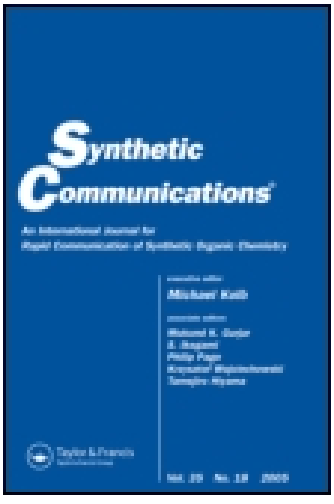

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Catalytic Oxidative Cyclocondensation of o-Aminophenols to 2-Amino-3H-phenoxazin-3-ones

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Catalytic Oxidative Cyclocondensation of *o*-Aminophenols to 2-Amino-3*H*-phenoxazin-3-ones

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Abstract: The catalytic oxidative cyclocondensation of the *o*-aminophenols **1a**–**f** was investigated. The oxidants used were air/laccase, H_2O_2/horseradish peroxidase, H_2O_2/ebselen (**3**), and TBHP/diphenyl diselenide **4**. The products obtained were 2-amino-3*H*-phenoxazin-3-one—questiomycin A, its derivative **2b**, and cinnabarinic acid and actinocin (**2c,d**). Substrates with methyl groups at 4 and 5 positions of benzene ring were converted to different dihydrophenoxazinones **2g,h**. Compounds having chlorine atoms at the same positions underwent oxidation to planar phenoxazinones **2e,f** with elimination of one hydrochloride molecule.

Keywords: *o*-aminophenols, 2-aminophenoxazin-3-ones, enzymes, hydroperoxides, organoselenium compounds, oxidation

2-Amino-3*H*-phenoxazin-3-one is a part of the chemical structure of actinomycin D, a known anticancer drug.[1] Compounds having such a skeleton occur in nature as insect pigments, fungal metabolites, antibiotics, and allelochemicals such as ommochromes, cinnabarines, and questiomycins.[2,3] Simple 2-aminophenoxazin-3-ones and 3-aminophenoxazin-2-one exhibit antitumor, antimicrobial, and antiviral activity in vitro and in vivo.[3–6]

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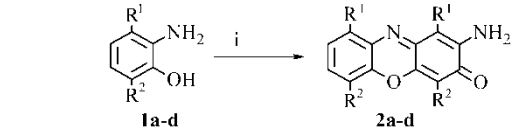
Aminophenoxazinones have been reported as *Streptomyces parvulus* and woodrotting fungi metabolites, cyclocondensation products of *o*-aminophenols with bovine erythrocyte hemolyzate, and bioconversion products of *Pseudomonas putida* grown on nitroarenes.[3,6–9] The traditional method for *o*-aminophenol cyclocondensation by chemical means employed quinones or lead and mercury heavy-metal reagents, which are not environmentally friendly.[5,10] Using hydrogen peroxide activated by cyclodextrin ketone or dioxygen as the oxidant in the presence of 1-oxyl-2,2,6,6-tetramethylpiperidine (TEMPO), cobalt, or copper catalysts, led to 2-aminophenoxazin-3-one in preparative yield up to 94%.[11–13]

In our previous article, we reported a convenient method for oxidative cyclocondensation of *o*-aminophenol to 2-amino-3*H*-phenoxazin-3-one (questiomycin A) with air-activated by Co(salen), *t*-butylhydroperoxide (TBHP) catalyzed by 3,3′-ditrifluoromethyldiphenyl diselenide, or hydrogen peroxide catalyzed by ebselen.[14] It corresponds to the modern trends in organic synthesis where the dioxygen, hydrogen peroxide, and TBHP are used as the reagents because they are cheap and environmentally friendly and are used on both laboratory and industrial scales.[15] Nevertheless, because these reagents are generally inactive or unselective toward most of the organic substrates, suitable activators must be used.[12–16]

In this work, we concentrated our study on the oxidative coupling of different ring-substituted *o*-aminophenols **1a**–**f** to aminophenoxazinones **2a**–**f** in both enzymatic and nonenzymatic conditions. The laccase was a catalyst for aerobic oxidation, whereas horseradish peroxidase was a catalyst for hydrogen peroxide oxidation. In the nonenzymatic conditions, the hydrogen peroxide activated by ebselen (2-phenylbenzisoselenazol-3(2*H*)-one) (**3**) and *t*-butylhydroperoxide in the presence of 3,3′-ditrifluoromethyldiphenyl diselenide (**4**) were used as reagents. Although the results strongly depended on the structure of substrate, oxidant used, and reaction conditions, the reaction has a synthetic value because of appreciable selectivity and moderate to high yields of the products.

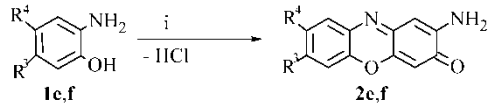
The aminophenols taken as the substrates fall into two different groups. There were *o*-aminophenol (**1a**) and its derivatives, having substituents in the vicinity to the amino and hydroxy groups **1b**–**d**, and *o*-aminophenols substituted at remote ring carbon atoms **1e**–**h**. The compounds **1a**–**h** were oxidized by chemical means with H_2O_2/**3** (method A) or TBHP/**4** (method B), or enzymatically with air/laccase (method C) or H_2O_2/horseradish peroxidase (method D). The results are presented in Scheme 1 and Table 1.

In all cases, the conversion of the substrates was complete (except carboxy derivatives **1c**), and desired products were isolated in the yields given in Table 1. The results of cyclocondensation depended on the substrate structure. The yields of the each product differed depending on the oxidant used.



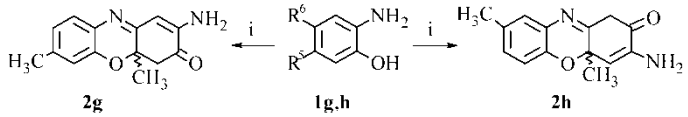
1a-d **2a-d**

R^1– R^2– H (**1a**, **2a**); (**2a**: questiomycin A)
R^1= CH_3, R^2= H (**1b**, **2b**);
R^1= COOH, R^2= H (**1c**, **2c**); (**2c**: cinnabarinic acid)
R^1= COOH, R^2= CH_3 (**1d**, **2d**) (**2d**: actinocin)



1e,f **2e,f**

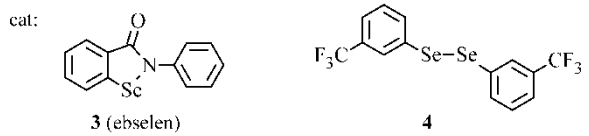
R^3– Cl, R^4– H (**1e**, **2e**); R^3– H, R^4– Cl (**1f**, **2f**)



2g **1g,h** **2h**

R^5– CH_3, R^6– H (**1g**); R^5– H, R^6– CH_3 (**1h**)

i: Method A: H_2O_2/cat. (**3**) 5.0 mol %, *t*-BuOH, 20°C, 20 h;
Method B: TBHP/cat. (**4**) 5.0 mol %, *t*-BuOH, 55°C, 20 h;
Method C: Air/laccase, H_2O, pH 5.0, 20°C, 0.5 – 23 h;
Method D: H_2O_2/horseradish peroxidase, H_2O, pH 5.0, 20°C, 0.3 – 15 h



3 (ebselen) **4**

Scheme 1.

In the chemical method, the reaction was carried out in *t*-butanol for 20 h, and the catalyst was used in the 5% molar amount (**3**,**4**). The reaction conditions were optimized. The reaction was faster when the molar ratio of H_2O_2 to the substrate **1a** was 4:1 and TBHP to **1a** was 5:1. In this way, *o*-aminophenol (**1a**) gave questiomycin A (**2a**) in good to excellent yield. 2-Amino-3-methylphenol (**1b**) was smoothly oxidized to the 1,9-dimethyl derivative of questiomycin A **2b** because the methyl group at the vicinity of the amino group strongly activates the substrate molecule. The product **2b** was formed in an excellent 93% yield even when the catalyst **3** was used in the amount of 0.10 mol %. In contrast, a strong electron-withdrawing carboxy substituent present at the same position of aminophenol **1c** inhibited the reaction, and cinnabarinic acid (**2c**) was not formed. The electron-donating methyl group in the

Table 1. Results of the oxidative cyclocondensation of *o*-aminophenols **1a–h**

Substrate	Method	Reaction time (h)	Product	Yield (%)[a]
1a	A	20	**2a**	92
	B	20		83[b]
	C	4.5		29 (39)[b]
	D	20 min		(40)[b]
1b	A	20	**2b**	92
	B	20		64
	C	23		24 (43)
	D	40 min		(52)
1c	A or B	20	**2c**	—
	C	22		38 (42)
	D	15		38
1d	A	20	**2d**	81
	C	1		(53)
1e	A	20	**2e**	44
	B	20		42
	C	3.5		51 (54)
1f	A	20	**2f**	—
	B	20		27
	C	7		35 (36)
1g	A	20	**2g**	42[c]
	B	20		55
	C	0.5		72 (84)
	D	0.5		(61)
1h	A	20	**2h**	1.5
	B	20		15
	C	1		60 (67)
	D	0.5		(15)

[a]Preparative yield. Data in parantheses refer to yields determined by UV/VIS analysis.

[b]See lit.[14]

[c]3 eq. of hydrogen peroxide was used (30%, 0.60 ml, 6.0 mmol).

vicinity to the hydroxyl group of 2-amino-3-carboxy-6-methylphenol **1d** made the substrate susceptible to cyclocondensation, and actinocin (**2d**) was the sole product. The chlorine atoms at remote ring carbon atoms in compounds **1e**,**f** entailed cyclocondensation to 2-aminophenoxazinones **2e**,**f** with elimination of one hydrochloride molecule. Methyl substituents present at the same positions in the aminophenols **1g**,**h** made the products more complex. The different nonplanar dihydrophenoxazinones **2g** and **2h** were produced in satisfactory to good yield. Generally, the yield of the produced phenoxazinones strongly depended on the electron character and position of substituents used. The results are given in Table 1.

Cyclocondensation of *o*-aminophenol and its derivatives in the presence of biocatalysts carried out in a phosphate buffer at room temperature yielded similar products. Enzymatic oxidation of *o*-aminophenol (**1a**) and its derivatives substituted in the vicinity of the amino group with electron-donating methyl group **1b** and electron-withdrawing carboxy group **1c,d** gave appropriate 2-aminophenoxazinones with quite satisfactory yield. *o*-Aminophenols with methyl groups substituted at remote ring carbon atom **1g**,**h** were converted to dihydrophenoxazinones **2g** and **2h** even up to 84%, and analogously substituted chloroaminophenols **1e,f** in aerobic condition (method C) gave 7- or 8-chloro-2-aminophenoxazinones **2e** and **2f**. A similar oxidation pattern of aminophenols by chemical means was observed.

The results presented here support our earlier hypothesis that formation of aminophenoxy radicals by oxidation of *o*-aminophenols by hydroperoxides in the presence of organoselenium compounds are crucial steps in their conversion to 2-aminophenoxazin-3-ones.[14] Our findings are also in accordance with well-known mechanism of action of laccase and peroxidase and other metaloenzymes.[17]

The cyclocondensation of *o*-aminophenols can be used successfully for preparation of phenoxazinones and dihydrophenoxazinones. The yield of the products strongly depends on the substrates and oxidant used. For the enzymatic method, more suitable is the air/laccase system, and for nonenzymatic conditions, more efficient as oxidant is hydrogen peroxide activated by ebselen. Both recommended catalysts are easy to obtain in the laboratory.[18]

We expect that 2-aminophenoxazinones with a planar structure of three condensed rings will be able to intercalate into cellular DNA similar to that described for anticancer actinomycin D drug.[1] Althought planar phenoxazinones and nonplanar dihydrophenoxazinones are suspected to be biologically active similar to that reported in Refs. 4–6, 8, and 19, the biological screening of compounds **2** and derivatives as potential cytostatics and virucides is in progress.

EXPERIMENTAL

Melting points were determined on a digital melting-point apparatus, Electrothermal IA 91100. ^{1}H NMR and ^{13}C NMR were measured with a Bruker DRX 300 spectrometer. IR (KBr pallets) was measured on a Perkin-Elmer 2000 FT spectrometer. UV/VIS was measured on a Jasco V-530 spectrofotometer. Because the physicochemical data reported in the previous papers had been incorrect, they are carefully revised.

t-Butylhydroperoxide (80% in di-*tert*-butylperoxide/water 3:2), 30% hydrogen peroxide, *o*-aminophenols **1a**–**c** and **1e**–**h**, horseradish peroxidase, and other commercial reagents and solvents were purchased from Aldrich, Fluka, or Sigma Co. Water to enzymatic transformations was distilled twice before using. Laccase was prepared as reported earlier in Ref. [18a]. 2-Amino-3-hydroxy-4-methylbenzoic acid (**1d**) was prepared by reduction of

nitro group of 3-hydroxy-4-methyl-2-nitrobenzoic acid with KBH_4 in the presence of Pd/C. 2-Phenylbenzisoselenazol-3(2*H*)-one (ebselen) was prepared from anthranilic acid.[18b] 3,3′-Ditrifluoromethyldiphenyl diselenide (**3**) was delivered by Ludwik Syper from our laboratory. The purity of the products was confirmed by comparison of their melting point with data given in literature for **2a**,[2a] **2b**,[20] and **2e,g**,[12] and measuring their NMR, IR, and UV/VIS spectra.

Preparation of 2-Amino-3-hydroxy-4-methylbenzoic Acid (1d)

To a suspension of 5% Pd/charcoal (50 mg) in distilled water (5.0 ml), methanol (10 ml), 2 drops of 4% sodium hydroxide in water and KBH_4 (500 mg, 9.27 mmol), 3-hydroxy-4-methyl-2-nitrobenzoic acid (0.986 g, 5.0 mmol) was added portionwise at 35–40°C over 20 min. The reaction was continued for an additional 2 h, filtered, and washed with methanol (5 ml). The filtrate was acidified with 2 M HCl to pH ca. 1 and washed with diethyl ether (3 × 150 ml). pH was adjusted to ca. 2.0 with sodium hydroxide and extracted with diethyl ether. The combined extracts were dried over anhydrous sodium sulfate; solvent was distilled off to give pure 2-aminophenol **1d** (0.710 g, 4.25 mmol, 85%). Mp 234–236°C (hydrochloride of **1d** mp 165–167°C, ref. [20] 162–163°C). ν_{max} (KBr) cm^{-1} 3400–2000 (broad, COOH, NH_2, OH), 1653 or 1612 (C=O), 1274, 1234, 1210 (C-N and/or C-O); ^1H NMR (DMSO-d_6) 10.11 (s, 1H, COOH), 8.29 (s, 2H, NH_2), 7.20 (d, $^3J = 8.1$ Hz, 1H, H-6), 6.31 (d, $^3J = 8.1$ Hz, 1H, H-5), 2.31 (s, 1H, OH), 2.15 (s, 3H, Me).

Oxidation of *o*-Aminophenols 1a–h by Chemical Means

To a mixture of *o*-aminophenol (**1a**–**h**) (2.0 mmol) and a selenium catalyst, ebselen (**3**) (for H_2O_2) or **4** (for TBHP) (0.10 mmol) in *t*-butanol (10 ml), 30% H_2O_2 (0.8 ml, 8.0 mmol), or TBHP (1.25 ml, 10 mmol) was added. The reaction mixture was magnetically stirred at room temperature (H_2O_2) or at 55°C (TBHP) for 20 h. After this period, the reaction was stopped by addition of a pinch of Pt/C and the solution of $NaHCO_3$ (1.25 g) and NaCl (4.0 g) in water (50 ml), and the mixture was vigorously stirred at room temperature until evolution of carbon dioxide and dioxygen ceased. The product was extracted with chloroform (until the red color disappear) and dried over anhydrous Na_2SO_4. Silica gel (70–230 mesh, 7.5 g) was added to the solution. The solvent was removed in vacuo, the residue was poured into a silica-gel column (70–230 mesh, 20 g), and products **2a**,**b**,**e**,**f**,**h** were isolated by eluting with chloroform–ethyl acetate (5:1) and **2g** with gradient (5:1–2:1). Actinocin (**2d**) crystallized directly on the reaction mixture and was filtered, washed with *t*-butanol, and dried in the air. The results are given in Table 1.

Data

2-Amino-3*H*-phenoxazin-3-one (2a) (Questiomycin A): Red powder. Mp 258°C (ref. [2a,12] mp 256–258°C). ^{1}H NMR (DMSO-d_6): 7.70 (dd, $^3J = 7.8$ Hz, $^4J = 1.5$ Hz, 1H, H-9); 7.50 (dd, $^3J = 8.2$ Hz, $^4J = 1.9$ Hz, 1H, H-6); 7.45 (ddd, $^3J = 8.2$ Hz, $^3J = 6.8$ Hz, $^4J = 1.5$ Hz, 1H, H-7); 7.38 (ddd, $^3J = 7.8$ Hz, $^3J = 6.8$ Hz, $^4J = 1.9$ Hz, 1H, H-8); 6.83 (s, 2H, NH_2); 6.36 (s, 1H, H-1 or H-4); 6.34 (s, 1H, H-1 or H-4). IR (KBr): $\gamma = 3413$, 3306 cm^{-1} (NH_2), 1587 cm^{-1}(br.) (C═O, C═N), 1273, 1203, 1115 cm^{-1} (C-N, C-O). UV/VIS (methanol): $\lambda_{max} = 434$ nm (log $\varepsilon = 4.252$).

2-Amino-1,9-dimethyl-3*H*-phenoxazin-3-one (2b): Red powder. Mp 247°C (ref. [21] mp 233°C). ^{1}H NMR (DMSO-d_6): 7.20–7.40 (m, 3H, H-6, H-7, H-8); 6.35 (s, 2H, NH_2); 6.23 (s, 1H, H-4); 2.59 (s, 1H, 1-CH_3 or 9-CH_3); 2.22 (s, 3H, 1-CH_3 or 9-CH_3). IR (KBr): $\nu = 3462$, 3389, 3359, 3248 cm^{-1} (NH_2 and N-H of tautomer), 1550–1639 cm^{-1} (C═O, C═N), 1250 cm^{-1} (C-N), 1201 cm^{-1} (C-O). UV/VIS (methanol): $\lambda_{max} = 422$ nm (log $\varepsilon = 4.379$).

2-Amino-1,9-dicarboxy-4,6-dimethyl-3*H*-phenoxazin-3-one (2d) (Actinocin): Dark red powder. Mp 259.5–260°C (ref. [18a] mp not reported). ^{1}H NMR (DMSO-d_6): 9.68 (s, 2H, COOH), 8.76 (s, 2H, NH_2), 7.85 (d, $^3J = 8.0$ Hz, 1H, H-8), 7.47 (d, $^3J = 8.0$ Hz, 1H, H-7), 2.50 (s, 3H, 4-CH_3 or 6-CH_3), 2.12 (s, 3H, 4-CH_3 or 6-CH_3). IR (KBr): $\nu = 2000$–3400 cm^{-1} (COOH and OH), 3390, 3251 cm^{-1} (NH_2), 1678 cm^{-1} (C═O), 1583 cm^{-1} (C═N), 1324, 1293, 1225 cm^{-1} (C-N and/or C-O). UV/VIS (methanol): $\lambda_{max} = 430$ nm (log $\varepsilon = 4.439$).

2-Amino-7-chloro-3*H*-phenoxazin-3-one (2e): Red powder. Mp 296–297°C (ref. [12] mp 288°C). ^{1}H NMR ($CDCl_3$): 7.70 (d, $^3J = 8.6$ Hz, 1H, H-9), 7.65 (d, $^4J = 2.2$ Hz, 1H, H-6), 7.43 (dd, $^3J = 8.6$ Hz, $^4J = 2.2$ Hz, 1H, H-8), 6.87 (s, 2H, NH_2), 6.36 (s, 2H, H-1 and H-4). IR (KBr): $\nu = 3453$ and 3364 cm^{-1} (NH_2), 1610 (broad) and 1578 cm^{-1} (C═O and C═N), 1284, 1181, 1073 cm^{-1} (C-N and/or C-O). UV/VIS (methanol): $\lambda_{max} = 436$ nm (log $\varepsilon = 4.424$).

2-Amino-8-chloro-3*H*-phenoxazin-3-one (2f): Red powder. Mp 270–271°C ($CHCl_3$). ^{1}H NMR (DMSO-d_6): 7.74 (d, $^4J = 2.4$ Hz, H-9); 7.54 (d, $^3J = 8.8$ Hz, 1H, H-6); 7.47 (dd, $^3J = 8.8$ Hz, $^4J = 2.4$ Hz, 1H, H-7); 7.01 (s, 2H, NH_2); 6.38 (s, 1H, H-1 or H-4); 6.32 (s, 1H, H-1 or H-4). IR (KBr): $\nu = 3345$, 3367 cm^{-1} (NH_2), 1607(br.), 1572 cm^{-1} (C═O, C═N), 1207, 1188, 1072 cm^{-1} (C-N, C-O). UV/VIS (ethanol): $\lambda_{max} = 439$ nm (log $\varepsilon = 4.345$). Found: C, 58.04; H, 3.10; Cl, 14.55. ($C_{12}H_7N_2O_2Cl$) 246.65 requires C, 58.44; H, 2.86; Cl, 14.37.

2-Amino-4,4α-dihydro-4α,7-dimethyl-3*H*-phenoxazin-3-one (2g): Mp 231°C (ref. [12] mp 178.5–179.5°C). ^{1}H NMR (DMSO-d_6): 7.08

(d, $^3J = 7.9$ Hz, 1H, H-9); 6.79 (dd, $^3J = 7.9$ Hz, $^4J = 1.0$ Hz, 1H, H-8); 6.71 (d, $^4J = 1.0$ Hz, 1H, H-6); 6.36 (s, 2H, NH_2); 6.04 (s, 1H, H-1), 3.19 (d, $^2J = 15.9$ Hz, 1H, H-4a); 2.97 (d, $^2J = 15.9$ Hz, 1H, H-4b); 2.25 (s, 3H, 7-CH_3); 1.09 (s, 3H, 4α-CH_3). IR (KBr): v = 3401, 3288, 3165 cm^{-1} (NH_2 and OH of tautomer), 1695, 1623 cm^{-1} (C═O, C═N). UV/VIS (methanol): $\lambda_{max} = 400$ nm (log $\varepsilon = 4.144$).

3-Amino-1,4α-dihydro-4α,8-dimethyl-2*H*-phenoxazin-2-one (2h): Mp 204°C (ref. [5] not reported). ^{1}H NMR ($CDCl_3$): 10.03 (s, 2H, NH_2); 7.05 (s, 1H, H-9); 6.88 (s, 2H, H-6, H-7); 6.14 (s, 1H, H-4); 3.33 (d, $^2J = 15.2$ Hz, 1H, H-1a); 3.26 (d, $^2J = 15.2$ Hz, 1H, H-1b); 2.34 (s, 3H, 8-CH_3); 1.42 (s, 3H, 4α-CH_3). UV/VIS (methanol): $\lambda_{max} = 349$ nm (log $\varepsilon = 4.250$).

Laccase-Catalyzed Aerobic Oxidation of *o*-Aminophenols (1a–h)

Aminophenol **1a**–**h** (0.50 mmol) in methanol (2 ml) was added to a vigorously stirred solution of laccase (0.6U for **1b**,**e**,**f**; 3U for **1h**; 4U for **1a**; 5U for **1g**; 6U for **1c**; 10U for **1d**)[22] in 5.0 pH phosphate buffer (100 ml, 0.066 M, Na_2HPO_4/KH_2PO_4) in an open vessel to supply oxygen for the period given in Table 1. The reaction was monitored using thin-layer chromatography (TLC). After the reaction was finished, products **2a**,**b** and **2e**–**h** were extracted with ethyl acetate, dried other anhydrous Na_2SO_4, and purified on a silica-gel column as described by chemical means (in this article).

For isolation of cinnabainic acid (**2c**) and actinocin (**2d**), the reaction mixture was acidified to pH ca. 2 with 0.15 M hydrochloric acid. The formed precipitate was washed several times with distilled water, collected by centrifugation, and crystallized from methanol–water to yield pure acids **2c**,**d**. The results are given in Table 1. Spectroscopic data are recorded.

Data

2-Amino-1,9-dicarboxy-3*H*-phenoxazin-3-one (2c) (cinnabarinic acid): Dark red powder. Mp > 330°C (ref. [18a] mp not reported). ^{1}H NMR (DMSO-d_6): 9.71 (s, 1H, COOH); 8.79 (s, 1H, COOH); 7.95 (dd, $^3J = 7.1$ Hz, $^4J = 1.1$ Hz, 1H, H-6 or H-8); 7.77 (dd, $^3J = 7.7$ Hz, $^4J = 1.1$ Hz, 1H, H-6 or H-8); 7.60 (dd, $^3J = 7.7$ Hz, $^3J = 7.1$ Hz, 1H, H-7); 6.60 (s, 1H, H-4); 5.75 (s, 2H, NH_2). UV/VIS (ethanol): $\lambda_{max} = 450$ nm (log $\varepsilon = 4.305$).

Horseradish Peroxidase–Catalyzed Hydrogen Peroxide Oxidation of *o*-Aminophenols

To the magnetically stirred solution of horseradish peroxidase (4U)[23] in pH 5.0 phosphate buffer (70 ml, 0.066 M, Na_2PO_4/KH_2PO_4), *o*-aminophenol (**1**)

(0.18 mmol) in methanol (1.0 ml) and 30% H_2O_2 (0.090 ml, 0.90 mmol) were added. The reaction was monitored by TLC. When the substrate vanished, the products were extracted with ethyl acetate and dried over anhydrous Na_2SO_4. Solvent was removed, and the residue was dissolved in ethanol. The amounts of **2a**,**b**,**g**,**h** were estimated by UV/VIS spectroscopy as the data given in chemical means and aerobic oxidation (in this article). For **2c**, the reaction mixture was acidified to pH 2 with hydrochloric acid, and the product was extracted with ethyl acetate to yield dark red powder of cinabarinic acid. The results are given in Table 1.

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