acorus calamus and Nardostachys jatamansi. We could not detect it in this fern.