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## Secondary metabolites from *Penicillium corylophilum* isolated from damp buildings

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**Abstract:** Indoor exposure to the spores and mycelial fragments of fungi that grow on damp building materials can result in increased non-atopic asthma and upper respiratory disease. The mechanism appears to involve exposure to low doses of fungal metabolites. *Penicillium corylophilum* is surprisingly common in damp buildings in USA, Canada and western Europe. We examined isolates of *P. corylophilum* geographically distributed across Canada in the first comprehensive study of secondary metabolites of this fungus. The sesquiterpene phomenone, the meroterpenoids citreohydrinol and andrastin A, koniginin A, E and G, three new alpha pyrones and four new isochromans were identified from extracts of culture filtrates. This is the first report of koniginins, meroterpenoids and alpha pyrones from *P. corylophilum*. These secondary metabolite data support the removal of *P. corylophilum* from *Penicillium* section *Citrina* and suggest that further taxonomic studies are required on this species.

**Key words:** alpha pyrone, indoor environment, isochroman, koniginin, meroterpenoid

### INTRODUCTION

*Penicillium corylophilum* Dierckx has been reported to grow on cereals, frozen fruit cakes, acid liquids and nuts mainly in temperate regions (Samson et al. 2010). However, it is surprisingly common in damp buildings. In a compendium of fungi isolated from moldy building materials in USA and Canada, *P. corylophilum* was common on paper-faced gypsum wallboard and also was reported from fibrous insulation, wood and manufactured wood (Miller et al. 2008). This species comprised ~5% of the penicillia isolated from both damp buildings in Scandinavia and countries in western Europe (Samson et al. 2010, Andersen et al. 2011) and is common in damp buildings in Japan (Ohnishi et al. 2002). In a

study of moldy crawl spaces in Sweden, *P. corylophilum* was found to be dominant (Bok et al. 2009).

Dampness and mold in buildings are associated with increased asthma and upper respiratory disease (WHO 2009, NIOSH 2012). Exposures to allergens triple helical  $\beta$ -(1, 3)-D-glucan, and low molecular weight compounds are thought to be responsible for some of these effects. The glucan and metabolites of fungi that are common on damp building materials have been shown to affect lung biology in vivo (Miller et al. 2010, Rand et al. 2010, Akpinar-Elci et al. 2013). *Penicillium corylophilum* has long been known to produce allergens leading to the serious lung disease hypersensitivity pneumonitis (Kremer et al. 1989, Ohnishi et al. 2002, Unoura et al. 2011).

There are few studies of secondary metabolites of *P. corylophilum*. Working with a soil isolate, Cutler et al. (1989a) isolated 3, 7-dimethyl-8-hydroxy-6-methoxyisochroman (DHMI) that caused etiolation of wheat coleoptiles. The synthetic derivatives, DHMI-8-acetoxy and DHMI-8-methoxy, also retained biological activity (Cutler et al. 1997). The isocoumarins (+) orthosporin and citreoisocoumarinol, as well as the sesquiterpene phomenone were isolated from *P. citreovirens*, a synonym of *P. corylophilum* (IFO 6030 = CBS 320.59; Lai et al. 1991, Frisvad and Filtenborg 1990). Malmström et al. (2000) examined the HPLC chromatograms of 10 *P. corylophilum* isolates. They were able to detect the two isocoumarins from all strains except two that were maintained too long in culture collections but could not confirm the presence of phomenone or furan-2-carboxylic acid.

To facilitate research on the effect of low molecular weight compounds on lungs, we have studied the metabolites of fungi that are common in buildings (Miller et al. 2003; Rand et al. 2005; de la Campa et al. 2007; Slack et al. 2009; McMullin et al. 2012, 2013). This is important for a number of reasons. In some cases the metabolites from building-associated strains have been different compared to strains from other environments. Second, the metabolite profiles of such fungi have been poorly studied, the strains not deposited in recognized culture collections or the spectroscopic data is of lower quality than feasible today. Finally, adequate amounts of well characterized, pure metabolites are required for toxicology studies. Because *P. corylophilum* is relatively common in damp buildings and known to cause human disease, the objective of this study was to conduct a

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TABLE I. *P. corylophilum* strains examined for metabolite production

| Strain      | Location     | Source                  | GenBank accession No. |
|-------------|--------------|-------------------------|-----------------------|
| DAOM 242288 | Ottawa, ON   | indoor air sample       | KF170363              |
| DAOM 242289 | Ottawa, ON   | indoor air sample       | KF170358              |
| DAOM 242290 | Ottawa, ON   | indoor air sample       | KF170357              |
| DAOM 242291 | Montreal, QC | moldy building material | KF170364              |
| DAOM 242292 | Calgary, AB  | indoor air sample       | KF170361              |
| DAOM 242293 | Halifax, NS  | moldy building material | KF170356              |
| DAOM 242294 | Ottawa, ON   | indoor air sample       | KF170362              |
| DAOM 242295 | Calgary, AB  | indoor air sample       | KF170360              |
| DAOM 242296 | Victoria, BC | indoor air sample       | KF170359              |

comprehensive examination of secondary metabolites from this fungus.

#### MATERIALS AND METHODS

**Cultures.**—*Penicillium corylophilum* isolates were obtained from Paracel Laboratories Ltd. (Ottawa, Ontario) and were collected directly from building materials or indoor air samples (SUPPLEMENTARY TABLE I). Cultures were grown on 2% malt extract agar (MEA) and Czapek yeast extract agar, and microscopic features were consistent with literature descriptions of this species (Samson et al. 2010). DNA was extracted from the mycelium of each strain with an UltraClean DNA Isolation Kit (MO BIO Laboratories 12224-250). PCR amplification and sequencing of the ITS1, 5.8S and ITS2 genes were performed by the National Fungal Identification Service, Ottawa, Ontario. The PCR primers used were UN-Up18S42 (5'-CGTAACAAGGTTT-CCGTAGGTGAAC-3') and UN-Lo28S22 (5'-GTTTCTTT-TCCTCCGCTTATTGATATG-3'). The PCR fragments were sequenced, aligned with MAFFT and compared to each other and the sequence of *P. corylophilum* with the BLASTN algorithm against the NCBI nucleotide collection database. *Penicillium corylophilum* is neotypified by FRR 802, the sequence of which was included in our search of the barcode locus. Identities were confirmed by the identification service at the National Mycological Herbarium (Agriculture Agri-Food Canada, Ottawa) and the strains were deposited with the Canadian Collection of Fungal Cultures (DAOM) (TABLE I). All strains were transferred to 2% MEA plates and slants. These were incubated at 25 °C until sufficient growth was observed. Afterward, they were sealed with Parafilm and stored at 4 °C until further use.

**Fermentation and metabolite screening.**—A slant of *P. corylophilum* DAOM 242291, randomly selected, was macerated in sterile distilled deionized water and an aliquot was used to inoculate (5% v/v) five Roux bottles each containing 200 mL yeast extract sucrose broth (YES; 20 g/L yeast extract [Difco], 150 g/L sucrose, 0.5 g/L MgSO<sub>4</sub> · 7 H<sub>2</sub>O), malt extract broth (20 g/L malt extract [Difco]) or Czapek Dox broth (30 g/L sucrose, 3 g/L NaNO<sub>3</sub>, 1 g/L K<sub>2</sub>HPO<sub>4</sub>, 0.5 g/L MgSO<sub>4</sub> · 7 H<sub>2</sub>O, 0.5 g/L KCl, 0.01 g/L FeSO<sub>4</sub> · 7 H<sub>2</sub>O, 5 g/L yeast extract). Cultures were incubated at 25 °C in the dark. To determine the time

course for metabolite production, one Roux bottle was extracted at 1 wk intervals. Fernbach flasks containing 560 mL YES were inoculated (5% v/v) and incubated in shake culture to examine the effect of aeration on metabolite production (NB Scientific triple-tier shaker, 3.5 cm throw, 220 rpm).

The liquid cultures were filtered (Whatman No. 4), and the volume of the culture filtrate and pH were measured. The mycelium was stored in a freezer at –20 °C. The culture filtrates were saturated with sodium chloride, exhaustively extracted with ethyl acetate and filtered (Whatman No. 1) through anhydrous sodium sulfate before being dried by rotary evaporation. The dried crude extract was redissolved in a minimal amount of HPLC grade methanol, filtered through a 13 mm PTFE 0.2 µm filter and dried under a gentle stream of nitrogen gas.

The dry *P. corylophilum* extracts each were dissolved in 1 mL HPLC-grade methanol and analyzed by liquid chromatography ultraviolet mass spectrometry (LC-UV-MS). This was achieved with a Waters 2795 separations module, Waters 996 diode array detector and Waters MicroMass Quattro LC mass spectrometer. Compounds were separated by a Phenomenex Kinetex C<sub>18</sub> (100 × 4.60 mm, 2.6 µm, 100 Å) column (Torrance, California) with a mobile phase consisting of acetonitrile-water (ACN-H<sub>2</sub>O) with formic acid (FA); (0.1% [v/v]). The solvent gradient was linear-programmed 5–100% ACN over 13 min with a flow of 1.0 mL/min.

Based on the UV and mass spectrometry data of the representative extracts, the optimal medium and fermentation time were determined for metabolite production by *P. corylophilum* DAOM 242291. All nine strains were inoculated as above into three Roux bottles containing 200 mL sterile YES media and grown in stationary culture for 2 wk in the dark at 25 °C. The culture filtrate and cells were treated as above.

Calibration plots for compounds (1–13) were constructed with the same LC conditions described above for screening extracts; however the gradient was linear programmed for 20 min instead of 13. The MS was operated in selected ion monitoring (SIM) mode with the [M + H]<sup>+</sup> ion for all compounds except 10 and 11 where the dehydrated ion, [M-H<sub>2</sub>O+H]<sup>+</sup> was used. Each standard solution was analyzed in triplicate, and the calibration plot was represented by 0.01, 0.1, 1.0 and 10 µg purified compound on the column.

The extracts also were screened by LC-MS for the production of citrinin by reference to an authentic standard (Sigma).

**Metabolite isolation.**—Larger scale fermentations of *P. corylophilum* DAOM 242293 were performed to produce sufficient quantities of metabolites for structural elucidation and toxicity assays. On a weight basis, this strain produced cleaner extracts on analysis compared to the other *P. corylophilum* strains examined. The optimal fermentation conditions determined during the screening process (YES broth, 25 °C, stationary in the dark) were used for larger scale fermentations in Glaxo bottles. Twenty 250 mL Erlenmeyer flasks containing 50 mL sterile YES broth were inoculated (5% v/v) and incubated with conditions that favored metabolite production until sufficient growth was observed (~ 3d). Starter cultures were macerated, individually transferred to Glaxo bottles containing 1 L the same medium and incubated as described above.

After extraction, purification of *P. corylophilum* metabolites was achieved by a combination of normal phase column chromatography, isocratic separations with Sephadex LH20 and reverse phase (RP) semi-preparative HPLC. The crude extract (5.2 g) was dissolved in a minimal amount of ethyl acetate (EtOAc) and methanol (MeOH) and fractionated by vacuum chromatography with a silica gel (40–63 µm) column that yielded two spectroscopically different fractions with EtOAc and MeOH solvent combinations. The more nonpolar fraction that eluted with EtOAc (1.5 g) was fractionated further by flash chromatography on silica gel with a MeOH-CHCl<sub>3</sub> gradient that increased 0–10% MeOH in 1% increments. Compounds were purified from these fractions by RP-HPLC with a Phenomenex Luna C<sub>18</sub> (250 × 10.00 mm, 5 µm, 100 Å) column and a mobile phase consisting of ACN- H<sub>2</sub>O. The linear gradient was programmed 10–80% ACN in H<sub>2</sub>O for 19 min with a flow rate of 4 mL/min. The nonpolar ethyl acetate fraction yielded compounds (1–8) that were subjected to spectroscopic analysis.

The more polar fraction that originally eluted in 50% EtOAc-MeOH (3.2 g) was applied to a silica gel column and washed with 10% MeOH-CHCl<sub>3</sub> to remove the less polar components. A MeOH-CHCl<sub>3</sub> gradient was employed with 10% MeOH increments. The 20% MeOH-CHCl<sub>3</sub> fraction (1.5 g) was collected, dried and chromatographed over Sephadex LH-20 with MeOH as the eluent. Compounds were purified by RP-HPLC with a Whatman Partisil 10 ODS-3 C<sub>18</sub> (250 × 9.40 mm, 10 µm) column and a linear gradient programmed 5–45% ACN in H<sub>2</sub>O over 19 min. The 20% MeOH-CHCl<sub>3</sub> fraction yielded the polar compounds 9–13.

NMR spectra of the metabolites were obtained on a Bruker Avance III 700 MHz NMR spectrometer equipped with a 5 mm QNP cryoprobe operating at 700.17 MHz (<sup>1</sup>H) and 176.06 MHz (<sup>13</sup>C) or Bruker Avance 400 Spectrometer at 400.13 (<sup>1</sup>H) and 100 MHz (<sup>13</sup>C) with a 5 mm auto-tuning broadband probe with a Z gradient. Secondary metabolites were dissolved in CD<sub>3</sub>OD (CDN Isotopes, Point Claire, QC) and were referenced to the solvent peak (δ<sub>H</sub> 3.30 and δ<sub>C</sub> 49.0). Chemical shifts were assigned based on <sup>1</sup>H, <sup>13</sup>C, <sup>1</sup>H/<sup>1</sup>H correlation spectroscopy (COSY), heteronuclear

single quantum coherence (HSQC), heteronuclear multiple bond correlation (HMBC) and distortionless enhancement by polarization transfer (DEPT-135) spectra. Metabolite purifications were achieved with an Agilent 1100 series HPLC equipped with quaternary pump and diode array detector (Mississauga, Ontario). High resolution ESI-MS data were acquired in positive mode on a QSTAR XL (AB Sciex, Foster City, California), and spectra were analyzed with Analyst SQ 2.0. UV spectra of isolated metabolites were obtained with a Varian Cary 3 UV-visible spectrophotometer scanning 190–800 nm. Optical rotations were determined with an Autopol IV polarimeter (Rudolph Analytical, New Jersey).

## RESULTS

LC-UV-MS chromatograms of the extracts revealed that YES in still culture at 25 °C proved to be optimal for metabolite production. The use of the other media tested (2% malt extract, Czapek-Dox) or increasing the aeration in YES cultures did not yield appreciable amounts of compounds or metabolite profiles of interest. DAOM 242293 was selected for large scale fermentation because it readily produced a high metabolite yield after the screening of each extract by LC-UV-MS (TABLE II). Fractionation of the crude extract into polar and nonpolar samples by silica gel chromatography yielded two fractions with different LC-UV-MS patterns. The known compounds phomenone (1), citreohybridonol (2), andrastin A (3) and koniginin A (4), E (5) and G (6) all were found in the nonpolar EtOAc fractions with two of the new alpha pyrones (7, 8). The new isochromans (10–13) and the other new alpha pyrone (9) were isolated from the more polar 20% MeOH- EtOAc fraction (FIG. 1, TABLE II). The spectroscopic data for new compounds (9–13) are reported by McMullin et al. (2014). Our chemical data agree with the observations of Smedsgaard (1997) that YES media is better compared to MEA or CYA for isochroman production.

**Phomenone:** (1*aR*,7*R*,7*aR*,7*bR*)-6-hydroxy-1*a*-(3-hydroxyprop-1-en-2-yl)-7,7*a*-dimethyl-4,5,6,7,7*a*,7*b*-hexahydronaphtho[1,2-*b*]oxiren-2(1*aH*)-one (1): (3.0 mg); light yellow powder; [α]<sub>D</sub> +170.7 (c 0.15, MeOH); UV (MeOH)/nm λ<sub>max</sub> (log ε) 203 (3.93), 240 (3.76); HRMS *m/z* 265.1436 [M+H]<sup>+</sup> (calculated for 265.1440). The structure was determined by X ray crystallography (Riche and Pascard-Billy 1975). Carbon and proton NMR data are reported (TABLE III). These <sup>1</sup>H NMR data are consistent with a literature report on various derivatives of phomenone (Capasso et al. 1986).

**Citreohybridonol:** (3*S*,6*R*,8*S*,10*R*,13*R*,14*R*)-methyl 3-acetoxy-15-hydroxy-4,4,8,12,13,16-hexamethyl-17,18-dioxo-1,2,3,4,5,6,7,8,9,13,14,17-dodecahydro-6,10-(epoxymethano) cyclopenta [*a*] phenanthrene-14-carboxylate

TABLE II. Metabolite production by *P. corylophilum* strains studied

| DAOM   | Compound |     |     |      |     |      |     |     |     |      |      |      |      |
|--------|----------|-----|-----|------|-----|------|-----|-----|-----|------|------|------|------|
|        | (1)      | (2) | (3) | (4)  | (5) | (6)  | (7) | (8) | (9) | (10) | (11) | (12) | (13) |
| 242288 | ++       | ++  | +   | +++  | ++  | ++   | ++  | +   | +   | +    | ++   | +++  | +    |
| 242289 | +        | ++  | ++  | +++  | ++  | ++   | +   | +   | +   | +    | +    | +    | +    |
| 242290 | +        | +   | ++  | +++  | ++  | +    | +   | +   | —   | +    | +    | +    | +    |
| 242291 | +        | +   | ++  | ++++ | +   | ++   | +   | +   | +   | +    | +    | +    | +    |
| 242292 | +        | ++  | +   | +++  | +   | +    | ++  | +   | +   | ++   | ++   | +++  | ++   |
| 242293 | +        | +   | +   | +++  | ++  | +++  | +   | +   | +   | ++   | ++   | +++  | +    |
| 242294 | +        | +   | ++  | ++++ | ++  | +++  | +   | +   | +   | +    | +    | +    | +    |
| 242295 | +        | ++  | +   | +++  | +   | +    | ++  | +   | +   | ++   | ++   | +++  | ++   |
| 242296 | +        | ++  | ++  | ++++ | ++  | ++++ | —   | +   | —   | +    | +    | +    | +    |

+ &lt; 1 mg/L.

++ &gt; 1 mg/L, &lt; 5 mg/L.

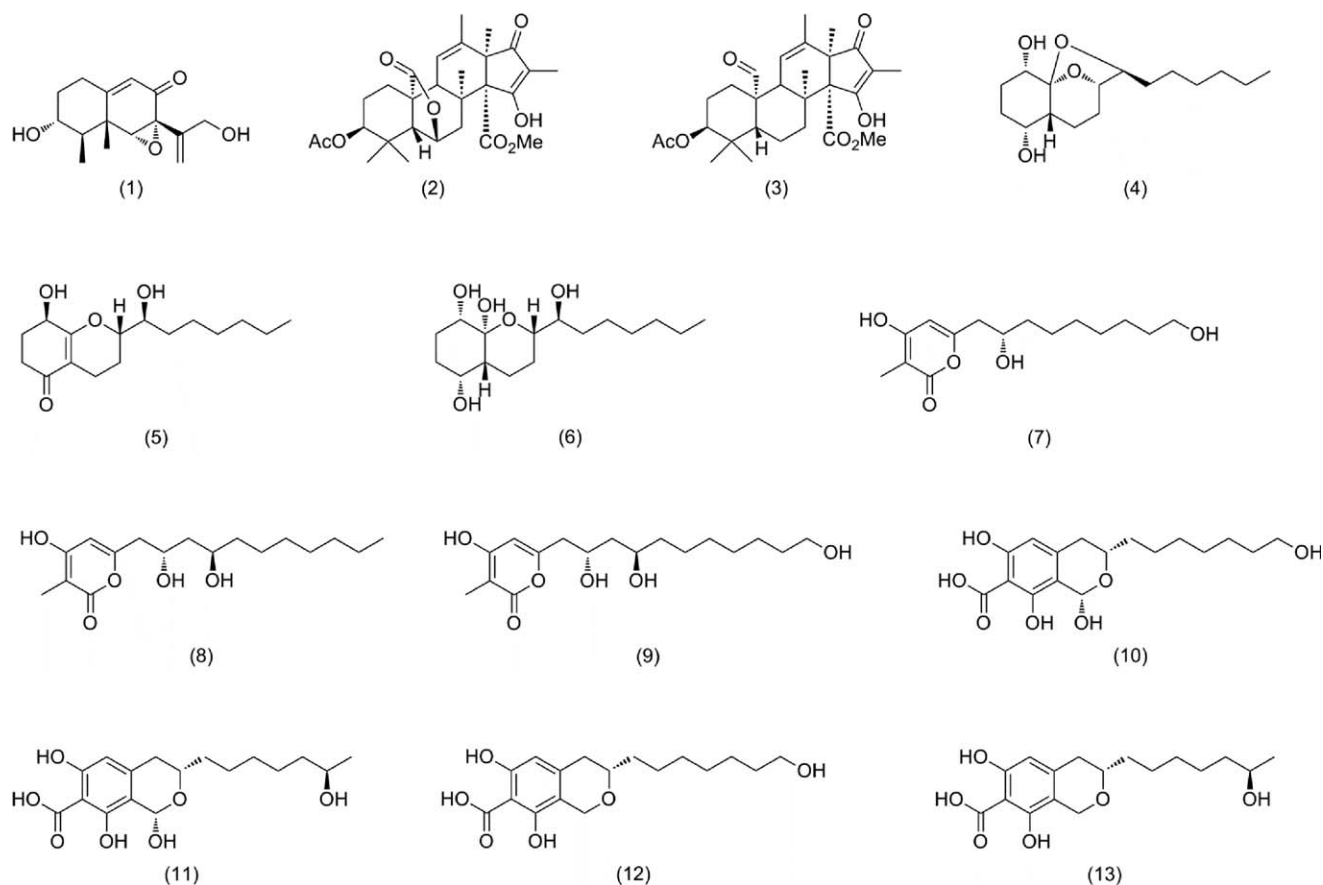
+++ &gt; 5 mg/L, &lt; 10 mg/L.

++++ &gt; 10 mg/L.

— not detected.

(2): (5.3 mg); clear gum[ $\alpha$ ]<sub>D</sub> +39.0 (c 0.20, MeOH); UV (MeOH)/nm  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (3.75), 279 (3.55); HRMS  $m/z$  501.2475 [M+H]<sup>+</sup> (calculated for 501.2488). <sup>1</sup>H and <sup>13</sup>C NMR data were consistent with published data (Kosemura et al. 1992).

*Andrastin A*: (3*S*,8*S*,10*S*,13*R*,14*R*)-methyl 3-acetoxy-10-formyl-15-hydroxy-4,4,8,12,13,16-hexamethyl-17-oxo-2,3,4,5,6,7,8,9,10,13,14,17-dodecahydro-1H-cyclopenta[*a*]phenanthrene-14-carboxylate (3): (3.4 mg); clear gum[ $\alpha$ ]<sub>D</sub> −30.0 (c 0.10, MeOH); UV (MeOH)/

FIG. 1. Secondary metabolites isolated from *P. corylophilum* from buildings.

nm  $\lambda_{\max}$  (log  $\epsilon$ ) 210 (4.05), 284 (3.75); HRMS  $m/z$  487.2723  $[M+H]^+$  (calculated for 487.2696).  $^1H$  and  $^{13}C$  NMR data were consistent with published data (Shiomi et al. 1996).

**Koninginin A:** (2*S*, 3*S*, 5*aR*, 6*R*, 9*S*, 9*aR*)-2-hexyloctahydro-2*H*-3, 9*a*-epoxy-1-benzoxepine-6, 9-diol (4): (22.0 mg); light yellow powder;  $[\alpha]_D -33.0$  (c 0.30, MeOH); UV (MeOH)/nm  $\lambda_{\max}$  (log  $\epsilon$ ) 220 (2.45), 262 (2.47); HRMS  $m/z$  285.2085  $[M+H]^+$  (calculated for 285.2066).  $^1H$  and  $^{13}C$  NMR data were consistent with published data (Cutler et al. 1989b).

**Koninginin E:** (2*S*, 8*R*)-8-hydroxy-2-([*S*]-1-hydroxyheptyl)-3, 4, 7, 8-tetrahydro-2*H*-chromen-5(6*H*)-one (5): (6.0 mg); light yellow powder;  $[\alpha]_D +8.3$  (c 0.30, MeOH); UV (MeOH)/nm  $\lambda_{\max}$  (log  $\epsilon$ ) 205 (2.94), 265 (3.40); HRMS  $m/z$  283.1927  $[M+H]^+$  (calculated for 283.1909).  $^1H$  and  $^{13}C$  NMR data were consistent with published data (Parker et al. 1995).

**Koninginin G:** (2*S*, 4*aR*, 5*R*, 8*S*, 8*aS*)-2-((*S*)-1-hydroxyheptyl) octahydro-2*H*-chromene-5, 8,8*a*-triol (6): (5.3 mg); light yellow powder;  $[\alpha]_D +61.3$  (c 0.30, MeOH); UV (MeOH)/nm  $\lambda_{\max}$  (log  $\epsilon$ ) 205(2.96), 264 (3.43); HRMS  $m/z$  302.2209  $[M+H]^+$  (calculated for 302.2171).  $^1H$  and  $^{13}C$  NMR data were consistent with published data (Cutler et al. 1999).

Regardless of geographic origin, the strains examined produced these compounds in yields of the individual compounds from 0.5 to >10 mg/L, with an average total yield of ~140 mg/L. We could not detect two of the three alpha pyrones from DAOM 242296 and one was not detected in DAOM 242290 (TABLE II). The calibration curves generated for each compound had an  $R^2$  value greater than 0.97 ( $n = 9$ ,  $P = 0.002$ ) over the entire concentration range based on triplicate analyses. The concentrations of compounds 1–13 are reported as the mean of triplicate fermentations in Roux bottles for each strain. The mycotoxin citrinin was not isolated and could not be detected by LC-UV-MS in each of the nine *P. corylophilum* extracts by comparison to an authentic standard.

#### DISCUSSION

The present report is the first comprehensive examination of secondary metabolites produced by *P. corylophilum* (FIG. 1, TABLE II). We confirm that this species produces phomenone (1), but in contrast to previous investigations our strains did not produce the isocoumarins (+) orthosporin and citreoisocoumarinol (Lai et al. 1991, Malmström et al. 2000). The latter authors were able to detect the two isocoumarins in all strains except two that were maintained too long in culture collections but could not confirm the presence of phomenone (1) or furan-2-carboxylic

TABLE III.  $^1H$  NMR Data (400 MHz) and  $^{13}C$  NMR Data (100 MHz) for phomenone (1) in  $CD_3OD$

| Position | $\delta_C$ , type | $\delta_H$ (J, Hz)                     |
|----------|-------------------|----------------------------------------|
| 1        | 32.0, $CH_2$      | 2.60, m<br>2.33, m                     |
| 2        | 36.4, $CH_2$      | 2.10, m<br>1.34, m                     |
| 3        | 71.3, CH          | 3.58, m                                |
| 4        | 46.0, CH          | 1.72, m                                |
| 5        | 42.0, C           |                                        |
| 6        | 70.4, CH          | 3.39, s                                |
| 7        | 62.8, C           |                                        |
| 8        | 194.6, C          |                                        |
| 9        | 121.3, CH         | 5.71, d (1.8)                          |
| 10       | 166.9, C          |                                        |
| 11       | 145.9, C          |                                        |
| 12       | 113.3, $CH_2$     | 5.25, m<br>5.20, m                     |
| 13       | 64.0, $CH_2$      | 4.34, br d (13.8)<br>4.27, br d (13.8) |
| 14       | 11.6, $CH_3$      | 1.30, s                                |
| 15       | 18.8, $CH_3$      | 1.23, d (6.82)                         |

acid due to lack of reference material. Complete NMR data for phomenone had not been reported and are presented herein (TABLE III). Other metabolites including fumiquinazolin F, decarestrictines A–D, epoxyagroclavine-I, agroclavine-I and quinocitrinin A and B were reported from *P. corylophilum* by Grabley et al. (1992), Silva et al. (2004) and Kozlovskii et al. (2013). The strains used in these studies were not deposited in culture collections for further examination. None of these compounds or structurally similar metabolites were produced by the present strains.

Phomenone (1) was isolated from pathogenic *Phoma* species and is a potent phytotoxin (Capasso et al. 1984). Since then it has been isolated from several taxonomically diverse fungi including *Drechslera gigantea* (Bunkers et al. 1990) and various strains of a *Xylaria* species (e.g. Isaka et al. 2000). Phomenone is structurally similar to other eremophilane sesquiterpenes including PR toxin produced by *P. roqueforti* (Capasso et al. 1984).

Our work also has resulted in a number of first metabolite reports. Citreohybridonol (2) originally was isolated from the mycelium of *P. citreonigrum* (formerly called *P. citreo-viride*) along with some related compounds (Kosemura et al. 1992). It appears to inhibit feeding by the diamond black moth, *Plutella xylostella*, a pest of some crucifers (Kosemura 2003). Another meroterpenoid, andrastin A (3), was isolated from these Canadian strains of *P. corylophilum*. Andrastin A is produced by various *Penicillium* species including the *P. roqueforti* complex isolated

from silage (Nielsen et al. 2005), *P. crustosum* (Sonjak et al. 2005) and *P. albocoremium* (Overy et al. 2005). Andrastin A is a fairly potent inhibitor of farnesyl transferase (Shiomi et al. 1996, Overy et al. 2005).

This is the first report of koniginins from any genus outside *Trichoderma*. Koninginin A (4), E (5) and G (6) were isolated from the culture filtrates of all nine strains of *P. corylophilum*. Koninginin A, the first reported metabolite from this natural products class, originally was characterized from a strain of *Trichoderma koningii* isolated from a wilting plant (Cutler et al. 1989b) and since has been reported from *T. harzianum* (Ghisalberti and Rowland 1993). Following this, koniginin E also from *T. koningii* (Parker et al. 1995) and koniginin G from *T. aureoviride* were reported (Cutler et al. 1999). These metabolites inhibit plant growth (Cutler et al. 1989b) and have modest antifungal activity (Ghisalberti and Rowland 1993). Other koniginins were present in these *P. corylophilum* extracts in minor amounts based on UV and MS data.

As noted, several new compounds produced by *P. corylophilum* strains were isolated from fractions of culture filtrate extracts not routinely examined (McMullin et al. 2014). The first alpha pyrones to be isolated from *P. corylophilum*, compounds 7–9, were detected in all but two of the nine strains investigated by LC-MS. At least one of these compounds was detected in each strain, indicating all strains examined produce this family of compounds (TABLE II). The failure to detect all compounds in each strain is possibly because several of the metabolites were present only in trace amounts. Citing unpublished HPLC UV data, Rahbaek et al. (2003) reported that they never observed alpha pyrones from *P. citreovirens* and *P. citreo-viride*. However, *P. citreo-viride* produces a wide variety of phenolics, meroterpenoids and alpha pyrones including citreoviridin (Lai et al. 1991, Kosemura 2003). Citreoviridin is a potent inhibitor of mitochondrial ATP-synthesis/hydrolysis as are some other pyrones and meroterpenoids (Sakabe et al. 1997, Kosemura 2003). Similar alpha pyrones were reported from *P. verrucosum* and the related ochratoxin-producing species *P. nordicum* and *P. olsonii* (Rahbaek et al. 2003). The alpha pyrones isolated from ochratoxin-producing fungi were isolated from the same medium used in the present study.

From a taxonomic perspective it is not surprising that isochromans (compounds 10–13) were produced by the strains investigated here. Cutler et al. (1989a) reported the isochroman DHMI as a plant growth regulator of *P. corylophilum* because it caused etiolation of wheat coleoptiles. However, this isochroman originally was characterized from a *P. steckii* strain isolated from moldy millet implicated in the

death of some cattle (Cox et al. 1979). DHMI and another related compound, 3, 7-dimethyl-1,8-dihydroxy-6-methoxyisochroman, were characterized from the polar extract fractions of a marine *P. steckii* isolate (Malmström et al. 2000). These isochromans are structurally similar to the mycotoxin citrinin. Malmström et al. (2000) did not observe any citrinin in the culture filtrate extracts of 10 *P. corylophilum* strains. Citrinin also was not observed in the extracts of the strains we tested, although some authors have reported citrinin from *P. corylophilum* (El-Kady et al. 1994, dos Santos et al. 2011). However, the isolates were not identified by *Penicillium* specialists and were not deposited in a culture collection. The absence of citrinin is consistent with the separation of *P. corylophilum* from citrinin-producing *Penicillium* species (Houbraken and Samson 2011).

Our investigation of secondary metabolites produced by *P. corylophilum* strains isolated from buildings from Halifax on the east coast to Victoria on the west coast has revealed a number of expected, unexpected and previously unknown compounds. The identification of isochromans and phomenone was consistent with the literature. The report of the meroterpenoid andrastin A is consistent with unpublished data from some European strains (Prof Jens Frisvad pers comm). The identification of the koniginins from *P. corylophilum* is remarkable, but there are other examples of secondary metabolites appearing in unrelated taxa, and these appear to be increasing in number. We had found that the use of larger scale fermentations with various culture media have produced unexpected metabolites even in well studied penicillia (Nielsen et al. 2006). *Penicillium corylophilum* was included in the *P. citrinum* series by Raper and Thom (1949) and series *Citrina* by Pitt (1979). The metabolite data presented here, notably the absence of citrinin, support the transfer of *P. corylophilum* from *Penicillium* section *Citrina* by Houbraken and Samson (2011) to their section *Exilicaulis*.

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Supplementary Table I.

|                                                 | Cultures                        |                                 |                                 |                                 |                                 |                                 |                                 |                                 |                                 |
|-------------------------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Paracel Laboratories Identification Number      | L6271-10                        | L5214-2                         | Louis-1                         | L5314-5                         | L5640-13                        | L5566-8                         | L5643-4d                        | L5379-5                         | L5566-7                         |
| Culture species:                                | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> |
| Habitat (soil, farm, forest, greenhouse, etc.): | indoor air sample               | indoor air sample               | indoor air sample               | indoor air sample               | moldy building material         | indoor air sample               | moldy building material         | indoor air sample               | indoor air sample               |
| Country:                                        | Canada                          | Canada                          | Canada                          | Canada                          | Canada                          | Canada                          | Canada                          | Canada                          | Canada                          |
| Province:                                       | BC                              | Ontario                         | Ontario                         | Ontario                         | Quebec                          | Alberta                         | Nova Scotia                     | Ontario                         | Alberta                         |
| City:                                           | Victoria                        | Ottawa                          | Ottawa                          | Ottawa                          | Montreal                        | Calgary                         | Halifax                         | Ottawa                          | Calgary                         |
| Name of collector of sample:                    | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            | Paracel Laboratories            |
| Date of first collection in field:              | 24-May-06                       | 27-Mar-06                       | 30-Mar-06                       | 29-Mar-06                       | 19-Apr-06                       | 13-Apr-06                       | 20-Apr-06                       | 05-Apr-06                       | 13-Apr-06                       |
| Name of isolator in lab:                        | Marcia Bertrand                 | Marcia Bertrand                 | Don Belisle                     | Marcia Bertrand                 | Marcia Bertrand                 | Marcia Bertrand                 | Marcia Bertrand                 | Marcia Bertrand                 | Marcia Bertrand                 |
| Date of isolation in lab:                       | 02-Jun-06                       | 06-Apr-06                       | 06-Apr-06                       | 06-Apr-06                       | 27-Apr-06                       | 27-Apr-06                       | 27-Apr-06                       | 13-Apr-06                       | 27-Apr-06                       |
| Media used for isolation:                       | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          | 2% MEA                          |
| Culture first identified by:                    | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     | Don Belisle                     |
| NFIS Code:                                      | 2012M_165                       | 2012M_169                       | 2012M_164                       | 2012M_163                       | 2012M_170                       | 2012M_167                       | 2012M_162                       | 2012M_168                       | 2012M_166                       |
| NFIS ID:                                        | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> | <i>Penicillium corylophilum</i> |
| Authors of NFIS ID:                             | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      | T. Barasubiye & K. Seifert      |
| Date of NFIS ID:                                | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       | 03-Jul-12                       |
| Accession number in CFCC:                       | DAOM 242296                     | DAOM 242288                     | DAOM 242289                     | DAOM 242290                     | DAOM 242291                     | DAOM 242292                     | DAOM 242293                     | DAOM 242294                     | DAOM 242295                     |