6-Chlorodehydrocurvularin, a New Metabolite from Cochliobolus spicifer

Emilio L. Ghisalberti, and Catherine Y. Rowland

More About This Article

The permalink http://dx.doi.org/10.1021/np50102a022 provides access to:

- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

6-CHLORODEHYDROCURVULARIN, A NEW METABOLITE FROM *COCHLIOBOLUS SPICIFER*

EMILIO L. GHISALBERTI* and CATHERINE Y. ROWLAND

Department of Chemistry, The University of Western Australia, Nedlands 6009, Australia

ABSTRACT.—The major lipophilic metabolite produced by a strain of *Cochliobolus spicifer* grown on potato dextrose broth has been identified as *E*-6-chloro-10,11-dehydrocurvularin [2]. The phytotoxic fungal metabolite, curvularin [1], is the major metabolite produced when the fungus is grown on Czapek-Dox medium.

Fungal species that have *Cochliobolus* as a perfect stage, e.g., *Bipolaris, Curvularia, Drechslera* (Deuteromycotina), include an important group of fungal plant pathogens which have been responsible for devastating disease epidemics, particularly on rice, corn, and sorghum (1). Some members of this group produce a related set of sesterterpenoids, the ophiobolins, which are phytotoxic to a wide range of plants. A knowledge of the various metabolites produced by different members of this group of fungi contributes to an understanding of their pathology (1). We had the opportunity of examining the metabolites elaborated by *Cochliobolus spicifer* R.R. Nelson [*Bipolaris spicifer* (Bainier) Subram. anamorph] (2) isolated from soil in which wheat seedlings were growing. The fungus, grown on Czapek-Dox medium, produced palmitic acid, linoleic acid, and curvularin [1] as major metabolites. When grown on potato dextrose broth (PDB), the major lipophilic metabolite produced was a new derivative of *E*-10,11-dehydrocurvularin incorporating a chlorine atom. Evidence for the structure of this metabolite is presented here.

RESULTS AND DISCUSSION

The liquid medium from cultures of *C. spicifer* (IMI 356287), grown on Czapek-Dox medium over a period of 3 months, was extracted with EtOAc. The mycelial mat was extracted with MeOH, and the EtOAc portion of the extract was recovered. Tlc analysis of the crude extracts showed that both contained two main components, which were separated by radial plate chromatography. The less polar fraction, after treatment with CH_2N_2, was shown to be a mixture of methyl palmitate and linoleate (1:13). The more polar fraction was identified as curvularin [1] (3,4).

The EtOAc-soluble extract of the liquid medium from a culture of *C. spicifer*, grown on 1/5 PDB medium, presented a more complex metabolic profile. Apart from the metabolites previously identified, curvularin being a minor metabolite this time, another compound, **2**, was detected as the major component.

Analysis of the ^{1}H- and ^{13}C-nmr spectral parameters of **2** showed that the compound was similar to curvularin with the following exceptions. The presence of an extra double bond in **2** was evidenced by a pair of mutually coupled protons at δ_H 6.68 (ddd, *J*=16.0, 3.5, 3.5 Hz) and 6.66 (d, *J*=16.0 Hz), replacing the signals at 3.21 and 2.75 attributable to the
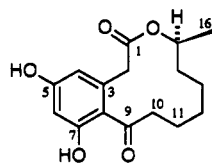
1
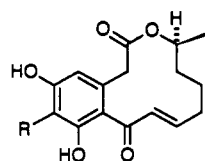
2   R=Cl
3   R=H

methylene protons α to the carbonyl (C-10) in curvularin, with corresponding doublets in the ^{13}C-nmr spectrum at δ_C 149.7 and 135.9. Furthermore, 2 contained only one aromatic proton (δ_H 6.48, br d) with demonstrable long range coupling to an AB quartet for the C-2 protons (δ_H 4.02 and 3.54). The nature of the extra substituent on the aromatic ring at C-6 was indicated by the ms, which showed the presence of a chlorine atom from the intensity of the ion at $[M+2]^+$. In addition, significant ions incorporating the aromatic ring observed in the ms of dehydrocurvularin, at m/z 203, 177, 167, and 150 (5), had their counterparts 34 amu higher in the ms of 2. A comparison of the ^{13}C-nmr spectral data between 2 and dehydrocurvularin [3] (Table 1) lends support to the assignment.

The negative value of the optical rotation for 2, $[\alpha]D$ −51°, is consistent with that observed for curvularin and the 10,11-dehydro analogue and is indicative of an identical configuration at the single asymmetric carbon (3). Thus, the structure of this new metabolite is E-6-chloro-10,11-dehydrocurvularin.

TABLE 1. ^{13}C-nmr Spectral Data for 2 and 3.

Carbon	Compound		
	2[a]	2[b]	3[c]
C-1	170.9	171.6	172.5
C-2	43.7	43.1	43.6
C-3	135.9	136.8	139.6
C-4	112.5	113.1	113.9
C-5	156.1	158.0	163.5
C-6	107.5	107.8	103.2
C-7	160.8	160.4	165.9
C-8	114.9	116.5	116.2
C-9	198.3	197.2	198.1
C-10	130.9	132.2	133.1
C-11	149.7	151.2	150.4
C-12	32.8	33.3	33.2
C-13	24.1	24.9	24.9
C-14	34.0	34.7	34.8
C-15	72.8	73.0	73.1
15-Me	20.0	20.3	20.2

[a]CDCl$_3$, 75.1 MHz.
[b]Me$_2$CO-d_6, 125.8 MHz.
[c]Me$_2$CO-d_6, 100.6 MHz (9).

The formation of 2 at the expense of curvularin when PDB is used as a medium is interesting. The involvement of chloride ions, present in PDB medium, in non-enzymatic production of 3-chlorogentisyl alcohol from epoxydon has been suggested (6). Because both media used contain chloride ion, it seems more likely that some other factor in PDB activates a haloperoxidase. The chloride radical thus produced apparently substitutes the aromatic ring of 2 in a process acting in competition with the conversion of 2 to curvularin. The nature of this factor remains to be established.

Curvularin and related compounds have been reported as metabolites produced by members of the *Curvularia* (5,7), *Alternaria* (8,9), and *Penicillium* (10–12) genera. Dehydrocurvularin co-occurs with curvularin in *Drechslera australiensis* (13). The non-specific phytotoxicity of 1 and 3 has been demonstrated and their selective antimicrobial activity has been noted (8,9). In addition, 1 and 3 effectively inhibit cell division in sea urchin embryonic development at concentrations of 2.5 and 1.2 μg/ml, respectively (12).

EXPERIMENTAL

ISOLATION OF METABOLITES FROM THE LIQUID CULTURE OF *COCHLIOBOLUS SPICIFER*.—*Culture grown on Czapek-Dox medium.*—The liquid medium (3 liters) of a 3-month-old culture of *C-spicifer* (CR229; culture deposited with the International Mycological Institute; IMI 356287), after filtration from the mycelial mat, was extracted repeatedly with EtOAc. The combined organic layer was dried over Na$_2$SO$_4$ and evaporated under reduced pressure to give a residue (189 mg). The

mycelial mat was extracted with MeOH, and the extract was washed with EtOAc to give a residue (266 mg). Tlc analysis [Si gel, CH_2Cl_2-EtOAc (1:1)] of both extracts showed them to be similar and to contain mainly two components. Radial plate chromatography (RPC) of the EtOAc-soluble portion of the mycelial extract using gradient elution (CH_2Cl_2 to EtOAc) gave two fractions. Fraction 1 (64 mg), R_f 0.78 [CH_2Cl_2-EtOAc(1:1)], appeared to be mainly linoleic acid from [1]H- and ^{13}C-nmr spectroscopy. The fraction was treated with CH_2N_2, and the methylated components were analyzed by gc/ms. The presence of methyl palmitate and linoleate (1:13) was confirmed by comparison with authentic samples. Fraction 2 (70 mg) was purified by cc on Si gel to give a crystalline sample of curvularin [**1**]: R_f 0.56 [CH_2Cl_2-EtOAc(1:1)], mp 203–205°; [α]D − 28.6° (c=0.4, EtOH) [lit. (11) mp 206–207°, lit. (8) [α]D − 33.9° (c=2.0; EtOH)]. The structure was confirmed by a single crystal X-ray diffraction studies (4).

Culture grown on potato dextrose broth (1/5 dilution).—A 3-month-old culture of *C. spicifer* grown on this medium (4 liters) was similarly processed to give two residues: EtOAc extract (234 mg) and EtOAc-soluble portion of mycelial extract (170 mg). Tlc analysis [Si gel, CH_2Cl_2- EtOAc (1:1)] of both fractions showed them to be similar. Rpc of the EtOAc extract (200 mg) and gradient elution [CH_2Cl_2-EtOAc(1:1) to EtOAc] yielded a fraction (16 mg) corresponding to the major component **2** as a solid.

6-Chloro-10,11-dehydrocurvularin [**2**].—Crystals from CH_2Cl_2/EtOAc as needles: mp 189–190°, [α]D − 51° (c=0.2; EtOH), [M]$^+$ 324; ^{1}H nmr (500 MHz, $CDCl_3$) δ 6.68 (1H, ddd, J=16.0, 3.5, 3.5 Hz, H-11), 6.66 (1H, br d, J=16.0 Hz, H-10), 6.48 (1H, br s, H-4), 4.85 (1H, ddq, J=2.0, 8.0, 6.5 Hz, H-15), 4.02 (1H, br d, J= 17.8 Hz, H$_a$-2), 3.54 (1H, br d, J=17.8, H$_b$-2), 1.25 (3H, d, J=6.5 Hz, H$_3$-16). The following assignments were made with the aid of decoupling techniques: 2.5 and 2.35 (H$_2$-12); 1.99 and 1.69 (H$_2$-13), 1.89 and 1.65 (H$_2$-14); ^{1}H nmr (500 MHz, Me$_2$CO-d$_6$) δ 6.77 (1H, d, J=15.4, H-11), 6.62 (1H, ddd, J=15.4, 8.9, 4.9 Hz, H-10), 6.57 (1H, br s, H-4), 4.74 (1H, m, H-15), 4.05 (1H, br d, J=17.6 Hz, H$_a$-2), 3.62 (1H, br d, J=17.6, H$_b$-2), 1.17 (3H, d, J=6.4 Hz, H$_3$-16); ^{13}C nmr see

Table 1; eims m/z 326 (15), [M]$^−$, 324 (36), 239 (29), 237 (9), 231 (6), 229 (14), 226 (16), 225 (11), 224 (24), 223 (18), 213 (12), 211 (37), 210 (31), 209 (32), 203 (12), 201 (37), 186 (15), 184 (46), 160 (24), 115 (26), 81 (60), 55 (100).

LITERATURE CITED

1. G. Strobel, D. Kenfield, and F. Sugawara, *Phytoparasitica*, **16**, 145 (1988).
2. A Sinevasan, *Mycological Papers*, **158**, 1 (1987).
3. J. Buckingham, Ed., "Dictionary of Organic Compounds," 5th Edition, 8th Supplement, Chapman and Hall, London, 1990.
4. E.L. Ghisalberti, D.C.R. Hockless, C. Rowland, and A.H. White, *Aust. J.Chem.*, **46**, 571 (1993).
5. H.D. Munro, O.C. Musgrave, and R. Templeton, *J. Chem. Soc. C*, 947 (1967).
6. K. Nabeta, A. Ichihara, and S. Sakamura, *Agric. Biol. Chem.*, **39**, 409 (1975).
7. R.G. Coombe, J.J. Jacobs, and T.R. Watson, *Aust. J. Chem.*, **21**, 783, 1968.
8. D.J. Robeson and G.A. Strobel, *Z. Naturforsch., Teil C*, **36C**, 1081 (1981).
9. K. Arai, B.J. Rawlings, Y. Yoshizawa, and J.C. Vederas, *J. Am. Chem. Soc.*, **111**, 3391 (1989).
10. H. Raistrick and F.A.H. Rice, *J. Chem Soc. C*, 3069 (1971).
11. A. Kobayashi, T. Hino, S. Yata, T.J. Itoh, H. Sato, and K. Kawazu, *Agric. Biol. Chem.*, **52**, 3119 (1988).
12. S. Lai, Y. Shizuri, S. Yamamura, K. Kawai, Y. Terada, and H. Furukawa, *Tetrahedron Lett.*, **30**, 2241 (1989).
13. W.B. Turner and D.C. Aldridge, "Fungal Metabolites II," Academic Press, London, 1983.
14. R.W. Dunlop, A. Simon, K. Sivasithamparam, and E.L. Ghisalberti, *J. Nat. Prod.*, **52**, 67 (1989).