DRYOFRAGIN AND ASPIDIN PB, PISCICIDAL COMPONENTS FROM *DRYOPTERIS FRAGRANS*

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The structures of dryofragin (**5**), a novel sesquiterpenoid connecting acylated filicinic acid, and a new aspidin analogue isolated as piscicidal components from the whole plant of *Dryopteris fragrans* have been elucidated by extensive spectroscopic analysis.

KEYWORDS *Dryopteris fragrans*; Aspidiaceae; filicinic acid; dryofragin; aspidin PB; piscicidal activity

Dryopteris species of Aspidiaceae are known to be rich in anthelmintic phloroglucinol derivatives. One of the species, *D. fragrans* (L.) Schott has been used as a remedy for skin diseases in Northeastern China. In the course of our screening for the natural piscicidal substances, we have isolated a new active compound with a unique carbon skeleton called dryofragin (**5**) together with a new phloroglucinol derivative, aspidin PB(**1**) , and three known homologues from the *n*-hexane extract of *D. fragrans*. This paper describes the structural elucidation of the new piscicidal substances.

The *n*-hexane extract of the dried whole plant of *D. fragrans* was subjected to a combination of column chromatography over silica gel and Sephadex LH-20, and fractionated by the guidance of toxicity to killie-fish (*Oryzyas latipes*; medaka) to yield aspidin PB (**1**) (5.9 x 10^{-3}% / dried materials) and dryofragin (**5**) (1.8 x 10^{-2}%) along with aspidins BB (**2**),[1] AB (**3**),[1] and albicanol (**4**)[2] as piscicidal components.

Aspidin PB (**1**), colorless prisms, mp 134-136°C, gave a pseudomolecular ion at *m/z* 447.2019 $(M+H)^+$ in high-resolution (HR) FAB-MS, establishing its molecular formula as $C_{24}H_{30}O_8$ [calcd for $(M+H)^+$ 447.2019]. The UV λ_{max}^{EtOH} [216 (log ε 4.47), 316 (sh) (4.12), 352 nm (4.25)] spectrum of **1** was similar to that of aspidins BB (**2**) and AB (**3**), indicating **1** to be an analogue of aspidins. The ^{1}H-NMR spectrum of **1** exhibited two primary [δ 0.99 and 1.17 (each 3H, t, *J*=7 Hz)] and three tertiary methyl [δ 1.45, 1.53 and 2.14 (each 3H, s)], and four methylene proton signals [δ 1.72 (sex, *J*=7 Hz), 3.08 (t, *J*=7 Hz), 3.20 (q, *J*=7Hz) and 3.57 (br s)].

Four exchangeable protons, among which a singlet at δ 18.49 is characteristic of a strongly hydrogen-bonded hydroxyl group of acylated filicinic acid,[3] were also observed. These spectral features of **1** including the appearance of signals due to small amounts of contaminants, two tautomers arising from a keto-enol tautomerization at filicinic acid moiety,[1] were very similar to those of aspidin BB (**2**) except for lacking one methylene group. Aspidin PB (**1**) thus has butyryl and propynyl substituents as an acyl side chain. The propynyl group was located at C-6 of the filicinic acid portion based on NOE's between C-10 methyl protons and hydroxyl proton at δ 18.49 and also between methoxyl protons and butylyl methylene (C-8') protons in the NOESY spectrum of **1**. The other NOE's, as illustrated by arrows in Fig. 1, were consistent with the structure of **1**.

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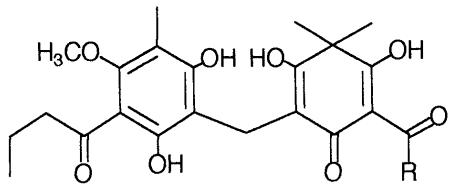


1: R=CH_2CH_3
2: R=$CH_2CH_2CH_3$
3: R=CH_3

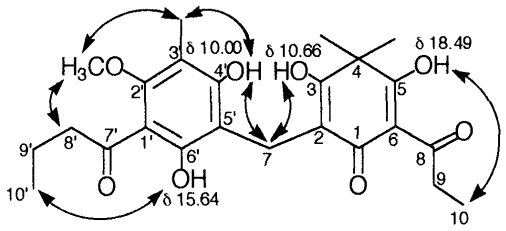
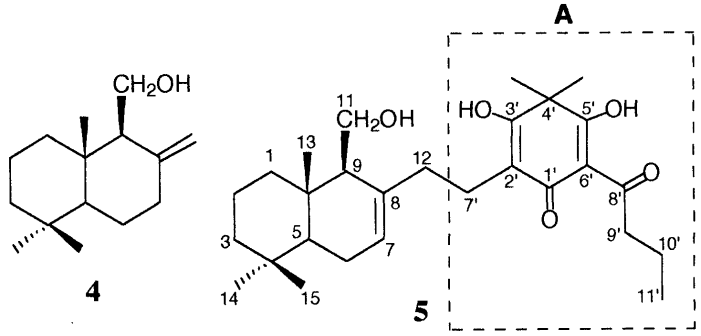


Fig. 1. NOE's Observed for **1**



4 **5**

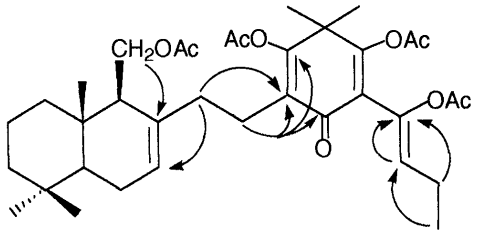


Fig. 2. Selected Long-Range Correlations in the HMBC Spectrum of **6**

Dryofragin (**5**), colorless fine needles, mp 180-181°C, $[\alpha]_D$ -30.1° (c = 1.7, $CHCl_3$), was shown to have the molecular formula $C_{28}H_{42}O_5$ (calcd for M+H; 459.3111) by the ion peak at m/z 459.3127 in HRFAB-MS. The UV λ_{max}^{EtOH}[222 (log ε 4.31), 354 nm (4.28)] spectrum, and the appearance of a hydrogen-bonded hydroxyl proton at δ 19.12 in the ^{1}H-NMR spectrum of **5** suggested the presence of an acylated filicinic acid moiety. The ^{1}H-NMR spectrum[4] also revealed signals due to one primary (δ 0.99), five tertiary methyl [δ 0.73, 1.34, 1.35, 0.88 (6H)], one olefinic (δ 5.64), and one methylol protons [δ 3.71 (dd, J=9, 11 Hz) and 4.08 (d, J=11 Hz)]. The ^{13}C-NMR[5] and ^{1}H-^{13}C COSY spectra of **5** exhibited eight sp^2 carbon resonances including two carbonyl carbons and twenty sp^3 carbon signals, among which three are attributed to quaternary carbons. Of these ^{13}C- NMR signals, thirteen (C-1'~C-11' and C_4'-dimethyl carbons) are accounted for by the butyryl filicinic acid part (partial structure A). The presence of a filicinic acid moiety in **5** was also evidenced by the observation of each signal accompanied by two small signals due to unisolable keto-enol tautomers as found in **3**.[1] The remaining ^{1}H- and ^{13}C-NMR data were in agreement with those of albicanol (**4**), although the signals of the exomethylene group in **4** were replaced by a trisubstituted olefin proton signal in **5**. These data indicate that dryofragin is composed of drimenol[6] (endo-isomer of **4**) and butyryl filicinic acid, which are linked to each other through a C-C bond.

Acetylation of **5** afforded a tetraacetate (**6**), ESI-MS m/z 627 $(M+H)^+$, which does not form an equilibrium mixture of keto-enol tautomers as in **5**. Its ^{1}H-NMR spectrum[7] showed a newly formed olefinic proton signal at δ 5.37 (t, J=7.0 Hz) coupled with H-10' methylene protons, indicating that an enol tautomerization at the butyryl residue of partial structure (A) occurred upon acetylation. The HMBC of **6** gave the evidence for the linking mode between the sesquiterpene and filicinic acid moieties, as shown in Fig. 2, leading to the planar structure of dryofragin (**5**) with a new carbon skeleton. The *cis*-relationship of the C-11 methylene and C-13 methyl groups was suggested by their NOE correlation in the NOESY spectrum of **6**. Taking into account the co-occurrence of **4** and **5** in the same plant and the same sign of optical rotation of **5** as that of drimenol ($[\alpha]_D$ -20°)[6], the absolute configurations at C-5, -9, and -10 were suggested to be the same as those of drimenol and **4**.

Sesquiterpenoids coupled with a phloroglucinol derivative through a C-C linkage are not rare, as exemplified by zonarol,[8] macrocarpals,[9),10] atrata-phloroglucinols A and B,[11] *etc.* However, to the best of our knowledge, dryofragin is the first sesquiterpenoid connecting with a filicinic acid moiety.

The isolated compounds **1**-**5** were toxic to killie-fish. Potent piscicidal activity was exhibited by aspidins BB (**2**), PB (**1**), and dryofragin (**5**) at TLm,[12] 1.2-1.5 ppm, which are comparable to buddledin B (TLm 1.2 ppm),[13] while albicanol (**4**) showed weaker toxicity (TLm 3.7 ppm). The biological activity of **1**-**5** is under further investigation from the viewpoint that some piscicidal substances often exhibit other biological activities such as cytotoxic, anti-ulcer, or anti-tumor promoting activities.[14]

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4) ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.73 (s, 13-H_3), 0.88 (s, 14- and 15-H_3), 0.99 (t, *J*=7.0 Hz, 11'-H_3), 1.34, 1.35 [each s, C4'-$(Me)_2$], 1.66 (sextet, *J*=7.0 Hz, 10'-H_2), 2.51 (t, *J*=7 Hz), 2.13 (m, 9-H, 12-H_2 and 7'-H_2), 2.98 (dt, *J*=7.0 Hz, 9'-H_2), 3.71 (dd, *J*= 11.0, 9.0 Hz, 11-H), 4.08 (d, *J*=11.0 Hz, 11-H), 5.64 (br s, 7-H), 19.12 (5'-OH).
5) ^{13}C-NMR (126 MHz, $CDCl_3$) δ: 14.0 (C-11'), 14.3 (C-13), 18.5(C-10'), 18.8 (C-2), 22.0 (C-15), 22.3 (C-12 or C-7'), 23.6, 24.5 (4'-diMe), 25.2 (C-6), 29.7. (C-7' or 12), 33.0 (C-4), 33.2 (C-14), 35.3 (C-1), 35.8 (C-3), 39.6 (C-10), 41.9 (C-9'), 42.1 (C-4'), 49.6 (C-5), 55.0 (C-9), 63.0 (C-11), 105.5 (C-2'), 106.8 (C-6'), 124.8 (C-7), 136.6 (C-8), 175.6 (C-3'), 189.0 (C-1'), 197.8 (C-5'), 203.7 (C-8').
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7) ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 0.80 (s, 13-H_3), 0.85 (s), 0.88 (s) (15- and 14-H_3), 1.01 (t, *J*=7.0 Hz, 11'-H_3), 1.27, 1.30 [each s, C4'-$(Me)_2$], 2.04 (s, OAc), 2.08 (m, 10'-H and 12-H), 2.11, (s, OAc), 2.16 (br s, 9-H), 2.20 (m, 7'-H and 12-H), 2.24, 2.29 (each s, OAc x 2), 2.40 (m, 7'-H), 4.08 (dd, *J*=12.0, 5.5 Hz, 11-H), 4.28 (dd, *J*=12.0, 3.0 Hz, 11-H), 5.37 (t, *J*=7.0 Hz, 9'-H), 5.41 (br s, 7-H).
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12) TLm: median tolerance limit after 24 h.
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