# CASTANEIOLIDE, ABSCISIC ACID AND MONORDEN, PHYTOTOXIC COMPOUNDS ISOLATED FROM FUNGI (*MACROPHOMA CASTANEICOLA* AND *DIDYMOSPORIUM RADICICOLA*) CAUSE "BLACK ROOT ROT DISEASE" IN CHESTNUT TREES

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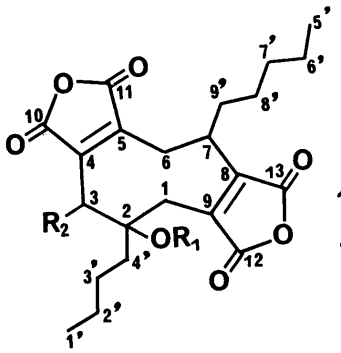
*Macrophoma castaneicola* and *Didymosporium radicicola* are pathogens causing chestnut black root rot disease. A new metabolite, castaneiolide, and abscisic acid were isolated from *M. castaneicola* and monorden was from *D. radicicola*. These metabolites were toxic to chestnut leaves.

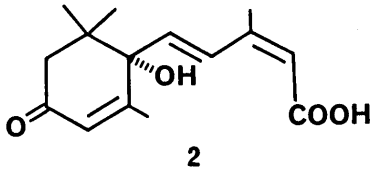
KEYWORDS castaneiolide; abscisic acid; monorden; *Macrophoma castaneicola*; *Didymosporium radicicola*; black root rot disease; phytotoxin

The "black root rot disease" has inflicted serious damage to chestnut plantations in the Noto area of Ishikawa, Japan. In this disease, leaves wither and roots are colored black and they rot in spite of normal appearance of the trunks. Ōishi isolated two species of fungi, *Macrophoma castaneicola* and *Didymosporium radicicola*, from the black granules formed on the surfaces of the diseased roots, and identified them as new pathogens of chestnut wilt.[1]

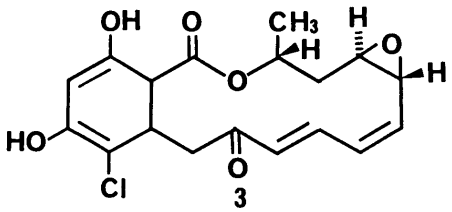
During the course of chemical studies of the biologically active metabolites of those pathogenic fungi, we isolated a new metabolite for which the name castaneiolide is proposed, together with abscisic acid from *M. castaneicola* and monorden from *D. radicicola*.



**1** $R_1$=H $R_2$=OH
**1a** $R_1$=Ac $R_2$=OAc
**4** $R_1$=H $R_2$=H



**2**



**3**

*M. castaneicola* was cultured on a corn meal medium at 27°C for 3 weeks. The fluorescent culture filtrate was stirred with Amberlite XAD-2 and the metabolites absorbed on resin were eluted with MeOH. The MeOH extract was subjected to silica gel column chromatography eluting with a mixture of benzene and ethyl acetate to give castaneiolide (**1**), mp 162-163°C, (11 mg/l of medium) as colorless needles, $[\alpha]_D$ -36.5° (c=0.11, EtOH), and abscisic acid (**2**), mp 167-168°C, (14 mg/l of medium).[2]

The molecular formula of **1** was established as $C_{22}H_{28}O_8$ by elemental analysis and MS. The IR spectrum showed anhydride bands at 1845, 1816 and 1760 $cm^{-1}$ and the UV spectrum had a maximum absorption at 247 nm ($\varepsilon$=7690). IR absorptions at 3540 and 3500 $cm^{-1}$ were attributed to two alcoholic hydroxyl groups. **1** was converted into a diacetate (**1a**) by treating it with acetic anhydride in the presence of zinc chloride.[3]

In the $^1$H-NMR spectrum of **1**, an AB pattern signal at $\delta$ 2.59 and 3.49 (d, $J$=15.2 Hz) was assigned to C(1)-methylene. A multiplet signal for C(7)-methine was observed at $\delta$ 3.33 (m) and signals at $\delta$ 3.00 (dd, $J$=13.1, 6.1 Hz) and 3.70 (t, $J$=13.1 Hz) were assigned to C(6)-methylene. Two signals of a three-proton triplet at $\delta$ 0.87 and 0.95 suggested the presence of two alkyl groups in **1**. The spectral data for **1** generally

paralleled and extended those reported for scytalidin, which has been isolated from *Scytalidium* species as a fungal toxic compound. Proton and carbon signals were assigned as shown in Table I by means of $^1H$-$^1H$ COSY and $^{13}C$-$^1H$ COSY spectra. Except the signal concerning C-3, the NMR signals of **1** is similar to those of scytalidin.[4] A signal for H-3 in **1** appeared as a singlet at a lower field ($\delta$ 4.70) and a signal for C-3 appeared at $\delta$ 69.89. This suggests that C-3 is substituted by a hydroxyl group. The arrangement of the alkyl groups in **1** was determined from the mass spectral fragmentation pattern.[5] The base peak, *m/z* 85, in the MS, corresponds to the ion $CH_3(CH_2)_3CO^+$ and it was apparent that it arose from the cleavage in the vicinity of the carbon atom bearing the tertiary hydroxyl group. The peak at *m/z* 57 ($C_4H_9$) indicates a *n*-butyl chain attached to the nonadride ring. The strong peak in the MS at *m/z* 141 ($C_6H_5O_4$), 195 ($C_{11}H_{15}O_3$), 226 ($C_{11}H_{14}O_5$) and 280 ($C_{16}H_{24}O_4$) resulted from the cleavages indicated in Chart 1. Any other substitution pattern in an isomeric structure would give rise to a number of other significant ion peaks from competing fragmentation pathways. These results were quite similar to those for scytalidin.[4]

From this spectral evidence, the structure of **1** was determined as shown in the Figure. Castaneiolide may be biosynthesized by the head-to-tail concerted cycloaddition of two $C_{11}$ units, derived by octanoic acid and oxaloacetic acid.[6]

The other metabolite was identified as abscisic acid by comparing it with the physical properties given in the literature. Abscisic acid is a well-known plant hormone and has been isolated as a phytotoxic principle from *Botrytis* species, a phytopathogenic fungus.[2]

TABLE I. $^{13}C$- and $^1H$-NMR Spectral Data for Castaneiolide (1) and Scytalidin (4)[a] ($\delta$, ppm)

| | $^{13}C$-NMR[b] | | $^1H$-NMR | |
|---|---|---|---|---|
| | 1[c] | 4[d] | 1[c] | 4[d] |
| 1 | 28.76(t) | 35.8 | 2.59(d, *J*=15.2), 3.49(d, *J*=15.2) | 2.46(d, *J*=13.3), 2.66(d, *J*=13.3) |
| 2 | 78.30(s) | 77.4 | 2.90 (OH) | variable |
| 3 | 69.89(d) | 35.1 | 4.70(s), 5.40(OH) | 2.62, 2.75(d, *J*=13.7) |
| 4[e] | 142.64(s) | 141.3 | - | - |
| 5[e] | 144.69(s) | 143.0 | - | - |
| 6 | 25.50(t) | 27.8 | 3.00(dd, *J*=13.1, 6.1), 3.70(t, *J*=13.1) | 2.80(dd, *J*=13.7, 4.0) 2.87(dd, *J*=13.7, 10.7) |
| 7 | 35.48(d) | 35.7 | 3.33(m) | 3.28(m) |
| 8[e] | 146.79(s) | 145.0 | - | - |
| 9[e] | 145.28(s) | 143.5 | - | - |
| 10[f] | 167.79(s) | 167.1 | - | - |
| 11[f] | 165.81(s) | 165.7 | - | - |
| 12[f] | 167.22(s) | 166.2 | - | - |
| 13[f] | 166.18(s) | 166.0 | - | - |
| 1'[g] | 14.36(q) | 13.7 | 0.95(t, *J*=8.0) | 0.97(t, *J*=7.1) |
| 2'[h] | 23.76(t) | 22.5 | 1.26-1.55(2H, m) | 1.12-1.45 (m) |
| 3'[h] | 25.50(t) | 27.3 | 1.71-1.82(m) | |
| 4' | 43.32(t) | 44.7 | 1.88(t, *J*=7.1) | |
| 5'[g] | 14.24(q) | 13.8 | 0.87(t, *J*=7.1) | 0.90(t, *J*=7.1) |
| 6'[h] | 23.04(t) | 23.4 | 1.26-1.55(6H, m) | 1.50-1.75 (m) |
| 7'[h] | 32.48(t) | 32.0 | | |
| 8'[h] | 23.76(t) | 26.0 | | |
| 9' | 36.27(t) | 34.5 | 1.62(m) | |

a) s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. b) The coupling pattern was characterized by off-resonance NMR. c) in acetone-$d_6$. d) in dioxane-$d_8$, refernce 4.
e), f), g), h) Assignements may be interchanged.

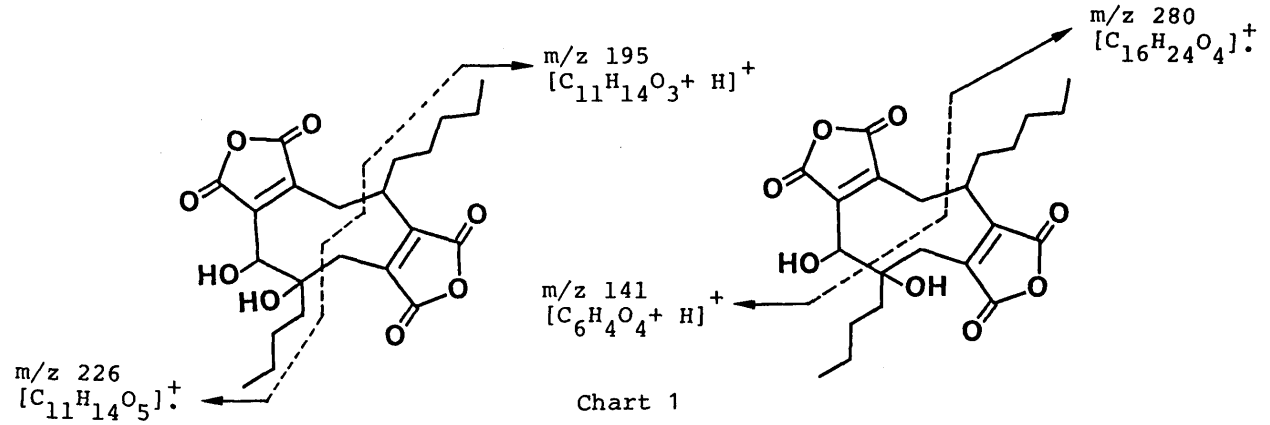



Chart 1

*D. radicicola* P7R was cultivated on potato extracted medium for 4 weeks. The culture broth was toxic to chestnut leaves. The ethyl acetate extract of the culture broth was chromatographed on a silica gel column eluting with chloroform to give monorden (**3**), mp 194-196°C, (265 mg/l of medium) as a major metabolite. Monorden has been isolated from *Monocillium nordinii*[7], *Penicillium luteo-auranitum*[8], *Nectria radicicola*[9] and *Neocosmospora tenuicristata*[10] and has antifungal and plant growth-regulating properties.

The phytotoxic effect of these metabolites on the chestnut leaves was examined.[11] Castaneiolide induced a few dark-colored spots at 100 ppm concentration. Wilting was began at 500 ppm and whole leaves were withered at 1000 ppm. Abscisic acid induced wilting at 1000 ppm and monorden caused a necrosis on chestnut leaves at 100 ppm concentration. These toxin-induced phenomena were similar to the symptoms of the disease. So it is assumed that these metabolites are the toxic principle of the disease.

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3) Mp 202-203°C, *Anal.* Calcd. for $C_{26}H_{32}O_{10}$: C, 61.89; H, 6.39. Found: C, 61.70; H, 6.44. EI-MS *m/z*: 504 ($M^+$), 462, 420. IR (KBr) $cm^{-1}$: 1829, 1775. $^1$H-NMR ($CDCl_3$) $\delta$ : 0.86-1.01 (6H, m), 1.33-1.41 (14H, m), 1.88, 2.16 (each 3H, s), 3.07-3.11 (5H, m), 6.25 (1H, s). $^{13}$C-NMR ($CDCl_3$) $\delta$ : 13.9 (q, Cx2), 20.3 (q), 21.3 (q), 22.4 (t), 22.7 (t), 24.5 (t), 26.6 (t), 27.2 (t), 27.8 (t), 31.4 (t), 35.0 (t), 35.8 (t), 36.2 (t), 68.0 (d), 83.6 (s), 139.4 (s), 140.6 (s), 144.2 (s), 145.6 (s), 162.9 (s), 163.7 (s), 165.1 (s), 168.1 (s), 168.7 (s). $[\alpha]_D^{21}$ -66.1° (c=0.12, $CHCl_3$).
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5) EI-MS *m/z*: 402 ($M^+$-18), 363, 330, 280, 262, 195, 141, 85, 57. CI-MS *m/z*: 421 ($M^+$+1), 403, 385, 280, 262, 195, 141, 85, 57. HI-MS *m/z*: 280.1594 (calcd. for $C_{16}H_{24}O_4$: 280.1672), 195.1014 (calcd. for $C_{11}H_{15}O_3$: 195.1019), 141.1511 (calcd. for $C_6H_5O_4$: 141.1879), 85.0648 (calcd.for $C_5H_9O$: 85.0653), 57.0782 (calcd. for $C_4H_9$ : 57.0704).
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11) The phytotoxic assay is based on the properties that cause dark coloration and wilting of chestnut leaves. A stalk-attached fresh young leaf is inserted into an aqueous solution containing a tested compound at 1000, 500, 250, 100 and 10 ppm concentration and compared with controls for one day in the dark at room temperature.

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