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BETULACHRYSOQUINONE HEMIKETAL: A *p*-BENZOQUINONE HEMIKETAL MACROCYCLIC COMPOUND PRODUCED BY *PHANEROCHAETE CHRYSOSPORIUM**

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Key Word Index—*Phanerochaete chrysosporium*; Aphyllophorales; *Betula lutea*; Betulaceae; yellow birch; *p*-benzoquinone; betulachrysoquinone hemiketal; betulachrysoquinone; fungal metabolite.

Abstract—Betulachrysoquinone hemiketal was isolated from pre-extracted wood of *Betula lutea* Michx. inoculated with *Phanerochaete chrysosporium* Burds. Acid-catalysed hydrolysis of betulachrysoquinone hemiketal produced betulachrysoquinone which was shown to be 2-hydroxy-6-(13′-hydroxytetradecanyl)-*p*-benzoquinone.

INTRODUCTION

We recently described various chemical and physical properties of heavily degraded, polymeric lignins isolated from wood partly decayed by white-rot fungi [1]. In other studies the complete conversion of ^{14}C-lignins to $^{14}CO_2$ by white-rot fungi was demonstrated [2, 3]. Neither of these investigations elucidated specific structures of intermediates of lignin degradation, and consequently it was considered desirable to investigate the low MW compounds extractable from white-rotted wood. We report here the characterization of the most abundant compound in the chloroform-soluble extractives from yellow birch wood decayed by *Phanerochaete chrysosporium*. The compound, a long-chain aliphatic derivative of *p*-benzoquinone, is a fungal metabolite rather than a product of lignin degradation.

RESULTS AND DISCUSSION

The pre-extracted wood tissues of *Betula lutea* (yellow birch) were inoculated with *P. chrysosporium* and maintained at 27° until loss in dry wt of wood reached *ca* 35%. The tissues were then extracted successively at room temperature with petrol, chloroform and methanol. From the chloroform-extract, betulachrysoquinone hemiketal (**1**) was isolated, yield *ca* 0.1% of the dry decayed wood.

The extractive obtained from sound wood of *B. lutea* did not contain **1**. Acid-catalyzed hydrolysis of **1** produced a long-chain alkyl derivative of *p*-benzoquinone, betulachrysoquinone (**3**). Several *p*-benzoquinones with long, saturated and unsaturated hydrocarbon side chains such as ubiquinone, boviquinone and mavioquinones have been identified as metabolites of micro-organisms [4–14]. This together with its absence in sound wood, indicates that **1** is a metabolite of *P. chrysosporium* rather than a constituent of *B. lutea*, or a lignin degradation product.

Betulachrysoquinone hemiketal (**1**), $C_{20}H_{32}O_4$ (M^+, *m/e* 336) was soluble in dilute KOH solution but insoluble in dilute $NaHCO_3$ solution; this showed the enolic nature of the compound. The IR spectrum (KBr) showed absorption bands corresponding to Me (2970 and 1370 cm^{-1}), methylenes (2920 and 2852 cm^{-1}), conjugate chelated enolic β-diketone (2710, 2650, 2540, 1640, and 1620 cm^{-1}) [15, 16], and trisubstituted ethylene (845 cm^{-1}). In THF, the compound absorbed at 3550 and 3500 cm^{-1} in the OH region and 1648 and 1620 cm^{-1} in the carbonyl region. The UV spectrum of the compound in methanol showed intense bands at λ_{max} 260 and 298 nm corresponding to the absorption bands of cross-conjugated 3-hydroxy-2,5-cyclohexadien-1-one derivatives [17–19]. The spectrum underwent a bathochromic shift upon addition of base to give intense bands at λ_{max} 271 and 298 nm. The PMR spectrum (CD_3COCD_3) of the compound was consistent with the structure proposed. In the olefinic region of the spectrum, two one-proton doublets at τ 3.72 and 3.80 (H-6 and H-3) constituted in AB spin-system with J_{AB} = 2.4 Hz. The rather small coupling constant of this spin-system must be due to a long range 4-bond π–π spin–spin interaction of a cross-conjugated β,β'-disubstituted $\alpha,\beta,\alpha',\beta'$-dienone group [20–22]. In the aliphatic region of the spectrum, a two-proton triplet at τ 7.09 (H-1′) is the A_2 part of an A_2X_2 spin-system with J_{AX} = 7.8 Hz. The X_2 part apparently merged with the large singlet at τ 8.73. This spin-system corresponds to a —CH_2—CH_2— group (H-1′ and H-2′) adjacent to an α,β-enone at β-C [23]. The one-proton multiplet at τ 6.37 (H-13′) and 3-proton doublet at τ 8.89 (H-14′) constituted the AX_3 part of an AM_2X_3 spin-system with $J_{AX} = J_{AM}$ = 6 Hz. The M_2 part also merged with the large singlet at τ 8.73. This spin-system corresponds to a Me—CH(R)—OR′ (R′ = H or C—). The fact that the compound gave a negative iodoform test excludes the possibility R′ = H. The 22-proton singlet at τ 8.73 corresponds to an unbranched —$(CH_2)_{11}$— chain.

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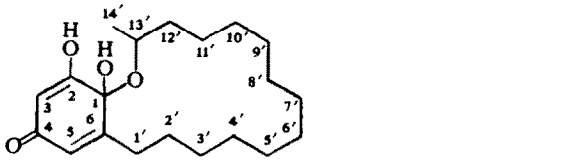
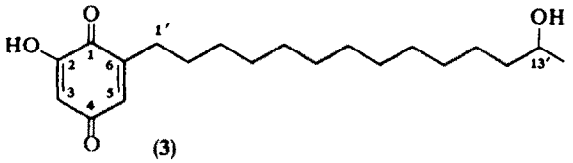
Treatment of **1** with ethereal diazomethane gave the O-methylated product **2**, $C_{21}H_{34}O_4$ (M^+, *m/e* 350). The IR spectrum (CCl_4) showed absorption bands corresponding to OH (3600 and 3300 cm^{-1}), enolic β-diketone ether (1655 and 1620 cm^{-1}), and enol ether (1260 cm^{-1}). In THF, the compound absorbed at 3475 cm^{-1} in the OH region and 1650 and 1618 cm^{-1} in the carbonyl region. The UV spectrum of the compound showed intense bands at λ_{max} 263 and 300 nm, corresponding to those of 3-methoxy-2,5-cyclohexadien-1-one derivatives [17–19]. The PMR spectrum of **2** was similar to that of **1** except for the presence of an additional 3-proton singlet at τ 6.09 (C-3 OMe). Thus, it is apparent that **1** is a derivative of 3-hydroxy-2,5-cyclohexadien-1-one with a $-(CH_2)_{12}$-chain substituted on C-5, an OH and a Me·CH(R)·O· group. When **1** was refluxed with dilute HCl in tetrahydrofuran, it underwent an acid-catalyzed hydrolysis to give the corresponding *p*-benzoquinone, betulachrysoquinone (**3**). The same reaction occurred when **1** was dissolved in warm trifloroacetic acid. This can be interpreted as evidence for an α-branched Et hemiketal group in **1**. Therefore, the structure **1**, is 2-hydroxy-6-(13′-hydroxytetradecanyl)-*p*-benzoquinone-1,13′-*O*-hemiketal.

The MS of **1** (Scheme 1) is consistent with the proposed structure. It exhibited prominent ion peaks at *m/e* 137 (**b**), 124 (**c**, base peak), 123 (**d**), 45 (**f**), and 44 (**g**), and less prominent ion peaks corresponding to M^+, (M^+ − Me), (M^+ − H_2O) (**a**), (67 + 14n) and (41 + 14n). The ion **c** corresponded to 3-hydroxy-*p*-benzoquinone. This ion was produced by a 1,4-elimination of water from the M^+ ion to the ion at *m/e* 318 (**a**) and a subsequent vinyl cleavage at C-6 with a H transfer, as indicated by the meta-stable ions at *m/e* 301 and 48.4. The same fragmentation process without H transfer gave the ion **d**, while an allylic cleavage of the ion **a** at C-1′ produced the ion **b**. The ion at *m/e* 44 (**g**) corresponded to acetaldehyde. The absence of the ion peak at *m/e* 44 in the MS of **3** then indicated that the ion **g** was produced from the M^+ by double cleavage of the α-branched Et hemiketal group. The same fragmentation process with a hydrogen transfer gave the ion **f**. Therefore, the occurrence of the ion **g** can be interpreted as further evidence for the presence of an α-branched Et hemiketal group in **1**.

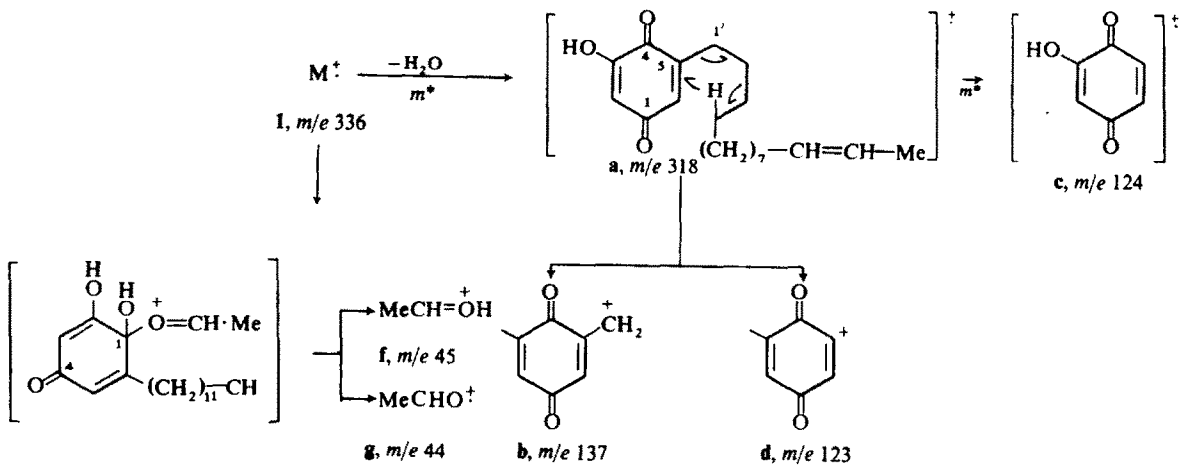
Betulachrysoquinone (**3**), $C_{20}H_{32}O_4$ (M^+, *m/e* 336), gave a positive iodoform test. This showed that the compound contained an α-branched ethanol group. The IR spectrum (KBr) showed absorption bands corresponding to H-bonded OH (3300 cm^{-1}), Me (2970 and 1375 cm^{-1}), methylenes (2920 and 2852 cm^{-1}), conjugated chelated enolic β-diketone (2730, 2670, 2540, and 1600 cm^{-1}) [15, 16], conjugated carbonyl (1630 cm^{-1}), and trisubstituted ethylenes (842 and 830 cm^{-1}). In THF, the compound absorbed at 3570 and 3495 cm^{-1} in the OH region and 1640, 1630, and 1605 in the carbonyl region. The UV spectrum of the compound in methanol showed intense bands at λ_{max} 274 and 280 nm and weak bands at λ_{max} 318, 430, and 448 nm corresponding to those of 2-hydroxy-*p*-benzoquinone derivatives [24]. The spectrum underwent a bathochromic shift on addition of base to give an intense band at λ_{max} 282 nm. This is similar to a *trans*-fixed enolic β-diketone [25]. In contrast to that of **1**, the olefinic region of the PMR spectrum (CD_3COCD_3) of **3** consisted of only a two-proton singlet at τ 3.84 (H-3 and H-5). In CD_3COCD_3, the H-3 and H-5 of 2,6-dimethoxy-*p*-benzoquinone



(**1**) R = H
(**2**) R = Me



(**3**)



Scheme 1. Major fragment pathways of betulachrysoquinone hemiketal.

resonate at τ 3.88; this compound was synthesized according to Baker [26]. The H-3 of 2-methoxy-5,6-dimethyl-*p*-benzoquinone resonates at τ 4.04 in CCl_4 [27] and the aliphatic region of the spectrum was similar to that of **1**. However, the A_2 part (H-1') of the A_2X_2 spin-system was shifted upfield to τ 7.58. The MS of **3** was similar to that of **1** except for the absence of the characteristic ion at *m/e* 44 corresponding to ion **g**. Thus, the structure **3** is 2-hydroxy-6-(13'-hydroxytetradecanyl)-*p*-benzoquinone.

EXPERIMENTAL

Culture conditions. Sound wood of *B. lutea* was air-dried, ground to pass a 20-mesh screen, and pre-extracted with C_6H_6–95% EtOH (2:1), then 95% EtOH, to remove extraneous substances. Into each of several 2 l. wide-mouth jars, 100 g of wood, 110 ml of H_2O and 60 ml of nutrient soln were added. The nutrient soln contained, per l. of H_2O: NH_4NO_3, 3 g; K_2HPO_4, 2 g; KH_2PO_4, 2.5 g; $MgSO_4 \cdot 7H_2O$, 2 g; $FeCl_3 \cdot 6H_2O$, 1 mg; $ZnSO_4 \cdot 7H_2O$, 0.5 mg; $MnSO_4 \cdot 4H_2O$, 0.5 mg; $CuSO_4 \cdot 5H_2O$, 0.5 mg; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 0.5 mg. The jars were capped with milk filters and then loosely with Al foil, and sterilized by autoclaving at 121° for 30 min. Each jar was seeded with 5 ml of a conidial suspension of *P. chrysosporium* ME-446 [3], and incubated at 27° and 70% RH. Contents were mixed weekly by shaking and after 2 weeks 30 ml of sterile H_2O were added to each jar. The cultures were terminated after 12 weeks and the contents dried, at which time the loss in dry wt of wood in each was 34 g (wt loss = 34%).

Extraction. The decayed wood meal (311 g) was extracted successively with petrol (bp 130–145°) 2 + 1 l.), $CHCl_3$ (2 × 1 l.) and MeOH (2 × 1 l.) at room temp. After removal of solvents, petrol, $CHCl_3$, and MeOH extractives (0.3 g, 0.95 g and 12.6 g, respectively) were obtained.

Betulachrysoquinone hemiketal (**1**). The $CHCl_3$ extractive was dissolved in hot MeOH (2 ml) and the soln kept a 0° for 48 hr. The crude crystals of **1** were filtered off and recrystallized from $CHCl_3$ to give colorless scales (290 mg), mp 135–136°; UV λ_{max} in MeOH 260 and 298 nm (ε 10180 and 4300). λ_{max} in 0.1N NaOMe–MeOH 271 and 298 nm (ε 12490 and 8250); IR (KBr) 2970 (Me), 2920 and 2852 (both *vs*, CH_2), 2710, 2650, 2540, 1640, 1620, and 1600, *sh*, (chelated enolic β-diketone), 1468 (CH_2), 1455 and 1370 (Me), 1260 (C=C—OH), 1175 (*t*-C—OH) and 845 (trisubstituted ethylene) cm^{-1}; PMR (CD_3COCD_3) τ 8.89 (3H, *d*, J_{AX} = 6 Hz, CH—C<u>H</u>$_3$), 8.73 (22H, *s*, aliphatic CH_2), 7.09 (2H, *t*, J_{AX} = 7.8 Hz, OC—C=C—C<u>H</u>$_2$—CH_2), 6.37 (1H, *m*, $J_{AX} = J_{AM}$ = 6 Hz, CH_2—C<u>H</u>OR—Me), 3.8 (1H, *d*, J_{AB} = 2.4 Hz, H-3), 3.72 (1H, *d*, J_{AB} = 2.4 Hz, H-5); MS (70 eV; temp. 120°), *m/e* (rel. int.) 336 (10, M^+), 318 (6), 301 (*m**), 137 (14), 136 (5), 125 (10), 124 (100), 123 (31). 69 (10), 67 (5), 57 (9), 55 (22), 48.4 (*m**), 45 (28), 44 (80), 43 (19), 41 (21); Analysis: calculated for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.52; H, 9.47. Treatment of **1** with CH_2N_2 in Et_2O gave the corresponding Me ether **2**, colorless oil; UV λ_{max} in MeOH 263 and 300 nm (ε 11000 and 4280); IR (CCl_4) 3600 and 3300 (OH), 3010 (C=CH), 2934 and 2862 (both *vs*, CH_2), 1655 and 1620 (enolic β-diketone ether), 1442 (CH_2) 1382 (Me), 1260 (C=C—OC) and 1178 (*t*-C—OH) cm^{-1}; PMR ($CDCl_3$) τ 8.83 (3H, *d*, J_{AX} = 6 Hz, CH—C<u>H</u>$_3$), 8.74 (20H, *s*, aliphatic CH_2), 7.19 (2H, *t*, J_{AX} = 7.8 Hz, OC—C=C—C<u>H</u>$_2$—CH_2), 6.26 (1H, *m*, CH_2—C<u>H</u>OR—Me), 6.13 (3H, *s*, OC<u>H</u>$_3$), 3.77 (1H, *d*, J_{AB} = 2.4 Hz, H-3), 3.71 (1H, *d*, J_{AB} = 2.4 Hz, H-5); M^+, *m/e* 350.

Betulachrysoquinone (**3**). A soln of **1** (60 mg) in 5 ml of N HCl in THF was refluxed for 1 hr. The reaction mixture was diluted with 15 ml H_2O, then extracted with $CHCl_3$ (5 × 20 ml). The $CHCl_3$ soln was washed, dried and the solvent removed. The crude product was recrystallized from $CHCl_3$ to give pale orangish-yellow crystals (47 mg), mp 126–128°; λ_{max} (MeOH) 274, 280, 318, *sh*, 430, and 448 nm (ε 1680, 1556, 282, 74, and 76), λ_{max} in 0.1N NaOMe–MeOH 282, 318, *sh*, 426, and 509 (ε 1890, 290, 61, and 52); IR (KBr) 3300 (OH), 2970 (Me), 2920 and 2952 (both *vs*, CH_2), 2730, 2670, 2540, and 1600 (chelated enolic β-diketone), 1630 (conj. C=O), 1468 (CH_2), 1348 (OH), 1162, 1002, 842, and 830 (trisubstituted ethylene) cm^{-1}; PMR (CD_3COCD_3) τ 8.89 (3H, *d*, J_{AX} = 6 Hz, CH—C<u>H</u>$_3$), 8.73 (22H, *s*, aliph. CH_2), 7.58 (2H, *t*, J_{AX} = 7.8 Hz, OC—C=C—C<u>H</u>$_2$CH$_2$), 6.36 (1H, *m*, $J_{AX} = J_{AM}$ = 6 Hz, CH_2—C<u>H</u>OH—Me), 3.84 (2H, *s*, H-3 and H-5); MS (70 eV; temp. 80°), *m/e* (rel. int.) 336 (6, M^+), 318 (5), 301 (*m**), 137 (15), 125 (17), 124 (100, M^+), 123 (25), 69 (6), 55 (14), 48.4 (*m**), 45 (22), 43 (8), 41 (15); Analysis. Calculated for $C_{20}H_{32}O_4$: C, 71.39; H, 9.59. Found: C, 71.46; H, 9.44. A soln of **1** (20 mg) in 0.5 ml of TFA was heated at 70–80° for a few min. After removal of acid, the residue was recrystallized from $CHCl_3$ to give pale orange–yellow crystals, mp 124–127°. The identity of the product was established by mmp and comparison of its spectroscopic data with those of **3** obtained previously.

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