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Main products of echinochrome A oxidative degradation

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In the last decade appeared a large number of publications on the therapeutic effectiveness of echinochrome A (**1**) and drug preparations based on it [1-5], and that caused the arousment of the interest in the whole world to search and investigation of the biologically active quinonoid pigments of sea urchins. Therefore, the study of the chemical properties of echinochrome A, in particular the stability of its solutions, is an urgent task for modern pharmaceuticals.

To investigate stability of echinochrome A in aqueous solutions, 200 mg of it was diluted 50-fold with distilled water saturated with atmospheric oxygen (pH 7.2), and separated on four parts that were stored for 85 days under following conditions: 1) in darkness without access of light and air; 2) in darkness with access of air; 3) in light without access of air; 4) in light with access of air. Content of echinochrome A in solutions 1-4 was determined photometrically at 468 nm (Figure 1). Stability of echinochrome A in studied solutions 1-4 as follows: 1>2>3>4.

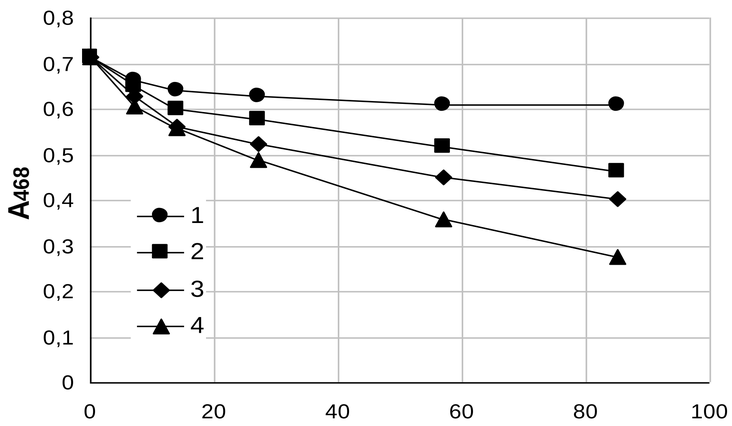


Figure 1. Stability of echinochrome A in aqueous solutions stored for 85 days under following conditions: 1) in darkness without access of light and air; 2) in darkness with access of air; 3) in light without access of air; 4) in light with access of air.

First of all echinochrome A oxidation products in solutions 1-4 were analyzed using LC-MS and HR-ESI-MS (Figure 2). On chromatogram of these solution only two peaks were detected, one of them belonged to echinochrome A, and another one – to its oxidation products. Peak of oxidation products in mass spectra contained five peaks of $[M-H]^-$ ions at *m/z* 237.0397 ($C_{11}H_9O_6$), 253.0356 ($C_{11}H_9O_7$), 263.0192 ($C_{12}H_7O_7$), 281.0294 ($C_{12}H_9O_8$) and 299.0399 ($C_{12}H_{11}O_9$). Structures for these compounds were proposed as shown on the Figure 2.

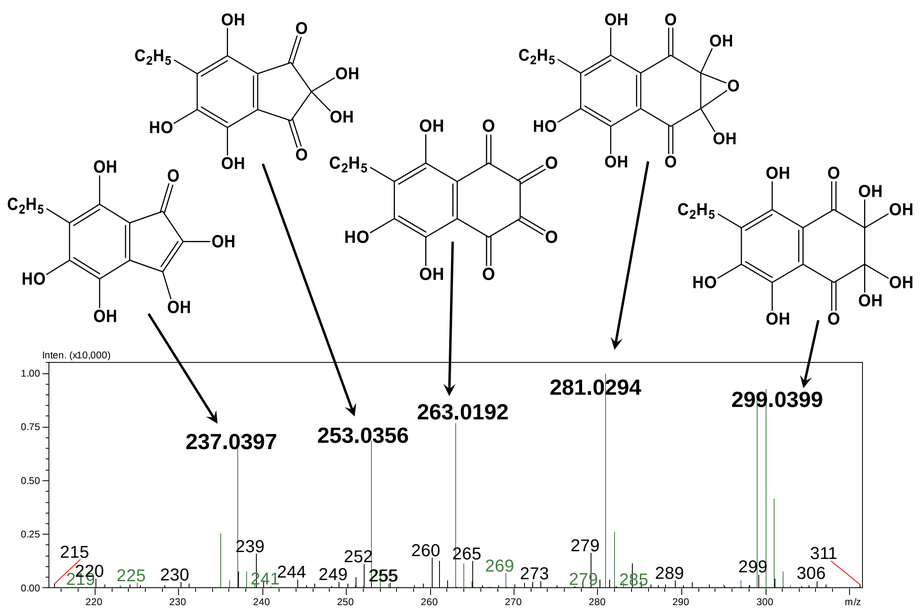
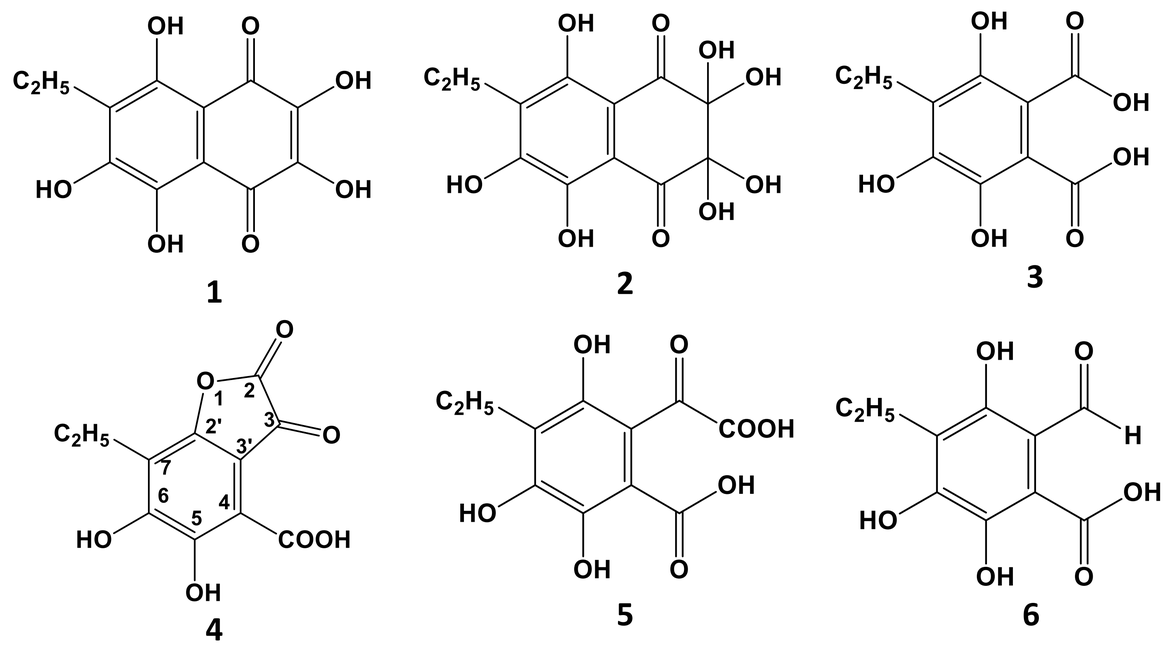


Figure 2. HR-ESI-MS of echinochrome A oxidation products.

To isolate products of echinochrome A oxidation in aqueous solutions, 200 mg of it was diluted 50-fold with distilled water saturated with atmospheric oxygen (pH 7.2), and was vigorously stirred at room temperature for 48 hours. Echinochrome A was removed from the reaction mixture by extraction with chloroform, its oxidation products were extracted with ethyl acetate and chromatographed on a Toyopearl HW-40 gel in a solvent system 20-50% EtOH containing 0.3% HCOOH. As a result, 7-ethyl-2,2,3,3,5,6,8-heptahydroxy-2,3-dihydro-1,4-naphthoquinone (**2**), echinolactone A (7-ethyl-5,6-dihydroxy-2,3-dioxo-2,3-dihydrobenzofuran-4-carboxylic acid) (**4**), and 4-ethyl-2-formyl-3,5,6-trihydroxybenzoic acid (**6**) were isolated. 4-Ethyl-3,5,6-trihydroxyphthalic acid (**3**) and 2-(carboxycarbonyl)-4-ethyl-3,5,6-trihydroxybenzoic acid (**5**) were isolated in etherified forms. The structures of compounds **2**, **3**, **5**, **6** were established using HR-ESI-MS and NMR, and of compound **4** – using X-Ray crystallography.



(**2**): *7-ethyl-2,2,3,3,5,6,8-heptahydroxy-2,3-dihydro-1,4-naphthoquinone*
UV/Vis λ_{max} (EtOH) nm: 256, 320, 391.
^{1}H NMR (300 MHz, aceton-d_6) δ_H: 1.16 (3H, t, J=7.5 Hz, CH_3), 2.78 (2H, q, J=7.5 Hz, CH_2), 5.91 (2H, br.s, OH), 5.96 (2H, br.s, OH), 9.50 (1H, br.s, OH), 11.28 (1H, s, OH), 11.88 (1H, s, OH).
^{13}C NMR (75 MHz, aceton-d_6) δ_C: 12.7, 17.0, 94.6, 94.7, 105.4, 111.4, 127.0, 145.4, 152.8, 157.6, 197.0, 198.9.
ESI-MS: *m/z* = 299 $[M-H]^-$.
HR-ESI-MS: *m/z* = 299.0399 $[M-H]^-$ (calcd. for $C_{12}H_{11}O_9$ 299.0409).

Ethyl ether of (**3**): *4-ethyl-3,5,6-trihydroxyphthalic acid* M.p. 132.5-133 °C
M.p. 140-141 °C.
UV/Vis λ_{max} (MeOH) nm: 232, 252, 272, 311, 378.
IR (CD_3CN) ν см$^{-1}$: 1733, 1703, 1667, 2978 .
^{1}H NMR (300 MHz, CD_3CN) δ_H: 1.11 (3H, t, J = 7.4, CH_3-10), 1.31 (3H, t, J= 7.1, CH_3-12), 2.67 (2H, q, J= 7.4, CH_2-9), 4.30 (2H, q, J= 7.1, CH_2-11).
^{13}C NMR (75 MHz, CD_3CN) δ_C: 171.3 (C-7), 168.0 (C-8), 156.2 (C-3), 150.7 (C-5), 136.8 (C-6),120.7 (C-4), 118.8 (C-1), 101.1 (C-1), 62.2 (C-11), 17.0 (C-9), 13.8 (C-12), 12.9 (C-10).
HMBC, HSQC: 1.11 (12.9) → 17.0, 120.7; 1.31 (13.8)→ 62.2; 2.67 (17.0) → 12.9, 120.7, 150.7, 156.2; 4.30 (62.2) → 13.8, 168.0.
ESI-MS: *m/z* = 269 $[M-H]^-$.

(**4**): *Echinolactone A (7-ethyl-5,6-dihydroxy-2,3-dioxo-2,3-dihydrobenzofuran-4-carboxylic acid)*
M.p.: 139-141 °C.
UV/Vis λ_{max} (EtOH) nm (logε): 215 (3.4), 331 (2.8), 385 (2.8).
IR ($CDCl_3$) ν см$^{-1}$: 3483, 1838, 1698, 1581.
^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 1.24 (3H, t, J=7.5 Hz, CH_3), 2.78 (2H, dd, J=7.5 Hz, CH_2), 5.24, (1H, s, OH), 12.88 (1H, s, OH).
^{13}C NMR (75 MHz, CD_3CN) δ_C: 12.0, 16.4, 105.5, 107.5, 120.6, 150.4, 157.2, 159.1, 160.8, 170.2, 177.0.
ESI-MS: *m/z* = 251 $[M-H]^-$, 253 $[M+H]^+$.
HR-EI-MS: *m/z* = 252.1905 $[M]^+$ (calcd. for $C_{11}H_8O_7$ 252.1863).
X-Ray: Crystal data for Orange-form: triclinic, *P*ī, *a* = 4.7823(6) Å, *b* = 7.9520(9) Å, c = 14.4705(17) Å, α = 86.271(3)°, β = 87.082(2)°, γ = 79.301(3)°, V = 539.16(11) Å^3, Z = 2, D_c = 1.664 Mg m^{-3}, T = 173(1) K, μ = 0.146 mm^{-1}, GOF on F^2 = 0.891, R_1 = 0.0407, wR_2 = 0.0929 ([I > 2σ(I)]).
Crystal data for Red-form: monoclinic, *P*2$_1$/*c*, *a* = 15.650(4) Å, *b* = 13.482(3) Å, *c* = 5.1857(12) Å, β = 92.781(6)°, V = 1092.9(5) Å^3, Z = 4, D_c = 1.642 Mg m^{-3}, T = 173(1) K, μ = 0.144 mm^{-1}, GOF on F^2 = 1.000, R_1 = 0.0512, wR_2 = 0.1270 ([I > 2σ(I)]).

(**5**): *2-(carboxycarbonyl)-4-ethyl-3,5,6-trihydroxybenzoic acid*
HR-MS: Mm = 270.0376 (calcd. for $C_{11}H_{10}O_8$ 270.0376).

(**6**): *4-ethyl-2-formyl-3,5,6-trihydroxybenzoic acid* Red plates, m.p. 157-158 °C.
UV/Vis λ_{max} (MeOH) nm: 230, 258, 305, 377.
IR (CD_3CN) ν см$^{-1}$: 1752, 1698, 1635, 2878, 2936.
^{1}H NMR (300 MHz, aceton-d_6) δ_H: 1.12 (3H, t, J = 7.5, CH_3), 2.73 (2H, q, J= 7.5, CH_2), 10.55 (H, s, COH), 13.46 (s, OH);
^{13}C NMR (75 MHz, aceton-d_6) δ_C: 196.7 (C-8), 171.8 (C-7), 159.2 (C-3), 152.3 (C-5), 146.2 (C-6), 123.6 (C-5), 109.9 (C-2), 108.8 (C-1), 16.7 (C-9), 12.7 (C-10).
EI-MS: *m/z* = 226 $[M]^+$.

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