Novel Radicinol Derivatives from Long-Term Cultures of *Alternaria chrysanthemi*

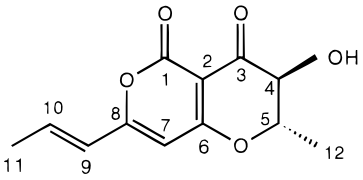
Helen Sheridan* and Ann-Marie Canning

Department of Pharmacognosy School of Pharmacy, Trinity College, Dublin 2, Ireland

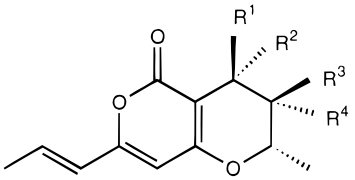
Received April 9, 1999

Cultures of *Alternaria chrysanthemi* normally produce radicinin (**1**) and radicinol (**2**) when cultured on Czapek−Dox medium or on potato dextrose broth. We have observed that long-term cultures of *A. chrysanthemi* grown on malt-extract broth produce 3-epiradicinol (**3**), the novel metabolites 3-methoxy-3-epiradicinol (**4**) and 9,10-epoxy-3-methoxy-3-epiradicinol (**5**), and (**2**).

Alternaria chrysanthemi (Deuteromycetes) is the causal fungus in leaf-spot disease of *Leucanthemum maximum* (Ramond) DC. (Compositae).[1] Chemical analyses of *A. chrysanthemi* have led to the isolation of the phytotoxins radicinin (**1**) and radicinol (**2**).[2,3] The phytotoxic dimer bisradicinin has been isolated from cultures of *A. chrysanthemi* used in biotransformation studies.[4] During the course of a long-term study into the production of metabolites by *Alternaria* species,[5] we observed trace compounds in addition to **1** and **2** in cultures of *A. chrysanthemi* grown on malt-extract broth.[6] The concentration of these compounds was found to increase when cultures were grown for longer than the normal 20 days. In this study, cultures of *A. chrysanthemi* grown for 100 days on malt-extract broth have been shown to produce radicinol (**2**) and 3-epiradicinol (**3**), together with the novel metabolites 3-methoxy-3-epiradicinol (**4**) and 9,10-epoxy-3-methoxy-3-epiradicinol (**5**). The phytotoxicity of these new compounds is under investigation.



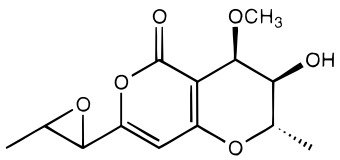
1



2 $R^1 = R^4 = H$, $R^2 = R^3 = OH$

3 $R^2 = R^4 = H$, $R^1 = R^3 = OH$

4 $R^2 = R^4 = H$, $R^1 = OCH_3$, $R^3 = OH$



5

Liquid cultures of *A. chrysanthemi* were incubated for 100 days on malt-extract broth and maintained in the dark without shaking. Column chromatography of an EtOAc extract of the growth medium led to isolation of the known phytotoxin **2**, which was characterized by spectroscopic means and by comparison with literature values.[2,3] The second compound recovered was an oil with the molecular formula $C_{12}H_{14}O_5$. The ^{1}H NMR spectrum for this compound was almost identical with that of **2** except for a slight shift in the signals for H-3, H-4, and the methyl at H-5. The coupling constant for the H-3 doublet ($J = 3.8$ Hz) was smaller than that for the corresponding proton in **2** ($J = 6.5$ Hz). The coupling constant of 3.8 Hz is in keeping with that observed by Nukina et al. for 3-epiradicinol, the diastereoisomer of **2** that has a 5*S*,4*R*,3*S* configuration.[3] Based on these data and a comparison with literature values,[4] **3** was identified as 3-epiradicinol, previously isolated from cultures of *A. chrysanthemi* and *A. longipipes*.[5]

The third compound (**4**) recovered from the growth medium, with the molecular formula $C_{13}H_{16}O_5$, was also an optically active oil. Absorbances in the IR spectrum at 3400, 1700, and 1400 cm^{-1} were similar to those observed in the spectra of **2** and **3**. The ^{1}H and 2D NMR spectra of **4** were almost identical with those of **3** in that they showed the presence of the dihydropyran ring and the propenyl side chain observed in **1**−**3** together with the H-7 resonance at δ 5.68. The relative stereochemistry of **4** was established with reference to the observed coupling constants. The coupling of the C-4 proton $J = 4.3$, 3.8 Hz was consistent with that observed for the two α,α protons observed in 3-epiradicinol (**3**). The main difference between the spectra of **4** and **3** lies in the presence of an additional methoxyl methyl resonance at δ 3.65 and an additional resonance at δ 55.2 in the ^{13}C NMR spectrum. An additional methoxyl group in the isolate is in keeping with the requirements of the molecular formula. Sequential decoupling experiments led to the positioning of the methoxyl group at C-3. This assignment was supported by a 2D NOESY experiment in which a very strong interaction between the doublet at δ 4.33 and the OCH_3 group was observed. Based on these spectral results, **4** was identified as the novel natural product 3-methoxy-3-epiradicinol (**4**).

The final compound (**5**) recovered from long-term cultures of *A. chrysanthemi* was an optically active oil with the molecular formula $C_{13}H_{16}O_6$. Analysis of the IR, ^{1}H and 2D NMR spectra of **5** showed that the compound had many similarities to **3**. The ^{1}H NMR spectrum supported the presence of a dihydropyran ring with a 3-methoxy sub-

* To whom correspondence should be addressed. Tel.: +353-1-6082802/3. E-mail: hsheridn@tcd.ie.

10.1021/np990154w CCC: $18.00
Published on Web 10/12/1999

stituent together with the H-7 resonance at δ 5.80. The main differences between the spectra of **5** and **4** were in the signals for the three-carbon side chain. For **2**−**4** the signals for the propenyl side chain are almost identical; however, for **5** all of the resonances moved upfield with the H-9 proton signal at δ 3.35, H-10 at δ 3.18, and the methyl group resonating as a doublet at δ 1.48. The shift in signals in the propenyl region, coupled with the molecular requirement for an additional oxygen, suggested the presence of an epoxide at C-9−C-10 in the three-carbon side chain. The epoxide assignment is further supported by the ^{13}C NMR spectrum and by the IR absorbance at 1250 cm^{-1} and was confirmed by the presence in the MS of ions at *m*/*z* 211 ($M^+ - C_3H_5O$) and *m*/*z* 57 corresponding to a C_3H_5O fragment. Based on these observations, **5** was identified as the novel compound: 9,10-epoxy-3-methoxy-3-epiradicinol.

In previous studies, when *A. chrysanthemi* was grown on modified Czapek−Dox medium[2] or potato dextrose broth,[4] **1** immediately appeared, followed after 10 days by **2**. The use of malt-extract broth as a long-term growth medium has yielded radicinol (**2**), together with the novel metabolites 3-epiradicinol (**3**) and its methoxyl derivatives **4** and **5**. It appears that the stereospecificity of enzymic reduction of **1** to **2** and/or **3** depends on growth conditions. This is in keeping with the observation that carbon source can influence the enzymes produced by a microorganism, and this, in turn, can lead to a difference in the stereochemical course of the reduction.[5,7] As the cultures of *A. chrysanthemi* age, it appears that selective methylation of **3** occurs at the 3-OH group. Partial epoxidation of **4** may be catalyzed by the aging cultures to yield **5**, or this isolate may be an artifact from atmospheric oxidation.

Experimental Section

General Experimental Methods. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC-400 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard. EIMS were recorded at 70 eV. Analytical TLC was carried out on precoated plastic sheets (0.2 mm) with Si gel 60F_{254} (E. Merck Darmstadt). Compounds were detected by visualization at UV 254 nm.

Organism and Culture. Liquid cultures of *A. chrysanthemi* (ex CBS Baarn) were prepared by inoculating malt-extract broth (150 mL in 250-mL Roux flasks) with four cores (4-mm diameter) of actively growing cultures of *A. chrysanthemi* grown on malt-agar plates. Cultures were incubated in the dark for 100 days.

Isolation of Compounds 2−5. Cultures of *A. chrysanthemi* were filtered, and the fungal growth medium (2 L) was extracted with EtOAc. This was dried over Na_2SO_4, filtered, and evaporated to dryness in vacuo. The resulting residue (530 mg) was chromatographed on a Si gel column using a $CHCl_3$, $CHCl_3$−MeOH (9:1 to 7:3) gradient as eluent. Four main fractions were chromatographed further to yield the following compounds, which were examined by TLC (Si gel, $CHCl_3$−MeOH−H_2O (10:1:0.1) and visualized under UV_{254}.

Radicinol (2): obtained as an oil (85 mg); ^{1}H and ^{13}C NMR ($CDCl_3$ 400 MHz) identical to literature;[1] HREIMS *m*/*z* 236.0838 (calcd for $C_{12}H_{14}O_5$, 236.0841).

3-Epiradicinol (3): obtained as an oil (7 mg); ^{1}H and ^{13}C NMR ($CDCl_3$ 400 MHz identical to the literature;[2] HREIMS *m*/*z* 236.0840 (calcd for $C_{12}H_{14}O_5$, 236.0841).

3-Methoxy-3-epiradicinol (4): obtained as an oil (23 mg); $[\alpha]_D$ −65° (*c* 5.8, $CHCl_3$); IR (neat) ν_{max} 3400, 1700, and 1400 cm^{-1}; ^{1}H NMR ($CDCl_3$ 400 MHz) δ 1.38 (3H, d, J = 6.9 Hz, H-12), 1.86 (3H, d, J = 7.5, H-11), 2.50 (1H, br s, OH), 3.40 (1H, dt, J = 3.8, 8.2 Hz, H-4), 3.65 (3H, s, OCH3) 4.12 (1H, dq, J = 6.9, 8.2 Hz, H-5), 4.33 (1H, d, J = 3.8 Hz, H-3), 5.68 (1H, s, H-7), 5.90 (1H, d, J = 13.9, H-10), 6.68 (1H, dq, J = 7.5, 13.9 Hz, H-10); ^{13}C NMR δ 17.1 (C-12), 18.4 (C-11), 55.2 (OCH_3), 68.0 (C-3), 72.5 (C-4), 76.8 (C-5), 99.1 (C-7), 100.4 (C-2), 122.7 (C-9), 135.7 (C-10), 158.9 (C-8), 165.4 (C-1); $[M]^+$ 252.1001 (calcd for $C_{13}H_{16}O_5$, 252.0998), *m*/*z* 238 (25), 197 (25), 181 (100), 69 (64).

9,10-Epoxy-3-methoxy-3-epiradicinol (5): isolated as an oil (15 mg), $[\alpha]_D$ −43° (*c* 0.5, $CHCl_3$); IR (neat) ν_{max} 3400, 1700, 1400, and 1250 cm^{-1}; ^{1}H NMR ($CDCl_3$ 400 MHz) δ 1.48 (3H, d, J = 6.4 Hz, H-11), 1.52 (3H, d, J = 6.9, H-12), 2.50 (1H, br s, OH), 3.18 (1H, dq, J = 3.1, 6.4, H-10), 3.35 (1H, d, J = 3.1 Hz, H-9), 3.41 (1H, dd, J = 3.8, 8.0 Hz, H-4), 3.52 (1H, d, J = 3.8, H-3), 3.60 (3H, s, OCH_3), 4.15 (1H, dq, J = 6.9, 8.0 Hz, H-5), 5.80 (1H, s, H-7); ^{13}C NMR δ 17.3 (C-12), 17.5 (C-11), 55.0 (OCH_3), 57.4 (C-10), 59.2 (C-9), 69.6 (C-3), 73.3 (C-4), 76.8 (C-5), 98.1 (C-2), 98.2 (C-7), 159.0 (C-8), 161.1 (C-1), 166.4 (C-6); M^+ 268.0936 (calcd for $C_{13}H_{16}O_6$, 268.0947) *m*/*z* 253 (20), 211 (45), 182 (100), 197 (18), 111 (48), 57 (80).

References and Notes

(1) Simmons, E. G. *Mycologia* **1965**, *57*, 140−143.
(2) Robeson, D. G.; Gray, G. R.; Strobel, G. A. *Phytochemistry* **1982**, *21*, 2359−2362.
(3) Nukina, M.; Marumo, S. *Tetrahedron Lett.* **1977**, *37*, 3271−3272.
(4) Canning, A. M.; Hook, I.; Sheridan, H.; James, P.; Kelly, D. *J. Nat. Prod.* **1992**, *55*, 487−490.
(5) Sheridan, H.; Smith, C.; Canning, A. M.; James, P. *J. Nat. Prod.* **1992**, *55*, 986−988.
(6) Canning, A. M. The Chemistry of *Alternaria chrysanthemi*. M.Sc. Thesis. Trinity College, Dublin, 1991.
(7) Favuve, A.; Veschambre, H. *Biocatalysis* **1990**, *3*, 95.

NP990154W