Spinazarin and ethylspinazarin, pigments of the sea urchin *Scaphechinus mirabilis*

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2,3,5,8-Tetrahydroxy-1,4-naphthoquinone (spinazarin) and 6-ethyl-2,3,5,8-tetrahydroxy-1,4-naphthoquinone (ethylspinazarin) were first isolated from the sea urchin *Scaphechinus mirabilis*. The structures of spinazarins were established based on analysis of spectroscopic data. A preparative synthetic route to ethylspinazarin was proposed.

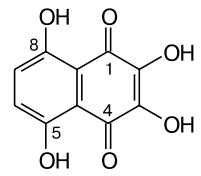
Key words: 5,8-dihydroxy-1,4-naphthoquinone, naphthazarin; 2,3,5,8-tetrahydroxy-1,4-naphthoquinone, spinazarin; 6-ethyl-2,3,5,8-tetrahydroxy-1,4-naphthoquinone, ethylspinazarin; sea urchin, *Scaphechinus mirabilis*.

Among natural products containing a 5,8-dihydroxy-1,4-naphthoquinonoid (naphthazarin) fragment, considerable interest is aroused by the group of echinodermata pigments (spinochromes).[1] Some spinochromes such as echinochrome and its synthetic analogs are known as biologically active compounds[2] and drugs.[3] However, no data on isolation of new compounds of this type have been reported during the last two decades.[4] Amine-containing polyhydroxynaphthazarins, echinamines A and B, isolated recently from the sea urchin *Scaphechinus mirabilis* are the only exception.

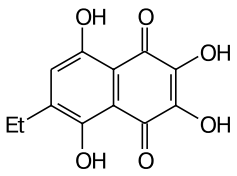
The sea urchin *S. mirabilis* is the main source of echinochrome, the substance of a cardiological and ophthalmological drug;[3] therefore, study of the pigments present in this material has not only a fundamental but also a practical value. Data on the structure and biological activities of echinochrome-accompanying pigments form the basis for standardization of a drug and a factor determining its quality.

To continue research along this line, using chromatography, we isolated two minor pigments from the alcohol extract of *S. mirabilis*. According to IR and NMR spectroscopy and mass spectrometry data, one of these was identified as 2,3,5,8-tetrahydroxy-1,4-naphthoquinone (spinazarin, **1**). The structure of the isolated pigment **1** was confirmed by direct comparison with a synthetic sample.[6] It is noteworthy that this pigment was isolated for the first time from a natural source.

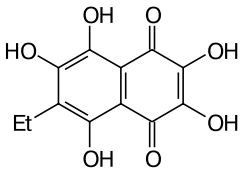
Unlike spinazarin (**1**), the molecule of the second compound isolated from the extract contained an ethyl fragment, as shown by ^{1}H NMR (δ 1.24 and 2.72) and mass spectrometry. The methylene protons of this fragment and the proton of the naphthazarin nucleus exhibit an allylic spin—spin coupling constant (J = 0.9 Hz), which



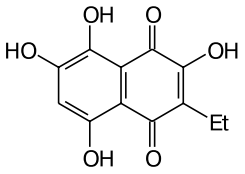
1
Spinazarin



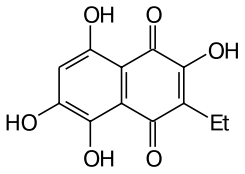
2
Ethylspinazarin



Echinochrome



Ethylmompain



Ethylisomompain

attests unambiguously to the 6-ethyl-2,3,5,8-tetrahydroxy-1,4-naphthoquinone structure (ethylspinazarin, **2**). Ethylspinazarin (**2**) is a new compound isolated from a natural material. The isomeric ethylmompain was previously isolated from the sea urchins *Echinothrix,*[7] while ethylisomompain was synthesized.[8]

We found that ethylspinazarin (**2**) is present as an impurity in synthetic echinochrome prepared from pyrogallol A (**3**).[9] The content of spinochrome **2** in the synthetic echinochrome samples varies within 0.12—0.63%.

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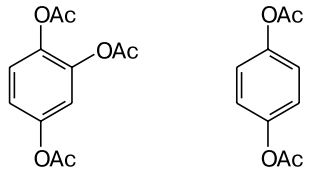
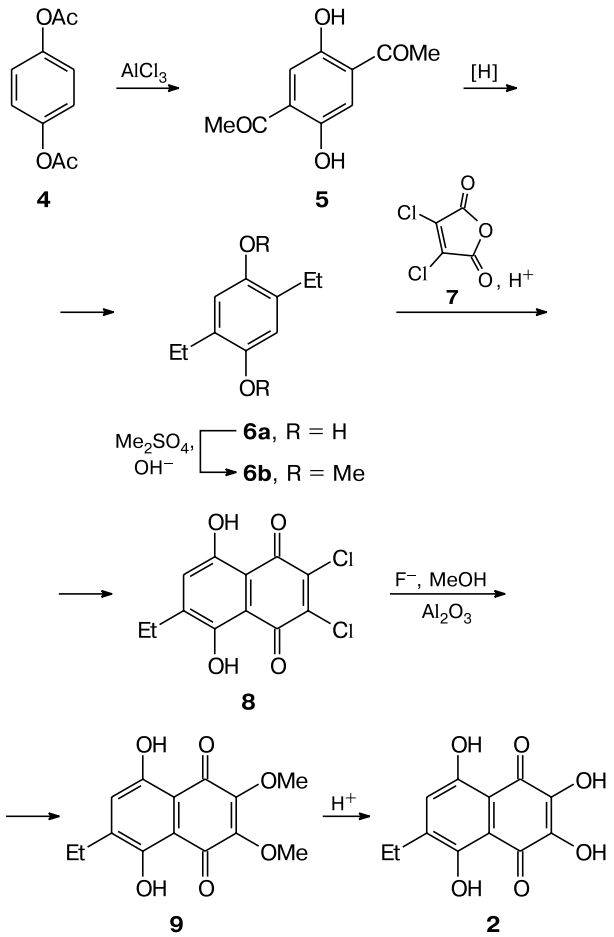


3 **4**

Spinochrome **2** is apparently formed from diacetate **4**, which is present in minor amounts in pyrogallol A (chemically pure grade) and undergoes the same transformations during the echinochrome synthesis as the major component **3** (Scheme 1).

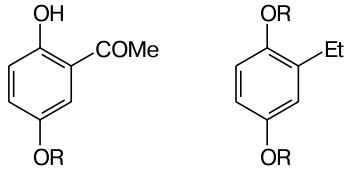
Scheme 1



According to the above presumptive scheme, hydroquinone diacetate (**4**) undergoes a double Fries rearrangement to give diacetylhydroquinone (**5**).[10] The Clemmensen reduction of intermediate **5** affords diethylhydroquinone (**6a**), which is methylated to yield diether **6b**. The double acylation of compound **6b** with dichloromaleic anhydride (**7**) is accompanied by elimination of one ethyl group[9] giving finally dichloroethylnaphthazarin **8**. The replacement of chlorine atoms in compound **8** by methoxy groups followed by hydrolysis of the resulting dimethyl ether **9** furnishes the final product **2**.

As noted above, pigments **1** and **2** are minor components, their content in *S. mirabilis* being ~0.0003%. Therefore, synthesis appears to be the only reliable source of these compounds in amounts required to study their biological properties. Our task was to develop the synthesis of ethylspinazarin (**2**), because the methods for the synthesis of pigment **1** have been described previously.[6]

While implementing Scheme 1 for this purpose, we found that over broad ranges of concentrations and temperatures, including those described previously,[10b] hydroquinone diacetate (**4**) is converted only into a 1 : 1 mixture of monoacyl derivative **10a** and acetylhydroquinone acetate **10b**.* However, reduction of compound **10b** under the Clemmensen reaction conditions is accompanied by hydrolysis of the ester group to give finally ethylhydroquinone (**11a**),[10b] which is methylated yielding dimethyl ether **11b**. The subsequent transformations **11b**→**8**→**9**→**2** proceeded in full agreement with Scheme 1. As was to be expected, the physicochemical characteristics of the synthetic product were identical to those of ethylspinazarin (**2**) isolated previously from the sea urchin *S. mirabilis*, which unambiguously conformed its structure.



10a,b **11a,b**

R = H (**10a, 11a**), Ac (**10b**), Me (**11b**)

To conclude, two new pigments were isolated for the first time from the sea urchin *S. mirabilis*: spinazarin (**1**) known previously as a synthetic product and ethylspinazarin (**2**). A preparative synthetic route to compound **2** was proposed.

Experimental

Melting points were determined on a Boetius hot stage and not corrected. IR spectra were measured on a Bruker Vector 22 spectrophotometer in $CHCl_3$. ^1H and ^{13}C NMR spectra were recorded on a Bruker AC-250 spectrometer (250.13 MHz for ^1H and 62.9 MHz for ^{13}C) in $CDCl_3$ and acetone-d_6 (Me_4Si as the internal standard). MS (EI) were run on an LKB-9000S instrument with direct sample inlet with an ionizing energy of 18 and 70 eV. The reactions were monitored and the purity of the products was checked by TLC on Merck 60F-254 plates in a 3 : 1 hexane—acetone system. The individual compounds were iso-

* This casts doubt on the correctness of product structure determination reported previously.[11]

lated from product mixtures by preparative TLC on plates (20×20 cm) with an unfixed silica gel layer (H^+-form),[11] 5—40 μm, or by column chromatography on a column with silica gel L 40/100 μm (H^+-form). The yields of the obtained compounds were not optimized. The elemental analysis was carried out on a Flash EA1112 C, H, N-analyzer. HPLC analysis was carried out on an Agilent 1100 chromatograph with a variable wavelength detector and an Eclipce® XDB-C8 ZORBAX column (150-mm long, an internal diameter of 4.6 mm, and 5 μm grain size). The column was maintained at 30 °C. The flow rate of the mobile phase was 1 mL min^{-1}. Solvent A: 1% acetic acid, solvent B: acetonitrile containing 1% (v/v) of acetic acid. Linear gradients with solvent B: 10→30% for 6 min, 30→70% from 6 to 20 min, 70→30% from 20 to 25 min, and column recovery for 5 min. The results were analyzed using the ChemStation^T program.

Spinazarin (**1**) and ethylspinazarin (**2**) were isolated from an alcohol extract of sea urchins *S. mirabilis* (2 kg) by a previously described procedure.[5,12] Commercially available dichloromaleic anhydride (Fluka) was used.

2,3,5,8-Tetrahydroxy-1,4-naphthoquinone (spinazarin, 1), yield 5 mg, red crystals, m.p. 268—270 °C (*cf.* Ref. 6: m.p. 279—279.5 °C (from AcOH)). UV ($CHCl_3$), λ_{max}/nm (logε): 230 (4.19), 246 (4.20), 302 sh (3.66), 460 (3.74), 489 (3.81), 525 sh (3.64). IR ($CHCl_3$), ν/cm^{-1}: 3520, 3433 (C—OH); 1601 (C=O, C=C). ^{1}H NMR (acetone-d_6), δ: 7.26 (s, 2 H, H_{arom}); 9.10 (br.s, 2 H, β-OH); 12.02 (s, 2 H, α-OH). MS (EI, 70 eV), *m/z* (I_{rel} (%)): 222 $[M]^+$ (43), 221 $[M - 1]^+$ (100), 193 $[M - COH]^+$ (100), 164 (21). MALDI spectrum: negative ions, 222 $[M]^-$ (100), 221 $[M - H]^-$ (50); positive ions, 224 $[M + 2H]^+$. Retention time 8.52 min.

6-Ethyl-2,3,5,8-tetrahydroxy-1,4-naphthoquinone (ethylspinazarin, 2), yield 3 mg, red crystals, m.p. 230—234 °C. Found (%): C, 57.73; H, 4.01. $C_{12}H_{10}O_6$. Calculated (%): C, 57.60; H, 4.03.UV (EtOH) λ_{max}/nm (logε): 204 (4.25), 253 (4.10), 292 (3.67), 460 (3.70), 485 (3.75), 510 sh (3.62), 522 sh (3.58). IR, ($CHCl_3$), ν/cm^{-1}: 3430, 3354 (C—OH); 2929, 2856, 1605 (C=O, C=C). ^{1}H NMR (acetone-d_6), δ: 1.24 (t, 3 H, Me, J = 7.6 Hz); 2.72 (qd, 2 H, CH_2, J_1 = 7.6 Hz, J_2 = 0.9 Hz); 7.12 (t, 1 H, H_{arom}, J = 0.9 Hz); 9.10 (br.s, 2 H, β-OH); 12.06, 12.57 (both s, 1 H each, α-OH). ^{1}H NMR ($CDCl_3$), δ: 1.26 (t, 3 H, Me, J = 7.6 Hz); 2.73 (qd, 2 H, CH_2, J_1 = 7.6 Hz, J_2 = 1.0 Hz); 6.66 (br.s, 1 H, β-OH); 6.69 (br.s, 1 H, β-OH); 7.07 (d, 1 H, H_{arom}, J = 1.0 Hz); 11.71, 12.16 (both s, 1 H each, α-OH). ^{13}C NMR ($CDCl_3$), δ: 183.1, 182.2, 157.9, 157.1, 147.5, 137.8, 137.5, 127.4, 108.1, 107.0, 23.2, 12.7. MS (EI, 18 eV), *m/z* (I_{rel} (%)): 251 $[M + 1]^+$ (18), 250 $[M]^+$ (100), 222 (9), 208 (27), 190 (7), 162 (7). Retention time 13.41 min.

Fries rearrangement of hydroquinone diacetate (4). Hydroquinone diacetate (31 g, 0.16 mol) (**4**) was added in portions with vigorous stirring at 140 °C to a melt of anhydrous $AlCl_3$ (145 g, 1.1 mol) and NaCl (27 g, 0.5 mol). The temperature of the mixture was raised to 195 °C and the melt was stirred for 9 min. The reaction mixture was cooled and hydrolyzed with a solution of conc. HCl (150 mL) in H_2O (2.0 L). The solid that precipitated within 12 h was separated, washed with hot H_2O (0.5 L), and dried to give 26 g of a mixture of acetylhydroquinone (**10a**) and acetylhydroquinone acetate (**10b**). ^{1}H NMR, **10a** ($CDCl_3$), δ: 2.60 (s, 3 H, Me); 4.62 (s, 1 H, C(4)OH); 6.89 (d, 1 H, H(6), J = 8.5 Hz); 7.03 (dd, 1 H, H(5), J_1 = 8.5, J_2 = 2.9 Hz); 7.19 (d, 1 H, H(3), J = 2.9 Hz); 11.81 (s, 1 H, C(1)OH). ^{1}H NMR, **10b** ($CDCl_3$), δ: 2.31 (s, 3 H, Me); 2.62 (s, 3 H, Me); 6.99 (d, 1 H, H(6), J = 8.5 Hz); 7.21 (dd, 1 H, H(5), J_1 = 8.5 Hz, J_2 = 2.9 Hz); 7.45 (d, 1 H, H(3), J = 2.9 Hz); 12.13 (s, 1 H, C(1)OH).

2-Ethylhydroquinone (11a). Solid zinc amalgam (160 g),[13] the mixture of compounds **10a** and **10b** (13 g), and conc. HCl (200 mL) were placed into a 3-L flask. The reaction mixture was heated to reflux with vigorous stirring and, after 30 min, an additional portion of the mixture of **10a** and **10b** (13 g) and conc. HCl (200 mL) were added. The mixture was stirred at reflux for additional 3 h, and the hot solution was decanted and left for 12 h. The white precipitate was separated, washed with ice water (30 mL), and dried to a constant weight to give 8.6 g of product **11a**, m.p. 94—95 °C. IR ($CHCl_3$), ν/cm^{-1}: 3604 (O—H); 1602, 1504 (C=C). ^{1}H NMR (acetone-d_6), δ: 1.16 (t, 3 H, Me, J = 7.6 Hz); 2.57 (q, 2 H, CH_2, J = 7.6 Hz); 6.49 (dd, 1 H, H(5), J_1 = 8.5 Hz, J_2 = 2.9 Hz); 6.61 (d, 1 H, H(3), J = 2.9 Hz); 6.65 (d, 1 H, H(6), J = 8.5 Hz); 7.59 (s, 1 H, OH); 7.64 (s, 1 H, OH). MS (EI, 70 eV), *m/z* (I_{rel} (%)): 138 $[M]^+$ (100), 123 $[M - Me]^+$ (57). Compound **11a** was used for the preparation of 1,4-dimethoxy-2-ethylbenzene (**11b**) without purification.

2-Ethyl-1,4-dimethoxybenzene (11b). Crude 2-ethylhydroquinone (**11a**) (8.6 g) was treated with 10% NaOH (60 mL) with vigorous stirring under nitrogen and then with Me_2SO_4 (14.3 g, 0.11 mol), the temperature of the mixture being maintained below 40 °C. After the addition of Me_2SO_4, the reaction mixture was heated for 30 min on a boiling water bath. The mixture was cooled, the organic layer was separated, and the aqueous layer was extracted with benzene. The combined organic solutions were washed with 5% NaOH and water, and dried with anhydrous $CaCl_2$. The solvent was removed at a reduced pressure. The residue was fractionated *in vacuo*. The fraction with b.p. 98—100 °C (7 Torr) was the target product **11b**, yield 7 g. IR ($CDCl_3$), ν/cm^{-1}: 1590, 1501 (C=C). ^{1}H NMR ($CDCl_3$), δ: 1.19 (t, 3 H, Me, J = 7.8 Hz); 2.64 (q, 2 H, CH_2, J = 7.8 Hz); 3.77, 3.76 (both s, 3 H each, OCH_3); 6.67 (dd, 1 H, H(5), J_1 = 8.8 Hz, J_2 = 2.9 Hz); 6.75 (d, 1 H, H(3), J = 2.9 Hz); 6.76 (d, 1 H, H(6), J = 8.8 Hz). MS (EI, 70 eV), *m/z* (I_{rel} (%)): 166 $[M]^+$ (40), 153 (10), 152 (100), 151 (17), 137 (41).

2,3-Dichloro-6-ethyl-5,8-dihydroxy-1,4-naphthoquinone (8). A mixture of 2-ethyl-1,4-dimethoxybenzene (**11b**) (7 g, 0.04 mol) and dichloromaleic anhydride (**7**) (12 g, 0.07 mol) was added at 140 °C with vigorous stirring to a melt of anhydrous $AlCl_3$ (64 g, 0.48 mol) and NaCl (12.6 g, 0.22 mol). The temperature of the mixture was raised to 195 °C and the melt was stirred for 5 min. The reaction mixture was cooled and hydrolyzed with a solution of concentrated HCl (75 mL) in H_2O (1.0 L). The precipitate formed within 12 h was separated, washed with hot H_2O (0.5 L), dried, and subjected to column chromatography using a 50 : 1 hexane—acetone mixture for elution to give 10.6 g (88%) of product **8**, red crystals, m.p. 123—125 °C. IR ($CDCl_3$), ν/cm^{-1}: 1617 (C=O); 1575, 1562 (C=C). ^{1}H NMR ($CDCl_3$), δ: 1.27 (t, 3 H, Me, J = 7.3 Hz); 2.73 (dq, 2 H, CH_2, J_1 = 7.3 Hz, J_2 = 1.4 Hz); 7.06 (t, 1 H, H(7), J = 1.4 Hz); 12.56, 12.90 (both s, 1 H each, α-OH). MS (EI, 70 eV), *m/z* (I_{rel} (%)): 286/288/290 $[M]^+$ (100), 285/287/289 $[M - 1]^+$ (21), 271/273/275 $[M - Me]^+$ (7), 268/270/272 $[M - H_2O]^+$ (8), 258/260/262 $[M - CO]^+$ (12), 257/259/261 (5).

6-Ethyl-5,8-dihydroxy-2,3-dimethoxy-1,4-naphthoquinone (9). A mixture of thoroughly dried substrate **8** (460 mg,

1.6 mmol), anhydrous CsF (1700 mg, 11.2 mmol), and activated Al_2O_3 (1630 mg, 16.0 mmol) in anhydrous MeOH (80 mL) was stirred at reflux under Ar for 6 h. Then the reaction mixture was cooled, and the sorbent was filtered off and washed with acetone (5 mL) with addition of 10% HCl (0.5 mL). The combined extract was concentrated *in vacuo* and the residue was diluted with water (10 mL) and extracted with $CHCl_3$. The organic solution was washed with water, dried with anhydrous Na_2SO_4, and concentrated. Column chromatography (hexane → hexane—benzene, 10 : 1) followed by preparative TLC (hexane—acetone, 3 : 1) gave compound **9**, 138 mg (31%), m.p. 68—70 °C. ^{1}H NMR ($CDCl_3$), δ: 1.25 (t, 3 H, Me, J = 7.6); 2.73 (dq, 2 H, CH_2, J_1 = 7.6 Hz, J_2 = 1.0 Hz); 4.11, 4.13 (both s, 3 H each, OMe); 7.06 (t, 1 H, H_{arom}, J = 1.0 Hz); 12.46, 12.90 (both s, 1 H each, α-OH). MS (EI, 70 eV), m/z (I_{rel} (%)): 279 (17), 278 $[M]^+$ (100), 277 (25), 263 (35), 262 (10), 260 (21), 249 (9), 248 (9). Apart from compound **8**, a mixture of **3-chloro-6-ethyl-2-methoxy-** and **2-chloro-6-ethyl-3-methoxynaphthazarins** was isolated (5%, 43 : 57 ratio). For 3-chloro-6-ethyl-2-methoxynaphthazarin, ^{1}H NMR ($CDCl_3$), δ: 1.27 (t, 3 H, Me, J = 7.6 Hz); 2.75 (q, 2 H, CH_2, J = 7.3 Hz); 4.33 (s, 3 H, OMe); 7.08 (br.s, 1 H, H_{arom}); 12.44, 13.03 (both s, 1 H each, α-OH). For 2-chloro-6-ethyl-3-methoxynaphthazarin, ^{1}H NMR ($CDCl_3$), δ: 1.27 (t, 3 H, Me, J = 7.6 Hz); 2.75 (q, 2 H, CH_2, J = 7.3 Hz); 4.30 (s, 3 H, OMe); 7.11 (br.s, 1 H, H_{arom}); 12.60, 12.84 (both s, 1 H, α-OH).*

6-Ethyl-2,3,5,8-tetrahydroxy-1,4-naphthoquinone (2). A solution of substrate **9** (60 mg, 0.22 mmol) was refluxed in a HBr—AcOH mixture (1 : 1, 10 mL) for 45 min, the course of the reaction being monitored by TLC. The reaction mixture was diluted with H_2O (200 mL) and extracted with ethyl acetate. The organic phase was dried with anhydrous Na_2SO_4 and concentrated. Preparative TLC (hexane—benzene—acetone, 3 : 1 : 1) gave 49 mg (91%) of product **2**.

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* The assignment is ambiguous.

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