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MINOR METABOLITES OF *MONOCILLIUM NORDINII*

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Key Word Index—*Monocillium nordinii*; fungal metabolites; monocillins; nordinone; nordinonediol; polyketides.

Abstract—Examination of the metabolites produced in liquid still culture by *Monocillium nordinii* resulted in the isolation and characterization of two new compounds, nordinone and nordinonediol, as well as the known compounds monorden, monocillins I–IV and sterigmatocystin. The transformation of monocillin I into monorden is reported.

INTRODUCTION

Monocillium nordinii (Bourchier) W. Gams is a destructive mycoparasite of the pine stem rusts *Cronartium coleosporoides* Arth. and *Endocronartium harknessii* (J. P. Moore) Y. Hiratsuka [1]. Outbreaks of these rusts in western Canada cause severe damage to hard pines.

Previous work [2] has shown that two of the metabolites produced by *M. nordinii* are active against pine stem rusts as well as other fungi, including *Ceratocystis ulmi*, the fungus responsible for Dutch elm disease. The two active components have been identified as monorden (**1**) [3–6] and monocillin I (**2**). Other compounds isolated and identified from the same culture filtrates of *M. nordinii* included monocillins II–V (**3–6**) [2]. Purification of the mycelium extract afforded monorden and the monocillins in addition to averufin (**7**) and the yellow pigment sterigmatocystin (**8**) [7]. In this paper we report the metabolites produced by a different isolate of *M. nordinii* when grown in liquid still culture. Two new compounds, which we have named nordinone (**9**) and nordinonediol (**10**), as well as compounds **1–5** were isolated from the broth extract. The mycelial extract gave the ubiquitous ergosterol *endo*-peroxide and sterigmatocystin (**8**) [7].

RESULTS AND DISCUSSION

During the course of obtaining further quantities of monorden (**1**) and monocillin I (**2**) for biological testing we have examined the metabolites produced by a strain of *M. nordinii* different from the one used in our initial study [2]. In addition to the previously reported metabolites, two new compounds, nordinone (**9**) and nordinonediol (**10**), have been isolated and identified.

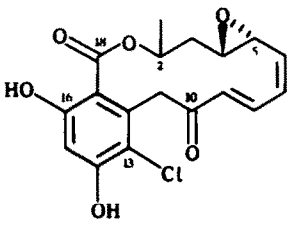
Nordinone (**9**), mp 130–134°, was obtained by preparative TLC purification of a mixture which contained monocillin II (**3**) as the major component. Compound **9** was less polar (R_f 0.59; Et_2O–Skellysolve B, 3:1) than monocillin II (**3**, R_f 0.47) on TLC. The high-resolution mass spectrum (HRMS of **9** showed a molecular ion peak at *m/z* 320 corresponding to a molecular formula of $C_{18}H_{24}O_5$, and a base peak at *m/z* 150 ($C_8H_6O_3$, **11**) characteristic of the monocillins [2]. The IR spectrum of **9** was very similar to that of monocillins IV (**5**) and V (**6**), displaying absorptions typical of a saturated ketone (1710 cm^{-1}) and of a hydrogen-bonded *o*-hydroxybenzoate (1645 cm^{-1}).

In the ^1H NMR spectrum of compound **9** there were signals for *meta*-coupled aromatic protons (δ 6.27 and 6.02, J = 2.5 Hz), a low-field proton (δ 5.35, *m*) coupled to a methyl doublet and benzylic methylene protons (δ 4.50 and 3.52). No vinylic protons were observed. These data are consistent with a structure having one less unsaturation than monocillin IV (**5**). The structure was confirmed by chemical correlation. Hydrogenation [6] of monocillin II (**3**) afforded a single product which was identical (TLC, IR, HRMS) with nordinone (**9**).

Nordinonediol (**10**), mp 194–196°, was isolated when the crude metabolites were separated by gel filtration through Sephadex LH-20 followed by flash chromatography. The high-resolution mass spectrum of compound **10** had a molecular ion peak at *m/z* 352 corresponding to the molecular formula $C_{18}H_{24}O_7$ (confirmed by CIMS; *m/z* 370, $M^+ + 18$; reagent gas ammonia) and showed fragments at *m/z* 334 ($C_{18}H_{22}O_6$) and *m/z* 316 ($C_{18}H_{20}O_5$) corresponding to the loss of one and two molecules of water, respectively, from the molecular ion. The base peak characteristic of monocillins at *m/z* 150 ($C_8H_6O_3$, **11**) was again observed. The IR spectrum of **10** showed absorptions characteristic of free (3615 cm^{-1}) and hydrogen-bonded (3400 cm^{-1}) hydroxyl groups [8], ketone carbonyl (1682 cm^{-1}) and the carbonyl of a hydrogen-bonded *ortho*-hydroxyester (1644 cm^{-1}).

The ^1H NMR spectrum of **10** indicated the presence of two secondary alcohol functions [one proton signals at δ 3.78 (*br s*) and 3.44 (*br s*)]. Acetylation of compound **10** (acetic anhydride–pyridine) afforded a tetraacetate derivative ($C_{26}H_{32}O_{11}$, M_r 520), whose IR spectrum did not display hydroxyl absorption. These data were consistent with the diol structure **10** derived by opening the epoxide ring in monocillin V (**6**), and was confirmed when nordinonediol (**10**) was obtained by perchloric acid treatment of **6**. Assuming a *trans*-opening of the epoxide, nordinonediol is a *cis*-diol, but we have not established whether opening occurred at C-4 or C-5 and thus cannot specify the absolute stereochemistry at these two centres.

Previous attempts to correlate monocillin I (**2**) with monorden (**1**) by chlorination of **2** have been unsuccessful [2]. However, chemical correlation has now been achieved by treatment of monocillin I (**2**) with *N*-chlorosuccinimide



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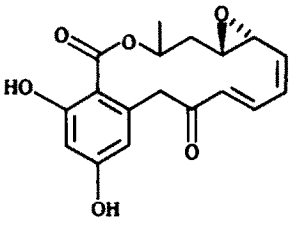

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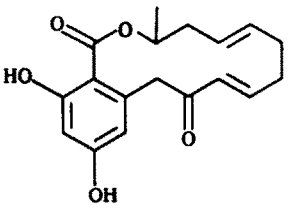

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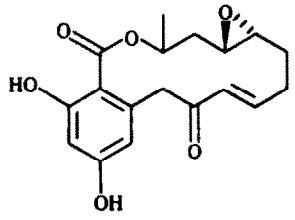

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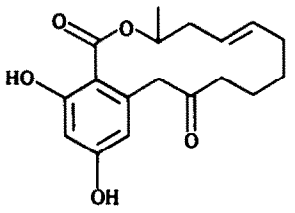

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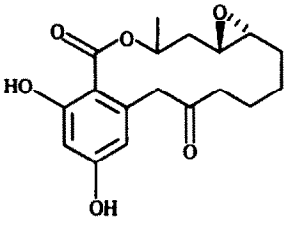

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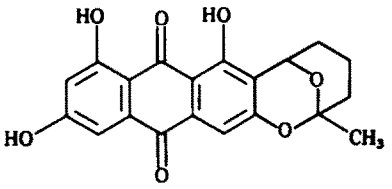

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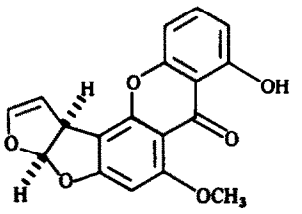

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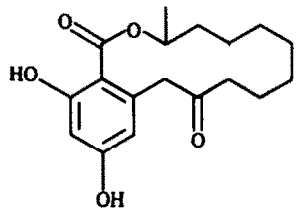

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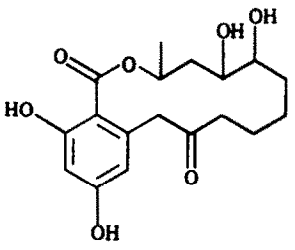

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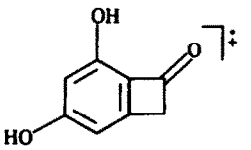

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in dimethyl formamide. The product of this reaction contained a single major component as shown by TLC. This compound is identical (TLC behaviour and spectroscopic data) with natural monorden (1).

EXPERIMENTAL

General procedures. Water for fungal culturing was distilled in an all-glass apparatus prior to use. Solvents for extraction and purification were distilled prior to use. Skellysolve B refers to Skelly Oil Company light petroleum, bp 62–70°. E. Merck silica gel 60 (70–230 mesh or 230–400 mesh) was used for CC. Analytical TLC was carried out on E. Merck precoated TLC plates of silica gel 60 F-254 (0.25 mm thickness). The chromatograms were examined under UV (254 or 350 nm) or by placing them in a chamber containing I_2 vapour. Visualization was completed by spraying with 30% H_2SO_4 followed by careful charring with a heat gun. Preparative TLC was carried out on E. Merck precoated TLC plates of silica gel 60 F-254 (20 × 20 cm, 0.25 mm thickness). Materials were detected by viewing under UV (254 or 350 nm). Mps were determined on a Leitz Weztlar microscope heating stage and are uncorr. IR spectra were recorded on a Nicolet 7199 FT interferometer. High-resolution electron impact mass spectra (EIMS) were recorded on an AEI MS-50 mass spectrometer coupled to a DS-50 data system. Chemical ionization mass spectra (CIMS) were obtained using an AEI MS-12 mass spectrometer with NH_3 as the reagent gas. Unless diagnostically significant, any peaks with intensities less than 20% of the base peak are omitted. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker WH-200 or WH-400 spectrometer with an Aspect 2000 computer system. All NMR measurements employed TMS as internal standard.

Plant material. The strain of *Monocillium nordinii* (C 690) isolated by A. Tsuneda in 1979 from western gall rust growing on Lodgepole pine in British Columbia and deposited in the Northern Forest Research Centre fungal culture collection [9] was used in this study. It was obtained from Y. Hiratsuka, Northern Forest Research Centre, Canadian Forestry Service, Edmonton. Cultures were maintained and liquid cultures prepared as previously described [2].

Separation and characterization of metabolites. Modified flash chromatography was employed to separate the crude broth extract which was prepared as described previously [2]. Broth extract (212 mg) was adsorbed on silica gel and placed on top of a silica gel column (20 × 3 cm). Final packing, air expulsion and flow rate control (2 in/min) were performed by passing Skellysolve B through the column (3X). Separation of the mixture was carried out using Et_2O–Skellysolve B (3:1). Fractions of 20 ml were collected, monitored by TLC and, if similar, combined, giving 10–15 final fractions.

The first metabolite eluted was monocillin IV (**5**), followed by monocillin II (**3**), nordinone (**9**), monocillin I (**2**), monorden (**1**), monocillin III (**4**) and nordinonediol (**10**). Usually monorden (**1**) and monocillin III (**4**) were obtained in pure, crystalline form. The other metabolites were obtained by further purification using preparative TLC. Ergosterol *endo*-peroxide and sterigmatocystin (**8**) were isolated from the mycelium extract.

Isolation of nordinone (**9**). The mixture of monocillin II (**3**) and a less polar component (40.2 mg) was separated by preparative TLC (20 × 20 cm; Et_2O–Skellysolve B, 3:1; 2 ×). The less polar compound (6 mg), identified as nordinone (**9**) (3,4,5,6,7,8,9,10-octahydro-14,16-dihydroxy-3-methyl-1H,2-benzoxacyclotetradecin-1,11(12H)-dione) was obtained a white powder, mp 130–134°. TLC: R_f 0.59 (Et_2O–Skellysolve B, 3:1). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3360, 1710, 1645, 1619 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 6.27 (1 H, *d*, J = 2.5 Hz), 6.02 (1 H, *d*, J = 2.5 Hz), 5.35 (1 H, *m*), 4.50 (1 H, *d*, J = 18 Hz), 3.52 (1 H, *d*, J = 18 Hz), 2.65–1.6 (10 H, *m*), 1.35 (3 H, *d*, J = 7 Hz). HRMS: *m/z* (rel. int.) calc. for $C_{18}H_{24}O_5$ [M]$^+$: 320.1624; found: 320.1614 (3), 150 (100).

Hydrogenation of monocillin II (**3**). Monocillin II (**3**, 28 mg) was dissolved in EtOH (10 ml) and Pd over $CaCO_3$ (10 mg) was added. The reaction mixture was stirred in a H_2 atmosphere for 50 min. TLC of the mixture did not show the presence of starting material. The catalyst was removed by gravity filtration and the solvent was removed *in vacuo* to yield the crude product (21.7 mg). Purification of this product by modified flash CC (Et_2O–Skellysolve B, 13:7; column 15 × 0.5 cm) afforded an amorphous white powder, mp 135–138°, identified as nordinone (**9**, 8 mg) on the basis of its TLC behaviour and spectroscopic data.

Isolation of nordinonediol (**10**). Gel filtration chromatography of the broth extract using Sephadex LH-20 as the adsorbent and MeOH as the eluant afforded five major fractions. Further purification of fraction 2 (276 mg) by flash chromatography ($CHCl_3$–MeOH, gradient elution 0.5–3%) yielded eight fractions. Nordinonediol (**10**, 7 mg) (3,4,5,6,7,8,9,10-octahydro-5,6,14,16-tetrahydroxy-3-methyl-1H,2-benzoxacyclotetradecin-1,11(12H)-dione) crystallized from fraction 7 as large colourless crystals, mp 194–196°. TLC: R_f 0.17 (Et_2O). IR ν_{max}^{KBr} cm^{-1}: 3515, 3400, 3182, 2965, 2935, 1682, 1644, 1469, 1265. ^1H NMR (400 MHz, DMSO-d_6): δ 10.38 (1 H, *s*), 9.92 (1 H, *s*), 6.20 (1 H, *d*, J = 2 Hz), 6.08 (1 H, *d*, J = 2 Hz); 4.9 (1 H, d, J = 2.5 Hz), 4.90 (1 H, *m*), 4.42 (1 H, *d*, J = 2.5 Hz), 4.04 (1 H, *d*, J = 18 Hz), 3.86 (1 H, *d*, J = 18 Hz), 3.78 (1 H, *br s*), 3.44 (1 H, *br s*), 2.3–1.4 (6 Hz, *m*), 1.04 (3 H, *d*, J = 6 Hz). HRMS: *m/z* (rel. int.) calc. for $C_{18}H_{24}O_7$ [M]$^+$: 352.1522; found: 352.1524 (13), 334 (4), 316 (4), 150 (100). CIMS (NH_3) *m/z* (rel. int.): 370 (100), 353 (30), 335 (26).

O,O,O,O-Tetraacetylnordinonediol. Nordinonediol (**10**, 3 mg) was treated with Ac_2O (3 ml) and pyridine (1.5 ml). The reaction mixture was stirred overnight at room temp. then poured over ice water (20 ml). The suspension was extracted with EtOAc (3 × 10 ml) and the organic extract worked up in the usual manner to yield 2.7 mg of crude product. Purification of the crude product by flash CC (Et_2O–Skellysolve B, 3:1; packed in a dispo pipette) afforded *O,O,O,O*-tetraacetylnordinonediol (1.2 mg) (3,4,5,6,7,8,9,10-octahydro-5,6,14,16-tetraacetoxy-3-methyl-1H,2-benzoxacyclotetradecin-1,11(12H)-dione). TLC: R_f 0.23 (Et_2O–hexane, 3:1; 2 ×). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 2920, 1774, 1736, 1370, 1259, 1022. HRMS *m/z* (rel. int.) calc. for $C_{26}H_{32}O_{11}$ [M]$^+$: 520.1944; found: 520.1947 (18), 478 (100), 460 (6), 436 (9), 418 (25), 376 (4), 358 (21), 316 (7), 150 (82).

Nordinonediol (**10**) *from monocillin V* (**6**). Monocillin V (**6**, 4.5 mg) was dissolved in a mixture of H_2O–Me_2CO (1:1, 3 ml) and the mixture acidified with $HClO_4$ (70%, 1 drop). The reaction was left at room temp. overnight then poured over H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 10 ml). The organic layer was separated, dried (dry Na_2SO_4) and concentrated to yield 3.5 mg (74.4%) of a yellowish solid which was purified by flash chromatography and identified as nordinonediol (**10**) on the basis of spectroscopic and TLC evidence.

Monorden (**1**) *from monocillin I* (**2**). Monocillin I (**2**, 25 mg) was dissolved in DMF (0.5 ml). *N*-Chlorosuccinimide (10.1 mg) in DMF (0.5 ml) was added and the mixture was stirred at room temp. for 48 hr. The reaction mixture was poured into H_2O (20 ml). The resulting suspension was extracted with CH_2Cl_2 (3 × 15 ml) and the extract washed with H_2O (4 × 15 ml). The extract was dried and concentrated under red. pres. to yield the crude product (23.7 mg). TLC of the product showed the presence of two major components. These were separated by preparative TLC (Et_2O–Skellysolve B, 3:1; 3 ×) and identified as monocillin I (**2**, 18 mg) and monorden (**1**, 4 mg) by comparison of TLC behaviour and spectroscopic data with those of authentic samples.

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REFERENCES

1. Tsuneda, A. and Hiratsuka, Y. (1980) *Phytopathology* **70**, 1103.
2. Ayer, W. A., Lee, S. P., Tsuneda, A. and Hiratsuka, Y. (1980) *Can. J. Microbiol.* **26**, 766.
3. Delmotte, P. and Delmotte-Plaqueé, J. (1953) *Nature (London)* **171**, 344.
4. McCapra, F., Scott, A. I., Delmotte, P. and Delmotte-Plaqueé, J. (1964) *Tetrahedron Letters* 869.
5. Mirrington, R. N., Ritchie, E., Shoppee, C. W., Taylor, W. C. and Sternhell, S. (1964) *Tetrahedron Letters* 365.
6. Mirrington, R. N., Ritchie, E., Shopee, C. W. and Taylor, W. C. (1966) *Aust. J. Chem.* **19**, 1265.
7. Ayer, W. A., Peña-Rodriguéz, L. and Vederas, J. C. (1981) *Can. J. Microbiol.* **27**, 846.
8. Nakanishi, K. and Solomon, P. H. (1977) in *Infrared Absorption Spectroscopy*, 2nd edn, p. 25. Holden-Day, San Francisco.
9. Tetu-Bernier, P., Maruyama, P. J. and Hiratsuka, Y. (1983) Information Report NOR-X-249, Northern Forest Research Centre, Canadian Forestry Service, Environment Canada, Edmonton.