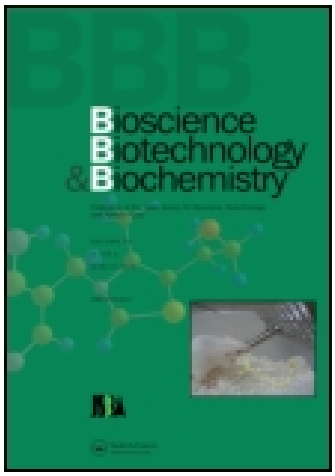

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Phytotoxic Metabolites Isolated from *Scolecotrichum graminis* Fuckel

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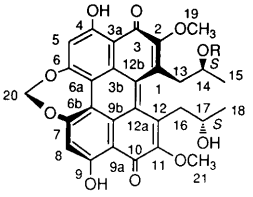
Two new cercosporins, (+)-isocercosporin (1) and (+)-14-acetylisocercosporin (2), were isolated from culture filtrates of *Scolecotrichum graminis* Fuckel, which is the causal fungus of a leaf streak disease in orchardgrass. The structure of each was elucidated by spectroscopic analysis, and (+)-isocercosporin (1) is the first isolated enantiomer of known (−)isocercorporin (8b). Compounds 1 and 8b had higher phytotoxic activity than that of (+)-cercosporin (a), an atropisomer of 8b. The results of the bioassay suggest that the phytotoxic activity of cercosporins depends on the relative configuration, and not on the absolute configuration. In addition, three phenol compounds, 1-(2,4-dihydroxy-6-methylphenyl)ethanone (3), isosclerone (4), and *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone (5), were isolated as minor components and identified. Compound 3 was isolated for the first time as a natural products.

In our continuing search for phytotoxic substances produced by this fungus, we have isolated and determined the structures of three new acetamido compounds, (2*S*)-2-acetamido-3-pentanone (**6**), (2*S*,3*R*)-2-acetamido-3-pentanol (**7a**), and (2*S*,3*S*)-2-acetamido-3-pentanol (**7b**), by spectroscopic analysis and chemical synthesis.[2] The present paper reports the further isolation and structural elucidation of two new cercosporins, (+)-isocercosporin (**1**)[3] and (+)-14-acetylisocercosporin (**2**), and the identification of three phenol compounds, 1-(2,4-dihydroxy-6-methylphenyl)ethanone (**3**), isosclerone (**4**), and *trans*-3,4-dihydro-3,4,8-trihydroxy-1(2*H*)-naphthalenone (**5**). Compound **3** was isolated for the first time as a natural products.

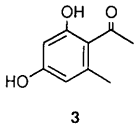
The extraction and isolation of metabolites **1**–**5** from culture filtrates of *Scolecotrichum graminis* Fuckel were carried out as described in the experimental section.

High-resolution EI-MS indicated the molecular formula of compound **1** to be $C_{29}H_{26}O_{10}$. The UV maxima at 476, 525 sh, and 564 nm, the IR absorbance at 1610 cm^{-1} and the signal due to a hydrogen-bonded proton at δ_H 14.91 in the ^{1}H-NMR spectrum show that compound **1** was a 4,9-dihydroxyperylene-3,10-quinone derivative. The color change from red to green in an alkaline solution also support the presence of this aromatic skeleton, because it is known that several red hydroxy quinones give a green coloration in an alkaline solution,[4] for instance, hypericin, erythrophins, elsinochromes,[4] (+)-cercosporin (**8a**),[5] and phleichrome.[6] In ^{1}H-NMR decoupling experiments the methine multiplet (2H) at δ_H 3.70, a methyl double-doublets (2H, 2H) at δ_H 2.87 and δ_H 3.51 collapsed to a singlet and a pair of doublets (J=13.3 Hz), respectively. These results indicate the presence of 2-hydroxypropyl side chains. The ^{1}H-NMR spectrum shows the presence of a methylenedioxy group at δ_H 5.73 and a two-methoxy group at δ_H 4.23 (Table I). From these spectral data and the color reaction, it is postulated that the chemical structure of pigment **1** was symmetrical and closely related to that of (+)-cercosporin (**8a**). The ^{13}C-NMR spectral data support this (Table II).

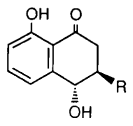
It is known that **8a** is readily isomerized by heating various solvents to give an equilibrium mixture of equal amounts of (+)-cercosporin (**8a**) and (−)-isocercosporin (**8b**), and that the same equilibration also occurs for **8b**.[7] (−)-Isocercosporin (**8b**) is the diastereomer of **8a** with the chirality axis formed by twisting the perylenequinone ring. The absolute configurations of the asymmetric carbons at C-14 and C-17, and the axial chirality of (+)-cercosporin (**8a**) have been established as *R* and *R*, respectively, on the basis of an X-ray analysis and chemical reactions.[7] Since the ^{1}H-NMR spectral data for **1** are identical with those for **8b** (Table I), indicating the same relative configuration for both compounds, and the CD spectral curve of **1** is antipodal to that of **8b** (Table III), compound **1** has the



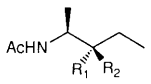
1; R = H
2; R = Ac



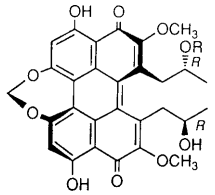
3



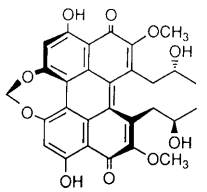
4; R = H
5; R = OH



6 ; R_1,R_2 = O
7a ; R_1 = H, R_2 = OH
7b ; R_1 = OH, R_2 = H



8a; R = H
9; R = Ac



8b

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Scolecotrichum graminis Fuckel is the causal fungus of a leaf streak disease of orchardgrass, and occurs on leaves of all ages throughout the growing season. The initial lesions appear as tiny (2 × 0.5 mm), elliptical, purplish-brown, water-soaked spots with a dark brown border that are visible on both leaf surfaces. Mature lesions appear as brown, elongated streaks.[1]

Table I. ^{1}H-NMR Spectral Data for Compounds **1**, **2**, **8a** and **8b** (500 MHz, $CDCl_3$)[a]

H	1	8a	8b	2	9[8]
4, 9-OH	14.91 (s)	14.86 (s)	14.91 (s)	14.88 (s), 14.77 (s)	14.82 (s), 14.76 (s)
5, 8	7.02 (s)	7.07 (s)	7.02 (s)	7.05 (s), 7.03 (s)	7.04 (s, 2H)
13, 16	2.87 (dd, 13.3, 8.2)	2.90 (dd, 13.1, 6.0)	2.87 (dd, 13.3, 8.1)	3.49 (dd, 10.6, 3.2), 3.52 (dd, 10.6, 3.1)	2.80–3.80 (m,
	3.51 (dd, 13.3, 3.4)	3.59 (dd, 13.1, 7.0)	3.51 (dd, 13.3, 3.4)	2.82 (dd, 13.3, 8.2), 2.99 (dd, 13.3, 9.3)	17-H, 13-H, 16-H)
14, 17	3.70 (m)	3.39 (m)	3.70 (m)	4.71 (m), 3.70 (m)	4.64 (m, 14-H)
15, 18	0.97 (d, 6.2)	0.65 (d, 6.1)	0.97 (d, 6.1)	1.05 (d, 6.2), 0.97 (d, 6.1)	0.64 (d), 0.56 (d)
19, 21	4.23 (s)	4.21 (s)	4.23 (s)	4.26 (s)	4.24 (s), 4.22 (s)
20	5.73 (s)	5.75 (s)	5.73 (s)	5.73 (s)	5.76 (s)
14-OAc				0.85 (s)	1.66 (s)

[a] Coupling constants (J in Hz) are given in parentheses.

Table II. ^{13}C-NMR Spectral Data for Compounds **1** and **2** (125 MHz, $CDCl_3$)

C	1	2
1, 12	136.6	136.5, 134.2
2, 11	152.2	152.5, 152.2
3, 10	181.7	181.9, 181.6
3a, 9a	108.4	108.5, 108.4
3b, 9b	127.6	127.7, 127.5
4, 9	167.6	167.5, 167.4
5, 8	109.1	109.2, 109.0
6, 7	163.4	163.4, 163.1
6a, 6b	113.0	112.6, 112.4
12a, 12b	131.6	131.3, 131.6
13, 16	42.3	42.2, 38.7
14, 17	69.2	71.2, 69.1
15, 18	23.8	23.8, 20.6
19, 21	61.0	60.99, 60.97
20	92.6	92.7
14-OAc		169.4, 19.9

Table III. CD Spectral Data for Compounds **1**, **2**, **8a**, **8b**, and **9**

1 nm ($\Delta\varepsilon$) (MeOH)	8a[7] nm ($\Delta\varepsilon$) (MeOH)	8b[7] nm ($\Delta\varepsilon$) (MeOH)	2 nm ($\Delta\varepsilon$) (EtOH)	9[8] nm ($\Delta\varepsilon$) (EtOH)
235 (+19.8)	245 (+24.0)	235 (−22.7)	245 (+22.1)	240 (+23.6)
282 (−15.2)	280 (−18.8)	280 (+17.7)	297 (−27.6)	297 (−42.2)
292 (−31.2)	296 (−44.8)	296 (+41.6)	349 (+1.6)	352 (+1.9)
325 (−5.3)	325 (−6.4)	323 (+7.4)	415 (−4.9)	410 (−8.0)
350 (+1.1)	350 (+2.4)	350 (−1.2)	500 (+6.6)	
410 (−5.3)	415 (−9.0)	410 (+7.6)	567 (+5.2)	
493 (+7.2)	500 (+8.2)	495 (−10.3)		
550 (+4.8)	550 (+7.2)	548 (−7.6)		
567 (+5.1)	570 (+8.4)	570 (−7.6)		
	590 (+4.0)	590 (−4.2)		

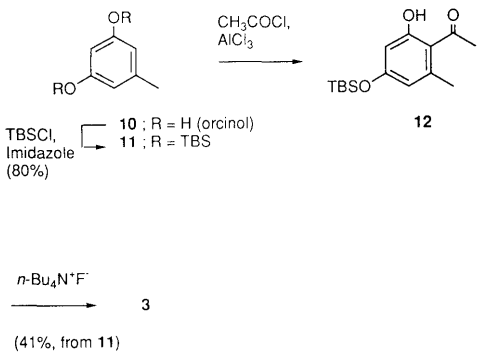
structure, 4,9-dihydroxy-2,11-dimethoxy-1,12-(2-hydroxypropyl)-6,7-methylenedioxyperylene-3,10-quinone, the absolute configuration of the asymmetric carbons of **1** being *S* and the axial chirality being confirmed as *R*. This compound **1**, named (+)-isocercosporin, is a diastereomer of (+)-cercosporin (**8a**), and the enantiomer of (−)-isocercosporin (**8b**), and is the first atropisomer of (−)-cercorporin isolated from natural sources.

Compound **2** has the molecular formula $C_{31}H_{28}O_{11}$ from EI-HRMS data. The UV maxima at 325, 476, and 564 nm, the IR absorbance at 1620 cm^{-1}, and the signals due to a hydrogen-bonded proton at δ_H 14.88 and 14.77 in the ^{1}H-NMR spectrum show that compound **2** was a 4,9-dihydroxyperylene-3,10-quinone derivative. The color reaction from red to green in an alkaline solution also support this. The ^{1}H-NMR spectrum shows the presence of two aromatic protons at δ_H 7.03 and 7.05, a methylenedioxy group at δ_H 5.72, two methoxy group at δ_H 4.26, a pair of 2-hydroxypropyl side chains at δ_H 2.82, 2.99, 3.49, 3.52, 3.70, and 4.71, and an acetyl group at δ_H 0.85 (Table I). From the foregoing results, it is postulated that the chemical structure of this pigment **2** is closely related to that of (+)-14-acetylcercosporin (**9**).[8] Since the CD spectral curve of **2** is similar to the pattern for **1** and the ^{1}H- and ^{13}C-NMR spectral data for **2** are very similar, but not identical with those for **9** (Tables I and III), compound **2** has the structure, 4,9-dihydroxy-2,11-dimethoxy-1-(2-acetoxypropyl)-12-(2-hydroxypropyl)-6,7-methylenedioxyperylene-3,10-quinone, the absolute configuration of the asymmetric carbons of **2** being *S*, in consideration of the biosynthetic pathway and the axial chirality being confirmed as *R*.

Compound **3** has the molecular formula $C_9H_{10}O_3$ from EI-HRMS data. The IR spectrum of **3** revealed absorption bands at 3180 (OH) and 1610 cm^{-1} (C=O). The ^{1}H-NMR spectrum shows the presence of a hydrogen-bonded phenol group at δ_H 13.41, two aromatic protons at δ_H 6.23 and 6.25, a phenol group at δ_H 5.29, an acetyl group at δ_H 2.63, and a methyl group at δ_H 2.56. The coupling constants of the aromatic protons (J=3.0 Hz) indicates compound **3** to have the 1,2,4,6-tetrasubstituted benzene structure. From these results, **3** was elucidated to be 1-(2,4-dihydroxy-6-methylphenyl)ethanone. Although this ethanone **3** is well known as a synthetic product and an important intermediate,[9] this is the first isolation as a natural product. The structure of compound **3** also confirmed by its synthesis from orcinol (**10**) (Scheme).

In addition, isosclerone (**4**)[10] and *trans*-3,4-dihydro-3,4,8-trihydro-1(2*H*)-naphthalenone (**5**)[11] were isolated as minor components and identified.

The effects of compounds **1**, **2**, **8a**, and **8b** on the growth inhibition of lettuce seeds are summarized in Table IV. It is known that (+)-cercosporin (**8a**) showes phytodynamic activity.[12] Although compound **1** extensively inhibited the growth of lettuce seeds at 20–40 ppm under light, **1** and **8b**, which are enantiomers of each other, had higher activity than **8a**, a diastereomer of **1** and **8b**. From these results,



Scheme. 1. Synthesis of Compound **3** from Orcinol.

Table IV. Effect of Compounds **1**, **2**, **8a**, and **8b** on the Root Growth Inhibition of Lettuce Seeds[a] (under the light, 25°C, 3 days)

Compound	1	2	8a	8b	Control
20 ppm	92%	66%	67%	86%	0%
40 ppm	99%	—	93%	96%	0%

[a] In the previous communication, the effect of cercosporins on the growth inhibition of lettuce seeds was estimated as lower than the mean value. High dilution of the samples with methanol for the bioassay might have caused decomposition.[3]

it is interesting to note that the phytotoxic activity of cercosporins depends on the relative configuration and not on the absolute configuration. Compound **3** had strong phytotoxic activity (100% inhibition) toward lettuce seeds at 500 ppm in the dark, but at concentrations lower than 100 ppm, the activity became weak (for example, 32% inhibition of hypocotyls at 100 ppm). The phytotoxic activity of compounds **4** and **5** couldn't be disclosed, since their amounts were not sufficient for bioassay. However, it is known that compound **4** stimulates the root elongation of rice seedlings by ca. 30% at concentrations of 1–10 ppm, and inhibites the growth of shoots and roots of rice seedlings (89% inhibition of roots at 100 ppm).[10] It has also been reported that, **5** reduced the growth of rice seedlings at high concentrations, but slightly stimulated their growth when seedlings were pretreated with a 100-ppm solution.[11]

Experimental

Instruments. EI-MS, JEOL DX-300; IR spectra, Hitachi model 285; UV spectra, Hitachi U-3210 spectrophotometer; ^{1}H- and ^{13}C-NMR spectra, Bruker AM-500 FT-NMR spectrometer; CD spectra, JASCO J-20A spectropolarimeter.

Fermentation, Extraction, and Isolation. Scolecotrichum graminis Fuckel, supplied by Hokkaido National Agricultural Experiment Station (Sapporo, Japan), was seeded into 500-ml Erlenmeyer flasks containing 150 ml of a potato extract-sucrose medium. After the fungus in 143 flasks had been incubated at 25°C for 30 days by surface culture, the culture filtrates (20 liters) were concentrated *in vacuo* to give a concentrate (1 liter), which was then extracted with ethyl acetate. The extracts were dried over Na_2SO_4 and concentrated to dryness, and the residue was fractionated by silica gel column chromatography, using $CHCl_3$–MeOH (9 : 1) as the eluent, into eight fractions (Fr. 1–8). Fraction 1 showed two red spots at $R_f = 0.34$ and 0.68 on silica gel TLC with $CHCl_3$–MeOH (95 : 5), and showed a yellow spot at $R_f = 0.45$ and a blue spot at $R_f = 0.21$ on silica gel TLC with benzene–ethyl acetate (8 : 2) by *p*-anisaldehyde–acetic acid–conc. sulfuric acid reagents. Fraction 1 was further fractionated by silica gel column chromatography, using $CHCl_3$–MeOH (95 : 5) as the eluent, into four fractions (Fr. 11–14). Finally, purification of Fr. 13 by silica gel preparative TLC, with benzene–ethyl acetate (1 : 1) as the eluent, gave compound **1** (4.1 mg). Fraction 11 was subjected to silica gel preparative TLC with $CHCl_3$–MeOH (97 : 3) to give compound **3** (0.4 mg) and compound **4** (0.5 mg). Fraction 2, which showed a red-purplish spot at $R_f = 0.33$ on silica gel TLC with ethyl acetate when applying a *p*-anisaldehyde reagent, was subjected to silica gel preparative TLC, eluting with ethyl acetate, to give compound **5** (0.4 mg).

(+)-*Isocercosporin* (**1**). UV λ_{max} (EtOH) nm (ε): 476 (21,300), 525sh (11,100), 564 (8,000); IR ν_{max} (film) cm^{-1}: 3480, 1720, 1610; ^{1}H-NMR (500 MHz, $CDCl_3$): see Table I; ^{13}C-NMR (125 MHz, $CDCl_3$): see Table II; CD λ_{ext} (EtOH) nm (ε): see Table III. EI-HRMS *m/z* (M^+): calcd. for $C_{29}H_{26}O_{10}$, 534.153; found, 534.154.

(+)-*14-Acetylisocercosporin* (**2**). UV λ_{max} (EtOH) nm (ε): 325 (1,920), 476 (13,800), 564 (4,680); IR ν_{max} (film) cm^{-1}: 3400, 1730, 1620; CD λ_{ext} (EtOH) nm (ε): see Table III; ^{1}H-NMR (500 MHz, $CDCl_3$): see Table I; ^{13}C-NMR (125 MHz, $CDCl_3$): see Table II. EI-HRMS *m/z* (M^+): calcd. for $C_{31}H_{28}O_{11}$, 576.163; found, 576.166.

Compound **3**. UV λ_{max} (EtOH) nm (ε): 259 (490), 282 (540), 322 (2,200); IR ν_{max} (film) cm^{-1}: 3180, 1610, 1560; ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 2.56 (3H, s, 6′-Me), 2.63 (3H, s, 2-H), 5.29 (1H, br. s, 4′-OH), 6.23 (1H, d, $J = 3.0$ Hz, 5′-H), 6.25 (1H, d, $J = 3.0$ Hz, 3′-H), 13.14 (1H, s, 2′-OH). EI-HRMS *m/z* (M^+): calcd. for $C_9H_{10}O_3$, 166.063; found, 166.060.

Silyl ether **11**. To a solution of orcinol-monohydrate (**10**; 95 mg, 0.67 mmol) in dry DMF (2.3 ml) were added imidazole (176 mg, 2.59 mmol) and *t*-butyldimethylsilyl chloride (355 mg, 2.36 mmol). After stirring at 40°C for 20 h, the mixture was added water, and extracted with ether (×3). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated *in vacuo*. Column chromatography of the residue on silica gel with *n*-hexane–ether (97 : 3) yielded silyl ether **11** (187 mg, 80%). ^{1}H-NMR (500 MHz, $CDCl_3$): δ_H 0.18 (12H, s, $SiMe_2 \times 2$), 0.97 (18H, s, Si-*t*-Bu × 2), 2.22 (3H, s, 1-Me), 6.14 (1H, s, 4-H), 6.28 (2H, d, $J = 2.1$ Hz, 2, 6-H). EI-HRMS *m/z* (M^+): calcd. for $C_{19}H_{36}OSi_2$, 352.225; found, 352.227.

Synthetic **3**. To a suspended solution of aluminium chloride (86 mg, 0.65 mmol) and acetyl chloride (40 mg, 0.40 mmol) in dry CH_2Cl_2 (0.3 ml) at 0°C was added a solution of silyl ether **11** (141 mg, 0.40 mmol) in dry CH_2Cl_2 (0.3 ml) dropwise over a 30-min period. After stirring at room temperature for 2 h, the mixture was added to ice-cooled water, and extracted with, dried (Na_2SO_4) and evaporated *in vacuo*. Crude product **12** (118 mg) obtained was employed in the next reaction without purification.

To a solution of crude product **12** (101 mg) in THF (1.5 ml) was added tetrabutylammonium fluoride (1 M in THF, 0.6 ml, 0.6 mmol) at −20°C. After stirring at 0°C for 20 min, the mixture was added cooled sat. $NaHCO_3$, and extracted with ethyl acetate (×3). The combined organic layers were dried (Na_2SO_4) and evaporated *in vacuo*. Column chromatography of the residue on silica gel with $CHCl_3$–acetone (98 : 2) yielded compound **3** (23 mg, 41% from **11**).

Bioassay (*growth inhibition of lettuce seeds*). A solution of a sample (0.06 and 0.12 mg) in ethyl acetate (1 ml) was poured on to a sheet of filter paper (7 cm in diameter, Toyo-Roshi No. 2) in a Petri dish (9 cm in diameter). After removing the solvent, 3 ml of a Tween-80 solution (100 ppm) was added to the dish to make up 20- and 40-ppm solutions of the sample. Lettuce seeds were placed on the filter paper and germinated in the dark at 25°C for 3 days (Table IV).

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