Hydroxycornexistin: A New Phytotoxin from *Paecilomyces variotii*

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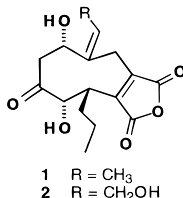
Shake-flask cultures of *Paecilomyces variotii* produce a phytotoxin with high activity against broadleaf weeds and selectivity to corn. The phytotoxin was purified and identified as 14-hydroxycornexistin (**2**), a new member of the nonadride family.

Cornexistin (**1**) is a phytotoxin produced by the fungus *Paecilomyces variotii* Bainier, strain SANK 21086.[1] Its herbicidal effects extend to a number of grass and broadleaf weed species with little phytotoxicity to corn.[2,3] As the mode of action of cornexistin appears to be novel[4] and few phytotoxins are known to possess a nonadride ring system[5] we pursued isolation and identification of other phytotoxins produced by this organism. During the course of these studies we unexpectedly discovered a material in the culture broth with significantly greater activity on broadleaf weeds than cornexistin.[6] This material was subsequently identified as 14-hydroxycornexistin (**2**).



Shake cultures of *Paecilomyces variotii* were highly phytotoxic after 6−14 days of growth. The cultures were harvested by centrifugation followed by extraction of the supernatant. The combined extracts were dried and fractionated by reversed-phase HPLC. When these fractions were subjected to biological testing, two distinct chromatographic regions were found with high levels of activity. The activity corresponding to the longer retention time was subsequently attributed to cornexistin, which was identified by comparison to published spectra.[1] However, the material eluting with a much shorter retention time was unknown. Preparative HPLC ultimately enabled isolation of sufficient material for detailed structural analysis.

2D NMR spectroscopy (COSY, HETCOR) was used to assign all protons and carbons. The physical characterization was consistent with hydroxycornexistin (**2**), a structure not previously described. The following observations were made: (a) ^{1}H NMR gave a similar spectrum to that reported for cornexistin except for an obvious single functionalization of the allylic methyl group (d, 1.7 ppm) to a methylene (m, 4.12 ppm), a shift consistent with the methylene carbon bearing a single heteroatom, particularly oxygen. (b) The olefinic proton of the exocyclic methylene remained at 5.8 ppm, as for cornexistin, but was reduced in multiplicity from a quartet to a triplet, consistent with a single functionalization of the allylic carbon. (c) ^{13}C NMR gave a similar spectrum as cornexistin, except for the change of the allylic methyl group at 13.5 ppm to a methylene at 58.4 ppm, a shift consistent with the methylene carbon bearing a single heteroatom. (d) An increase in polarity as demonstrated by HPLC retention time and TLC was consistent with a free primary hydroxyl substitution. (e) Negative-ion mass spectroscopy gave a fragmentation pattern identical to that of cornexistin + 16 amu. This confirmed oxygen as the heteroatom. (f) There were no additional protons or carbons present in the molecule by NMR. (g) Direct chemical ionization mass spectroscopy gave 324 (consistent with hydroxycornexistin) as the parent, with confirming M + 29 and M + 41 due to the methane carrier gas. (h) An NOE study confirmed the olefin regiochemical assignment to be the same as cornexistin (methylene syn to the hydroxyl). Proton NMR coupling constants indicated the same relative stereochemistry of all chiral centers as cornexistin.

The herbicidal activity of cornexistin and hydroxycornexistin were compared in postemergence applications (Table 1). A cross section of weeds that are problems for corn production were included in this greenhouse evaluation.

Although the level of activity varied by species for cornexistin and hydroxycornexistin, the activity of the latter was particularly strong on broadleaf weeds. The species *Xanthium stumarium* (cocklebur), *Chenopodium album* (lambsquarter), *Ipomoea hederaceae* (morning glory), and *Polygonum convolvulus* (wild buckwheat) are very aggressive weeds yet appear quite sensitive to low concentrations of hydroxycornexistin.

Hydroxycornexistin represents the second phytotoxin of the nonadride family to be identified in cultures of *Paecilomyces variotii*. Its high activity against weeds and low phytotoxicity to corn suggest other nonadrides may be of interest to pursue and evaluate as potential herbicides.

Experimental Section

General Experimental Procedures. ^{1}H-NMR spectra were taken on a Varian Unity 300 or Bruker AM-400 NMR with TMS as internal standard. Analytical HPLC was performed on Kromasil KR100-10-C18 (4.6 × 250 mm, 5 μ particle size, purchased from Richard Scientific) with dual wavelength UV detection at 220 nm and 254 nm using a step gradient of MeCN/H_2O (20% for 5 min, 30% for 5 min, 40% for 10 min at 2 mL/min). Cornexistin (**1**) gave t_R = 14.7 min and hydroxycornexistin (**2**) gave t_R = 10.3 min under these condi-

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Table 1. Herbicidal Activity of Cornexistin and Hydroxycornexistin[a]

	cornexistin (ppm)			hydroxycornexistin (ppm)		
test species	31.25	15.63	7.81	31.25	15.63	7.81
broadleaf weeds						
Xanthium strumarium	20	10	0	100	80	80
Chenopodium album	30	30	30	90	80	20
Ipomoea hederaceae	0	0	0	85	0	0
Amaranthus sp.	0	0	0	80	60	0
Abutilon theophrasti	30	60	0	70	40	40
Polygonum convolvulus	90	40	20	100	100	40
crops						
Zea mays	30	20	0	20	0	0
Triticum aestevium	85	0	0	40	20	35
grass weeds						
Echinochloa crus-galli	95	80	0	40	40	0
Setaria faberi	20	20	20	70	0	0
Sorghum bicolor	30	0	0	0	0	0
Avena sativa	100	0	0	45	25	30
Digitaria sanguinalis	20	20	0	80	20	20

[a] Injury was visually assessed on a scale of 0−100 where 100 represents complete plant death and 0 represents no effect.

tions. All HPLC conditions used H_2O containing 0.1% v/v trifluoroacetic acid (TFA), pH = 2.0. Purity was verified as >95% by NMR and HPLC.

Culture of Organism. The organism *Paecilomyces variotii* Bainier SANK 21086 was obtained from the Fermentation Research Institute, Agency of Industrial Science and Technology, Ministry of International Trade and Industry, Japan (deposit number FERM BP-1351). The organism grows well on a number of solid media (for example, malt extract agar media) and was routinely maintained on this medium at 24−26 °C. The organism was subcultured weekly to maintain a healthy and viable source culture for inoculation of liquid cultures.

Liquid cultures were established in 500-mL baffled shake flasks utilizing 80 mL/flask of the medium: glycerol (5%), dehydrated potato flakes (0.9%), malt extract (0.25%), yeast extract (0.25%), and deionized H_2O (93.6%). The media were prepared, adjusted to pH 6.0, and sterilized by autoclave in baffled shake flasks equipped with polypropylene caps. Inoculation was achieved by adding a few drops of sterile H_2O to the surface of a malt extract agar solid culture and transferring 1 loopful of H_2O, now containing spores, to the liquid culture.

Incubation of the liquid cultures was at 24−26 °C with a shaker speed of 200 rpm. After 3−5 days of growth, detectable activity was present upon harvesting and processing of the culture. Optimal levels of activity, however, were normally not reached until 6−14 days after inoculation. The flasks were routinely harvested as described below after 9 days of growth.

Isolation of Hydroxycornexistin. The shake-flask medium and mycelial mass were transferred to 1-L containers and centrifuged at 3000−5000 rpm for 30 min. The supernatant was removed, refrigerated, and extracted with EtOAc as described below. The mycelial pellet was resuspended in a small amount of H_2O (pH = 9.0) and centrifuged as described above. The resulting supernatant was combined with the original and refrigerated.

The supernatant obtained above was adjusted to pH 12.0 and extracted with two volumes of EtOAc. The EtOAc was discarded and the aqueous phase adjusted to pH 2.0. Extraction was repeated with two volumes of EtOAc. The above "base wash" step is optional; satisfactory results have been obtained by acidifying the supernatant and directly extracting with EtOAc. The EtOAc was dried under reduced pressure to provide a dark oil (crude extract). Typical yields were 0.25−2.0 g of crude extract per liter of culture broth.

Crude extract (12 g) was fractionated via preparative HPLC using a three-step binary gradient (Kromasil KR100-10-C18, 5 μm particle size, purchased from Richard Scientific, 80 mm × 215 mm; 260 mL/min; 20% $MeCN/H_2O$ at 0 min, 30% $MeCN/H_2O$ at 10 min, 40% $MeCN/H_2O$ at 20 min). Fractions from 18.5 min to 20.0 min were collected, combined, saturated with NaCl, extracted with EtOAc (3 × 500 mL) and concentrated *in vacuo* (rotary evaporator) to give 760 mg of >50% pure **2**.

The material was dissolved in 1 N NaOH (1 mL) and the pH readjusted to 8.0 with 1 N HCl (1 mL) followed by NaH_2PO_4/NaOH buffer (3 mL 50 mM, pH 8). Further purification was achieved by preparative HPLC (Dynamax 42.4 × 300 mm C_{18} ODS, 8 μm particle size, purchased from Rainin; 54 mL/min flow rate, isocratic 50 mM NaH_2PO_4/NaOH buffer, pH = 8.0). A broad peak from 5 min to 15 min was collected, acidified to pH = 2.0 (1 N HCl), saturated with NaCl, and extracted with EtOAc. The extract was concentrated to give 110 mg of >95% pure **2** as an off-white amorphous solid: IR (CD_3CN) 3514, 3402 (br), 2957, 2919, 2851, 1767, 1705, 1695, 1680, 1460, 1273, 1200 cm^{-1}; ^{1}H NMR (CD_3CN, 300 MHz) δ 5.80 (1H, t, J = 6.4 Hz) 4.75 (1H, dd, J = 8.8, 4.9 Hz) 4.12 (2H, m) 3.81 (1H, d, J = 9.5 Hz) 3.44−3.34 (3H, m) 0.89 (3H, t, J = 7.3 Hz); ^{13}C NMR (^{1}H decoupled, CD_3CN, 100 MHz) δ 212.5, 167.0, 166.3, 146.8, 142.6, 137.4, 135.6, 80.8, 68.3, 58.4, 44.7, 41.1, 30.6, 28.1, 21.7, 14.1; Negative ion EIMS m/z (rel int) 437 (100), 405 (15), 383 (20), 343 (10), 323 (M-1, 40), 307 (100), 289 (15), 279 (40), 249 (70), 227 (35), 213 (10), 183 (20), 153 (10); positive ion DCIMS m/z 324.

Evaluation of Postemergence Herbicidal Activity. Seeds of the desired test species were planted in a commercial soil preparation having a pH range of 6.0 to 6.8 and an average organic matter content of 30%. The plants were grown for 7−21 days in a greenhouse with a 15-h photoperiod and with the temperature maintained at 23−29 °C during the day and 22−28 °C during the night. Supplemental lighting was provided, when necessary, with overhead 1000 W metal halide lamps. Plants were treated with test compounds after they reached the 1−2-true-leaf stage.

A 3.75-mg sample of each test chemical was weighed into a 20-mL glass vial. A concentrated stock solution was made by adding 4 mL of Me_2CO/DMSO, (97:3 v/v) to each weighed sample. Spray solutions were formulated at three rates as indicated in Table 1. The spray solutions were made by injecting aliquots of the stock solution into the surfactant solution comprised of Me_2CO−H_2O−isopropyl alcohol−DMSO−Atplus 411F−Triton X-155 (48.5:39:10:1.5:1:0.02 v/v).

Solutions were sprayed onto the foliage of test plants with a DeVilbiss atomizer driven by compressed air at a pressure of 2−4 psi. Approximately 1.5 mL of spray solution was applied to the plants in each pot. The atomized spray provided thorough plant coverage. Control plants were sprayed with the surfactant solution alone. In this test an application rate of 1 ppm results in the application of approximately 1 g/Ha.

The treated plants and control plants were placed in a greenhouse as described above and watered by subirrigation to prevent wash-off of the test compounds. Nutrients were added on a regular basis to maintain vigorous plant growth. Assessments of weed control and crop injury were made 2 weeks after application of the test chemicals. Plant injury was visually assessed on a scale of 0−100 by comparison to controls.

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