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BIOLOGICALLY ACTIVE PHENOLIC METABOLITES OF A *VERTICICLADIELLA* SPECIES

WILLIAM A. AYER, LOIS M. BROWNE and SARAH H. LOVELL

Department of Chemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2G2

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Key Word Index—*Verticicladiella* sp.; Deuteromycotina; blue-stain fungus; phytotoxin; phenols; methylorcinol rhamnoside; phenol monomethyl ethers; antiseptics.

Abstract—The metabolites produced when a *Verticicladiella* species (Canadian Forestry Service strain C728), the causative agent of the black stain root disease of many conifers, is grown in liquid culture have been investigated. Orcinol, orcinol monomethyl ether, 1,3,6,8-tetrahydroxyanthraquinone, and the α-L-rhamnopyranosides of orcinol and orcinol methyl ether have been isolated and identified. Orcinol methyl ether and its rhamnoside both show antibacterial activity and orcinol methyl ether also inhibits the growth of pine germlings. The general antibacterial activity of 5-alkylresorcinols and their monomethyl ethers is reported.

INTRODUCTION

Black stain root disease, which imparts longitudinal dark stains to the root and butt wood of conifers, is caused by fungi belonging to the genus *Verticicladiella*. The disease, which in Canada occurs mainly on Douglas fir, lodgepole pine and western hemlock, is debilitating to the tree and frequently results in mortality. However, the means by which the fungal disease kills host trees and the manner in which the disease spreads are poorly understood [1].

In western North America, black stain root disease is generally attributed to *Verticicladiella wagnerii* Kendrick although in western Canada two species or forms of *Verticicladiella* may cause this disease. We have now studied several virulent isolates of *Verticicladiella*, some of which are responsible for damage to conifers in our National Parks. One of these, *Verticicladiella* sp. C728, when grown on liquid medium produces phenolic compounds which show antibiotic and phytotoxic activity. We report herein the isolation and identification of the antibiotic and phytotoxic compounds.

RESULTS AND DISCUSSION

The biologically active constituents of the culture broth of *Verticicladiella* sp. C728 were readily extracted with ethyl acetate. Examination of the TLC of this extract showed the presence of one major and three minor metabolites. Chromatographic separation over Si gel led to the isolation of orcinol monomethyl ether (**1a**), orcinol (**2a**), an orange pigment. $C_{14}H_8O_6$ (**3**) and a weakly bioactive component. Orcinol (**2a**) and its monomethyl ether (**1a**) were unambiguously identified by comparison with authentic samples [2]. Compound **1a** comprises *ca* 40% of the crude extract of *Verticicladiella*.

A comparison of the antibiotic activity of **1a** with that of the crude extract using the disc-agar diffusion method [3] is presented in Table 1. From this data it appears that the antibiotic activity of the extract must be due primarily to the presence of orcinol monomethyl ether (**1a**).

Table 1. Antibiotic screening* of some extracts of *Verticicladiella* C728

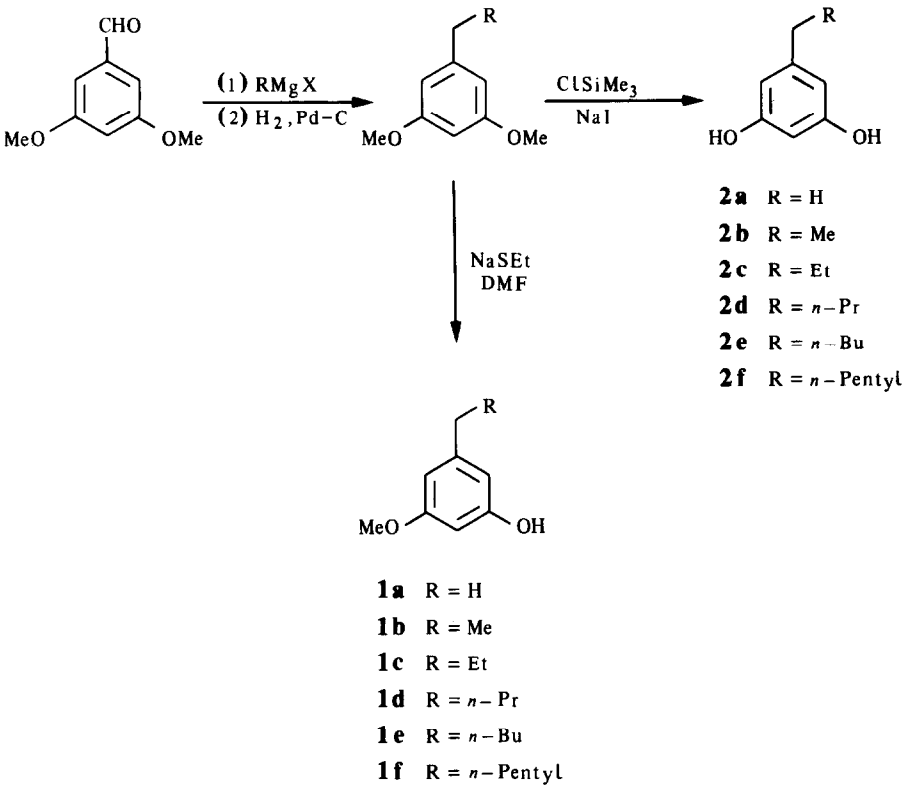
Micro-organism	ATCC No.	Crude extract†	Orcinol monomethyl ether (**1a**)	
		10%	5%	2%
Enterobacter cloacae	23355	16	—	N
Escherichia coli	25922	18	16	8
Klebsiella pneumoniae	13883	16	—	N
Proteus vulgaris	13315	17	26	10.5
Pseudomonas aeruginosa	27853	13	—	—
Salmonella typhimurium	14028	16	28	7
Serratia marcescens	8100	12	24	—
Staphylococcus aureus	25923	13	—	N
Candida albicans	UAH 3468	24	20	9.5

*Zone diameters of inhibition expressed in mm.

†The authors thank Katherine D. Ayer and J. Robin Browne for this data which was taken from a Special High School Biology report.

—, Values not documented. N, No activity.

The bactericidal effect of phenols is well-established. They must possess lipid solubility since most interact with the cell membrane of bacteria. Structural modifications of phenols that increase lipid solubility (e.g. alkylation) tend to increase biological activity [4]. For example, 4-hexylresorcinol finds use as a topical antiseptic and astringent in many proprietary products. However, the enhanced biological activity of a monomethyl ether of an alkyl substituted resorcinol appears not to be documented. In order to determine the scope of this phenomenon we prepared a series of 5-alkylresorcinols and 5-alkylresorcinol monomethyl ethers as outlined in Scheme 1 and described in the Experimental. The results of antibiotic



2a R = H
2b R = Me
2c R = Et
2d R = *n*-Pr
2e R = *n*-Bu
2f R = *n*-Pentyl

1a R = H
1b R = Me
1c R = Et
1d R = *n*-Pr
1e R = *n*-Bu
1f R = *n*-Pentyl

Scheme 1.

screening of the 5-alkylresorcinols and their monomethyl ether derivatives using the disc agar diffusion method are presented in Table 2. These data reveal the general trend that the monomethyl-5-alkylresorcinols possess stronger bactericidal properties than the 5-alkylresorcinol itself when alkyl equals methyl to propyl (**1a**–**1c** compared with **2a**–**2c**). When alkyl equals butyl the bactericidal properties of the phenol and its monomethyl ether are about the same (**1d**, **2d**) whereas increasing the alkyl chain to hexyl results in enhanced biological activity of the resorcinol derivative over its monomethyl ether (**1e**, **1f** compared with **2e**, **2f**). It is interesting to note that 5-hexylresorcinol (**1f**) is less potent than 4-hexylresorcinol under these bioassay conditions.

The orange pigment **3** was present in very small quantities (2–3 mg/l.) in the broth and the mycelial extracts. Compound **3** was difficult to purify even after several chromatographic separations of the broth extract. This problem was readily overcome by first defatting the mycelium by extraction with Skellysolve B, followed by extraction with diethyl ether, then ethyl acetate. Chromatography of this diethyl ether extract over Si gel led to isolation of pure **3** whose spectral properties showed it to be 1,3,6,8-tetrahydroxy-9,10-anthraquinone

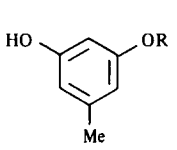
Table 2. Antibiotic screening* of some 5-alkylresorcinols and their monomethyl ethers

	Compound (2% soln)												
Micro-organism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**	4-Hexylresorcinol
C. albicans	9.5	11	20	17	15.5	14	N	7	10	12	18	19	21
C. cloacae	N	12.5	11	9	—	N	N	7	17	11	10	11	15
E. coli	8	10	9.5	9.5	7.5	N	N	N	9	16	16	14	15
K. pneumoniae	N	9	9	N	N	N	N	N	8	15	18	10	13
P. vulgare	10.5	13.5	15	18.5	—	9.5	N	N	13	20	22	25	31
S. typhimurium	7	8	8	N	N	N	N	N	N	N	11	11	13
S. aureus	N	9	13.5	12.5	13	12	N	N	11	15	16	16	21
S. epidermis	N	N	12.5	11	11.5	11.5	N	N	N	14	17	19	20

*Zone diameters of inhibition expressed in mm.
N, No activity.

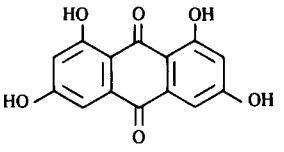
[5, 6]. Comparison of orange pigment **3** with an authentic sample of 1,3,6,8-tetrahydroxy-9,10-anthraquinone revealed their identity. This pigment, which had previously been known only as a synthetic compound, has recently been isolated from *Aspergillus versicolor* [7].

The weakly bioactive component was isolated from the crude metabolites by CC, and although it showed a single spot by TLC in several solvent systems, its spectral data revealed that it was a mixture of compounds containing hydroxyl functionality. Thus, the component was acetylated in the usual way. The acetylated material was separated by TLC into a minor (**4**) and a major (**5**) component (ratio 1:2). The mass spectral data for each compound shows that they differ from one another by 28 mass units (a carbon and an oxygen) and that both compounds fragment to a common ion at *m/z* 273 below which the mass spectral fragmentation patterns are very similar. The fragment which is lost from the minor compound (**4**) is that derived from orcinol monomethyl ether, while from the major compound (**5**) is that from orcinol monoacetate.

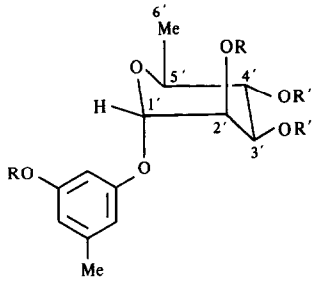


1a R = Me

2a R = H



3



4 R = Me, R' = COMe

5 R = R' = COMe

6 R = Me, R' = H

7 R = R' = H

The ^{1}H NMR of compound **4** is very similar to that of **5** except that in **5** an additional methoxycarbonyl signal replaces the methoxyl signal. The ^{1}H NMR of **4** shows signals expected for the orcinol monomethyl ether moiety, three acetoxy singlets and a methyl doublet coupled to one of five low-field methines. Spin decoupling experiments allowed the unequivocable assignment of coupling partners and showed that the common fragment in these closely related compounds was a 6-deoxy sugar bearing an axial oxygen substituent at C-2'. Thus, the weakly bioactive component was a mixture of glycosides which differed by the presence of a methoxyl (**6**) or hydroxyl (**7**) substituent in the aglycone. Acetylation of the mixture of **6** and **7** led to the isolation of their acetyl derivatives **4** and **5**.

The stereochemistry of the glycosidic linkage is based on the ^{13}C NMR C-1'–H-1' coupling constant since the H-1'–H-2' coupling constant of the anomeric proton signal of rhamnosides, which has an axial OH-2' group in its stable conformation, generally appears as a broadened singlet and thus is not diagnostic for determination of anomeric configuration. Bock and Pederson [8] have shown that the C–H coupling constant of C-1' ($^1J_{C\text{-}1'\text{-}H\text{-}1'}$) of hexapyranoses are characteristic of the anomeric configuration: $^1J_{C\text{-}1'\text{-}H\text{-}1'}$ is consistently *ca* 10 Hz smaller when H-1' is axial than when it is equatorial. The ^{13}C NMR spectra were determined for compounds **6** and **7** (obtained from **4** and **5**, respectively, by triethylamine-catalysed hydrolysis). The $^1J_{C\text{-}1'\text{-}H\text{-}1'}$ of 168.5 Hz observed in the ^{13}C NMR spectrum of both **6** and **7** shows that in each case the H-1' proton is equatorial and, thus, each compound is an α-rhamnoside. These values compare well with that reported for the α-L-rhamnoside of *p*-nitrophenol ($^1J_{C\text{-}1'\text{-}H\text{-}1'}$ = 168 Hz) [9].

Orcinol monomethyl ether (**1a**) shows phytotoxic activity in two different bioassays. *Chlorella* sp. ATCC 7516 cells swabbed on agar medium become colorless and die when filter paper discs containing **1a** were placed on the medium [10]. A similar inhibition has been observed for fomannosin, the toxic metabolite of the wood-decay fungus *Fomes annosus* [11]. Compound **1a** significantly inhibits growth of *Pinus barksiana* seedlings in a root growth bioassay. Germlings of *P. barksiana* with 5 mm radicals, when incubated with a 1% aqueous solution or orcinol monomethyl ether (**1a**), showed 40% reduction in growth and darkening of the radical when compared to control germlings [Mallett, K. and Hiratsuka, Y., personal communication]. Further biological testing is in progress.

EXPERIMENTAL

General procedures. High resolution MS were recorded on an AEI MS-50 mass spectrometer, coupled to a DS 50 computer. ^{1}H NMR spectra were determined using $CDCl_3$ as solvent unless otherwise specified. ^{13}C NMR spectra were determined with TMS as internal standard.

Si gel TLC plates were 0.5 mm Si gel G (E. Merck, Darmstadt) containing 1% electronic phosphor (General Electric, Cleveland). Compounds were detected by viewing under UV light or by spraying with 30% H_2SO_4 and charring. All solvents were distilled before use. Unless otherwise specified, dry $MgSO_4$ was used as a drying agent and Si gel 60 (BDH Chemicals, 70–230 mesh) as adsorbant for CC. V-8 juice is a juice of eight garden vegetables available from Campbell Soup, Canada.

Plant materials. The strain of *Verticicladiella* species (C728) (isolated from Douglas fir, Radium Hot Springs, B. C.) used in this study was obtained from Y. Hiratsuka, Northern Forest Research Center, Edmonton, and cultures were maintained at 4° in slant tubes containing Difco potato dextrose agar. An aq. suspension of mycelium was used to inoculate two agar plates (10% filtered V-8 juice, 1% glucose, 2% agar). After 7–10 days at room temp. the culture was blended in a Waring blender with *ca* 200 ml sterile media (10% filtered V-8 juice, 1% glucose) and *ca* 20 ml aliquots were used to inoculate 10 × 1 l. sterile medium in 2 l. flasks. After inoculation the still cultures were kept at room temp. for 6 weeks. The culture broth was decanted from the mycelium, concd *in vacuo* to *ca* 2 l. and continuously extracted with EtOAc for 24 hr. The EtOAc extract was dried and concd to an oil (0.75 g) which was separated as described below. The mycelium was subjected to successive continuous extractions in a Soxhlet extractor with Skellysolve B, Et_2O and EtOAc. The

organic extracts were dried, concd and separated as described below.

Separation and characterization of metabolites. The crude broth extract (0.75 g) was subjected to gradient flash chromatography over Si gel. Orcinol monomethyl ether (**1a**) (0.33 g, R_f 0.38, pentane–Et_2O, 4:1), orcinol (**2a**) (0.02 g, R_f 0.4, pentane–Et_2O, 3:2), 1,3,6,8-tetrahydroxyanthraquinone (**3**) (0.005 g, R_f 0.5, Et_2O, isolated as orange crystals) and component A (0.025 g, R_f 0.3, Et_2O–EtOAc, 1:1) were isolated. Component A was shown by spectroscopic data to be a mixture of two very similar compounds and attempted separation by Si gel or Al_2O_3 chromatography using a variety of solvent systems was unsuccessful.

Component A (0.020 g) was acetylated in the usual way. This product was separated into two oils: compound B (0.004 g, R_f 0.44, pentane–Et_2O, 3:2 double elution) and compound C (0.008 g, R_f 0.36, pentane–Et_2O, 3:2 double elution) were isolated by prep. TLC over Si gel (1 mm) eluting with Skellysolve B–EtOAc (7:3).

Compound B was identified as 3-methoxy-5-methylphenoxy-*O,O,O*-triacetyl-α-L-rhamnopyranoside (**4**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1750 (C=O); ^1H NMR: δ6.52 (1H, *s*, ArH), 6.46 (1H, *s*, ArH), 6.43 (1H, *s*, ArH), 5.51 (1H, *dd*, J = 3.2, 10 Hz, H-3′), 5.44 (1H, *d*, J = 2 Hz, H-1′), 5.42 (1H, *dd*, J = 2, 3.2 Hz, H-2′), 5.15 (1H, *t*, H-4′), 4.00 (1H, *dq*, J = 2.8, 8.8 Hz, H-5′), 3.78 (3H, *s*, OMe), 1.21 (3H, *d*, J = 2.8 Hz, H-6′); ^{13}C NMR: δ170.0 (3, OCOMe), 160.7 (*s*), 157.0 (*s*), 140.5 (*s*), 109.5 (*d*), 109.4 (*d*), 99.7 (*d*), 95.1 (*d*), 71.1 (*d*), 69.8 (*d*), 69.0 (*d*), 67.2 (*d*), 55.3 (*q*), 21.7 (*q*), 20.9 (*q*). Exact mass calc. for $C_{20}H_{26}O_9$: 410.1576. Found (MS): 410.1590.

Compound C was identified as 3-acetoxy-*O,O,O*-triacetyl-5-methylphenoxy-α-L-rhamnopyranoside (**5**). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1749 (C=O); ^1H NMR: δ6.78, 6.65, 6.61 (three 1Hs, ArH), 5.48 (1H, *dd*, J = 3.6, 10 Hz, H-3′), 5.44 (1H, *d*, J = 2 Hz, H-1′), 5.39 (1H, *dd*, J = 2.36 Hz, H-2′), 5.14 (1H, *t*, J = 10 Hz, H-4′), 3.96 (1H, *dq*, J = 2.6, 6 Hz, H-5′), four 3Hs at 2.33, 2.20, 2.07, 2.04 (OCOMe), 2.29 (3H, *s*, ArMe), 1.21 (3H, *d*, J = 6 Hz, H-6′); ^{13}C NMR: δ170.0 (*s*, 4 × OCOMe), 156.4 (*s*), 151.4 (*s*), 140.6 (*s*), 116.6 (*d*), 114.6 (*d*), 107.2 (*d*), 95.7 (*d*), 71.0 (*d*), 69.7 (*d*), 68.9 (*d*), 67.3 (*d*), 21.5 (*q*), 21.1 (*q*), 20.8 (*q*), 20.7 (× 2, *q*), 17.4 (*q*). Exact mass calc. for $C_{21}H_{26}O_{10}$: 438.1526. Found (MS): 438.1515.

Hydrolysis of compound **4**. Compound **4** (0.022 g) and excess NEt_3 (0.2 ml) in MeOH (0.08 ml), H_2O (2 ml) was allowed to stir at room temp. overnight. The reaction mixture was concd and dried *in vacuo* (0.1 τ, 24 hr) to give the free rhamnoside **6** as a glass (0.019 g). $[\alpha]_D^{25}$ −57.8 (MeOH; *c* 0.18); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3380 (OH); ^1H NMR: three 1Hs at δ6.47, 6.44, 6.39 (ArH), 5.50 (1H, *s*, H-2′), 4.16 (1H, *s*, $W_{1/2}$ = 5.4 Hz, H-1′), 4.02 (1H, *d*, J = 7.5 Hz, H-3′), 3.78 (1H, *m*, H-5′), 3.76 (*s*, OH), 3.62 (1H, *t*, J = 7.5 Hz, H-4′), 2.26 (3H, *s*, OMe), 1.30 (3H, *d*, J = 8 Hz, H-6′); ^{13}C NMR (pyridine-d_5): δ136.0, 110.1, 108.9, 100.7, 100.0 (*d*, $J_{C\text{-}1'\text{-}H\text{-}1'}$ = 168.5 Hz, C-1′), 73.8 (*d*), 72.6 (*d*), 72.1 (*d*), 70.9 (*d*), 55.2 (*q*), 21.7 (*q*), 18.7 (*q*). Exact mass calc. for $C_{14}H_{20}O_6$: *m/z* 284.1260. Found: *m/z* 284.1257.

Hydrolysis of compound **5**. Compound **5** (0.060 g) was hydrolysed as described above to give the free rhamnoside **7** (0.040 g) as an oil. $[\alpha]_D^{25}$ −76.9° (MeOH; *c* 0.36); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3300 (OH); ^1H NMR: three 1Hs at δ6.39, 6.36, 6.34 (ArH), 5.43 (1H, *d*, J = 1.5 Hz, H-1′), 4.56 (*br s*, OH), 4.05 (1H, *dd*, J = 1.5, 3.5 Hz, H-2′), 3.91 (1H, *dd*, J = 3.5, 9.5 Hz, H-3′), 3.73 (1H, *dq*, J = 6 Hz, H-5′), 3.51 (1H, *t*, J = 9.5 Hz, H-4′); ^{13}C NMR (pyridine-d_5): δ160.0, 140.4, 135.7, 111.0, 108.9, 102.2, 100.0 (*d*, $J_{C\text{-}1'\text{-}H\text{-}1'}$ = 168.5 Hz, C-1′), 73.8 (*d*), 72.6 (*d*), 72.0 (*d*), 70.7 (*d*), 21.6 (*q*), 18.5 (*q*). Exact mass calc. for $C_{13}H_{18}O_6$: *m/z* 270.1102. Found: *m/z* 270.1102.

3,5-Dimethoxybenzaldehyde. A soln of $(COCl)_2$ (5 ml) in C_6H_6 was added dropwise to a cooled stirred mixture of 3,5-dimethoxybenzoic acid (4.5 g) in C_6H_6 (100 ml). The reaction mixture was allowed to stir at room temp. for 2 hr, heated under reflux for 1 hr, then concd. Distillation at red. pres. (107°/0.5 mm Hg) gave 3,5-dimethoxybenzoyl chloride (4.42 g, 89% yield). A pressure vessel, charged in order with dry toluene (100 ml), dry NaOAc (5.0 g), dry 10% Pd–C catalyst (1.0 g) and 3,5-dimethoxybenzoyl chloride (4.0 g), was flushed with H_2 in a Parr hydrogenation apparatus and pressurized to 3.3 kg/cm^2. The vessel was shaken for 1 hr, then heated to *ca* 60° and shaken overnight. The reaction mixture was filtered through celite and concd to give 3,5-dimethoxybenzaldehyde (2.34 g, 70% yield).

General Grignard reaction [12]. To a stirred mixture of Mg turnings (0.014 mol, 0.34 g), and dry Et_2O (10 ml) was added dropwise 0.013 mol of the alkyl bromide reagent in 5 ml Et_2O. The reaction was initiated by adding two drops MeI. Addition was continued with gentle refluxing for *ca* 3 hr. 3,5-Dimethoxybenzaldehyde (0.01 mol in Et_2O) was added dropwise at room temp. then heated under reflux for 1 hr. The reaction mixture was cooled and diluted with ice H_2O. The organic layer was washed with 3 N H_2SO_4 (2 × 25 ml), 10% K_2CO_3 (2 × 25 ml), H_2O (25 ml), brine (25 ml), dried and concd. The product was purified by chromatography over Si gel (yields 30–70%). The Grignard product (2–6 mmol) in 50 ml EtOAc containing two drops conc. H_2SO_4 was hydrogenated at room temp. and 4 kg/cm^2 over 10% Pd–C (*ca* 0.2 g) catalyst for 3–4 hr. The mixture was filtered through celite and the celite washed with EtOAc. The filtrate was washed with satd $NaHCO_3$ soln, dried and concd to give the 5-alkylresorcinol dimethyl ethers (yields 55–90%).

General selective monodemethylation to give the monomethyl ether (**1**) [2]. A soln of 0.002 mol ethanethiol in 5 ml dry DMF was added dropwise over 10 min to 0.0025 mol NaH (50% dispersion) in 5 ml dry DMF stirred under N_2 and cooled in an ice bath. The ice bath was removed and the mixture allowed to warm to room temp. A soln of 0.001 mol of the 5-alkylresorcinol dimethyl ether in 5 ml dry DMF was added in one lot. The mixture was heated under reflux for 3 hr, then allowed to cool, poured into 75 ml cold water, and extracted with Skelly B which was discarded. The aq. layer was acidified with ice cold 4 N HCl, then extracted with Et_2O. The Et_2O extracts were washed with brine, dried and concd to give the 5-alkylresorcinol monomethyl ether (**1b–1f**). Yields were not maximized.

General demethylation to give resorcinol (**2**) [13]. To a soln of 5-alkylresorcinol dimethyl ether (1 mmol) and NaI (4 mmol) in MeCN (2 ml) was added $ClSiMe_3$ (4 mmol) slowly with continuous stirring under N_2. The soln was heated under reflux for 36 hr, quenched with 2 ml H_2O, and extracted with Et_2O. The Et_2O extracts were washed with 10% $Na_2S_2O_3$ to remove iodine and brine, dried and concd to give the 5-alkylresorcinol (**2b–2f**). Yields were not maximized.

Biological assay of Verticicladiella *extracts, 5-alkylresorcinols and derivatives.* The susceptibility of nine micro-organisms (Table 1) to antibiotic substances present in *Verticicladiella* extracts and the 5-alkylresorcinols and derivatives was determined by the disc agar diffusion method. Filter paper discs (0.25 in., Schleicher and Schuell) were soaked in metabolite extracts of known conc and air dried. The discs were placed on Mueller Hinton agar plates which had been swabbed with the test organism and the plates were incubated at 37°. Inhibition zone diameters (mm) were recorded after 18 hr.

A 4-day old culture of *Chlorella* sp. ATCC 7156 maintained in a sporulation media was impregnated on sporulation agar (ATCC 5). Filter paper discs (0.5 in.) were soaked in an aq. MeOH soln of **1a** (1:100), air dried, applied to the agar plate and incubated in the light at room temp. Chlorophyllytic activity was observed after 3 days.

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REFERENCES

1. Hunt, R. S. and Morrison, D. J. (1979) *Canadian Forestry Service Pest Leaflet*, FPL 67.
2. Mirrington, R. S. and Feutrill, G. I. (1973) *Org. Synth.* **53**, 90.
3. Bauer, A. W., Kirby, W., Sherris, J. C. and Turck, M. (1966) *Am. J. Clin. Pathol.* **45**, 493.
4. Harvey, S. C. (1980) in *The Pharmacological Basis of Therapeutics* (Gilman, A. G., Goodman, L. S. and Gilman, A., eds.) Chap. 14. MacMillan, Toronto.
5. Castonguary, A. and Berger, Y. (1978) *Org. Mag. Reson.* **11**, 375.
6. Castonguary, A. and Brassard, P. (1977) *Can. J. Chem.* **55**, 1324.
7. Berger, Y. (1980) *Phytochemistry* **19**, 2779.
8. Bock, K. and Pedersen, C. (1975) *Acta. Chem. Scand. Ser. B* **29**, 258.
9. Kasai, R., Okihara, J., Asakawa, J., Mizutani, K. and Tanaka, O. (1979) *Tetrahedron* **35**, 1427.
10. Warren, J. R. and Winstead, N. N. (1965) *Phytopathology* **55**, 244.
11. Bassett, C., Sherwood, R. T., Kepler, J. A. and Hamilton, P. B. (1967) *Phytopathology* **57**, 1046.
12. Ridlley, D. D., Ritchie, E. and Taylor, W. C. (1968) *Aust. J. Chem.* **21**, 2979.
13. Olah, G. A., Narang, S. C., Gupta, B. G. B. and Malhotra, R. (1979) *J. Org. Chem.* **44**, 1247.