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J. Nat. Prod., **1994**, 57 (2), 317-319• DOI:
10.1021/np50104a021 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 57, No. 2, pp. 317-319, February 1994

ANTHRAQUINONES AND A 10-HYDROXYANTHRONE FROM *PHIALOPHORA ALBA*

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ABSTRACT.—The metabolites produced by the fungus *Phialophora alba*, isolated from healthy aspen, have been examined. 6,8-Dihydroxy-1-methoxy-3-methylanthraquinone [**1**] and its 5-chloro- and 7-chloro derivatives **2** and **3** were obtained, along with the dihydro derivative **4** of the 5-chloro compound. Compounds **2** and **4** are new, and **1** has not been reported previously as a natural product.

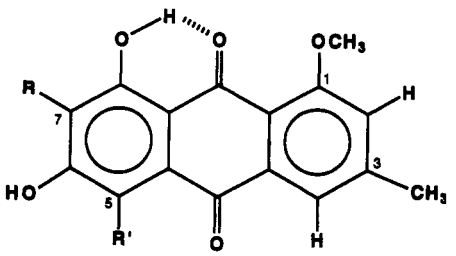
Recently we have reported on the metabolites of several fungi associated with healthy and decayed aspen (1–3). The fungus *Phialophora alba* von Beyma frequently has been isolated from aspen that is not infected with other fungi (L.J. Hutchison and Y. Hiratsuka, Northern Forestry Centre, Forestry Canada, Edmonton, personal communication.) The possibility that this fungus might protect the aspen from attack by other fungi prompted us to investigate its metabolites and to test their activity against aspen decay-causing fungi.

P. alba was cultured on a medium of 2% malt extract containing 0.1% added yeast extract. The culture broth was filtered, concentrated and extracted with EtOAc. The crude extract was subjected to flash chromatography followed by recrystallization or prep. tlc to afford the pure metabolites.

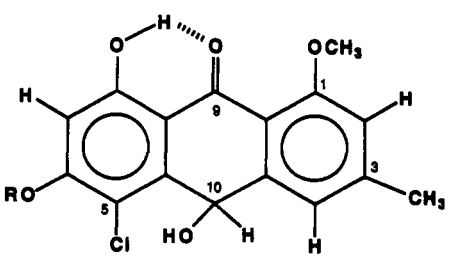
6,8-Dihydroxy-1-methoxy-3-methylanthraquinone (1-*O*-methylemodin [**1**]) was obtained as an orange solid, mp 257–260°, with spectroscopic properties identical with those reported in the literature (4). The nOe enhancements of the signals of H-2 and H-4 on irradiation of the Me hydrogens and the enhancement of the signal of H-2 on irradiation of the CH_3O hydrogens are consistent with structure **1**. Compound **1** has been obtained previously as a degradation product of anthraquinone glucosides but has not been reported previously as a natural product.

5-Chloro-6,8-dihydroxy-1-methoxy-3-methylanthraquinone [**2**] was obtained as a red-orange solid, mp 250–254°. The molecular formula $C_{16}H_{11}ClO_5$ is based on hreims. The location of the chlorine at C-5 is based on the chemical shift of H-7 (6.91 ppm) and on the chemical shifts of H-2 and H-4, which are similar to those in compound **1**. In addition, compound **2** has been correlated with compound **4** (see below).

7-Chloro-6,8-dihydroxy-1-methoxy-3-methylanthraquinone [**3**] was isolated as an orange solid. It is isomeric with compound **2** as shown by hreims. The chemical shift of H-5 (at 7.34 ppm) and of H-2 and H-4 (very similar to those



1 R=H, R′=H
2 R=H, R′=Cl
3 R=Cl, R′=H



4 R=H
5 R=CH_3

in **1** and **2**) in the ^{1}H-nmr spectrum indicated that the chlorine atom is located at C-7. This compound has been isolated previously from a lichen (5).

5-Chloro-6,8,10-trihydroxy-1-methoxy-3-methyl-9(10H)-anthracenone [**4**] was obtained as a yellow solid, $[\alpha]_D$ +17.3°. The molecular formula $C_{16}H_{13}ClO_5$ was determined by hreims and the optical activity suggested that it is a dihydroanthraquinone. A series of nOe enhancements revealed that ring C has the same substituents as in compounds **1** and **2**, and that the benzylic hydrogen is located at C-10, not at C-9. When kept in a MeOH solution at room temperature for several days, compound **4** underwent aerial oxidation to give the anthraquinone **2**. Treatment of compound **4** with diazomethane gave the methyl derivative **5**.

Compounds **1** and **4** showed ca. 50% inhibition of the growth (agar cultures) of the aspen decay-causing fungus *Phellinus tremulae* at a concentration of 1 mg/ml.

EXPERIMENTAL

CULTURE OF *P. ALBA* AND ISOLATION OF METABOLITES.—Cultures of *P. alba* (strain NOF 1598) were obtained from Dr. Y. Hiratsuka, Forestry Canada, Northern Forestry Centre, Edmonton, and are deposited at the University of Alberta Microfungus Herbarium (UAMH 7232). One 2% malt extract agar plate culture was blended in a Waring blender with 200 ml of H_2O and ca. 10 ml of the mycelial suspension was used to inoculate each of ten 2-liter Erlenmeyer flasks containing 1 liter of sterilized medium (20 g of malt extract, 1 g of yeast extract and 1 liter of distilled H_2O). After four weeks of shaking at 23° the culture broth was filtered and the mycelium washed with distilled H_2O and air-dried. The filtered broth was concentrated under reduced pressure to ca. 2 liter and extracted with EtOAc (3×800 ml). The organic solvent was removed under reduced pressure and the residue (0.97 g of red oil) was subjected to flash chromatography over Si gel with the eluent petroleum ether-EtOAc (9:1 to 1:2). The first orange fraction (35 mg) was recrystallized from EtOAc/petroleum ether to afford pure **1** (12.4 mg). The second fraction was further purified by prep. tlc with $CHCl_3$-petroleum ether-EtOAc-MeOH (54:22:22:2) to give pure 2-(*p*-hydroxyphenyl)ethanol. The third (yellow-orange) fraction (16 mg) was purified by prep. tlc, using as eluent $CHCl_3$-petroleum ether-EtOAc-MeOH (70:19:10:1) (threefold development). The yellow zone (R_f 0.50) was eluted with CH_2Cl_2-MeOH (85:15) to afford pure **4** (15.0 mg). The colorless but uv-active zone at R_f 0.25 gave daidzein (1.8 mg).

The air-dried mycelium was ground and extracted with MeOH by stirring at room temperature to afford 5.1 g of a red-brown oil, which was subjected to cc over Si gel with petroleum ether-EtOAc (2:1 to 1:10) followed by EtOAc-petroleum ether-MeOH (86:9:5 to 85:5:10). In this way three orange fractions were collected. These were recrystallized from MeOH. The first and the third fraction provided additional amounts of **1** and **4**, respectively, while the second gave pure **3** (5.6 mg).

6,8-Dihydroxy-1-methoxy-3-methylanthraquinone (1-O-Methylemodin, [**1**]).—Orange solid, mp 257–260° [lit. mp 265°, (4)]; R_f 0.70 [$CHCl_3$-petroleum ether-EtOAc-MeOH (70:20:8:2)]; uv (EtOH), ir (KBr), and ms spectra identical with those data reported (4); ^{1}H-nmr (400 MHz, Me_2CO-d_6) δ 2.50 (3H, s, CH_3), 4.00 (3H, s, OCH_3), 6.62 (1H, d, J=2.5, H-7), 7.17 (1H, d, J=2.5, H-5), 7.42 (1H, br s, H-2), 7.66 (1H, br s, H-4), 9.82 (1H, br s, HO-6), 13.40 (1H, s, HO-8); nOe observations: irradiation at δ 2.50 resulted in enhancement of the signals at δ 7.42 and 7.66; irradiation at δ 4.00 resulted in enhancement of the signal at δ 7.42.

5-Chloro-6,8-dihydroxy-1-methoxy-3-methylanthraquinone [**2**].—Red-orange microcrystals (from EtOAc), mp 250–254°; R_f 0.65 [$CHCl_3$-petroleum ether-EtOAc-MeOH (70:20:8:2)]; ^{1}H nmr (360 MHz, $CDCl_3$) δ 2.52 (3H, s, CH_3), 4.06 (3H, s, OCH_3), 6.70 (1H, br s, HO-6), 6.91 (1H, s, H-7), 7.14 (1H, br s, H-2), 7.73 (1H, br s, H-4), 13.06 (1H, s, HO-8); hreims: $[M+2]^+$, 320.0265 (34) ($C_{16}H_{11}{}^{37}ClO_5$ requires 320.0262), $[M]^+$, 318.0295 (100) ($C_{16}H_{11}{}^{35}ClO_5$ requires 318.0291), $[M+2-H_2O]^+$ 302 (19), $[M-H_2O]^+$ 300 (54), 291 (8), 290 (6), 289 (19), $[M-Cl]^+$ 283 (3), 275 (20), 274 (24), 273 (16), 272 (69), 197 (7), 139 (11).

7-Chloro-6,8-dihydroxy-1-methoxy-3-methylanthraquinone [**3**].—Orange crystals (from MeOH), mp 270° [lit. mp 279–281°, (5)]; R_f 0.65 [$CHCl_3$-petroleum ether-EtOAc-MeOH (70:20:8:2)]; ir ν max ($CHCl_3$) 3466, 3060, 2960, 2920, 2842, 1658, 1619, 1600 cm^{-1}; uv λ max (EtOH, ε) 257 (15,000), ca. 300 sh (8,900), 321 (10,500), 424 sh (2,400), ca. 496 (2,600) nm; ^{1}H-nmr (360 MHz, $CDCl_3$) δ 2.52 (3H, s, CH_3), 4.07 (3H, s, OCH_3), 6.32 (1H, HO), 7.17 (1H, br s, H-2), 7.44 (1H, s, H-5), 7.78 (1H, br s, H-4), 13.38 (1H, s, HO-8); ^{1}H nmr (360 MHz, Me_2CO-d_6) 2.51 (3H, s, CH_3), 4.02 (3H, s, OCH_3), 7.45 (1H, s, H-2), 7.34 (1H, br s, H-5), 7.67 (1H, br s, H-4), 11.20 (1H, s, HO-

8); hreims $[M+2]^+$ 320.0270 (33) ($C_{16}H_{11}{}^{37}ClO_5$ requires 320.0262), $[M]^+$ 318.0288 (100) ($C_{16}H_{11}{}^{35}ClO_5$ requires 318.0291), $[M+2-H_2O]^+$ 302 (21), $[M-H_2O]^+$ 300 (61), 291 (7), 290 (9), 289 (24), 288 (12), 287 (12), 284 (7), $[M-Cl]^+$ 283 (3), 275 (25), 274 (35), 273 (24), 272 (94), 245 (15), 197 (13), 145 (24), 139 (19).

5-Chloro-6,8,10-trihydroxy-1-methoxy-3-methyl-9(10H)-anthracenone [**4**].—Yellow solid (from Me_2CO-Et_2O), which turned dark at ca. 210° forming needles which sublimed at 245–248°; R_f 0.20 [$CHCl_3$-petroleum ether-EtOAc-MeOH (70:20:8:2)]; $[\alpha]_D$ 17.3° (*c*=0.24, MeOH); cd $\Delta\epsilon$ nm (*c*=0.012, MeOH) 258 (+3.36), 294 (−0.62), 325 (+0.80), 358 (−0.64), 386 (+0.80); ir ν max ($CHCl_3$) 3400 br, 3045, 3020, 2980, 2940, 2860, 1626, 1610, 1560, 1465 cm^{-1}; uv λ max (MeOH, ϵ) 227 (20,000), 259 (8,300), 273 sh (6,400), 308 sh (5,000), 372 (11,500) nm; ^1H nmr (400 MHz, Me_2CO-d_6) δ 2.43 (3H, s, CH_3), 3.92 (3H, s, OCH_3), 4.96 (1H, br s, HO-10), 5.82 (1H, s, H-10), 6.52 (1H, s, H-7), 7.02 (1H, s, H-2), 7.14 (1H, s, H-4), 13.50 (1H, s, HO-8); nOe observations: irradiation at δ 2.43 resulted in enhancement of the signals at δ 7.02 and 7.14; irradiation at δ 3.92 resulted in enhancement of the signal at δ 7.02; irradiation at δ 5.82 resulted in enhancement of the signal at δ 7.14; ^{13}C-nmr (75.47 MHz, Me_2CO-d_6) δ 22.0 (CH_3), 56.4 (OCH_3), 65.4 (C-10), 104.3, 114.0, 123.8 (C-2, C-4, C-7), 111.6, 113.0 (C-5, C-8a, C-9a), 141.6, 146.2, 147.5 (C-3, C-4a, C-10a), 160.0, 161.9, 164.1 (C-1, C-6, C-8), 188.5 (C-9); hreims: $[M+2]^+$ 322.0454 (43) ($C_{16}H_{13}{}^{37}ClO_5$ requires 322.0422), $[M]^+$ 320.0440 (100) ($C_{16}H_{13}{}^{35}ClO_5$ requires 320.0451), 307 (9), 306 (17), 305 (43), 304 (27), 303 (52), 302 (15), 291 (14), 289 (12), 287 (11), $[M-Cl]^+$ 285 (10), 277 (19), 275 (29), 274 (16), 268 (10), 267 (21), 239 (12), 238 (12), 139 (10).

5-Chloro-8,10-dihydroxy-1,6-dimethoxy-3-methyl-9(10H)-anthracenone [**5**].—Amorphous yellow-orange solid, R_f 0.80 [$CHCl_3$-petroleum ether-EtOAc-MeOH (70:20:8:2)]; ^1H nmr (360 MHz, $CDCl_3$) δ 2.47 (3H, s, CH_3), 3.96, 4.01 (each 3H, s, 2×OCH_3), 4.90 (1H, br s, HO-10), 5.94 (1H, s, H-10), 6.55 (1H, s, H-7), 6.87 (1H, s, H-2), 7.13 (1H, s, H-4), 13.05 (1H, s, HO-8); hreims: $[M+2]^+$ 336.0580 (41) ($C_{17}H_{15}{}^{37}ClO_5$ requires 336.0596), $[M]^+$ 334.0554 (100) ($C_{17}H_{15}{}^{35}ClO_5$ requires 334.0626), 333 (13), 332 (37), 321 (10), 319 (38), 318 (14), 317 (33), 316 (15), 314 (21), 305 (15), 303 (21), 301 (14), $[M-Cl]^+$ 299 (27), 298 (40), 291 (25), 290 (14), 289 (28), 288 (15), 286 (19), 282 (14), 281 (32), 280 (21), 275 (12), 273 (13), 271 (18), 269 (16), 254 (10), 252 (32), 245 (12), 231 (12), 209 (11), 152 (15), 139 (24).

ACKNOWLEDGMENTS

The financial support of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged. We thank Y. Hiratsuka and P. Chakravarty, Northern Forestry Centre, for cultures of *P. alba* and for assistance in the bioassays, and M. Anstee for technical assistance.

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Received 24 August 1993