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PHENOLIC AND POLYKETIDE METABOLITES OF THE ASPEN BLUE STAIN FUNGUS *OPHIOSTOMA CRASSIVAGINATA*

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Key Word Index—*Ophiostoma crassivaginata*; microascales; aspen; *Populus tremoloides*; Salicaceae; blue stain disease; 4-ethyl-2H-pyran-2-one-6-carboxylic acid.

Abstract—The metabolites produced in liquid culture by the blue stain fungus *Ophiostoma crassivaginata* have been identified. 4-Ethyl-2H-pyran-2-one-6-carboxylic acid is found for the first time as a natural product. Fifteen other known compounds are reported.

INTRODUCTION

Recently we reported on the metabolites of several fungi associated with aspen (*Populus tremuloides* Michaux) [1–6]. Several of the compounds showed antifungal activity and in one instance sesquiterpenes with a new skeleton were found [4]. *Ophiostoma crassivaginata* (H. D. Griffin, Upadhyay, NOF 1509; = *Ceratocystiopsis crassivaginata*) is one of the most prevalent so-called blue stain fungi on aspen. It is known that in some cases the discoloration of the wood is due to the formation of complexes between metabolites and Fe(III) ions [7]. Accordingly, our search was focused on metabolites which give a $FeCl_3$ positive reaction.

RESULTS AND DISCUSSION

The fungus was grown in V-8 juice liquid shake culture. The culture broth was filtered, concentrated and extracted with EtOAc or was passed through Amberlite XAD-2 and the metabolites were eluted with MeOH. Flash chromatography on silica gel followed by preparative TLC, afforded pure compounds. Occasionally the crude fractions were treated with diazomethane. Compounds **1**–**10** and **12**–**15** are known compounds. (*R*)-3-Phenyllactic acid was also identified which, unlike the (*S*)-enantiomer, has not been reported as a fungal metabolite. In addition, succinic acid, 2-furoic acid and tryptophol were isolated.

The production of 4-ethylpyrocatechol (**12**) and 4-ethylpyran-2-one-6-carboxylic acid (**16**, isolated and identified as the methyl ester **17**) was found to be dependent on the age of the culture. Thus, a 36 day culture contained only **12**, while a 50 day culture contained only **16**. Compound **16** is presumably derived from **12** as the result of enzymatic oxidation of the aromatic ring. Compound **16** was reported recently as a synthetic product [8], but it has not been found as a natural product. Some pyran-2-one-6-carboxylic acid derivatives have been reported as fungal metabolites. All of them are derived from tyrosine and the α-amino acid functionality is preserved [9].

All the above-mentioned phenolic compounds, especially **12**, **13**, and **14**, gave an intense colour reaction with $FeCl_3$ and could be, at least in part, responsible for the wood discoloration caused by this fungus.

EXPERIMENTAL

General. Mp: measured on a Fisher-Johns apparatus and uncorr. ^1H and ^{13}C NMR: 360 and 75 MHz, respectively. Chemical shifts give in δ(ppm) and referenced in $CDCl_3$ to the residual $CHCl_3$, 7.26 ppm for ^1H and 77.06 ppm for ^{13}C, respectively. Flash chromatography was performed on silica gel 230–400 mesh (General Intermediates, Canada). Prep. TLC was performed on E. Merck precoated 20 × 20 cm glass plates on silica gel 60 F-254.

Extraction and isolation. Ophiostoma crassivaginata was obtained from Y. Hiratsuka, Forestry Canada, Northern Forestry Centre, Edmonton, Canada. One 2% malt extract agar plate culture was blended with 200 ml of water and *ca* 10 ml of the mycelial suspension was used to inoculate each of 12 2 l flasks containing 1 l sterilized medium [100 ml V-8 juice filtered through Celite, glucose (10 g) and 1 l of distilled H_2O]. A control experiment established that the compounds mentioned below were not present in the culture medium. After 36 days of shaking at 23° the culture broth was filtered and the mycelium washed with distilled H_2O. The filtrate was

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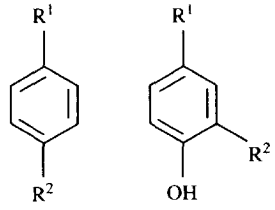
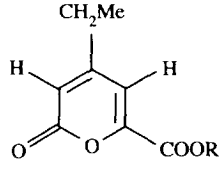


	R^1	R^2
1	CHO,	OH
2	CH_2COOMe,	OH
3*	CH_2COOMe,	OH
4	CH_2CH_2OH,	H
5	CH_2CH_2OH,	H
6*	CH_2CH_2OH,	OMe
7	$CH\overset{E}{=}CHCOOH$,	OH
8*	$CH\overset{Z}{=}CHCOOMe$,	OH
9	CH_2CH_2COOMe,	OMe
10*	CH_2CH_2COOMe,	OMe
11*	$CH_2CH(OH)COOMe$,	H

*After treatment with CH_2N_2.

	R^1	R^2
12	CH_2Me,	OH
13	COOH,	OMe
14	CH_2COOH,	OH
15*	H,	CH_2COOMe



16	RH
17*	RMe

reduced to 2 l under vacuum and the residue was extracted with EtOAc (4 × 600 ml). The organic extract was dried over $MgSO_4$ and the solvent removed at red. pres. to afford 780 mg of a red oil. The latter was subjected to flash chromatography with petrol–EtOAc–CH_2Cl_2–MeOH (63:16:16:5) and CH_2Cl_2–MeOH (17:3) to give 2 frs. Fr. 1 was flash chromatographed with petrol–EtOAc (9:1 → 2:1) to give phenylethanol (**4**) (5.1 mg), 4-ethylpyrocatechol (**12**) (3.1 mg), and *p*-hydroxybenzaldehyde (**1**) (1.5 mg). Fr. 2 was flash chromatographed with CH_2Cl_2–EtOH–HOAc (98:1.5:0.5) to afford 3,4-dihydroxyphenylacetic acid (**14**) (1.0 mg), 2-furoic acid (1.1 mg), *p*-hydroxydihydrocinnamic acid (**9**) (*ca* 0.5 mg), *trans*-*p*-hydroxycinnamic acid (**7**) (*ca* 0.5 mg), 2-(*p*-hydroxyphenyl)ethanol (**5**) (25 mg), *p*-hydroxybenzoic acid (**2**) (3 mg), 4-hydroxy-3-methoxybenzoic acid (**13**) (1.2 mg), and a polar fr. which was treated with diazomethane and then sepd by prep. TLC (petrol–EtOAc 4:1, 3-fold development) to afford methyl *cis*-*p*-hydroxycinnamate (**8**) (0.9 mg), methyl *p*-hydroxyphenylacetate (**3**) (3.5 mg), and a Cl-containing compound (1.1 mg, $C_{12}H_{13}ClO_5$).

A second batch (22 l) was prepd in the same medium and the mycelium was harvested after 50 days. The filtered culture broth was passed through Amberlite XAD-2. The resin was washed with H_2O (1.5 l) and then with MeOH (2 l). The MeOH was removed under vacuum and the residue (2.15 g) chromatographed on RP-18 column with 25–100% MeOH. Fr. 1 was recrystallized from acetone to give succinic acid (90 mg). Fr. 2 was treated with diazomethane and flash chromatographed with petrol–EtOAc (33:17) to afford frs which were further purified by prep. TLC with petrol–$PhCH_3$–EtOAc–MeOH (25:21:3:1) to give methyl *o*-hydroxyphenylacetate (**15**) (44 mg), methyl (*R*)-3-phenyllactic acid (**11**) (32 mg), methyl *p*-methoxydihydrocinnamate (**10**) (2.0 mg), methyl *p*-hydroxyphenylacetate (**3**) (6.5 mg), *p*-methoxyphenylethanol (**6**) (12 mg), methyl 4-ethyl-2H-pyran-2-one-6-carboxylate (**17**) (6.2 mg), and 2-(*p*-hydroxyphenyl)ethanol (**5**) (38 mg). The mother liquor was prep. TLC chromatographed with petrol–EtOAc (33:17) to give tryptophol (5.0 mg).

Methyl 4-ethyl-2H-pyran-2-one-6-carboxylate (**17**). Crystals, mp 101.0–103.0°. R_f 0.12 (petrol–$PhCH_3$–EtOAc–MeOH, 50:42:7:1); IR ν_{max}^{KBr} cm^{-1}: 3079, 2978, 2954, 2925, 1710, 1437, 1304, 1275, 1117, 876; ^{1}H NMR (360 MHz, $CDCl_3$): δ1.23 (3H, *t*, *J* = 7.4, $MeCH_2$), 2.52 (2H, *q*, *J* = 7.4, $MeCH_2$), 3.93 (3H, *s*, COOMe), 6.31 (1H, *d*, *J* = 1.3, H-3), 7.00 (1H, *d*, *J* = 1.3, H-5); ^{13}C NMR (125 MHz, $CDCl_3$): δ12.4 ($MeCH_2$), 28.5 ($MeCH_2$), 53.5 (COOMe), 112.1 (C-5), 116.3 (C-3), 148.3 (C-6), 159.5 (C-4), 160.2 (COOMe), 160.5 (C-2); HMBC: H-3 → C-2, C-5, C-7; H-5 → C-3, C-6, C-7, C-9; H-7 → C-3, C-4, C-5, C-8; H-8 → C-4, C-7; H-10 → C-9; HR-EIMS *m/z* (rel. int.): 182.0577 $[M]^+$ (17). ($C_9H_{10}O_4$ requires 182.0575), 123.0446 [M – COOMe] (100) ($C_7H_7O_2$ requires 123.0446), 67.0558 $[C_5H_7]^+$ (64) (requires 67.0568).

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REFERENCES

1. Trifonov, L. S., Chakravarty, P., Hiratsuka, Y. and Ayer, W. A. (1992) *Eur. J. For. Path.* **22**, 441.
2. Ayer, W. A. and Miao, S. C. (1993) *Can. J. Chem.* **71**, 487.
3. Ayer, W. A. and Cruz, E. (1993) *Tetrahedron Letters* **34**, 1589.
4. Ayer, W. A. and Cruz, E. (1993) *J. Org. Chem.* **58**, 7529.
5. Ayer, W. A. and Miao, S. C. (1993) *J. Nat. Prod.* **56**, 2046.
6. Ayer, W. A. and Trifonov, L. S. (1994) *J. Nat. Prod.* **57**, 317.
7. Ayer, W. A., Attah-Poku, S. K., Browne, L. M. and Orszanska, H. (1987) *Can. J. Chem.* **65**, 765.
8. Mazaki, M., Takeda, H., Hashimoto, K., Myake, M., Ishida, M. and Shizonaki, A. (1989) *Jpn. Kokai Tokkyo Koho* JP 01, 151, 569 [89, 151, 569] (Cl.C07D309/38), 14.6.1989 (CA 112, P35682).
9. Dickinson, J. M. (1993) *Nat. Prod. Reports* **10**, 71.