Preparation of 2-((4-Methylpentan-2-yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione (6PPD-quinone), an Environmental Hazard for Salmon

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ABSTRACT

A recent report has identified 6PPD-quinone as an environmental compound responsible for drastic declines in coho salmon (*Oncorhynchus kisutch*) populations. As it is derived by oxidation of the preservative 6PPD that is found in tire rubber, this compound is not commercially available for further investigation of its biological properties. This work provides a brief synthesis of the oncorhynchicide 6PPD-quinone that can be used for that purpose.

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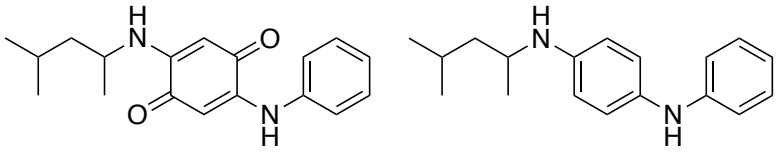
1. Introduction

A recent report showed an environmental bis-aminobenzoquinone (**1**) is highly toxic to coho salmon.[1] It is derived from a *p*-phenylenediamine anti-oxidant **2** that is included in vehicle tire rubber to combat ozone degradation. The physicochemical characterization of **1** was unambiguous, but it was derived from **2** by an oxidative route paralleling that observed in the environment. A directed synthesis of **1** would offer a much more reliable supply of this key compound to understand the molecular basis of its toxicity. We therefore developed this preparation of 6PPD-quinone.

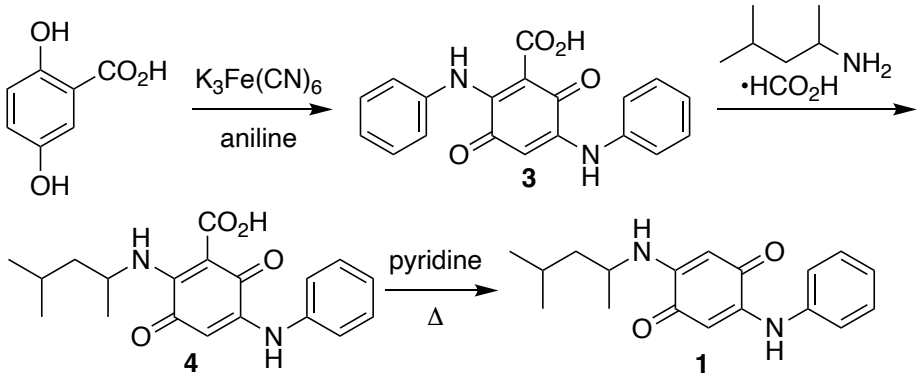


6PPD-quinone **1** 6PPD **2**

Chart 1

2. Results

The synthesis begins with gentisic acid, which can be oxidized and oxidatively coupled to aniline to yield **3** in ca. 90% yield. This process parallels two reported methods using periodate or ferricyanide as the oxidant.[2,3] The replacement of one aniline residue in this intermediate[3] with 4-methylpentan-2-amine is facile, delivering **4** in 94% yield. Decarboxylation of **4** occurs upon heating in pyridine solution, yielding the target in 93% yield. The NMR and mass spectroscopic properties of our final product were compared to those in the initial report of Kolodziej,[1] and they proved identical.



Scheme 1. Preparation of 6PPD-quinone

3. Discussion

This concise synthesis of 6PPD-quinone has enabled the preparation of significant quantities of material, which should be sufficient to supply much further study of its actions on both model organisms and species of agronomic importance. This is a versatile synthetic route that enables structural variants to be generated, which could be useful in target identification or the development of sensitive assays for 6PPD-quinone.

Biological researchers who wish to receive samples of 6PPD-quinone for further study should inquire of these authors. We look forward to opportunities for collaboration.

4. Experimental

3,6-Dioxo-2,5-bis(phenylamino)cyclohexa-1,4-diene-1-carboxylic acid (3). 2,5 Dihydroxybenzoic acid (200 mg, 1.30 mmol) was dissolved in pH 7.2 phosphate buffer (1 M, 20 mL). Potassium ferricyanide (854.5 mg, 2.600 mmol, 2 eq) was added followed by aniline (326 μL, 3.90 mmol, 3 eq), and the reaction mixture was stirred overnight. The solution was extracted with CH_2Cl_2 (3 × 25 mL) and the organic layers were combined, washed with dil HCl (3 × 25 mL) and brine (2 × 25 mL), and dried with sodium sulfate. The solution was concentrated in vacuo, yielding the crude product as a light brown solid (398 mg, 92%) that includes the known

compound **3** as well as some impurities. This material was used without further purification. ^{1}H NMR (500 MHz, $CDCl_3$): δ 13.85 (s, 1H), 13.32 (s, 1H), 8.04 (s, 1H), 7.42 (m, 4H), 7.35 (m, 2H), 7.27 (m, 2H), 7.18 (d, *J* = 7.6 Hz, 2H), 5.99 (s, 1H). HRMS (ESI) *m*/*z* [M-H]$^-$ calcd for $C_{19}H_{13}N_2O_4$: 333.0875, found: 333.0905.

2-((4-Methylpentan-2-yl)amino)-3,6-dioxo-5-(phenylamino)cyclohexa-1,4-diene-1-carboxylic acid (4). In a 20 mL scintillation vial, crude compound **3** (125 mg, 374 μmol) was dissolved in 10 mL of CH_2Cl_2. To this solution was added sodium carbonate (79.0 mg, 748 μmol, 2 eq). 4-Methylpentan-2-aminium formate (55 mg, 374 μmol, 1 eq) was dissolved in 2 mL of CH_2Cl_2 and the solution was added to the reaction mixture by pipette. It was stirred overnight, diluted with 13 mL CH_2Cl_2, and washed with dil HCl (3 × 25 mL) and brine (25 mL). The solution was dried with sodium sulfate and evaporated in vacuo. The crude product was purified by flash column chromatography (CH_2Cl_2, R_f = 0.37). The resulting red oil was stirred in hexanes overnight to precipitate a bright red solid, which was isolated by filtration to give the title compound (120 mg, 94% for the two steps). Recrystallization gave red plates. ^{1}H NMR (500 MHz, $CDCl_3$): δ 14.20 (s, 1H), 12.33 (s, 1H), 8.12 (s, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.28 (m, 1H), 7.26 (m, 2H), 6.05 (s, 1H), 5.17 (m, 1H), 1.65 (m, 2H), 1.44 (m, 1H), 1.33 (d, *J* = 6.3 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.88 (d, *J* = 6.4 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 178.9, 178.3, 170.9, 155.7, 146.5, 136.5, 129.8, 126.7, 123.2, 99.6, 95.8, 51.3, 46.6, 25.3, 22.5, 22.3, 21.6. IR (neat) 3244, 2956, 2925, 2870, 1737, 1681, 1614, 1579, 1513, 1466, 1442, 1388, 1366, 1346, 1302, 1230, 1105, 808, 753, 692, 636, 613, 563, 530, 515 cm^{-1}. HRMS (ESI) *m*/*z* [M-H]$^-$ calcd for $C_{19}H_{21}N_2O_4$: 341.1507, found: 341.1533. mp (hexanes) 166.7 °C.

2-((4-Methylpentan-2-yl)amino)-5-(phenylamino)cyclohexa-2,5-diene-1,4-dione (1). Compound **4** (109.8 mg, 0.321 mmol) was dissolved in 20 mL of pyridine and heated at reflux

for 5 h. The solution was evaporated in vacuo and the residue was dissolved in CH_2Cl_2 and washed three times with 0.1 M HCl and once with brine. The organic layer was dried with sodium sulfate and concentrated in vacuo, yielding the product as a pink solid (89 mg, 93%). Recrystallization from hexanes gave pink needles. The proton NMR spectrum was consistent with the earlier report.[1] ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.27 (d, *J* = 7.7 Hz, 2H), 7.23 (t, *J* = 7.7 Hz, 1H), 6.41 (d, *J* = 7.5 Hz, 1H), 5.99 (s, 1H), 5.45 (s, 1H), 3.57 (m, 1H), 1.69 (m, 1H), 1.55 (dt, *J* = 14.1, 7.1 Hz, 1H), 1.40 (dt, *J* = 14.1, 7.1 Hz, 1H), 1.24 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.6 Hz, 3H). ^{13}C NMR (125 MHz, $CDCl_3$): δ 180.1, 178.5, 149.6, 147.7, 137.4, 129.7, 126.1, 122.8, 95.7, 92.8, 46.8, 45.6, 25.1, 22.6, 20.1. IR (neat) 3263, 3227, 2953, 1638, 1556, 1486, 1442, 1355, 1290, 1263, 1209, 1161, 1124, 1078, 1026, 978, 919, 886, 860, 826, 813, 763, 727, 693, 601, 574, 550, 542, 534 cm^{-1}. HRMS (ESI) *m/z* [M-H]$^-$ calcd for $C_{18}H_{21}N_2O_2$: 297.1603, found: 297.1623. mp (hexanes) 190.7 °C.

5. References

1. Tian, Z.; Zhao, H.; Peter, K. T.; Gonzalez, M.; Wetzel, J.; Wu, C.; Hu, X.; Prat, J.; Mudrock, E.; Hettinger, R.; Cortina, A. E.; Biswas, R. G.; Kock, F. V. C.; Soong, R.; Jenne, A.; Du, B.; Hou, F.; He, H.; Lundeen, R.; Gilbreath, A.; Sutton, R.; Scholz, N. L.; Davis, J. W.; Dodd, M. C.; Simpson, A.; McIntyre, J. K.; Kolodziej, E. P. A ubiquitous tire rubber-derived chemical induces acute mortality in coho salmon. *Science* **2021**, *371*, 185-189. DOI: 10.1126/science.abd6951

2. Suto, M. J.; Stier, M. A.; Winters, R. T.; Turner, W. R.; Pinter, C. D.; Elliott, W. E.; Sebolt-Leopold, J. S. Synthesis and evaluation of a series of 3,5-disubstituted benzisoxazole-4,7-diones. Potent radiosensitizers in vitro. *J. Med. Chem.* **1991**, *34*, 3290-3294.

3. Pardo, M.; Joos, K.; Schäfer, W. 2-Alkylamino-5-arylamino-1,4-benzoquinones. *J. Chem. Res. (S)* **1978**, 177.