Pergamon

0031-9422(95)00897-9

Phytochemistry, Vol. 42, No. 2, pp. 365–368, 1996

0031-9422/96 $15.00 + 0.00

PLANT GROWTH PROMOTING AND FUNGICIDAL 4-QUINOLINONES FROM *PSEUDOMONAS CEPACIA*

SURK-SIK MOON, PYENG MO KANG, KYUNG SEOK PARK* and CHOONG HOE KIM*

Department of Chemistry, Kongju National University, Kongju 314-701, Korea; *Division of Plant Pathology, Agricultural Science and Technology Institute, Suwon 441-707, Korea

(*Received in revised form* 23 *October* 1995)

Key Word Index—*Pseudomonas cepacia*; antifungal; plant growth promoting; 4-quinolinones; red pepper; *Capsicum annuum*.

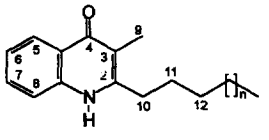
Abstract—Three new 4-quinolinone metabolites were isolated from *Pseudomonas cepacia* PC II, along with the known 2-(2-heptenyl)-3-methyl-4-quinolinone (HMQ) and 3-methyl-2-(2-nonenyl)-4-quinolinone, and their structures were established as 3-methyl-2-pentyl-4-quinolinone, 2-heptyl-3-methyl-4-quinolinone and 3-methyl-2-nonyl-4-quinolinone. HMQ showed antifungal and red pepper growth promoting activities.

INTRODUCTION

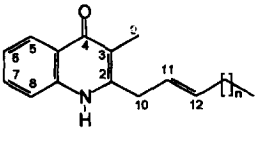
The phytophthora blight of red pepper (*Capsicum annuum*) is a troublesome plant disease, especially in continuous red pepper cropping areas. The blight is caused by *Phytophthora capsici*, a plant pathogen [1]. Since the pathogen is primarily soil-borne, the application of fungicides cannot provide efficient control of the disease. However, the bacterization of planting materials as a practical technique to control the root diseases is becoming attractive [2].

To control phytophthora blight biologically, an attempt was made to isolate mciroorganisms antagonistic to *P. capcisi* from rhizosphere and non-rhizosphere soil samples of healthy red pepper plants in the phytophthora blight-affected area. We selected an antagonistic bacterial strain PC-II, that was identified as *Pseudomonas cepacia* by morphological and physiological characteristics [3]. The mycelial growth and zoosporangial germination of *P. capsici* were remarkably inhibited, and the growth of the red pepper plant was significantly promoted, when this strain was inoculated in the plants in conventional seed bed soil [4].

In this paper, we report the isolation and characterization of the plant growth promoting and fungicidal substances (PSC-A, B, C, D and E) produced by *P. cepacia* PC-II.



PSC-A: n = 1
PSC-C: n = 3
PSC-E: n = 5



PSC-B: n = 3
PSC-D: n = 5

RESULTS AND DISCUSSION

The methanolic filtrate obtained by diluting the culture broth with methanol was fractionated by reverse phase silica gel chromatography with an elution gradient from H_2O–methanol (4:1) to methanol. Fractions active against *Pythium ultimum*, *P. capsici*, *Rhizoctonia solani*, *Fusarium oxysporum* and *Trychophyton mentagrophyte* were further purified on silica gel with ethyl acetate–acetonitrile elution. Trituration of concentrated active fractions with ethyl acetate gave a crude white solid material. From the crude sample, five active compounds (PSC-A, PSC-B, PSC-C, PSC-D and PSC-E) in the ratio of 2:67:3:26:2 were obtained by semi-preparative reverse phase HPLC. The compounds were fairly soluble in methanol, slightly soluble in chloroform and ethyl acetate, and almost insoluble in hexane and water. Recrystallization from aqueous methanol gave white flakes or thick lobe-shaped crystalline solids.

Isolate PSC-B, a major metabolite, was fully characterized by spectral studies, i.e. mass spectrometry HREI and 2D-NMR spectroscopy. The molecular formula of PSC-B was deduced as $C_{17}H_{21}NO$ from analysis of the HREI mass spectrum at *m/z* 255.1634 $[M]^+$ and from the proton and carbon count from the ^1H and ^{13}C NMR spectra. The UV maximum absorption at 213, 244, 322 and 335 nm, and IR absorption at 1639, 1608 and 1559 cm^{-1} were indicative of a quinolinoid system. The complete structure of PSC-B was deduced from H–H COSY and HMQC experiments. The alkenyl portion (C-11, C-12) was deduced

to be *trans*, based on the coupling constant (J = 16.3 Hz) between two alkenyl protons in the ^{1}H NMR spectrum. The position of the C-9 methyl group was clarified from analysis of the HMBC experiment. The C-9 methyl protons (2.22 ppm) correlate to a C-4 carbonyl carbon (177.3 ppm) and a C-2 quaternary carbon (148.8 ppm). The C-2 quaternary carbon also correlates with the protons (C-10 and C-11) at 3.57 and 5.53 ppm. All of the quaternary carbons were unambiguously assigned from the HMBC experiment. It is worth noting that the H-11 and H-12 protons almost coalesced in DMSO-d_6, showing an AB spin pattern. The spectral data indicated that the compound PSC-B was identical with HMQ [2-(2-heptenyl)-3-methyl-4-quinolinone], which was previously identified from a pseudomonad [5, 6].

The molecular mass (*m/z* 257 for $C_{17}H_{23}NO$) of compound PSC-C was 2 amu more than that of PSC-B, and the UV spectrum was slightly different in that the maximum absorption of PSC-B at 244 nm was shifted to 239 nm in PSC-C. Two alkenyl proton signals of PSC-B disappeared in PSC-C, and four more protons appeared in PSC-C in the range 1.40–1.73 ppm in the ^{1}H NMR spectrum. Therefore, the structure of PSC-C was deduced to be a reduced form of PSC-B with a saturated side chain, 2-heptyl-3-methyl-4-quinolinone. The structure was further confirmed by chemical synthesis, from the reaction of aniline with ethyl 2-methyl-3-oxodecanoate [7, 8]. PSC-C was known from the reduction of HMQ [5], but was not reported from nature.

Compound PSC-D showed similar spectral patterns (UV and IR) to PSC-B. The EI and HREI mass spectra revealed a molecular formula of $C_{19}H_{25}NO$ (283.1936 amu), indicating two more methylene groups than in PSC-B, and the fragmentation pattern (*m/z* 212, 198 and 184) was quite similar to that of PSC-B. The ^{1}H NMR spectrum also indicated four more protons (two methylenes) in the range 1.25–1.36 ppm. These observations suggest that the compound PSC-D is 3-methyl-2-(2-nonenyl)-4-quinolinone (known as NMQ), and supported by literature comparison [6].

The molecular weight of compound PSC-E was determined to be 285 (HREI mass spectrum at *m/z* 285.2087, $C_{19}H_{27}NO$), 2 amu more than that of PSC-D. The ^{1}H NMR spectrum showed no alkenyl proton signals, but instead four more protons in the range 1.38–1.73 ppm than in PSC-D. These observations suggest the structure of PSC-E is a reduced form of PSC-D. By the similar spectral properties (UV, IR and NMR) with PSC-C, except two more methylene signals in the range 1.27–1.34 ppm in the ^{1}H NMR spectrum than in PSC-C, the compound PSC-E was determined to be 3-methyl-2-(nonyl)-4-quinolinone. This was further confirmed by chemical synthesis.

Compound PSC-A was the least retained on a C-18 HPLC column. The HREI mass spectrum gave *m/z* 229.1459, corresponding to a molecular formula of $C_{15}H_{19}NO$, and the fragmentation pattern (*m/z* 200, 186, 173 and 144) was similar to those of PSC-C and PSC-E. The ^{1}H NMR spectrum showed only four methylene signals in the range 1.40–2.81 ppm, together with two methyl groups (0.93 and 2.15 ppm) and the quinolinoid system. The IR and UV spectra were almost the same as those of PSC-C and PSC-E. Therefore, PSC-A was determined to be 3-methyl-2-pentyl-4-quinolinone. An antibiotic compound, 2-pentyl-4-quinolinone, similar to PSC-A, was reported from a marine pseudomonad, *P. bromoutilis* [9].

The minimum inhibitory concentration [10] of PSC-B was 32–128 μg ml^{-1} against several plant pathogens, i.e. *P. capsici*, *F. oxysporum*, *P. ultimum* and *R. solani*. Interestingly, when red pepper seeds coated with celite containing PSC-B were planted in autoclaved pot soil, growth of the resulting seedlings was significantly enhanced. Seed treatment with 9.7–485 μg of PSC-B per gram of red pepper seeds resulted in a 40–76% increase in weight and 16–22% in height of plants after 30 days [4]. This is the first report of plant growth promoting effect of 4-quinolinone compounds.

The compounds PSC-A, PSC-C and PSC-E are newly isolated minor metabolites from *P. cepacia* PC-II and chemically related to the antifungal compounds, PSC-B and PSC-D, which were previously characterized from a culture broth of a pseudomonad [5, 6]. Several antibiotic 4-quinolinone compounds have been reported from fluorescent pseudomonads [11], i.e. cepacin [12], and from other sources, i.e. aurachins [13]. PSC-E was reported to show NADH-ubiquinone oxidoreductase inhibitory activity [14]. Anitfungal activity against several other plant pathogens, and the plant growth promoting effects of each metabolite, are presently under evaluation. The detailed biological properties of these quinolinone metabolites will be reported elsewhere.

EXPERIMENTAL

General. Mps are uncorr. ^{1}H and ^{13}C NMR spectra were recorded at 500 and 125.77 MHz, respectively. HR and LR mass spectra were obtained using tandem MS–MS by EI. Chromatography was performed on silica gel 60 (70–230 mesh, Merck) and LiChroprep RP-18 (40–63 μm, Merck).

Isolation. The culture broth (10 l) [3] was diluted with MeOH (20 l) and solid debris was filtered. The MeOH filtrate was concd and chromatographed on a RP-18 silica gel by successive elution with each eluent (200 ml) as follows: H_2O–MeOH (4:1, 3:2, 1:4), MeOH. H_2O–MeOH (1:4) and MeOH eluates showed activity against *P. ultimum*, *P. capsici*, *R. solani*, *F. oxysporum* and *T. mentagrophyte*. The combined active frs were chromatographed on silica gel using EtOAc–CH_3CN (3:2) as eluent. The active frs were cond, triturated with EtOAc, and filtered to afford a crude solid powder (1.144 g). The filtrate was concd and trituration of the conc. filtrate with EtOAc afforded an additional amount of a pale orange solid (100 mg). A portion of the crude solid powder (150 mg) was sepd on a reversed phase HPLC column [C-18, Deltapak, 19 × 300 mm, MeOH–H_2O (3:1), 5 ml min^{-1}, UV 225 nm) to afford the solids PSC-A (3 mg), PSC-B

(95 mg), PSC-C (3 mg), PSC-D (36 mg) and PSC-E (4 mg) (RR_t 21.6, 33.3, 37.1, 60.8, 75.3 min, respectively). An analyt. sample was obtained by recrystallization from aq. MeOH. They showed the following physicochemical properties.

PSC-A (*3-methyl-2-pentyl-4-quinolinone*). Mp 222–223°; EIMS *m/z* 229 $[M]^+$; HREIMS *m/z* (rel. int.) 229.1459 $[M]^+$ (47) (calc. 229.1467 for $C_{15}H_{19}NO$), 200 (90), 186 (76), 173 (100), 144 (16); UV (85% MeOH–H_2O) λ_{max}: 213, 239, 321, 334 nm; IR (KBr) ν_{max}: 3353, 2910, 1637, 1608, 1590, 1550, 1505, 1480, 1366, 1360, 760, 690 cm^{-1}; ^1H NMR (CD_3OD): δ 0.93 (*t*, 3H, $J = 7.0$ Hz, H-14), 1.40–1.44 (*m*, 4H), 1.68–1.74 (*m*, 2H), 2.15 (*s*, 3H, H-9), 2.81 (*t*, 2H, $J = 8.0$ Hz, H-10), 7.33 (*ddd*, 1H, $J = 8.2$, 6.9, 1.1 Hz, H-6), 7.53 (*ddd*, 1H, $J = 8.4$, 1.1, 0.6 Hz, H-8), 7.62 (*ddd*, 1H, $J = 8.4$, 6.9, 1.4 Hz, H-7), 8.22 (*ddd*, 1H, $J = 8.2$, 1.4, 0.6 Hz, H-5) ppm; ^{13}C NMR (CD_3OD): δ 179.6 (C-4), 153.3 (C-2), 140.6 (C-8a), 132.6 (C-7), 126.2 (C-5), 124.47 (C-6), 124.42 (C-4a), 118.6 (C-8), 116.2 (C-3), 33.4 (C-10), 32.8 (C-12), 29.6 (C-11), 23.5 (C-13), 14.3 (C-14), 10.8 (C-9).

PSC-B (*2-(2-heptenyl)-3-methyl-4-quinolinone*). Mp 225–227°; EIMS *m/z* 255 $[M]^+$; HREIMS *m/z* (rel. int.) 255.1634 $[M]^+$ (44) (calc. 255.1623 for $C_{17}H_{21}NO$), 212 (100), 198 (74), 184 (43), 172 (10); UV (85% MeOH–H_2O) λ_{max}: 213, 244, 322, 335 nm; IR (KBr) ν_{max}: 3443, 2920, 1639, 1608, 1593, 1559, 1481, 1360, 1258, 996, 969, 750, 691 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.86 (*t*, 3H, $J = 7.5$ Hz, H-16), 1.25–1.30 (*m*, 4H, H-14, H-15), 1.97–2.01 (*m*, 2H, H-13), 2.22 (*s*, 3H, H-9), 3.57 (*d*, 2H, $J = 6.2$ Hz, H-10), 5.53 (*dt*, 1H, $J = 16.3$, 6.3 Hz, H-11), 5.62 (*dt*, 1H, $J = 16.3$, 6.3 Hz, H-12), 7.30 (*dt*, 1H, $J = 8.3$, 1.0 Hz, H-6), 7.54 (*dt*, 1H, $J = 8.3$, 1.3 Hz, H-7), 7.68 (*d*, 1H, $J = 8.3$ Hz, H-8), 8.41 (*d*, 1H, $J = 8.3$ Hz, H-5), 11.22 (*bs*, 1H, NH) ppm; ^{13}C NMR ($CDCl_3$): δ 177.3 (C-4), 148.8 (C-2), 139.2 (C-8a), 135.5 (C-12), 131.2 (C-7), 125.7 (C-5), 123.39 (C-4a), 123.36 (C-6), 123.2 (C-11), 117.9 (C-8), 115.5 (C-3), 35.6 (C-10), 32.2 (C-13), 31.3 (C-14), 22.2 (C-15), 13.8 (C-16), 10.6 (C-9).

PSC-C (*2-heptyl-3-methyl-4-quinolinone*). Mp 227–228°; EIMS *m/z* 257 $[M]^+$; HREIMS *m/z* (rel. int.) 257.1795 $[M]^+$ (45) (calc. 257.1780 for $C_{17}H_{23}NO$), 200 (76), 186 (61), 173 (100), 144 (24); UV (MeOH) λ_{max}: 215, 239, 322, 335 nm; IR (KBr) ν_{max}: 3355, 2900, 1632, 1608, 1603, 1590, 1552, 1500, 1479, 1369, 1358, 1252, 995, 751, 690 cm^{-1}; ^1H NMR (CD_3OD): δ 0.89 (*t*, 3H, $J = 7.0$ Hz, H-16), 1.30–1.35 (*m*, 4H, H-14, H-15), 1.34–1.42 (*m*, 2H, H-13), 1.40–1.46 (*m*, 2H, H-12), 1.68–1.73 (*m*, 2H, H-11), 2.15 (*s*, 3H, H-9), 2.81 (*t*, 2H, $J = 8.0$ Hz, H-10), 7.33 (*ddd*, 1H, $J = 8.2$, 6.9, 1.1 Hz, H-6), 7.53 (*ddd*, 1H, $J = 8.4$, 1.1, 0.6 Hz, H-8), 7.62 (*ddd*, 1H, $J = 8.4$, 6.9, 1.4 Hz, H-7), 8.22 (*ddd*, 1H, $J = 8.2$, 1.4, 0.6 Hz, H-5); ^{13}C NMR (CD_3OD): δ 179.6 (C-40, 153.3 (C-2), 140.6 (C-8a), 132.6 (C-7), 126.2 (C-5), 124.47 (C-6), 124.42 (C-4a), 118.6 (C-8), 116.2 (C-3), 33.4 (C-10), 32.9 (C-14), 30.5, 30.1, 29.9, 23.6 (C-15), 14.3 (C-16), 10.8 (C-9).

PSC-D (*3-methyl-2-(2-nonenyl)-4-quinolinone*). Mp 197–198°; EIMS *m/z* 283 $[M]^+$; HREIMS *m/z* (rel. int.) 283.1952 $[M]^+$ (37) (calc. 283.1936 for $C_{19}H_{25}NO$), 212 (100), 198 (69), 184 (53), 173 (10); UV (85% MeOH–H_2O) λ_{max}: 213, 243, 321, 335 nm; IR (KBr) ν_{max}: 3450, 2930, 1639, 1608, 1593, 1560, 1503, 1482, 1361, 1260, 1000, 969, 758, 698 cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.87 (*t*, 3H, $J = 7.0$ Hz, H-18), 1.25–1.32 (*m*, 6H), 1.31–1.36 (*m*, 2H, H-14), 2.00–2.05 (*m*, 2H, H-13), 2.23 (*s*, 3H, H-9), 3.61 (*d*, 2H, $J = 6.4$ Hz, H-10), 5.54 (*dt*, 1H, $J = 15.3$, 6.4 Hz, H-11), 5.69 (*dt*, 1H, $J = 15.3$, 6.7 Hz, H-12), 7.33 (*t*, 1H, $J = 8.3$ Hz, H-6), 7.57 (*t*, 1H, $J = 8.3$ Hz, H-7), 7.67 (*d*, 1H, $J = 8.3$ Hz, H-8), 8.45 (*d*, 1H, $J = 8.3$ Hz, H-5), 10.79 (*bs*, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 176.5 (C-4), 148.0 (C-2), 138.9 (C-8a), 136.5 (C-12), 131.5 (C-7), 125.7 (C-5), 123.8 (C-6), 122.9 (C-4a), 122.9 (C-11), 117.9 (C-8), 115.7 (C-3), 35.5 (C-10), 32.5 (C-13), 31.6 (C-16), 29.1 (C-14), 28.9 (C-15), 22.6 (C-17), 14.0 (C-18), 10.6 (C-9).

PSC-E (*3-methyl-2-nonyl-4-quinolinone*). Mp 215–216°; EIMS *m/z* 285 $[M]^+$; HREIMS *m/z* (rel. int.) 285.2087 $[M]^+$ (39) (calc. 285.2093 for $C_{19}H_{27}NO$), 200 (46), 186 (65), 173 (100), 144 (9); UV (MeOH) λ_{max}: 214, 239, 322, 335 nm; IR (KBr) ν_{max}: 3350, 2830, 1639, 1608, 1593, 1557, 1503, 1481, 1397, 1360, 1000, 758, 694 cm^{-1}; ^1H NMR (CD_3OD): δ 0.88 (*t*, 3H, $J = 7.1$ Hz, H-18), 1.27–1.34 (*m*, 8H), 1.32–1.38 (*m*, 2H, H-13), 1.38–1.46 (*m*, 2H, H-12), 1.70–1.73 (*m*, 2H, H-11), 2.15 (*s*, 3H, H-9), 2.81 (*t*, 2H, $J = 8.0$ Hz, H-10), 7.33 (*ddd*, 1H, $J = 8.2$, 6.9, 1.0 Hz, H-6), 7.53 (*dd*, 1H, $J = 8.4$, 1.0 Hz, H-8), 7.62 (*ddd*, 1H, $J = 8.4$, 6.9, 1.4 Hz, H-7), 8.22 (*dd*, 1H, $J = 8.2$, 1.4 Hz, H-5); ^{13}C NMR (CD_3OD): δ 179.6 (C-4), 153.3 (C-2), 140.6 (C-8a), 132.6 (C-7), 126.2 (C-5), 124.47 (C-7), 124.42 (C-4a), 118.6 (C-8), 116.2 (C-3), 33.4 (C-10), 33.0 (C-16), 30.6, 30.5, 30.4, 30.3, 30.0, 23.7 (C-17), 14.4 (C-18), 10.8 (C-9).

Synthesis of PSC-C. A soln of ethyl 2-methyl-3-oxodecanoate (20 mmol), aniline (20 mmol) and *p*-toluenesulphonic acid (0.02 g) in C_6H_6 (50 ml) was heated to reflux with stirring for 5 hr. The H_2O formed was removed by using a Dean–Stark apparatus. The reaction mixt. was concd and diluted with diphenyl ether (50 ml) at 200° in a dropwise fashion. The reaction mixt. was stirred at this temp. for 30 min, cooled, diluted with EtOAc, and chromatographed on silica gel with hexane–EtOAc (1:1) to remove diphenyl ether, followed by MeOH elution to afford the main product. Concn, trituration with EtOAc and filtration afforded the 4-quinolinone as a yellowish solid in 65% yield. An analyt. sample was obtained as a crystalline solid by recrystallization from aq. MeOH. Physicochemical properties were the same as the natural product. HPLC RR_t 6.7 min [Phenomenex μ-Bondapak C-18, 3.9 × 300 mm, UV 225 nm, 1 ml min^{-1}, MeOH–H_2O (3:1)].

Synthesis of PSC-E. The above method was applied for the synthesis of PSC-E, starting from ethyl 2-methyl-3-oxododecanoate. PSC-E was obtained as a yellowish solid in 60% yield. An analyt. sample was obtained as a crystalline solid by recrystallization from aq. MeOH. Physicochemical properties were the same

as the natural product. HPLC RR_t 11.5 min [Phenomenex μ-Bondapak C-18, 3.9×300 mm, UV 225 nm, 1 ml min^{-1}, MeOH–H_2O (3:1)].

Acknowledgements—This work was supported in part by the Korea Research Foundation (Non Directed Research Fund), 1993. We thank Dr J. S. Yoo (Korea Basic Science Center, Taejon) for the mass spectra, and Miss E.-J. Bang (Korea Basic Science Center, Seoul) for NMR spectra.

REFERENCES

1. Hwang, B. K. and Kim, C. H. (1995) *Plant Disease* **79**, 221.
2. Cook, R. J. and Baker, K. F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens*. American Phytopathological Society, St Paul, MN.
3. Park, K. S., Hagiwara, H. and Kim, C. H. (1993) *Korean J. Plant Pathol.* **9**, 1.
4. Park, K. S. (1994) Ph.D. Dissertation. Chungbuk National University, Korea.
5. Hashimoto, M. and Hattori, K. (1967) *Chem. Pharm. Bull.* **15**, 718.
6. Homma, Y., Sato, Z., Hirayama, F., Konno, K., Shirahama, H. and Suzui, T. (1989) *Soil Biol. Biochem.* **21**, 723.
7. Conrad, M. and Limpach, L. (1887) *Ber.* **20**, 944.
8. Somanathan, R. and Smith, K. M. (1981) *J. Heterocyclic Chem.* **18**, 1077.
9. Wratten, S. J., Wolfe, M. S., Andersen, R. J. and Faulkner, D. J. (1977) *Antimicro. Agents Chemother.* **11**, 411.
10. Hewitt, W. and Vincent, S. (1988) *Theory and Application of Microbiological Assay*, pp. 19–20. Academic Press, San Diego, CA.
11. Leisiniger, T. and Margraff, R. (1979) *Microbiol. Rev.* **43**, 422.
12. Parker, W. L., Rathnum, M. L., Seiner, V., Trejo, W. H., Principe, P. A. and Sykes, R. B. (1984) *J. Antibiot.* **37**, 431.
13. Kunze, B., Hofle, G. and Reichenbach, H. (1987) *J. Antibiot.* **40**, 258.
14. Chung, K. H., Cho, K. Y., Takahashi, N. and Yoshida, S. (1991) *J. Korean Agric. Chem. Soc.* **34**, 43.