Full Papers

Synthesis of Echinamines A and B, the First Aminated Hydroxynaphthazarins Produced by the Sea Urchin *Scaphechinus mirabilis* and Its Analogues†

Nataly D. Pokhilo,‡ Maria I. Shuvalova,§ Maxim V. Lebedko,§ Galina I. Sopelnyak,‡ Alla Ya. Yakubovskaya,‡ Natalia P. Mischenko,‡ Sergey A. Fedoreyev,‡ and Victor Ph. Anufriev*,‡

Pacific Institute of Bioorganic Chemistry, Russian Academy of Sciences, 159, Prospect 100 let Vladivostoku, Vladivostok 690022, Russian Federation, and Institute of Chemistry and Applied Ecology, Far Eastern National University, 27, Oktyabr'skaya Street, Vladivostok 690600, Russian Federation

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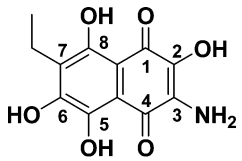
The first total synthesis of two marine aminated hydroxynaphthazarins, echinamines A (3-amino-7-ethyl-2,5,6,8-tetrahydroxy-1,4-naphthoquinone) and B (2-amino-7-ethyl-3,5,6,8-tetrahydroxy-1,4-naphthoquinone), produced by the sea urchin *Scaphechinus mirabilis* is described. This was achieved from 1,2,4-triacetoxybenzene (**13**) through a sequence involving double Fries rearrangement of **13**, reduction of 3,5-diacetyl-1,2,4-trihydroxybenzene (**14**), methylation of 3,5-diethyl-1,2,4-trihydroxybenzene (**15**), simultaneous double acylation of 3,5-diethyl-1,2,4-trimethoxybenzene (**16**) with a dichloromaleic anhydride−ethyl radical elimination process, methylation of 6,7-dichloro-3-ethyl-2-hydroxynaphthazarin (**17**), nucleophilic substitution of a chlorine atom by the methoxy group in 6,7-dichloro-3-ethyl-2-methoxynaphthazarin (**18**), introduction of an amino group via direct substitution of a chlorine atom in 7-chloro-3-ethyl-2,6-dimethoxy- (**11**) and 7-chloro-2-ethyl-3,6-dimethoxynaphthazarins (**12**) by an azido group, and functional group deprotection. The synthesis of amino analogues of spinazarin and spinochrome D is also described.

More than 100 years have passed since the first isolation of quinonoid pigments (spinochromes) from the sea urchin (*Echinoidea*). Some spinochromes and their synthetic analogues are known today as biologically active compounds that possess high antimicrobial,[1] antialgal,[2] antioxidant,[3] and cardioprotective activity[4] or are actual drugs.[5,6] From the sea urchin *Scaphechinus mirabilis* (Agassiz) we have recently isolated two novel spinochromes named echinamines A (**1**) and B (**2**).[7] Echinamines A and B are the first polyhydroxylated naphthazarins[8] (5,8-dihydroxy-1,4-naphthoquinones) having an amino constituent. In in vitro experiments, compounds **1** and **2** were found to be highly effective antioxidants.[7] However, echinamines A and B are not easily accessible on a preparative scale for extended bioassays due to their very low natural abundance and separation difficulties.



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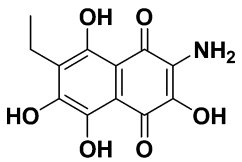

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Results and Discussion

Two approaches to the synthesis of echinamines A and B have been investigated. The first is based on the application of a conjugated addition reaction of hydrazoic acid to 1,4-naphthoquinones as the key step. In this case the corresponding 2-amino-1,4-naphthoquinones were obtained directly (without the need of a reduction step of azido derivatives) via an overall intramolecular oxidation−reduction mechanism.[9] However, in our experiments, mompain dimethyl ether **3**, a model substrate, did not add hydrazoic acid. In the case of naphthopurpurin monomethyl ether **4**, the reaction resulted in an inseparable mixture of products **5** and **6** (Scheme 1).

In our second approach, we introduced an amino group into a naphthazarin nucleus via direct substitution of a chlorine atom by an azido group (Scheme 2). The nucleophilic substitution of a chlorine atom by an azido group in 2-methoxy-3-chloronaphthazarin (**7**) prepared from dichloronaphthazarin **8** (see below) by the action of excess NaN_3 in DMSO, followed by treatment with water, gave the expected 3-amino-2-methoxynaphthazarin (**9**) in moderate yield. Product **9** is the result of reduction of the corresponding azido-1,4-naphthoquinone (derived from **7**) with hydrazoic acid that arises during treatment of the reaction mixture with water (Scheme 2).[9] Aminomethoxynaphthazarin **9** was easily converted into the amino analogue spinazarin (**10**) by the action of concentrated HBr in acetic acid.

The second method was employed to obtain echinamines A (**1**) and B (**2**). The necessary chloromethoxynaphthazarins **11** and **12** were obtained from triacetoxybenzene **13** by the route summarized in Scheme 3, as follows. A double Fries rearrangement of triacetoxybenzene **13** in rigid conditions (melt $AlCl_3$−NaCl) gave the green-yellow diacetyl derivative **14** in quantitative yield. Clemmensen reduction then afforded the corresponding diethyltrihydroxybenzene **15**, and methylation produced trimethoxy derivative **16**. Intermediate **17** was formed as a result of double acylation of the trisubstituted hydroquinone derivative **16** with dichloromaleic anhydride. During the course of this reaction, elimination of ethyl radical at position 5 takes place. This method of forming the naphthazarin system constitutes a useful addition to existing approaches.[10] Methylation of hydroxynaphthazarin **17** with trimethyl

† Dedicated to the memory of Professor George B. Elyakov, who has been an inspiration to us over the years.

* To whom correspondence should be addressed. Tel: 7(4232)-346669. Fax: 7(4232)-314050. E-mail: anufriev@piboc.dvo.ru.

‡ Pacific Institute of Bioorganic Chemistry.

§ Institute of Chemistry and Applied Ecology, Far Eastern National University.

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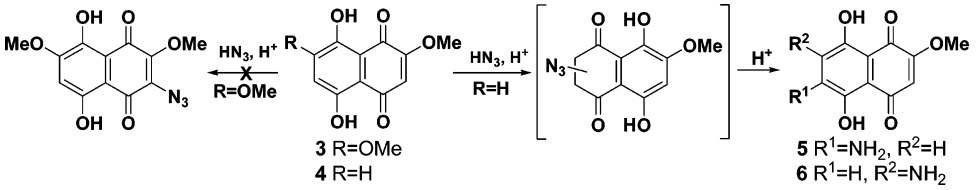
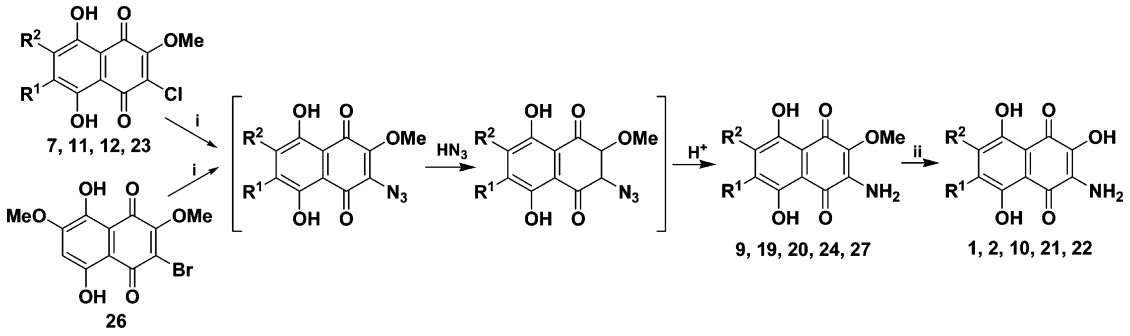
Scheme 1

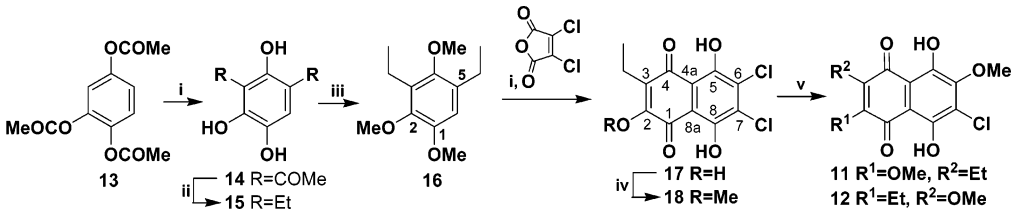


Scheme 2[a]



[a] **1** R^1 = OH, R^2 = Et; **2** R^1 = Et, R^2 = OH; **7** R^1 = R^2 = H; **9**, **10** R^1 = R^2 = H; **11** R^1 = OMe, R^2=Et; **12** R^1 = Et, R^2 = OMe; **19** R^1 = OMe, R^2 = Et; **20** R^1 = Et, R^2 = OMe; **21** R^1 = OH, R^2 = H; **22** R^1 = H, R^2 = OH; **23** R^1 = OMe, R^2 = H; **24** R^1 = OMe, R^2 = H; **27** R^1 = H, R^2 = OMe. (i) NaN_3, DMSO, 50 °C, then H_2O; (ii) HBr−HOAc, reflux.

Scheme 3[a]



[a] (i) $AlCl_3$−NaCl, 190 °C; (ii) Zn/Hg, HCl, reflux; (iii) Me_2SO_4, NaOH; (iv) $(MeO)_3CH$, reflux; (v) MeOH/CsF/Al_2O_3, reflux.

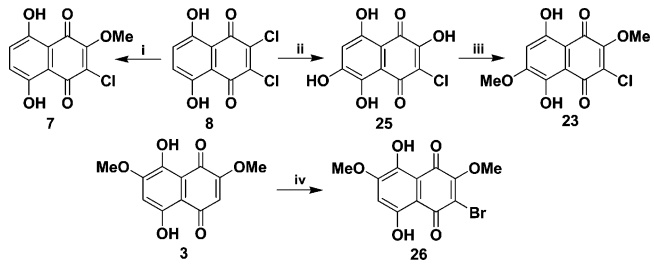
orthoformate gave the corresponding methyl ether **18**, and nucleophilic substitution of chlorine by a methoxy group in that compound afforded a mixture of 3-chloro-7-ethyl-2,6-dimethoxy- (**11**) and 3-chloro-6-ethyl-2,7-dimethoxynaphthazarins (**12**) in high yield. Isomers **11** and **12** were separable, on a preparative scale, and were purified by column chromatography. The structures of dimethoxynaphthazarins **11** and **12** were assigned on the basis of spectral analysis and direct comparison with authentic samples.[11]

Dimethoxynaphthazarins **11** and **12** underwent amination (**11** → **19**, **12** → **20**) via direct substitution of chlorine by an azido group and reduction of the corresponding azido-1,4-naphthoquinones (Scheme 2). Demethylation of dimethyl ethers **19** and **20** gave the expected aminohydroxynaphthazarins **1** and **2**. Synthetic compounds **1** and **2** are identical in all respects with echinamines A and B isolated from the sea urchin *S. mirabilis*.[7]

The same approach was used for the synthesis of amino analogues of spinochrome D (**21** and **22**), which were never reported as a natural product, but are presumed to be present among the minor pigments of the sea urchin *S. mirabilis*. The necessary substrate **23** for the conversion **23** → **24** → **21** (Scheme 2) was prepared by oxidation of dichloronaphthazarin **8** with sulfuric acid followed by methylation of dihydroxynaphthazarin **25** with diazomethane according to Scheme 4. Compound **26**, in turn, was obtained by direct bromination of dimethyl ether **3**.

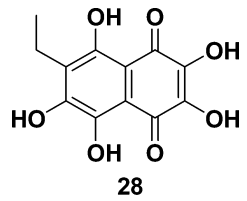
It should be noted that when echinamines A and B were worked up using acids (the standard procedure for separation of spinochromes from sea urchins), the formation of echinochrome (**28**), a major pigment of the sea urchin *S. mirabilis*,[7] was observed. The apparent ease of conversion of compounds **1** and **2** to **28** under acidic

Scheme 4[a]



[a] (i) MeOH/CsF/Al_2O_3, reflux; (ii) H_2SO_4−H_3BO_3, 200 °C; (iii) CH_2N_2, Et_2O; (iv) Br_2, AcOH.

conditions suggests that echinochrome is, at least in part, an artifact of the isolation procedure.



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In this paper, we describe the first total synthesis of two marine aminated hydroxynaphthazarins, echinamines A and B, which are produced by the sea urchin *S. mirabilis*. The basis for this synthesis is simultaneous double acylation of 3,5-diethyl-1,2,4-trimethoxybenzene with dichloromaleic anhydride followed by an ethyl radical elimination process and the introduction of an amino group via direct substitution of a chlorine atom in a naphthazarin moiety by an azido group. The general utility of this latter transformation has

been demonstrated with amino analogues of spinazarin and spinochrome D.

Experimental Section

General Experimental Procedures. All melting points were determined with a Boetius apparatus and are uncorrected. The IR absorption spectra were recorded on a Vector 22 IR-FT spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE DPX-300 and DRX-500 NMR spectrometers at ^{1}H and ^{13}C frequencies of 300 and 75 MHz, or 500 and 125 MHz, respectively. Chemical shifts in δ are relative to TMS as an internal reference ($\delta = 0$). Mass spectra were taken on a LKB-9000S spectrometer (direct sample inlet, ionizing energy 70 eV, and elevated temperature). Elemental analysis was performed with a Flash EA1112 CHN/MAS200. The course of reactions was monitored and the purity of the compounds obtained were checked by TLC (Merck Kieselgel 60F-254 plates were preliminarily treated with 0.05 M tartaric acid in MeOH and dried at ~50 °C for 2−3 h; a 3:1 *n*-hexane/acetone mixture was used as an eluent). Preparative TLC and column chromatography were performed on silica gel L (Chemapol, Czechia), 5/40 and 40/100 μm, respectively, using *n*-hexane/acetone. Yields were not optimized.

The following starting compounds were prepared according to previously described procedures: 5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (**3**),[11] 5,8-dihydroxy-2-methoxy-1,4-naphthoquinone (**4**),[12] 2,3-dichloro-5,8-dihydroxy-1,4-naphthoquinone (**8**).[10]

6-Amino-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone (5) and 7-Amino-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone (6). To a stirred solution of naphthopurpurin monomethyl ether **4** (220 mg, 1.0 mmol) in 100 mL of methanol under argon was added a solution of sodium azide (740 mg, 11.4 mmol) in 10 mL of water, acidified to pH ~4 (with 1 N HCl). The reaction mixture was stirred at 50 °C for 20 h. Then the mixture was extracted with EtOAc, and the organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The mixture of products **5** and **6** in a 1:1.7 ratio was isolated by preparative TLC (*n*-hexane/acetone, 3:1) as a brown powder (31 mg, 13%): R_f 0.12; IR ($CHCl_3$) ν_{max} 3515 m, 3395 m (NH_2), 1645 w, 1629 m, 1618 m (C═O), 1593 s, 1564 s (NH_2, C═C) cm^{-1}.

Compound 5: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.97 (3H, s, OCH_3), 5.33 (2H, br s, NH_2), 5.99 (1H, s, H_{arom}), 6.42 (1H, s, H_{arom}), 12.73, 13.81 (each 1H, s, α-OH).[13]

Compound 6: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.94 (3H, s, OCH_3), 5.12 (2H, br s, NH_2), 6.02 (1H, s, H_{arom}), 6.55 (1H, s, H_{arom}), 12.49, 13.47 (each 1H, s, α-OH).[13]

General Procedure 1. Nucleophilic Substitution of Chlorine Atoms in Chloronaphthazarins 8 and 18. A mixture of the corresponding well-dried substrate (1 mmol), anhydrous CsF (6−7 mmol), activated neutral alumina (Aldrich, ~150 mesh, for chromatography) (1.0−1.5 g), and absolute MeOH (100 mL) was stirred in a closed flask under reflux for 1 h. After being cooled the absorbent was separated by filtration and washed successively with 5% HCl (2 mL) and acetone (5 mL) or hot alcohol. The combined filtrate was concentrated in vacuo, and the residue was treated with $CHCl_3$. The organic layer was washed successively with water and brine, dried (Na_2SO_4), filtered, and concentrated. The products were isolated by column chromatography followed by crystallization from EtOH.

In accordance with general procedure 1, **3-chloro-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone** (**7**) was obtained as brown-red crystals from **8** (180 mg, 71%): mp 141−144 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 4.34 (3H, s, OCH_3), 7.25, 7.29 (each 1H, d, $J = 9.6$ Hz, H_{arom}), 12.25, 12.44 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.9 (C, C-1), 181.5 (C, C-4), 159.2 (C, C-8), 158.2 (C, C-5), 157.2 (C, C-2), 130.5 (CH, C-6), 129.6 (CH, C-7), 128.5 (C, C-3), 110.8 (C, C-8a), 110.1 (C, C-4a), 62.2 (CH_3, OCH_3); EIMS *m*/*z* 255/257 [M + 1]$^+$ (13), 254/256 [M]$^+$ (100), 236/238 (26); *anal.* C 52.03%, H 2.81%, calcd for $C_{11}H_7ClO_5$, C 51.89%, H 2.77%.

General Procedure 2. Reaction of Chloronaphthazarins 7, 11, 12, 23, and 26 with NaN_3. To a stirred solution of the corresponding naphthazarin (0.5 mmol) in 40 mL of DMSO was added NaN_3 (3.0 mmol). The reaction mixture was stirred at 60−70 °C and monitored by TLC. Then the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated, and the product was isolated by preparative TLC (*n*-hexane/acetone, 3:1).

In accordance with general procedure 2, **3-amino-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone** (**9**) was obtained as a brown powder from **7** (50 mg, 43%): R_f 0.34; mp >260 °C (dec); IR ($CHCl_3$) ν_{max} 3514 m, 3398 m (NH_2), 2857 m, 1648 w, 1625 w, 1595 s (C═O), 1569 m, 1556 s (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 4.03 (3H, s, OCH_3), 5.15 (2H, br s, NH_2), 7.10, 7.20 (each 1H, d, $J = 9.4$ Hz, H_{arom}), 11.97, 12.87 (each 1H, s, α-OH); EIMS *m*/*z* 236 [M + 1]$^+$ (46), 235 [M]$^+$ (100), 192 (41), 189 (26), 165 (34), 164 (20); *anal.* C 56.64%, H 3.72%, N 6.00%, calcd for $C_{11}H_9NO_5$, C 56.17%, H 3.86%, N 5.96%.

General Procedure 3. Hydrolysis of Aminomethoxynaphthazarins 9, 19, 20, 24, and 27. The methyl ether of aminomethoxynaphthazarin (0.2 mmol) in concentrated HBr/HOAc, 1:1 (20 mL), was refluxed for 0.5−1 h. The reaction mixture was diluted with H_2O (100 mL) and extracted with EtOAc. The extract was concentrated, and the product was isolated by preparative TLC (*n*-hexane/acetone, 2:1).

In accordance with general procedure 3, **3-amino-2,5,8-trihydroxy-1,4-naphthoquinone** (**10**) was obtained as an orange-brown powder from **9** (37 mg, 85%); R_f 0.27; mp >300 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 6.36 (2H, br s, NH_2), 7.14, 7.20 (each 1H, d, $J = 9.3$ Hz, H_{arom}), 8.31 (1H, s, β-OH), 12.01, 12.66 (each 1H, s, α-OH); EIMS *m*/*z* 223 [M + 2]$^+$ (10), 222 [M + 1]$^+$ (65), 221 [M]$^+$ (100), 194 (39), 193 (32); *anal.* C 54.46%, H 3.07%, N 6.40%, calcd for $C_{10}H_7NO_5$, C 54.31%, H 3.19%, N 6.33%.

3,5-Diacetyl-1,2,4-trihydroxybenzene (14). At 140 °C, 1,2,4-triacetoxybenzene (**13**) (60 g, 0.24 mol) was added with vigorous stirring to a melt consisting of anhydrous $AlCl_3$ (234 g, 1.75 mol) and NaCl (44 g, 0.75 mol). The temperature of the mixture was increased to 195 °C, and the melt was stirred for an additional 5 min. The reaction mixture was cooled, hydrolyzed with 5% HCl (2 L), and allowed to stand for 12 h. From the resulting crude product **14** was separated as yellow powder and washed with 5% HCl (1 L) and hot H_2O (1 L): yield 48.0 g (96%); mp 180−185 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 2.56, 2.79 (each 1H, s, $COCH_3$), 5.47 (1H, br s, OH), 7.43 (1H, s, H_{arom}), 14.39, 14.81 (each 1H, s, OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 206.1 (C, C-5-*C*O*C*H$_3$), 203.0 (C, C-3-*C*O*C*H$_3$), 161.8 (C, C-4), 158.6 (C, C-2), 137.1 (C, C-1), 119.7 (CH, C-6), 110.3 (C, C-3), 109.5 (C, C-5), 33.2 (CH_3, C-5-CO*C*H$_3$), 26.2 (CH_3, C-3-CO*C*H$_3$); EIMS *m*/*z* 210 [M]$^+$ (90), 195 (100).

3,5-Diethyl-1,2,4-trihydroxybenzene (15). In a three-necked 3 L round-bottom flask equipped with a mechanical stirrer and a reflux condenser diacetyltrihydroxybenzene **14** (24 g, 0.11 mol), solid zinc amalgam (500 g), and concentrated HCl (330 mL) were mixed. The mixture was stirred under reflux for 0.5 h, then a second portion of substrate **14** (24 g, 0.11 mol) and concentrated HCl (330 mL) was added, and the mixture was heated to reflux for 3 h. The hot solution was decanted and allowed to stand for 15 h. The solid was separated, washed with 35 mL of cold H_2O, and successively dried in vacuo. According to the ^{1}H NMR data, the resulting product, a white amorphous solid, contained 80% (33 g) of diethyltrihydroxybenzene **15**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18, 1.20 (each 3H, t, $J = 7.8$ Hz, CH_3), 2.52, 2.68 (each 2H, q, $J = 7.8$ Hz, CH_2), 4.35, 4.54 (each 1H, br s, OH), 5.15 (1H, br s, OH), 6.56 (1H, s, H_{arom}). Crude product **15** was used in the next synthetic step without purification.

3,5-Diethyl-1,2,4-trimethoxybenzene (16). To a mechanically stirred dark mixture of 10% aqueous NaOH (290 g) and 3,5-diethyl-1,2,4-trihydroxybenzene (**15**) (33 g, 0.18 mol) under argon was added dropwise Me_2SO_4 (68.6 g, 0.54 mol), maintaining the temperature of the mixture below 40 °C. After Me_2SO_4 was added, the reaction mixture was heated for 30 min in a boiling water bath to decompose the excess Me_2SO_4. After the mixture was cooled, the organic layer was separated, and the aqueous layer extracted with benzene. The combined organic layers were washed with 5% aqueous NaOH and H_2O, dried over anhydrous $CaCl_2$, and filtered, and the solvent was removed using a rotary evaporator. The resulting product, a colorless oil, was purified by vacuum distillation (33.3 g, 82%): bp 133−139 °C, 7 mmHg; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.19, 1.24 (each 3H, t, $J = 7.8$ Hz, CH_3), 2.64, 2.66 (each 2H, q, $J = 7.8$ Hz, CH_2), 3.71, 3.82, 3.83 (each 3H, s, OCH_3), 6.60 (1H, s, H_{arom}).

6,7-Dichloro-3-ethyl-2,5,8-trihydroxy-1,4-naphthoquinone (17). At 140 °C, a mixture of 1,2,4-trimethoxy-3,5-diethylbenzene (**16**) (1.75 g, 7.8 mmol) and dichloromaleic anhydride (3 g, 17.9 mmol) was added with vigorous stirring to a melt consisting of anhydrous $AlCl_3$ (16 g, 120 mmol) and NaCl (3.2 g, 55 mmol). The temperature of the mixture was increased to 195 °C, and the melt was stirred for an additional 5

min. The reaction mixture was cooled, hydrolyzed with 5% HCl (200 mL), and allowed to stand for 12 h. The resulting crude product was separated, washed with 100 mL of hot H_2O, dried, and purified by column chromatography to give **17** as wine-red needles (1.39 g, 59%): mp 156−158 °C (lit.[14] mp 156−158 °C); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.18 (3H, t, J = 7.7 Hz, CH_3), 2.66 (2H, q, J = 7.7 Hz, CH_2), 7.42 (1H, br s, β-OH), 12.07, 13.60 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 187.5 (C, C-4), 181.5 (C, C-1), 154.6 (C, C-5), 154.3 (C, C-8), 153.5 (C, C-2), 135.3 (C, C-6), 131.3 (C, C-7), 127.7 (C, C-3), 109.3 (C, C-4a), 109.0 (C, C-8a), 16.5 (CH_2, *C*H_2CH$_3$), 12.5 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 302/304/306 [M]$^+$ (71), 286/288/290 (83), 285/287/289 (65), 267/269 (45), 252/254 (35), 244/246 (31), 230 (100); *anal*. C 47.64%, H 2.71%, calcd for $C_{12}H_8Cl_2O_5$, C 47.55%, H 2.66%.

6,7-Dichloro-3-ethyl-5,8-dihydroxy-2-methoxy-1,4-naphthoquinone (18). A mixture of hydroxynaphthazarin **17** (400 mg, 1.32 mmol) and trimethyl orthoformate (30 mL) was refluxed for 12 h. After being cooled in the refrigerator to 8 °C, the crystals were separated and dried in vacuo to give methoxynaphthazarin **18** as wine-red prisms (360 mg). The filtrate was evaporated and the residue was purified by preparative TLC using *n*-hexane/acetone (2:1) to give 40 mg of the same product. Total yield of **18** was 94%; mp 138−141 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.14 (3H, t, J = 7.1 Hz, CH_3), 2.65 (2H, q, J = 7.1 Hz, CH_2), 4.17 (3H, s, OCH_3), 12.86, 13.32 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.9 (C, C-4), 177.4 (C, C-1), 159.5 (C, C-8), 158.8 (C, C-5), 157.2 (C, C-2), 138.3 (C, C-3), 135.5 (C, C-7),[15] 134.1 (C, C-6),[15] 110.0 (C, C-8a), 108.9 (C, C-4a), 61.8 (CH_3, OCH_3), 17.0 (CH_2, *C*H$_2$-CH$_3$), 13.2 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 316/318/320 [M]$^+$ (100), 301/303/305 (73), 283/285/287 (10), 273/275/277 (15); *anal*. C 49.54%, H 3.23%, calcd for $C_{13}H_{10}Cl_2O_5$, C 49.23%, H 3.18%.

In accordance with general procedure 1, products **11** and **12** were obtained as red solids.

3-Chloro-7-ethyl-5,8-dihydroxy-2,6-dimethoxy-1,4-naphthoquinone (11): 156 mg (50%); R_f 0.62; mp 137−139 °C (acetone); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.15 (3H, t, J = 7.5 Hz, CH_3), 2.69 (2H, q, J = 7.5 Hz, CH_2), 4.12, 4.25 (each 3H, s, OCH_3), 13.09, 13.14 (each 1H s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.5 (C, C-4), 170.6 (C, C-1), 168.4 (C, C-5), 166.2 (C, C-8), 156.6 (C, C-2), 156.2 (C, C-6), 137.0 (C, C-3), 126.0 (C, C-7), 108.3 (C, C-8a), 108.5 (C, C-4a), 61.9 (CH_3, C-6-O*C*H$_3$), 61.6 (CH_3, C-2-O*C*H$_3$), 17.0 (CH_2, *C*H$_2$CH$_3$), 13.4 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 312/314 [M]$^+$ (100), 311/313 (92), 297/299 (12), 296/298 (25), 294 (10), 293 (11), 256 (9); *anal*. C 53.79%, H 4.30%, calcd for $C_{14}H_{13}ClO_6$, C 53.84%, H 4.20%.

6-Chloro-3-ethyl-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (12): 81 mg (26%); R_f 0.59; mp 129−131 °C (acetone); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.17 (3H, t, J = 7.5 Hz, CH_3), 2.71 (2H, q, J = 7.5 Hz, CH_2), 4.11, 4.22 (each 3H, s, OCH_3), 12.83, 13.34 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 171.8 (C, C-4), 169.3 (C, C-5), 169.0 (C, C-1), 167.8 (C, C-8), 156.1 (C, C-2), 155.3 (C, C-7), 138.2 (C, C-3), 127.6 (C, C-6), 110.3 (C, C-8a), 106.6 (C, C-4a), 61.8 (CH_3, C-7-O*C*H$_3$), 61.6 (CH_3, C-2-O*C*H$_3$), 17.3 (CH_2, *C*H$_2$CH$_3$), 13.5 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 312/314 [M]$^+$ (100), 311/313 [M − 1]$^+$-(70), 297/299 (27), 296/298 (30), 278 (12), 223 (16); *anal*. C 53.77%, H 4.28%, calcd for $C_{14}H_{13}ClO_6$, C 53.84%, H 4.20%.

In accordance with general procedure 2, products **19** and **20** were obtained as yellow-brown needles.

3-Amino-7-ethyl-5,8-dihydroxy-2,6-dimethoxy-1,4-naphthoquinone (19): obtained from **11** (63 mg, 43%); R_f 0.32; mp 300 °C (dec); IR ($CHCl_3$) ν_{max} 3514 m, 3398 m (NH_2), 1684 w, 1641 m, 1616 m (C═O), 1593 s, 1556 s (NH_2, C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.17 (3H, t, J = 7.6 Hz, CH_3), 2.74 (2H, q, J = 7.6 Hz, CH_2), 4.00, 4.01 (each 3H, s, OCH_3), 5.06 (2H, br s, NH_2), 12.52, 13.48 (each 1H, s, α-OH); EIMS *m*/*z* 293 [M]$^+$ (100), 292 (30), 278 (76), 263 (22), 250 (31), 248 (37), 235 (27), 221 (26); *anal*. C 57.25%, H 5.25%, N 4.90% calcd for $C_{14}H_{15}O_6N$, C 57.32%, H 5.16%, N 4.78%.

3-Amino-6-ethyl-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (20): obtained from **12** (66 mg, 45%); R_f 0.36; mp 118−120 °C; IR ($CHCl_3$) ν_{max} 3514 m, 3396 m (NH_2), 1639 m, 1618 m (C═O), 1590 s, 1555 (NH_2, C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 1.15 (3H, t, J = 7.5 Hz, CH_3), 2.69 (2H, q, J = 7.5 Hz, CH_2), 3.99, 4.06 (each 3H, s, OCH_3), 5.17 (2H, br s, NH_2), 12.67, 13.56 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 181.1 (C, C-1), 181.2 (C, C-4), 159.6 (C, C-5), 156.3 (C, C-7), 153.4 (C, C-8), 140.5 (C, C-3), 136.5 (C, C-2), 133.5 (C, C-6), 108.7 (C, C-8a), 106.4 (C, C-4a), 61.4 (CH_3, C-7-O*C*H$_3$), 60.4 (CH_3, C-2-O*C*H$_3$), 17.0 (CH_2, *C*H$_2$CH$_3$), 13.6 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 293 [M]$^+$ (100), 292 (86), 279 (22), 278 (86), 263 (25), 250 (23), 248 (24); *anal*. C 57.27%, H 5.22%, N 4.66%, calcd for $C_{14}H_{15}O_6N$, C 57.32%, H 5.16%, N 4.78%.

In accordance with general procedure 3, products **1** and **2** were obtained as dark brown powders (acetone).

3-Amino-7-ethyl-2,5,6,8-tetrahydroxy-1,4-naphthoquinone (echinamine A, 1): obtained from **19** (48 mg, 91%); R_f 0.17; mp 245−246 °C; IR ($CHCl_3$) ν_{max} 3522 m, 3445 w, 3379 m (NH_2, β-OH), 1650 m, 1603 m (C═O), 1589 s, 1562 s (NH_2, C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.14 (3H, t, J = 7.5 Hz, CH_3), 2.69 (2H, q, J = 7.5 Hz, CH_2), 5.36 (2H, br s, NH_2), 8.49, 9.20 (each 1H, br s, β-OH), 12.62, 13.03 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 181.7 (C, C-1), 177.4 (C, C-4), 161.0 (C, C-8), 154.0 (C, C-5), 152.3 (C, C-6), 137.0 (C, C-2), 132.4 (C, C-3), 126.5 (C, C-7), 108.6 (CH, C-4a), 102.6 (C, C-8a), 16.3 (CH_2, *C*H$_2$CH$_3$), 12.9 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 266 [M + 1]$^+$ (44), 265 [M]$^+$ (100), 264 (15), 223 (12), 222 (40).

2-Amino-7-ethyl-3,5,6,8-tetrahydroxy-1,4-naphthoquinone (echinamine B, 2): obtained from **20** (37 mg, 69%); R_f 0.19; mp 265−267 °C; IR ($CHCl_3$) ν_{max} 3518 m, 3460 w, 3398 m (NH_2, β-OH), 1664 m, 1603 m (C═O), 1580 m, 1560 s (NH_2, C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.13 (3H, t, J = 7.5 Hz, CH_3), 2.67 (2H, q, J = 7.5 Hz, CH_2), 5.81 (2H, br s, NH_2), 8.36, 9.44 (each 1H, br s, β-OH), 13.02 (2H, s, α-OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 178.7 (C, C-4), 176.6 (C, C-1), 163.2 (C, C-8), 154.0 (C, C-5), 151.4 (C, C-6), 135.1 (C, C-3), 134.8 (C, C-2), 124.4 (C, C-7), 107.6 (CH, C-4a), 103.9 (C, C-8a), 16.6 (CH_2, *C*H$_2$CH$_3$), 12.9 (CH_3, CH$_2$*C*H$_3$); EIMS *m*/*z* 266 [M + 1]$^+$ (32), 265 [M]$^+$ (77), 250 (25), 237 (17), 222 (100).

3-Bromo-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (26). To a stirred solution of 5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (**3**) (250 mg, 1.0 mmol) in 60 mL of AcOH was added dry bromine (325 μL). The reaction mixture was kept at room temperature for 48 h and monitored by TLC (*n*-hexane/acetone, 2:1). Then the reaction mixture was diluted with H_2O and extracted with EtOAc. The organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated, and the product was isolated by preparative TLC (*n*-hexane/acetone, 2:1) as a red-brown solid (118 mg, 36%): R_f 0.42; mp >230 °C (dec); IR (CCl_4) ν_{max} 2856 s, 1614 s, 1602 s (C═O), 1570 m, 1554 m, 1550 m (C═C) cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.96, 4.18 (each 3H, s, OCH_3), 6.34 (1H, s, H_{arom}), 12.67, 13.37 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.7 (C, C-4), 171.4 (C, C-1), 164.4 (C, C-5), 163.4 (C, C-8), 159.7 (C, C-2), 156.0 (C, C-7), 120.2 (C, C-3), 111.3 (C, C-8a), 108.9 (CH, C-6), 105.5 (C, C-4a), 61.5 (CH_3, C-7-O*C*H$_3$), 56.9 (CH_3, C-2-O*C*H$_3$); EIMS *m*/*z* 328/330 [M]$^+$ (92), 330 (33), 237 (62), 236 (100).

3-Chloro-2,5,6,8-tetrahydroxy-1,4-naphthoquinone (25). A mixture of dichloronaphthazarin **8** (2.0 g, 7.9 mmol), H_3BO_3 (1.0 g, 16.1 mmol), and 20 mL of concentrated H_2SO_4 was heated at 210−220 °C for 20 min. After being cooled the reaction mixture was diluted with H_2O. The solid was separated, dried in vacuo, and diluted with EtOAc (1 L). Then this solution was filtered through the column with silica gel and dried over Na_2SO_4, and the product was isolated by column chromatography (*n*-hexane/acetone, 2:1) as a dark wine-red powder (1.4 g, 69%): mp 227−230 °C (dec); ^{1}H NMR (acetone-d_6, 300 MHz) δ 6.62 (1H, s, H_{arom}), 10.07, 10.52 (each 1H, br s, β-OH), 12.29, 13.00 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 180.1 (C, C-4), 174.8 (C, C-1), 166.0 (C, C-6), 159.3 (C, C-8), 157.6 (C, C-2), 154.1 (C, C-5), 116.8 (C, C-3), 110.4 (C, C-4a), 109.1 (CH, C-7), 105.1 (C, C-8a); EIMS *m*/*z* 256/258 [M]$^+$ (100), 228/230 (42), 200 (5), 193 (12), 188 (6), 186 (17), 158 (7); *anal*. C 47.02%, H 2.02%, calcd for $C_{10}H_5ClO_6$, C 46.81%, H 1.96%.

7-Chloro-5,8-dihydroxy-2,6-dimethoxy-1,4-naphthoquinone (23): obtained by reaction of corresponding naphthazarin **25** (0.5 mmol) in Et_2O with a solution of CH_2N_2 in Et_2O monitored by TLC. The reaction mixture was concentrated in vacuo to give a residue, which was purified by preparative TLC (*n*-hexane/acetone, 3:1). Compound **23**: dark red needles; yield 110 mg (78%); R_f 0.43; mp 190−194 °C; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 3.97, 4.26 (each 3H, s, OCH_3), 6.35 (1H, s, H_{arom}), 13.03, 13.09 (each 1H, s, α-OH); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 177.4 (C, C-4), 168.6 (C, C-1),[15] 167.2 (C, C-8),[15] 163.6 (C, C-5), 160.2 (C, C-2), 156.3 (C, C-6), 124.9 (C, C-7), 108.6 (C, C-8a), 108.3 (CH, C-3), 107.7 (C, C-4a), 61.9 (CH_3, C-6-O*C*H$_3$), 56.9 (CH_3, C-2-O*C*H$_3$); EIMS *m*/*z* 284/286 [M]$^+$ (100), 283/285 [M − 1]$^+$ (92), 266/268 (25), 255 (19), 249 (12), 236 (9); *anal*. C 50.50%, H 3.45%, calcd for $C_{12}H_9ClO_6$, C 50.63%, H 3.19%.

In accordance with general procedure 2, products **24** and **27** were obtained as yellow-brown powders.

3-Amino-5,8-dihydroxy-2,6-dimethoxy-1,4-naphthoquinone (24): from **23** (15% yield); R_f 0.29; mp 190−194 °C; IR ($CHCl_3$) ν_{max} 3514 m, 3399 s (NH_2), 2854 m, 1647 w, 1620 sh m, 1597 s (C═O), 1580 sh m, 1557 m (C═C), cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 3.97, 4.26 (each 3H, s, OCH_3), 6.35 (1H, s, H_{arom}), 13.03, 13.10 (each 1H, s, α-OH); EIMS *m*/*z* 266 $[M + 1]^+$ (46), 265 $[M]^+$ (100); *anal*. C 54.25%, H 4.40%, N 5.20%, calcd for $C_{12}H_{11}NO_6$, C 54.34%, H 4.18%, N 5.28%.

3-Amino-5,8-dihydroxy-2,7-dimethoxy-1,4-naphthoquinone (27): from **26** (29% yield); R_f 0.29; mp 100−110 °C; IR (CCl_4) ν_{max} 3520 m, 3400 m (NH_2), 2856 m, 1650 m, 1619 m, 1591 s (C═O), 1580 sh s, 1545 m (C═C) cm^{-1}; ^1H NMR (acetone-d_6, 300 MHz) δ 3.89, 3.98 (each 3H, s, OCH_3), 6.48 (2H, br s, NH_2), 6.58 (1H, s, H_{arom}), 12.67, 13.83 (each 1H, s, α-OH); EIMS *m*/*z* 266 $[M + 1]^+$ (17), 265 $[M]^+$ (100), 250 (20), 247 (14); *anal*. C 54.12%, H 4.52%, N 5.07%, calcd for $C_{12}H_{11}NO_6$, C 54.34%, H 4.18%, N 5.28%.

In accordance with general procedure 3, products **21** and **22** were obtained as dark brown powders.

3-Amino-2,5,7,8-tetrahydroxy-1,4-naphthoquinone (21): from **24** (66% yield); R_f 0.05; mp >300 °C (dec); ^1H NMR (acetone-d_6, 300 MHz) δ 5.90 (2H, br s, NH_2), 6.53 (1H, s, H_{arom}), 8.32, 9.84 (1H each, both br s, β-OH), 12.54, 12.58 (1H each, both s, α-OH); EIMS *m*/*z* (%) 238 (12) $[M + 1]^+$, 237 (8) $[M]^+$, 236 (50), 235(100), 223 (7), 218 (5), 205 (5); *anal*. C 50.40%, H 3.10%, N 5.70%, calcd for $C_{10}H_7NO_6$, C 50.64%, H 2.97%, N 5.91%.

3-Amino-2,5,6,8-tetrahydroxy-1,4-naphthoquinone (22): from **27** (48% yield); R_f 0.06; mp >300 °C (dec); ^1H NMR (acetone-d_6, 300 MHz) δ 5.90 (2H, br s, NH_2), 6.46 (1H, s, H_{arom}), 8.32, 9.84 (1H each, both br s, β-OH), 12.50, 13.03 (1H each, both s, α-OH); EIMS *m*/*z* (%) 240 (13), 239 (39), 238 (92) $[M + 1]^+$, 237 (100) $[M]^+$, 236 (19), 211 (12), 210 (25), 209 (26); *anal.* C 50.75%, H 3.00%, N 6.10%, calcd for $C_{10}H_7NO_6$, C 50.64%, H 2.97%, N 5.91%.

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