Pergamon

Tetrahedron Vol. 51, No. 29, pp. 7907-7918, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4020/95 $9.50+0.00

0040-4020(95)00423-8

Phytocassanes A, B, C and D, Novel Diterpene Phytoalexins from Rice, *Oryza sativa* L.

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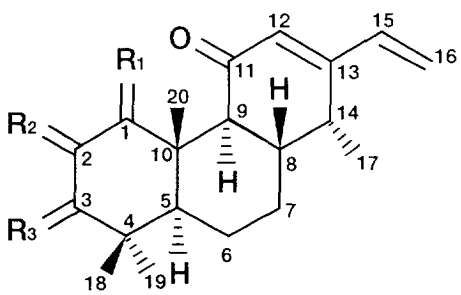
Abstract: Four novel phytoalexins, designated phytocassanes A, B, C and D, were isolated from rice (*Oryza sativa* L.) leaves infected with *Magnaporthe grisea* (previously designated *Pyricularia oryzae*) and from rice stems infected with *Rhizoctonia solani*. The structures of the phytocassanes, classified as cassane-type diterpenes, were determined by spectroscopic methods. Phytocassanes had high antifungal activity against the pathogenic fungi, *M. grisea* and *R. solani*. The ED_{50} values of phytocassanes A, B, C and D in prevention of spore germination of *M. grisea* were 20, 4, 7 and 25 μg/ml, respectively. Furthermore, large amounts of phytocassanes were produced in rice infected with the pathogenic fungi.

Phytoalexins are antifungal compounds synthesized in plant cells after their exposure to plant pathogenic fungi. Phytoalexin production is thought to be involved in plant defense mechanism against the pathogenic fungi. In rice plants, momilactones A and B[1,2], oryzalexins A-F[3-7] and S[8,9], and sakuranetin[10] have been reported as phytoalexins. Momilactones A and B, isolated initially as plant growth inhibitors from rice husks[11], were subsequently found to be produced in blast-infected or UV-irradiated rice leaves[1,2]. Independently, oyrzalexins A-F[3-7], S[8,9] and sakuranetin[10] were isolated from rice leaves



	R_1	R_2	R_3	
1	H, H	α-H, β-OH	O	Phytocassane A
2	α-H, β-OH	α-H, β-OH	α-H, β-OH	Phytocassane B
3	α-H, β-OH	H, H	α-H, β-OH	Phytocassane C
4	H, H	O	α-H, β-OH	Phytocassane D

Fig. 1 Structures of phytocassanes A, B, C and D

infected with blast fungus, *Magnaporthe grisea*. Momilactones A and B and oyrzalexins A–F are classified as pimarane-type diterpenes. Oryzalexin S and sakuranetin are classified as a stemarane-type diterpene and a flavanone, respectively. However, it remains unclear which phytoalexins are predominantly involved in defense mechanisms against the pathogenic fungi. Therefore, a large number of phytoalexins produced in rice leaves that had been infected with *M. grisea* were screened for the high antifungal activity. As a result, four novel diterpenes with a cassane skeleton were isolated. We report here the structures and antifungal activities of these novel phytoalexins, designated phytocassanes A, B, C and D (Fig. 1).

To identify the phytoalexins with the high antifungal activity among all the phytoalexins, EtOAc extracts from rice leaves infected with *M. grisea* (incompatible race 031^{12}) were fractionated by HPLC on an ODS–120A column with elution with a linear gradient of 20–100 % EtOH. Each fraction was subjected to an assay of the inhibitory activity on spore germination of *M. grisea*. The fraction with the highest antifungal activity was further purified by the procedure shown in Fig. 2 to yield six compounds, numbered **1**–**6**. The limited amounts of the compounds obtained from rice leaves infected with *M. grisea*

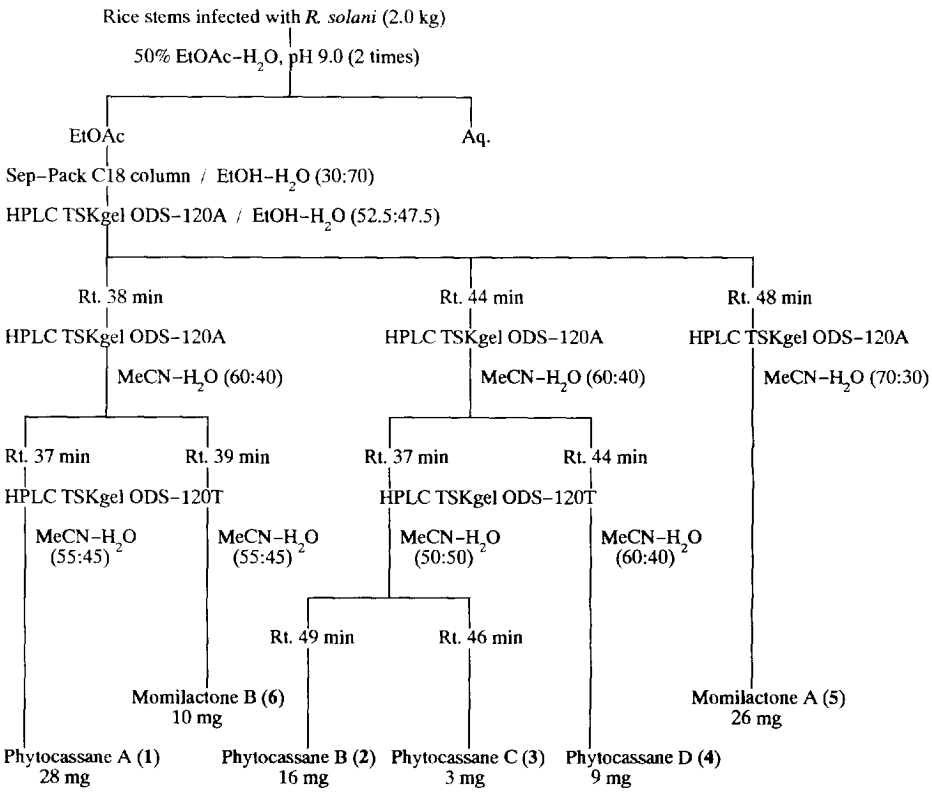


Fig. 2 Isolation procedure of phytocassanes and momilactones from rice stems infected with *Rhizoctonia solani* (Rt., retention time)

hampered determinations of their structures. Therefore, to obtain large amounts of compounds **1–6**, we used rice stems (2 kg) that had been infected with *Rhizoctonia solani* as starting material. Since in such tissues, high levels of phytoalexins were contained and the contents of the hydrophobic compounds such as chlorophyll were extremely lower than in rice leaves, it was easy to purify compounds **1–6**. As a result of purification by the procedure shown in Fig. 2, 28 mg of compound **1**, 16 mg of compound **2**, 3 mg of compound **3**, 9 mg of compound **4**, 26 mg of compound **5** and 10 mg of compound **6** were obtained. Compounds **1** and **4** had maximum UV absorption at 268 nm, compound **2** and **3** showed maximum UV absorption at 274 nm, and compounds **5** and **6** showed no absorption maximum above 210 nm, suggesting that compounds **1–4** and compounds **5** and **6** are different types of phytoalexin.

^{13}C NMR and the low-resolution mass spectroscopic data (Experimental) identified compounds **5** and **6** as momilactones A and B[11,13], respectively, known phytoalexins that had been isolated previously from blast-infected or UV-irradiated rice leaves[1,2]. However, ^{13}C NMR and the low-resolution mass spectroscopic data indicated that compounds **1**, **2**, **3** and **4** were new phytoalexins, different from known phytoalexins from rice.

The IR spectrum of compound **2** showed the presence of an α, β, γ, δ-unsaturated ketone at 1,635 cm^{-1} and a hydroxyl group at 3,240 cm^{-1}. The identification of 20 carbons in the ^{13}C NMR (Table 1) and DEPT experiment, and the high-resolution mass spectrum, which showed a molecular ion $[M+H]^+$ at m/z 335.2286 indicated a molecular formula of $C_{20}H_{30}O_4$. The ^1H NMR spectrum ($CDCl_3$) of compound **2** (Experimental) showed signals for four methyl groups (δ 1.03, 1.04, 1.10, 1.13), three protons

Table 1 ^{13}C NMR (125.8 MHz) Data of Phytocassanes A, B,C and D in $CDCl_3$

C	Phytocassane A	Phytocassane B	Phytocassane C	Phytocassane D
1	50.5	77.2	75.8	52.7
2	68.6	73.6	36.6	211.1
3	219.0	76.6	75.4	82.3
4	45.8	38.7	39.4	45.1
5	51.6	51.6	52.5	53.8
6	22.4	20.2	20.5	21.1
7	30.4	31.5	31.6	30.9
8	38.3	39.0	39.4	38.3
9	56.4	58.8	58.7	56.5
10	36.8	44.0	45.2	44.0
11	200.3	204.6	204.6	200.6
12	128.4	128.3	128.5	128.4
13	160.7	163.9	163.5	161.1
14	32.9	34.1	34.1	33.2
15	136.2	135.6	135.6	136.2
16	120.9	122.3	122.1	120.8
17	13.5	13.3	13.4	13.5
18	19.4	17.5	15.2	16.4
19	29.6	30.4	28.3	29.2
20	16.7	12.0	11.1	16.0

geminal to hydroxyl groups (δ 3.23, 3.54, 4.19) and four olefinic protons (δ 5.58, 5.76, 5.90, 6.39). The connectivities between each of the ^{13}C signals and the related ^{1}H signals were established by the ^{1}H–^{13}C COSY spectrum. The connectivities between ^{13}C signals were established by the ^{1}H–^{1}H COSY spectrum and the HMBC experiments, as shown in Fig. 3. The hydroxyl group-bearing carbon signals at δ 73.6, 76.6 and 77.2 (Table 1), the chemical shifts (δ 3.23, 3.54, 4.19) of the protons at C-1, C-2 and C-3 and the IR spectrum revealed that the three hydroxyl groups should be at C-1, C-2 and C-3. The carbonyl group-bearing carbon signal at δ 204.6 and the IR spectrum revealed that the α, β, γ, δ-unsaturated ketone should be at C-11. The olefinic carbon signals at δ 122.3, 128.3, 135.6 and 163.9 and the chemical shifts (δ 5.90, 6.39, 5.58, 5.76) of the olefinic protons at C-12, C-15 and C-16 revealed that the olefinic carbons should be at C-12, C-13, C-15 and C-16. The stereochemistry was confirmed by NOE experiments. The irradiation of H-1 showed effect with H-2, H-3, H-5 and H-9; H-2 showed effect with H-1 and H-3; H-3 showed effect with H-2 and H-5; H-5 showed effect with H-3 and H-9; H-9 showed effect with H-1 and Me-17; Me-17 showed effect with H-9 and H-14; H-8 showed effect with H-14 and Me-20; H-14 showed effect with H-8, H-16 and Me-17; and Me-20 showed effect with H-8. Consequently, compound **2** was confirmed to be 1β,2β,3β-trihydroxy-12,15-cassadien-11-one or its enantiomer, and it was designated phytocassane B (Fig. 1).

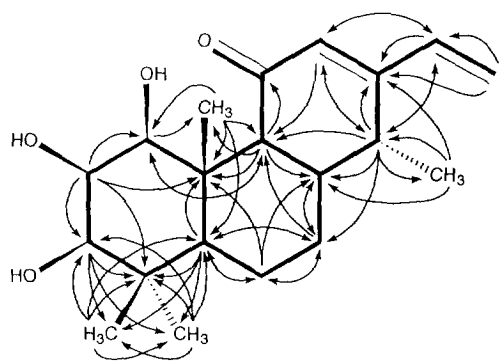


Fig. 3 HMBC correlation map of phytocassane B (**2**)

The IR spectrum of compound **3** showed the presence of an α, β, γ, δ-unsaturated ketone at 1,637 cm^{-1} and a hydroxyl group at 3,304 cm^{-1}. The identification of 20 carbons in the ^{13}C NMR experiment and the high-resolution mass spectrum which showed a molecular ion $[M]^+$ at m/z 318.2164 indicated a molecular formula of $C_{20}H_{30}O_3$. The ^{1}H NMR spectrum, ^{13}C NMR spectrum, ^{1}H–^{13}C COSY spectrum, HMBC correlations and NOE effects of compound **3** were very similar to those of compound **2**. Consequently, compound **3** was confirmed to be 1β,3β-dihydroxy-12,15-cassadien-11-one or its enantiomer, and it was designated phytocassane C (Fig. 1).

The IR spectrum of compound **4** showed the presence of an α, β, γ, δ-unsaturated ketone at 1,655 cm^{-1}, a carbonyl group at 1,713 cm^{-1} and a hydroxyl group at 3,488 cm^{-1}. The identification of 20

carbons in the ^{13}C NMR experiment and the high-resolution mass spectrum, which showed a molecular ion $[M]^+$ at m/z 316.2048 indicated a molecular formula of $C_{20}H_{28}O_3$. The carbonyl group-bearing carbon signal at δ 211.1 (Table 1), the IR spectrum, ^1H–^{13}C COSY spectrum, and HMBC correlations revealed that the carbonyl group should be at C-2. The other features of compound **4** were very similar to those of compound **2**. Consequently, compound **4** was confirmed to be 3β-hydroxy-12,15-cassadien-2,11-dione or its enantiomer, and it was designated phytocassane D (Fig. 1).

The IR spectrum of compound **1** showed the presence of an α, β, γ, δ-unsaturated ketone at 1,657 cm^{-1}, a carbonyl group at 1,713 cm^{-1} and a hydroxyl group at 3,484 cm^{-1}. The identification of 20 carbons in the ^{13}C NMR experiment and the high-resolution mass spectrum, which showed a molecular ion $[M]^+$ at m/z 316.2052 indicated a molecular formula of $C_{20}H_{28}O_3$. The carbonyl group-bearing carbon signals at δ 219.0 (Table 1), the IR spectrum, ^1H–^{13}C COSY spectrum and HMBC correlations revealed that the carbonyl group should be at C-3. The stereochemistry was confirmed by NOE experiments. The irradiation of H-1α showed effect with H-2 and H-9; H-2 showed effect with H-1α, H-5 and Me-19; H-5 showed effect with H-2 and H-9; and Me-19 showed effect with H-2, H-5 and H-6α. The other features of compound **1** were very similar to those of compound **2**. Consequently, compound **1** was confirmed to be 2β-hydroxy-12,15-cassadien-3,11-dione or its enantiomer, and it was designated phytocassane A (Fig. 1).

The IR bands (1,635 and 1,637 cm^{-1}) for α, β, γ, δ-unsaturated ketones of phytocassanes B and C showed different values from those (1,657 and 1,655 cm^{-1}) for α, β, γ, δ-unsaturated ketones of phytocassanes A and D. The C-11 signals at δ 204.6 (Table 1) of phytocassanes B and C were slightly shifted to a lower field as compared with those of phytocassanes A and D which appeared at δ 200.3 and 200.6, respectively. The OH-1 signals of phytocassanes B and C were remarkably shifted downfield (δ 7.72 and δ 7.22, see Experimental). Furthermore, in the stereostructures of phytocassanes B and C, the proton of the OH-1 group was in close spatial proximity to the carbonyl group at C-11. These common features, observed in phytocassanes B and C, suggest that the hydroxyl group at C-1 forms a intramolecular hydrogen bond to the carbonyl group at C-11 (Fig. 4). The slight differences between the overall ^{13}C NMR signals of phytocassanes B, C and A, D can be explained by this intramolecular hydrogen bonding.

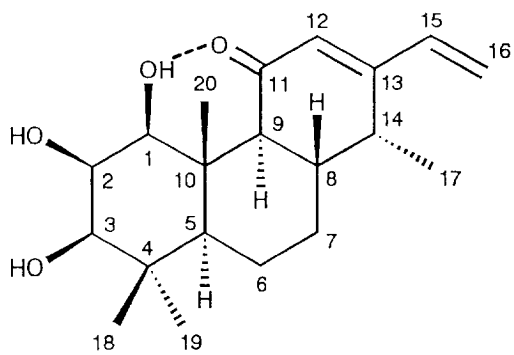
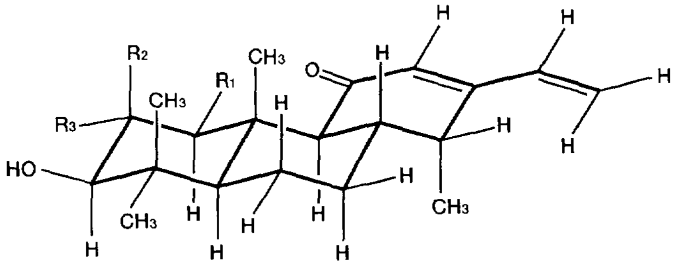


Fig. 4 Intramolecular hydrogen bonding in phytocassane B

The overall spectral data of phytocassane A were very similar to those of the other phytocassanes. However, there was a significant difference in their stereostructures. Analysis of the vicinal coupling constants between the C–1 protons and the C–2 proton in phytocassane A ($J_{1ax-2ax}$ = 11.5; $J_{1eq-2ax}$ = 7.9) revealed that the C–2 proton was in an axial orientation and that the cyclohexanone ring (C–1, C–2, C–3, C–4, C–5, C–10) adopted a boat conformation (Fig. 5). This result was also supported by the fact that NOE effects were observed between the C–2 proton and the C–5 or C–19 protons in phytocassane A. NOE effects were observed between the C–3 proton and the C–1 or C–5 protons in phytocassanes B, C and D, suggesting that the cyclohexane rings (C–1, C–2, C–3, C–4, C–5, C–10) were in a chair conformations (Fig. 5). Since phytocassane A was unstable in basic solutions and was converted non-enzymatically to phytocassane D (Experimental), it may be in a highly reactive configuration.

To the best of our knowledge, phytocassanes A, B, C and D have not previously been found as diterpenes and this is the first report of phytoalexins with a cassane skeleton from rice.



<table>
<tr><th></th><th>R1</th><th>R2</th><th>R3</th></tr>
<tr><td>Phytocassane B</td><td>OH</td><td>OH</td><td>H</td></tr>
<tr><td>Phytocassane C</td><td>OH</td><td>H</td><td>H</td></tr>
<tr><td>Phytocassane D</td><td>H</td><td colspan="2">O</td></tr>
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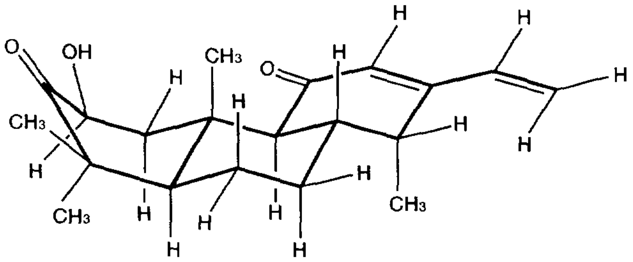

Phytocassane A

Fig. 5 Stereostructures of phytocassanes A, B, C and D

Phytocassanes A, B, C and D had high antifungal activity against the pathogenic fungi, *M. grisea* and *R. solani*. The ED_{50} values of phytocassanes A, B, C and D in prevention of spore germination of *M. grisea* were 20, 4, 7 and 25 μg/ml, respectively. The ED_{50} values of phytocassanes A, B, C and D in prevention of germ tube growth of *M. grisea* were 5, 1.5, 3 and 10 μg/ml, respectively. These results indicate that the number of hydroxyl groups at the C–1, C–2 and C–3 positions or the intramolecular hydrogen bonding between the hydroxyl group at C–1 and the carbonyl group at C–11 is the main determinant of the antifungal activity in phytocassanes. Phytocassanes B and C almost completely inhibited hyphal growth of *R. solani* at 10 μg/ml. Thus, the phytocassanes exhibited high antifungal activity as compared with known phytoalexins. The levels of total phytocassanes in rice infected with *M. grisea* and *R. solani* were 68.8 and 97.4 μg/g fresh weight of plant, respectively (Table 2). Furthermore, the amounts of phytocassanes in rice leaves infected with *M. grisea*, incompatible race 031, were higher than those in rice leaves infected with compatible race 007. These results indicate that phytocassanes occupy dominant proportion in the rice phytoalexins and are specifically synthesized in association with defense reactions in rice plants.

Table 2 Amounts of Phytoalexins in Rice Plants Infected with *Magnaporthe grisea* and with *Rhizoctonia solani*

		Amounts (μg/g fresh weight) of					
		phytocassane				momilactone	
		A	B	C	D	A	B
Rice leaves (Jukkoku)	Uninfected	ND	ND	ND	ND	ND	ND
	Infected with *M. grisea* (compatible race 007[12])	17.1	6.0	1.9	8.8	43.2	8.5
	Infected with *M. grisea* (incompatible race 031[12])	37.7	10.9	4.4	15.8	40.2	12.7
Rice stems (Koshihikari)	Uninfected	ND	ND	ND	ND	ND	ND
	Infected with *R. solani*	58.2	20.7	2.6	15.9	49.9	19.3

ND, Not detectable.

EXPERIMENTAL

General procedures Infrared (IR) spectra were recorded on a Nippondenshi JIR–100 FT–IR spectrophotometer. ^1H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on a JOEL JNM–GSX 500 spectrometer (500.2 MHz for ^1H, 125.8 MHz for ^{13}C), using $SiMe_4$ and $CDCl_3$ as internal

standards. Electron impact mass spectra (EI-MS) were recorded on an Nippondenshi DX-303 mass spectrometer. Fast atom bombardment mass spectra (FAB-MS) were recorded on a VG ZAB-HF mass spectrometer using glycerol as a matrix. CD measurements were performed on a JASCO J-500 A spectropolarimeter. For analytical high performance liquid chromatography (HPLC), TSKgel ODS-120A and ODS-120T (4.6 mm i.d. x 30 cm; TOSOH Co., Ltd., Japan) were used as reverse phase HPLC columns at a flow rate of 1.2 ml/min at 50 °C. For preparative HPLC, TSKgel ODS-120A and ODS-120T (21.5 mm i.d. x 37.5 cm; TOSOH) were used as reverse phase HPLC columns at a flow rate of 10 ml/min at 40 °C. For preparative work before the HPLC purification, Sep-Pak Vac 35cc (10 g) C18 cartridges (Waters) was used.

Plant material and microorganisms Rice plants (*Oryza sativa*, L. cv. Jukkoku) were cultivated in a phytotron and, at the fifth-leaf stage, the leaves were sprayed with the spore suspension of *M. grisea* (race 007 or 031), prepared as described[14]. After the rice plants had been kept in a moist chamber (100% humidity) at 24 °C for 24 hours, they were removed and cultivated in the phytotron at 23 °C as described[14]. Seven days after inoculation, the lesions on the fourth and fifth leaves were collected. Rice stems (*Oryza sativa* L. cv. Koshihikari) infected with *R. solani* (2 kg) were collected in a paddy field of Konokan High school, Niigata-ken, Japan, in September 1993. *M. grisea* (race 007) was kindly provided by Dr. M. Iwata (Meiji Seika Kaisha, Ltd.) and *M. grisea* MAFF 305494 (race 031) was obtained from the Ministry of Agriculture, Forestry and Fisheries, Tsukuba-shi, Ibaraki, Japan.

Isolation of phytocassanes and momilactones for elucidation of structures For elucidation of structures, rice stems infected with *R. solani* (2 kg) were used as plant material. Isolation of phytocassanes and momilactones was achieved by the method that is shown schematically in Fig. 2.

Quantitative analysis of phytocassanes and momilactones from rice leaves or stems Rice leaves infected with *M. grisea* or rice stems infected with *R. solani* were collected and cut into pieces. The sample (0.2 g) was shaken with 5 ml of EtOAc and 5 ml of 0.1 N Na_2CO_3 (pH 10.5) for 16 hours to yield EtOAc and aqueous fractions. The EtOAc fraction was evaporated to yield a crude extract. The extract was dissolved in 1.5 ml of 0.02 N HCl containing 55% EtOH and centrifuged at 15,000 x *g* for 20 min. The supernatant (1.4 ml) was subjected to HPLC on a TSKgel ODS-120A column (4.6 mm i.d. x 30 mm) under the following conditions: mobile phase, EtOH-H_2O (55:45 v/v); flow rate, 1.2 ml/min; temperature, 50 °C; with an ultraviolet detector. The fraction containing the peak amounts of phytocassanes and momilactones was collected and evaporated *in vacuo*. The residue was dissolved in 0.45 ml of 40% EtOH and the sample (0.15 ml) was subjected to the HPLC on a TSKgel ODS-120T column (4.6 mm i.d. x 30 mm) under the following conditions: mobile phase, $MeCN_3$-H_2O (45:55 v/v); flow rate, 1.2 ml/min; temperature, 50 °C; with an ultraviolet detector. Phytocassanes were monitored at 280 nm and momilactones were monitored at 215 nm. The elution profiles of phytocassanes and momilactones from rice leaves infected with *M. grisea* (incompatible race 031) are shown in Fig. 6. The fractions corresponding to the peaks of phytocassanes and momilactones were collected and the structures of the compounds were confirmed by ^{13}C NMR.

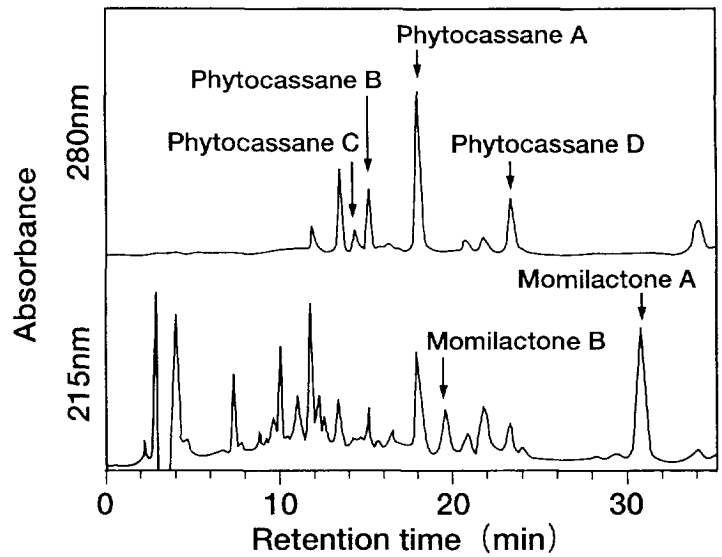

Fig. 6 HPLC elution profiles of phytocassanes and momilactones from rice leaves infected with *Magnaporthe grisea* (race 031)

Nonenzymatic conversion of phytocassane A to phytocassane D 30 μM of phytocassane A was incubated in 50 mM Na_2CO_3 and 0.01% Tween 20 solutions, pH 10, at 50 °C for 60 min. After incubation, the reaction mixture was extracted with EtOAc and the extract was analyzed by HPLC on a TSKgel ODS-120T column as described previously. The HPLC fraction corresponding to the reaction product was collected and the structure of the compound was confirmed by ^{13}C NMR. Under the above conditions, 50 % of phytocassane A was non-enzymatically converted to phytocassane D, while at pH values below 7, phytocassane A was stable and was hardly converted to phytocassane D.

Assays of antifungal activity During the isolation of phytoalexins, antifungal activity was estimated by observing inhibitory effects on spore germination and germ tube growth of *M. grisea* by the previously described method[15,16]. Phytocassanes A, B, C and D were dissolved in 10 mM potassium phosphate and 0.02% Tween 20, pH 7 and were subjected to the assays of antifungal activity.

***Phytocassane* A (1)** Colorless gum; CD λ_{max} (EtOH) 369 nm ($\Delta\varepsilon$ = −2.78), 319 nm ($\Delta\varepsilon$ = −0.71), 289 nm($\Delta\varepsilon$ = −8.74), 263 nm ($\Delta\varepsilon$ = +2.28); UV λ_{max} (EtOH) 268 nm; IR ν_{max} 3484, 2974, 2939, 2916, 2868, 1713, 1657, 1624, 1589 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.80 (3H, s, Me-20), 1.12 (3H, d, J=7.2 Hz, Me-17), 1.14 (3H, s, Me-18), 1.17 (3H, s, Me-19), 1.45 (1H, dddd, J=13.0, 12.5, 12.2, 3.3 Hz, H-6β), 1.56 (1H, dddd, J=12.5, 12.5, 12.5, 3.3 Hz, H-7α), 1.68 (1H, dddd, J=13.0, 3.3, 3.3, 3.3 Hz, H-6α), 1.77 (1H, dddd, J=12.5, 3.3, 3.3, 3.3 Hz, H-7β), 1.94 (1H, dd, J=12.2, 3.3 Hz, H-5), 2.06 (1H, dddd, J=13.0, 12.5, 4.3, 3.3 Hz, H-8), 2.23 (1H, d, J=13.0 Hz, H-9), 2.32 (1H, dd, J=14.7, 7.9 Hz, H-1β),

2.68 (1H, dq, J=7.2, 4.3 Hz, H-14), 2.83 (1H, br dd, J=14.7, 11.5 Hz, H-1α), 3.51 (1OH, br, OH-2), 4.63 (1H, dd, J=11.5, 7.9 Hz, H-2), 5.51 (1H, br d, J=10.9 Hz, H-16), 5.69 (1H, br d, J=17.7 Hz, H-16), 5.78 (1H, s, H-12), 6.36 (1H, dd, J=17.7, 10.9 Hz, H-15); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS m/z (%): 316 $[M]^+$ 316 (60), 301 (9), 288 (13), 272 (9), 255 (6), 243 (98), 229 (14), 215 (16), 201 (24), 187 (19), 173 (21), 161 (55), 148 (62), 135 (100), 121 (18), 108 (35), 91 (42), 79 (51), 69 (39), 55 (40), 41 (80); HREIMS $[M]^+$ at m/z 316.2052 (calc. for $C_{20}H_{28}O_3$, 316.2065).

***Phytocassane B* (2)** Colorless gum; CD λ_{max} (EtOH) 345 nm ($\Delta\varepsilon$ = −5.36), 277 νμ ($\Delta\varepsilon$ = +4.41), 228 nm ($\Delta\varepsilon$ = −4.17); UV λ_{max} (EtOH) 274 nm; IR ν_{max} 3240, 2970, 2944, 2916, 2876, 1635, 1624, 1594 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.85 (1H, dd, J=11.4, 2.2 Hz, H-5), 1.03 (3H, s, Me-18), 1.04 (3H, s, Me-19), 1.10 (3H, d, J=7.2 Hz, Me-17), 1.13 (3H, s, Me-20), 1.51 (1H, m, H-7), 1.57 (1H, m, H-6), 1.67 (1OH, br, OH-3), 1.77 (1H, m, H-7), 1.84 (1H, m, H-6), 2.14 (1H, d, J=13.1 Hz, H-9), 2.27 (1H, dddd, J=13.1, 12.0, 4.3, 3.7 Hz, H-8), 2.66 (1H, dq, J=7.2, 3.4 Hz, H-14), 3.09 (1OH, br, OH-2), 3.23 (1H, br d, J=3.6 Hz, H-3), 3.54 (1H, br s, H-1), 4.19 (1H, br dd, J=3.8, 3.6 Hz, H-2), 5.58 (1H, br d, J=10.8 Hz, H-16), 5.76 (1H, br d, J=17.7 Hz, H-16), 5.90 (1H, s, H-12), 6.39 (1H, dd, J=17.7, 10.8, H-15), 7.72 (1OH, br, OH-1); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS m/z (%): 316 $[M-18]^+$ 316 (32), 301 (14), 283 (8), 255 (6), 244 (19), 229 (9), 216 (18), 203 (25), 189 (9), 175 (15), 161 (42), 148 (100), 135 (73), 121 (14), 107 (18), 91 (29), 79 (30), 69 (19), 55 (33), 41 (50); HRFABMS $[M+H]^+$ at m/z 335.2286 (calc. for $C_{20}H_{31}O_4$, 335.2223).

***Phytocassane C* (3)** Colorless gum; CD λ_{max} (EtOH) 347 nm ($\Delta\varepsilon$ = −5.17), 278 nm ($\Delta\varepsilon$ = +4.84), 230 nm ($\Delta\varepsilon$ = −3.25); UV λ_{max} (EtOH) 274 nm; IR ν_{max} 3304, 2974, 2944, 2916, 2876, 1637, 1633, 1595 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.79 (1H, dd, J=11.3, 2.3 Hz, H-5), 0.80 (3H, s, Me-18), 0.94 (3H, s, Me-20), 1.01 (3H, s, Me-19), 1.08 (3H, d, J=7.2 Hz, Me-17), 1.50 (1H, m, H-7), 1.52 (1H, m, H-6), 1.64 (1OH, br, OH-3), 1.72 (H, ddd, J=13.1, 12.5, 11.6, H-2β), 1.77 (1H, m, H-7), 1.82 (1H, m, H-6), 2.03 (1H, ddd, J=13.1, 4.4, 4.1, H-2α), 2.15 (1H, d, J=13.1 Hz, H-9), 2.24 (1H, dddd, J=13.1, 13.1, 4.0, 3.6 Hz, H-8), 2.64 (1H, dq, J=7.2, 3.6 Hz, H-14), 3.33 (1H, dd, J=12.5, 4.1, H-3), 3.65 (1H, dd, J=11.6, 4.4 Hz, H-1), 5.57 (1H, br d, J=10.8 Hz, H-16), 5.74 (1H, br d, J=17.7 Hz, H-16), 5.89 (1H, s, H-12), 6.38 (1H, dd, J=17.7, 10.8, H-15), 7.22 (1OH, br s, OH-1); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS m/z (%): 318 $[M]^+$ 318 (8), 300 (5), 285 (10), 267 (6), 246 (8), 231 (13), 213 (4), 203 (19), 187 (9), 175 (36), 161 (42), 148 (97), 135 (100), 121 (18), 107 (22), 91 (34), 79 (36), 69 (27), 55 (40), 41 (62); HREIMS $[M]^+$ at m/z 318.2164 (calc. for $C_{20}H_{30}O_3$, 318.2133).

***Phytocassane D* (4)** Colorless gum; CD λ_{max} (EtOH) 368 nm ($\Delta\varepsilon$ = −3.27), 271 nm ($\Delta\varepsilon$ = +3.97); UV λ_{max} (EtOH) 268 nm; IR ν_{max} 3488, 2974, 2947, 2916, 2877, 1713, 1655, 1624, 1589 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.70 (3H, s, Me-18), 0.91 (3H, s, Me-20), 1.10 (3H, d, J=7.2 Hz, Me-17), 1.20 (3H, s, Me-19) 1.49 (1H, dddd, J=12.9, 12.5, 12.5, 3.3 Hz, H-6β), 1.58 (1H, dd, J=12.5, 3.0 Hz, H-5), 1.59 (1H, dddd, J=12.5, 12.5, 12.5, 4.0 Hz, H-7α), 1.81 (1H, dddd, J=12.5, 3.3, 3.3, 3.3 Hz, H-7β), 1.87 (1H, dddd, J=12.9, 4.0, 3.3, 3.0 Hz, H-6α), 2.15 (1H, d, J=13.0 Hz, H-9), 2.19 (1H, dddd, J=13.0, 12.5, 3.7, 3.3 Hz, H-8), 2.45 (1H, br d, J=13.3 Hz, H-1α), 2.65 (1H, dq, J=7.2, 3.7 Hz, H-14), 3.50

(1OH, br, OH-3), 3.82 (1H, d, J=13.3 Hz, H-1β), 3.94 (1H, d, J=1.4 Hz, H-3), 5.50 (1H, br d, J=10.7 Hz, H-16), 5.69 (1H, br d, J=17.5 Hz, H-16), 5.77 (1H, s, H-12), 6.35 (1H, dd, J=17.5, 10.7 Hz, H-15); ^{13}C NMR ($CDCl_3$), see Table 1; EIMS m/z (%): 316 $[M]^+$ 316 (65), 301 (10), 288 (13), 272 (9), 255 (7), 243 (100), 229 (12), 215 (16), 201 (23), 187 (18), 173 (20), 161 (50), 148 (58), 135 (91), 123 (18), 108 (33), 91 (39), 79 (48), 69 (38), 55 (38), 41 (73); HREIMS $[M]^+$ at m/z 316.2048 (calc. for $C_{20}H_{28}O_3$, 316.2058).

Momilactone A ^{13}C NMR ($CDCl_3$) δ 21.5, 21.8, 22.0, 24.0, 31.2, 32.5, 34.9, 37.3, 40.1, 46.5, 47.5, 50.2, 53.6, 73.2, 110.2, 114.1, 148.0, 149.0, 174.3, 205.1; EIMS m/z (%): 314 $[M]^+$ 314 (100), 299 (56), 286 (17), 271 (68), 255 (64), 243 (19), 232 (41), 213 (53), 199 (100), 187 (40), 173 (31), 157 (38), 145 (47), 133 (77), 119 (54), 105 (64), 91 (100), 81 (100), 67 (67), 55 (98), 41 (96).

Momilactone B ^{13}C NMR ($CDCl_3$) δ 19.0, 21.9, 24.8, 26.5, 28.9, 30.8, 37.3, 40.0, 43.0, 44.8, 47.5, 50.4, 72.7, 73.7, 96.6, 110.2, 114.1, 146.7, 148.9, 180.4; EIMS m/z (%): 330 $[M]^+$ 330 (12) 312 (24), 294 (12), 285 (20), 266 (15), 257 (86), 239 (75), 226 (31), 211 (35), 197 (55), 183 (69), 171 (56), 157 (54), 143 (60), 129 (57), 117 (46), 105 (55), 91 (90), 79 (59), 67 (37), 55 (69), 41 (100).

ACKNOWLEDGMENTS

We are most grateful to Dr. Shuichi Gomi (Pharmaceutical research center, Meiji Seika Kaisha, Ltd.) for the determination of structures of phytocassanes B and C, and for many helpful advice. We wish to thank Mr. Tarozaemon Kera (Konokan High school) for providing many plant materials and for helpful advice and critical suggestions. Thanks are also due to Dr. Michiaki Iwata (Pharmaceutical research center, Meiji Seika Kaisha, Ltd.) for technical advice.

REFERENCES AND NOTES

1. Cartwright, D.; Langcake, P.; Pryce, R. J.; Leworthy, D. P.; Ride, J. P. *Nature* **1977**, *267*, 511–513.
2. Cartwright, D. W.; Langcake, P.; Pryce, R. J.; Leworthy, D. P.; Ride, J. P. *Phytochem.* **1981**, *20*, 535–537.
3. Akatsuka, T.; Kodama, O.; Kato, H.; Kono, Y.; Takeuchi, S. *Agric. Biol. Chem.* **1983**, *47*, 445–447.
4. Kono, Y.; Takeuchi, S.; Kodama, O.; Akatsuka, T. *Agric. Biol. Chem.* **1984**, *48*, 253–255.
5. Sekido, H.; Endo, T.; Suga, R.; Kodama, O.; Akatsuka, T.; Kono, Y.; Takeuchi, S. *J. Pesticide. Sci.* **1986**, *11*, 369–372.
6. Kato, H.; Kodama, O.; Akatsuka, T. *Phytochem.* **1993**, *33*, 79–81.
7. Kato, H.; Kodama, O.; Akatsuka, T. *Phytochem.* **1994**, *36*, 299–301.
8. Kodama, O.; Li, W. X.; Tamogami, S.; Akatsuka, T. *Biosci. Biotech. Biochem.* **1992**, *56*, 1002–1003.

9. Tamogami, S.; Mitani, M.; Kodama, O.; Akatsuka, T. *Tetrahedron* **1993**, *49*, 2025–2032.
10. Kodama, O.; Miyakawa, J.; Akatsuka, T.; Kiyosawa, S. *Phytochem.* **1992**, *31*, 3807–3809.
11. Kato, T.; Kabuto, C.; Sasaki, N.; Tsunagawa, M.; Aizawa, H.; Fujita, K; Kato, Y.; Kitahara, Y. *Tetrahedron Lett.*, **1973**, *39*, 3861–3864.
12. Rice plants (*Oryza sativa*, L. cv. Jukkoku and Koshihikari) are resistant to *M. grisea* (incompatible race 031), but are sensitive to *M. grisea* (compatible race 007). Sekizawa, Y.; Haga, M.; Hirabayashi, E.; Takeuchi, N.; Takino, Y. *Agric. Biol. Chem.* **1987**, *51*, 763–770.
13. Matsuyama, N. *Ann. Phytopath. Soc. Japan* **1983**, *49*, 200–205.
14. Matsumoto, K.; Suzuki, Y.; Mase, S.; Watanabe, T.; Sekizawa, Y. *Ann. Phytopath. Soc. Japan* **1980**, *46*, 307–314.
15. Akatsuka, T.; Kodama, O.; Sekido, H.; Kono, Y.; Takeuchi, S. *Agric. Biol. Chem.* **1985**, *49*, 1689–1694.
16. Shimura, M.; Iwata, M.; Tashiro, N.; Sekizawa, Y.; Suzuki, Y. Mase, S.; Watanabe, T. *Agric. Biol. Chem.* **1981**, *45*, 1431–1435.

(*Received in Japan* 12 *April* 1995; *accepted* 25 *May* 1995)