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SIMPLE PREPARATIVE SYNTHESIS OF SPINOCHROME E, A PIGMENT FROM SEA URCHINS OF THE GENUS *Echinothrix*

K. L. Borisova and V. F. Anufriev*

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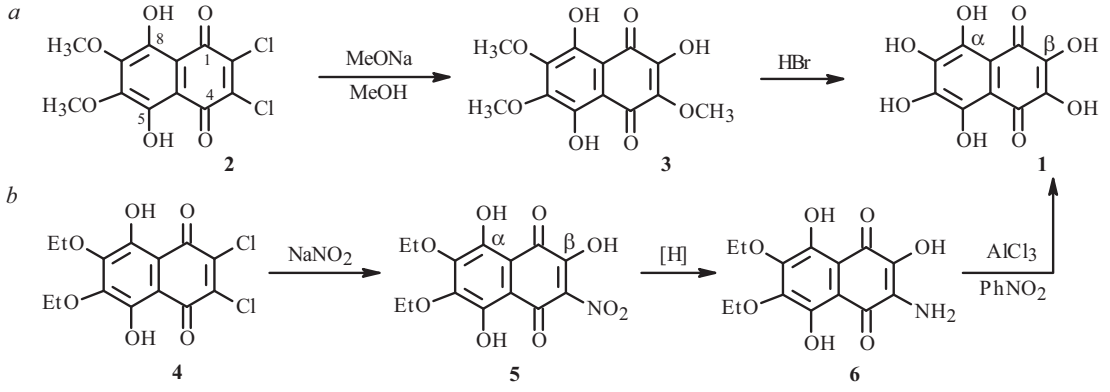
A preparative synthesis of spinochrome E (2,3,5,6,7,8-hexahydroxy-1,4-naphthoquinone, **1***), a metabolite of sea urchins of the genus* Echinothrix*, is proposed starting from 2,3-dichloro-6,7-diethoxynaphthazarine (***4***) with simultaneous substitution of the Cl atoms by hydroxyl- and nitro-groups, reduction of the latter, and subsequent removal of alkoxy groups and hydrolysis of the amine in the resulting 3-amino-2-hydroxy-6,7-diethoxynaphthazarine (***6***).*

Keywords: spinochrome E, 2,3,5,6,7,8-hexahydroxy-1,4-naphthoquinone, 2,3,6,7-tetrahydroxynaphthazarine, sea urchins of the genus *Echinothrix*.

Among natural products containing the 5,8-dihydroxy-1,4-naphthoquinoid (naphthazarine) moiety,* spinochrome pigments are rather widely distributed in nature [1]. Several spinochromes such as echinochrome and its synthetic analogs are known as biologically active compounds [2] and drugs [3].

Preparative methods for producing polyhydroxynaphthazarines must be developed in order to expand their biological testing. This relates primarily to the synthesis of spinochrome E (**1**), a pigment of sea urchins of the genus *Echinothrix* [1a] and a very close analog of echinochrome, the drug substance of a series of histochromes [4].

Spinochrome E was previously synthesized according to Scheme 1a, the key step in which was the nucleophilic substitution reaction of Cl by a methoxy in 2,3-dichloro-6,7-dimethoxynaphthazarine (**2**) by the action of NaOMe in MeOH. Ionization of the hydroxyls on C-5 and C-8 of **2** had an inhibiting effect on the occurrence of the nucleophilic substitution reaction. Therefore, a huge excess of the reagent was required to carry it out. Thus, dichloronaphthazarine **2** (80 mg, 0.25 mmol) had to be refluxed in saturated NaOMe solution (800 mL, 3125 mmol) in MeOH for 2 d in order to replace the Cl atoms by methoxyls [5]. All attempts to improve this ratio gave negative results [6]. The product of this reaction was trimethyl ether **3** (34%), which was hydrolyzed into spirochrome E (**1**) by conc. HBr.



Scheme 1

*Structures of naphthazarine derivatives are given only for one of all possible tautomers.

G. B. Elyakov Pacific Institute of Bioorganic Chemistry, Far-East Branch, Russian Academy of Sciences, 690022, Vladivostok, Prosp. 100-Letiya Vladivostoka, 159, Russian Federation, fax: (4232) 31 40 50, e-mail: anufriev@piboc.dvo.ru.
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This method was unsuitable for preparation of the amounts of spinochrome E required for biological testing because of the exceedingly unfavorable substrate–reagent ratio in the **2→3** conversion step. An approach that was previously described for sequential conversion of 2,3-dichloro-1,4-naphthoquinones into 2-hydroxy-3-nitro- and then 3-amino-2-hydroxy-1,4-naphthoquinones [7] and was later adapted to the synthesis of the 2-hydroxy-3-nitro- and 3-amino-2-hydroxynaphthazarines, respectively, [8] (Scheme 1b) turned out to be more efficient for the preparative synthesis of spinochrome E.

According to Scheme 1b, the starting substrate in the synthesis of spinochrome E (**1**) was dichlorodiethoxynaphthazarine **4**, which was readily transformed in aqueous alcoholic $NaNO_2$ into hydroxynitro derivative **5** in good yield. Reduction of **5** by $Na_2S_2O_4$ gave aminohydroxynaphthazarine **6**. However, attempts at direct conversion of **6** into **1** by conc. HBr or HBr–HOAc gave complicated product mixtures including those that were not naphthazarines. A much more suitable reagent for this was a solution of anhydrous $AlCl_3$ in nitrobenzene. Product **1** obtained as a result of the hydrolysis of **6** by this reagent was in all respects identical to the pigment that was isolated earlier from sea urchins of the genus *Echinothrix* [9].

EXPERIMENTAL

Melting points of products were determined on a Boetius heating stage and are uncorrected. PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ and DMSO-d_6 solutions with TMS internal standard (δ, ppm, J/Hz) at 30°C on Bruker Avance-300 (300 and 75 MHz, respectively) and Bruker Avance-700 (700 and 176 MHz, respectively) spectrometers. Mass spectra (EI) were recorded in an AMD 604S instrument with direct introduction at ionizing electron energy 70 eV.

The course of reactions and purity of products were monitored by TLC on Merck 60F-254 plates impregnated with an alcohol solution of tartaric acid (0.05 M) [10] using hexane:acetone (1:1, system 1; 2:1, system 2). Pure compounds were isolated from their mixtures by column chromatography (silica gel, 40–100 μm) and PTLC on plates (20 × 20 cm) with a loose layer (silica gel, 5–40 μm). Silica gel acidified by dilute HCl to pH ≈ 5 was used for chromatographic separation of the products. R_f values were determined by chromatography of products on Merck 60F-254 plates using systems 1 and 2. The synthesis of starting dichlorodiethoxynaphthazarine **4** has been reported [11].

2,5,8-Trihydroxy-3-nitro-6,7-diethoxy-1,4-naphthoquinone (5). A solution of **4** (1.04 g, 3 mmol) and $NaNO_2$ (2.07 g, 30 mmol) in EtOH:H_2O (2:1, 100 mL) was refluxed for 1 h, cooled, acidified by HCl (5%) to pH ~ 6, and extracted with EtOAc (3 × 10 mL). The extract was dried over anhydrous Na_2SO_4 and evaporated at reduced pressure. The crude product was purified by column chromatography with elution by hexane:acetone to afford **5** (0.78 g, 77%), mp 118–120°C (acetone), R_f 0.60 (system 1). PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.44 (3H, t, J = 7.0, C$\underline{H}_3$CH_2O), 1.45 (3H, t, J = 7.0, C$\underline{H}_3$CH_2O), 4.34 (2H, q, J = 7.0, CH_3C$\underline{H}_2$O), 4.47 (2H, q, J = 7.0, CH_3C$\underline{H}_2$O), 12.39 (1H, s, α-OH), 13.35 (1H, s, α-OH). Mass spectrum (*m*/*z*, I_{rel}, %): 339 (53) $[M]^+$, 309 (40), 276 (100), 248 (57), 195 (24), 167 (11). High-resolution mass spectrum: found *m*/*z* 339.0599 $[M]^+$, $C_{14}H_{13}NO_9$, calcd: MW 339.05903.

3-Amino-2,5,8-trihydroxy-6,7-diethoxy-1,4-naphthoquinone (6). A mixture of **5** (680 mg, 2 mmol) and $Na_2S_2O_4$ (700 mg, 4 mmol) in H_2O (60 mL) was stirred for 0.5 h (based on the published method [8]). The resulting precipitate was separated by filtration, washed with H_2O, and dried in air. The product was purified by column chromatography with elution by hexane:acetone to afford **6** (440 mg, 71%), mp 125–127°C (acetone), R_f 0.52 (system 2). PMR spectrum (700 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.42 (3H, t, J = 7.0, C$\underline{H}_3$CH_2O), 1.42 (3H, t, J = 7.0, C$\underline{H}_3$CH_2O), 4.28 (2H, q, J = 7.0, CH_3C$\underline{H}_2$O), 4.32 (2H, q, J = 7.0, CH_3C$\underline{H}_2$O), 4.74 (2H, br.s, NH_2), 6.41 (1H, br.s, β-OH), 12.47 (1H, s, α-OH), 12.60 (1H, s, α-OH). Mass spectrum (*m*/*z*, I_{rel}, %): 309 (12) $[M]^+$, 280 (8), 255 (100), 191 (16), 159 (37), 127 (42), 95 (11), 63 (42). High-resolution mass spectrum: found *m*/*z* 309.0854 $[M]^+$, $C_{14}H_{15}NO_7$, calcd: MW 309.08485.

2,3,5,6,7,8-Hexahydroxy-1,4-naphthoquinone (**1**, spinochrome E). A saturated solution of anhydrous $AlCl_3$ in anhydrous $PhNO_2$ (2 mL) was stirred, treated with an equal volume of **6** (30 mg, 0.1 mmol) in the same solvent, held at 70°C for 6 h, and cooled. The product was extracted with HCl (5%, 3 × 10 mL). The combined extract was washed with hexane (3 × 10 mL) to remove traces of nitrobenzene. The resulting HCl solution was refluxed for 10 min to destroy the Al complex of the product and to hydrolyze the amine. The solution was cooled and extracted with EtOAc (3 × 10 mL). The extract was washed with H_2O (2 × 10 mL), dried over anhydrous Na_2SO_4, and evaporated at reduced pressure. The crude product was purified by PTLC with elution by hexane:acetone:conc. HCl (10:10:1) to afford spinochrome E (**1**, 10 mg, 43%), mp >360°C (acetone); lit. [5] mp 300–320°C (dec), [9] 320°C (MeOH); R_f 0.46 (system 1). PMR spectrum (700 MHz, DMSO-d_6, δ, ppm): 10.17 (br.s, β-OH), 12.83 (s, α-OH). ^{13}C NMR spectrum (700 MHz, DMSO-d_6): 102.31, 140.72, 166.41.

Mass spectrum (m/z, I_{rel}, %): 254 (100) $[M]^+$, 226 (55), 205 (46), 176 (16), 148 (27), 128 (21), 98 (22). High-resolution mass spectrum: found m/z 254.0053 $[M]^+$, $C_{10}H_6O_8$, calcd: MW 254.00627.

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