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Synthesis and Properties of Water-Soluble 5,8-Dihydroxy-1,4-naphthoquinone Thioglucosides Structurally Related to Echinochrome

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Abstract—Acetylated hydroxynaphthazarin di- and trithioglucosides structurally related to echinochrome were synthesized. Their deacetylation by the action of sodium methoxide in methanol and the effect of hydroxy groups on this process were studied. Water-soluble echinochrome thioglucosides were synthesized for the first time.

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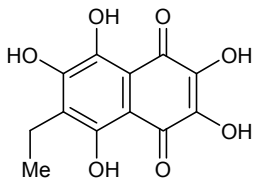
Naphthoquinones are widespread in nature, and they exhibit diverse physiological activity [1, 2]. Among these compounds, polyhydroxynaphthoquinones of the naphthazarin (5,8-dihydroxy-1,4-naphthoquinone) series occupy a particular place. Due to specificity of their structure, they are capable of effectively trapping free radicals and binding Fe^{2+} ions responsible for the formation of active oxygen species [3, 4]; excess concentration of the latter in an organism promotes development of various pathological states [5].

Echinochrome (**I**, 6-ethyl-2,3,5,7,8-pentahydroxy-1,4-naphthoquinone) is a pigment isolated from the sea urchin shell (*Echinodea*); it was used as active substance of the drug *Gistokhrom* for the treatment of eye injuries and burns, as well as of myocardial infarction [6]. The results of recent experiments in animals showed that Gistokhrom is also effective in the treatment of hemorrhagic stroke [7, 8]. Unfortunately, echinochrome is poorly soluble in water. In order to improve the solubility and bioavailability, echinochrome was converted into the corresponding tris-*O*-glucoside [9]. However, the latter turned out to be unstable: it decomposed shortly after isolation.

S-Glycosides are known to be more stable toward acids and bases than their *O*-glycoside analogs [10]. It was shown previously that chlorine atoms in the naphthazarin core are readily replaced by acetylglucoside residues under conditions of base catalysis [11]. It was also found that nucleophilic addition of chlorine atoms is accompanied by Michael 1,4-addition of thiols to the quinoid ring [12].

The present article describes the synthesis of acetylated naphthazarin *S*-glucosides that are structurally related to echinochrome and deacetylation of the acetylglucosides by the action of sodium methoxide, which afforded stable water-soluble thioglucosides of the echinochrome series; the effect of hydroxy groups in the naphthazarin core on the deacetylation process was also studied.



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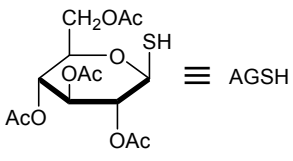
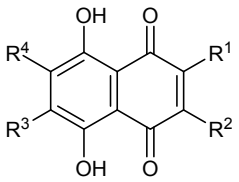


II

Commercially available tetra-*O*-acetyl-1-sulfanyl-β-D-glucopyranose (**II**, AGSH) was used as thiol, and the substrates for nucleophilic substitution were chloronaphthazarins **III**–**VII** that are intermediate products in the total synthesis of echinochrome [13, 14]. Initial chloronaphthazarins **III**–**V** possess hydroxy groups of two types: acid β-OH groups ($pK \approx 4$–5) in the quinoid ring and phenolic α-OH groups ($pK \approx 10$–12) [15, 16]. The hydroxy groups undergo deprotonation by the action of bases; as a result, electron density in the

naphthazarin core increases, which could affect the state of its tautomeric equilibrium.

The reactions of β-hydroxychloronaphthazarins **III–V** with AGSH (Table 1; run nos. 1–3) involved fast formation of poorly soluble salt of the initial quinone, which then reacted in 10–30 min with acetylthioglucose **II** to give the corresponding acetylated bis-thioglucosides **VIII–X** in 81–87% yield. The condensation of chloroquinones **VI** and **VII** with AGSH (run nos. 4, 5) also smoothly afforded bis- and tris-glucosides **XI** and **XII** in 84 and 89% yield, respectively.



III–XV, **XVIa**, **XVIb**, **XVIIIa**, **XVIIIb**, **XIX**, **XX**

III, R^1 = OH, R^2 = H, R^3 = R^4 = Cl; **IV**, R^1 = OH, R^2 = Me, R^3 = R^4 = Cl; **V**, R^1 = OH, R^2 = Et, R^3 = R^4 = Cl; **VI**, R^1 = R^2 = Cl, R^3 = Et, R^4 = H; **VII**, R^1 = R^2 = R^4 = Cl, R^3 = Et; **VIII**, R^1 = OH, R^2 = H, R^3 = R^4 = AGS; **IX**, R^1 = OH, R^2 = Me, R^3 = R^4 = AGS; **X**, R^1 = OH, R^2 = Et, R^3 = R^4 = AGS; **XI**, R^1 = R^2 = AGS, R^3 = Et, R^4 = H; **XII**, R^1 = R^2 = R^4 = AGS, R^3 = Et; **XIII**, R^1 = OH, R^2 = H, R^3 = R^4 = GS; **XIV**, R^1 = OH, R^2 = Me, R^3 = R^4 = GS; **XV**, R^1 = OH, R^2 = Et, R^3 = R^4 = GS; **XVIa**, R^1 = MeO, R^2 = AGS, R^3 = Et, R^4 = H; **XVIb**, R^1 = AGS, R^2 = MeO, R^3 = Et, R^4 = H; **XVIIIa**, R^1 = MeO, R^2 = GS, R^3 = Et, R^4 = H; **XVIIIb**; R^1 = GS, R^2 = MeO, R^3 = Et, R^4 = H; **XIX**, R^1 = R^2 = GS, R^3 = Et, R^4 = H; **XX**, R^1 = R^2 = R^4 = GS, R^3 = Et; GS = β-D-glucopyranosylsulfanyl.

The structure of the newly synthesized acetylthioglucosides was determined on the basis of their IR and ^{1}H NMR spectra. The IR spectra contained absorption bands in the region 1755–1756 cm^{-1}, which are typical of acetate groups in carbohydrates, and bands at 1595–1618 cm^{-1} due to stretching vibrations of the carbonyl groups in the quinoid ring [17]. The β-configuration of the glycoside bond follows from the coupling constant for the anomeric 1′-H proton (J = 10.0–10.3 Hz), and the site of addition, from the presence of signals from the C^5OH and C^8OH groups in the ^{1}H NMR spectra. The β-hydroxy groups in acetylthioglucosides **VIII–X** give rise to strong IR absorption bands in the region 3414–3421 cm^{-1}.

Naphthazarins are potentially tautomeric systems. The structure of glycoside **VIII** as particular tautomer was assumed on the basis of the position of the 3-H signal which appeared in the ^{1}H NMR spectrum at δ 6.45 ppm, i.e., in the resonance region typical of quinoid protons (δ 6.40–6.50 ppm). The structure of compound **XI** was assigned taking into account the chemical shift of 7-H (δ 7.07 ppm), which is characteristic of aromatic protons. Acetylglucosides **IX** and **X** having no protons attached directly to the naphthazarin carbon skeleton were identified by analogy with glucoside **VIII**; in addition, the presence of β-hydroxy group in the quinoid ring was considered to determine the state of tautomeric equilibrium [18].

Table 1. Reactions of acetylthioglucose (**II**, AGSH) with chloronaphthazarins (quinones) **III–VII** in acetonitrile

Run no.	Quinone, mmol	AGSH, mmol	K_2CO_3, mmol	Reaction time, min	Product, %
1	**III**, 0.50	1.01	4.20	20	**VIII**, 87
2	**IV**, 0.68	1.38	5.77	25	**IX**, 87
3	**V**, 0.33	0.67	2.80	10	**X**, 81
4	**VI**, 0.33	0.70	2.80	20	**XI**, 84
5	**VII**, 0.33	1.10	3.20	20	**XII**, 89

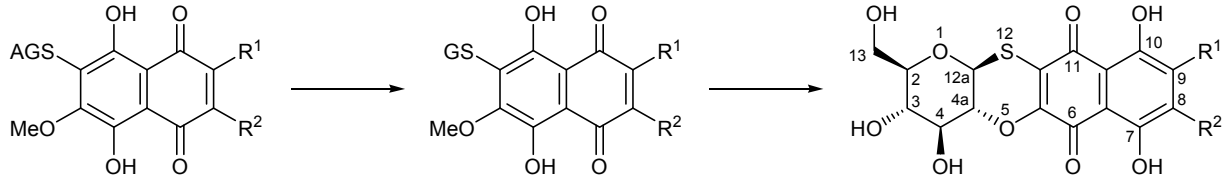
Another parameter indicating the position of tautomeric equilibrium in naphthazarin thioglucosides may be position of signals from anomeric protons. The assumed structure of thioglucoside tautomer **XII** is confirmed by similarity of the chemical shifts of the 1′-H protons in **XII** (δ 5.60 and 5.62 ppm) and **XI** (δ 5.73 and 5.63 ppm).

In the next step the obtained acetylthioglucosides were subjected to hydrolysis. It was reported previously that deacetylation of acetylated naphthoquinone *O*-glucosides with sodium methoxide in methanol is accompanied by nucleophilic replacement of the glycoside residue by methoxide ion with rupture of the *O*-glycoside bond [19, 20]. As shown later in [9, 21], the hydroxy group neighboring to the glycoside moiety is deprotonated in basic medium, thus hampering replacement of the latter, and 2,3-dihydroxynaphthazarin and 2,3-dihydroxy-1,4-naphthoquinone *O*-monoglycosides are stable under these conditions.

Treatment of acetylated 1,4-naphthoquinone 2-thioglucoside and its 3-substituted derivatives possessing readily departing groups (Cl, MeO, SGA) with sodium methoxide in methanol resulted in intramolecular nucleophilic substitution of those groups and formation of linearly fused quinone–carbohydrate tetracycle [22].

Hydrolysis of β-hydroxynaphthazarin thioglucosides **VIII–X** smoothly afforded 63–96% of the corresponding deacetylated thioglucosides **XIII–XV** (Table 2; run nos. 1–3). Presumably, the observed selectivity is also determined by ionization of the α- and

Scheme 1.



XVIa, **XVIb** **XVIIIa**, **XVIIIb** **XVIIa**, **XVIIb**

R^1 = Et, R^2 = H (**a**); R^1 = H, R^2 = Et (**b**).

β-hydroxy groups in the naphthazarin core. Deacetylation of bis-thioglucoside **XI** having no β-OH group was less selective. Treatment of **XI** with an equimolar amount of MeONa (run no. 4) led to the formation of a mixture of colored products. The product mixture was subjected to preparative thin-layer chromatography to isolate its main components: weakly polar fraction with R_f 0.86 (it was chromatographically more mobile than initial bis-thioglucoside **XI**), two medium-polarity fractions with R_f 0.61 and 0.59, and polar fraction with R_f 0.10.

The ^{1}H NMR spectrum of the weakly polar fraction contained signals assignable to two structurally similar acetylated naphthazarin monoglucosides and fairly distant signals from AGSH with an intensity ratio of ~1 : 1 : 0.6. Monoglycoside mixture free from AGSH was obtained by repeated chromatography of that fraction. It displayed in the ^{1}H NMR spectrum closely located signals from methoxy groups, aromatic 6-H and 7-H protons, anomeric protons in the acetylthioglucoside residues, and signals from two ethyl groups; therefore, the monoglycosides were assigned structures **XVIa** and **XVIb** as products of substitution of one acetylglucoside radical by methoxy group.

Table 2. Deacetylation of naphthazarin *O*-acetylthioglucosides (quinones) **VIII**–**XII** with MeONa in methanol

Run no.	Quinone, mmol	MeONa, mmol	Reaction time, min	Products (mol %)
1	**VIII**, 0.30	0.91	25	**XIII** (80)
2	**IX**, 0.30	0.91	30	**XIV** (82)
3	**X**, 0.30	0.91	20	**XV** (75)
4	**XI**, 0.20	0.21	60	**XVIa**/**XVIb** (20), **XVIIa**/**XVIIb** (47), **XVIIIa**/**XVIIIb** (6), **XIX** (8)
5	**XI**, 0.23	1.06	60	**XVIIa**/**XVIIb** (10.4), **XVIIIa**/**XVIIIb** (6), **XIX** (60)
6	**XII**, 0.20	1.06	20	**XX** (61)

In the ^{1}H NMR spectrum of the fraction with R_f 0.61 four α-OH singlets were present in the region δ 11–13 ppm (δ 11.95, 12.23, 12.46, and 12.75 ppm; intensity ratio ~4 : 5 : 5 : 4). Two singlets were observed in the aromatic region at δ 7.21 and 7.26 ppm, and "twinned" upfield signals from ethyl protons were characterized by the same intensity ratio. These data indicated that the fraction with R_f 0.61 consists of two structurally similar compounds of the naphthazarin series. Three one-proton broadened singlets due to hydroxy protons were located in the region δ 3.0–6.0 ppm (δ 5.02, 5.41, and 5.68 ppm), one-proton doublet at δ 5.02 ppm had a coupling constant J of 7.7 Hz, and four groups of multiplets were observed at δ 3.75 (1H), 3.61 (2H), 3.51 (2H), and 3.32 ppm (1H), the latter being overlapped by the water signal. Correlations between these groups of protons were determined by ^{1}H–^{1}H COSY experiments. Components of the above fraction were assigned tetracyclic structures **XVIIa** and **XVIIb** (Scheme 1) on the basis of the intensity ratio of signals from the quinoid and carbohydrate fragments, the presence of only three signals from hydroxy protons in the carbohydrate fragment, and good agreement of the chemical shifts with those reported in [22] for fused 1,4-naphthoquinoid tetracyclic compound.

The ^{1}H NMR spectrum of the fraction with R_f 0.59 (Table 2, run no. 4) contained signals at δ 12.17, 12.66, 12.74, and 12.88 ppm, which are typical of α-OH protons in the naphthazarin core, two singlets from aromatic 6-H and 7-H protons at δ 7.23 and 7.24 ppm, two doublets at δ 5.26 and 5.29 ppm (J = 9.8 and 9.5 Hz, respectively) from anomeric protons in the thioglucoside residues, two singlets from methoxy protons at δ 4.14 and 4.17 ppm, and characteristic multiplet signals from two ethyl groups, the signal intensity ratio being ~5 : 4. The lack of acetate proton signals in the region δ 1.90–2.20 ppm, as well as of

carbonyl absorption bands in the IR spectrum (1755–1756 cm^{-1}) made it possible to identify the fraction with R_f 0.59 as deacetylated monoglucosides **XVIIIa** and **XVIIIb**.

The polar fraction with R_f 0.10 was targeted bis-glucoside **XIX**. It showed in the ^1H NMR spectrum singlets at δ 12.26 and 12.77 ppm due to protons in the hydroxy groups on C^5 and C^8, doublets at δ 5.41 and 5.46 ppm (J = 7.5 and 7.7 Hz) from two anomeric protons, four broadened two-proton singlets from the carbohydrate hydroxy groups at δ 4.39, 4.98, 5.17, and 5.56 ppm, a singlet at δ 7.25 ppm due to 6-H, and ethyl group signals at δ 2.69 and 1.21 ppm. The IR spectrum of **XIX** contained a strong absorption band with its maximum at 3417 cm^{-1} due to stretching vibrations of hydroxy groups and absorption band at 1616 cm^{-1} due to stretching vibrations of the carbonyl groups in the naphthazarin fragment. These spectral data were very consistent with the assumed structure of **XIX**.

Treatment of acetylated bis-thioglucoside **XI** with 4 equiv of sodium methoxide (Table 2, run no. 5) resulted in predominant formation of compound **XIX**, whereas tetracyclic derivatives **XVIIa** and **XVIIb** were formed as minor products. Presumably, the α-hydroxy groups in initial compound **XI** are deprotonated to a stronger extent, which does not favor intramolecular heterocyclization to tetracyclic structure **XVII**. The absence of intramolecular heterocyclization products in the hydrolysis of tris-glucoside **XII** (Table 2, run no. 6) is likely to be related to the effect of the third thioglucoside radical on tautomeric transformations in the naphthazarin fragment.

Acetylglycosides **V**–**XII** are moderately soluble in chloroform, acetone, and ethanol and insoluble in water. Deacetylation of **V**–**XII** leads to bis- and tris-thioglucoside derivatives **XIII**–**XV**, **XVIa**, **XVIb**, **XVIIIa**, **XVIIIb**, **XIX**, and **XX** which are readily soluble in water and moderately soluble in DMSO, methanol, and ethanol. Tetracyclic compounds **XVIIa** and **XVIIb** are insoluble in water and poorly soluble in DMSO and methanol.

EXPERIMENTAL

The melting points were determined on a Boetius melting point apparatus and are uncorrected. The IR spectra were measured on a Bruker Vector-60 spectrometer. The ^1H NMR spectra were recorded on a Bruker DRX-500 instrument using tetramethylsilane as internal reference. The progress of reactions was monitored by thin-layer chromatography on Silufol UV-254 plates using the following solvent systems as eluents: hexane–benzene–acetone, 2:1:1 (by volume) (A); benzene–ethyl acetate–methanol, 2:1:1 (B); toluene–ethyl acetate–methanol, 4:1:1 (C). To reduce residual adsorption of quinones, chromatographic plates were preliminarily saturated with gaseous hydrogen chloride. Individual substances were isolated by crystallization and preparative thin-layer chromatography on silica gel (Silicagel 60, 0.040–0.063 mm) which was preliminarily treated with a boiling mixture of concentrated nitric and hydrochloric acids (1:3 by volume), dried, and activated by heating at 120°C.

2,5,8-Trihydroxy-6,7-bis(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (VIII). Quinone **III**, 0.138 g (0.5 mmol), was dissolved in 20 ml of anhydrous acetonitrile, 0.367 g (1.01 mmol) of acetylthioglucose **II** and 0.588 g (4.2 mmol) of finely powdered potassium carbonate were added, and the mixture was stirred at room temperature. After addition of K_2CO_3, the originally red mixture turned red–brown, and a dark brown solid separated. The precipitate gradually dissolved, and the mixture turned bright blue. According to the TLC data (solvent system A), the reaction involved intermediate formation of monosubstituted product, R_f ~0.62; cf. R_f 0.33 for compound **VIII**. The mixture was stirred for 20 min until complete transformation of the initial quinone into bis-thioglucoside **VIII**. The precipitate (inorganic salts) was filtered off and washed with acetonitrile, the filtrate was combined with the washings and neutralized with KU-2 (H^+) exchanger until the solution turned red. The ion-exchange resin was filtered off, the solvent was removed under reduced pressure, and the residue was crystallized from benzene–anhydrous methanol and dried. Yield 0.408 g (87%), dark brown crystals, mp 212–215°C. IR spectrum ($CHCl_3$), ν, cm^{-1}: 3414 (OH), 1756 (MeCO), 1606 (C=O), 1368, 1249, 1191, 1093, 1044. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.99 (Ac), 2.00 (Ac), 2.01 (2Ac), 2.02 (2Ac), 2.08 (Ac), 2.12 (Ac), 3.61 m (1H, 5′-H), 3.68 m (1H, 5′-H), 4.01 m (2H, 6′-H), 4.18 m (2H, 6′-H), 5.11 m (4H, 2′-H, 4′-H), 5.24 m (3H, 1′-H, 3′-H), 5.68 d (1H, 1′-H, J = 10.3 Hz), 6.45 s (3-H), 12.39 s (α-OH), 13.84 s (α-OH). Found, %: C 49.21; H 4.61; S 6.72. $C_{38}H_{42}O_{23}S_2$. Calculated, %: C 49.03; H 4.55; S 6.89.

Compounds **IX**–**XII** were synthesized in a similar way. For reaction conditions (reactant ratio and reaction time), see Table 1.

2,5,6-Trihydroxy-3-methyl-6,7-bis(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (IX). Yield 0.560 g (87%), light red needles, mp 248–251°C. IR spectrum ($CHCl_3$), ν, cm^{-1}: 3421 (OH), 2958, 1756 (MeCO), 1622, 1601 (C=O), 1379, 1367, 1326, 1255, 1241, 1235, 1218, 1210, 1189, 1138, 1090, 1042. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 2.00 (2Ac), 2.01 (Ac), 2.02 (3Ac), 2.09 (3-CH_3), 2.12 (Ac), 2.13 (8Ac), 3.60 m (1H, 5′-H), 3.65 m (1H, 5′-H), 3.99 m (2H, 6′-H), 4.19 m (2H, 6′-H), 5.12 m (4H, 2′-H, 4′-H), 5.24 m (3H, 1′-H, 3′-H), 5.59 d (1H, 1′-H, J = 10.0 Hz), 12.38 s (α-OH), 14.00 s (α-OH). Found, %: C 49.69; H 4.75; S 6.63. $C_{39}H_{44}O_{23}S_2$. Calculated, %: C 49.58; H 4.69; S 6.79.

3-Ethyl-2,5,6-trihydroxy-6,7-bis(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (X). Yield 0.256 g (81%), dark red crystals, mp 260–262°C. IR spectrum ($CHCl_3$), ν, cm^{-1}: 3421 (OH), 1755 (MeCO), 1601 (C=O), 1539, 1433, 1368, 1329, 1247, 1138, 1044. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.16 t (3H, $CH_2C\mathbf{H}_3$, J = 7.4 Hz), 2.00 (2Ac), 2.01 (Ac), 2.02 (3Ac), 2.09 (Ac), 2.02 (8Ac), 2.64 q (2H, $C\mathbf{H}_2CH_3$, J = 7.4 Hz), 3.59 m (1H, 5′-H), 3.66 m (1H, 5′-H), 4.01 m (2H, 6′-H), 4.19 m (2H, 6′-H), 5.12 m (4H, 2′-H, 4′-H), 5.24 m (3H, 1′-H, 3′-H), 5.59 d (1H, 1′-H, J = 10.2 Hz), 12.38 s (α-OH), 14.05 s (α-OH). Found, %: C 49.14; H 4.69; S 6.73. $C_{38}H_{42}O_{23}S_2$. Calculated, %: C49.03; H 4.55; S 6.89.

6-Ethyl-5,8-dihydroxy-2,3-bis(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (XI). No solid separated from the solution after addition of K_2CO_3. The filtrate was evaporated, the residue was subjected to preparative TLC using solvent system A, and subsequent crystallization gave an additional portion, 0.056 g, of compound **XI**. Overall yield 0.260 g (84%), dark red crystals, R_f 0.33 (A), mp 222–225°C. IR spectrum ($CHCl_3$), ν, cm^{-1}: 1756 (MeCO), 1603 (C=O), 1434, 1369, 1329, 1248, 1191, 1089, 1059. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.26 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 1.95 (Ac), 1.96 (Ac), 2.01 (4Ac), 2.08 (Ac), 2.09 (8Ac), 2.73 q (2H, $C\mathbf{H}_2CH_3$), 3.71 m (2H, 5′-H), 4.04 m (2H, 6′-H), 4.15 m (2H, 6′-H), 5.10 m (4H, 2′-H, 4′-H), 5.25 m (2H, 3′-H), 5.63 d (1H, 1′-H, J = 10.3 Hz), 5.73 d (1H, 1′-H, J = 10.0 Hz), 7.07 s (1H, 7-H), 12.76 s (α-OH), 13.14 s (α-OH). Found, %: C 51.06; H 5.03; S 6.72. $C_{40}H_{46}O_{22}S_2$. Calculated, %: C 50.95; H 4.92; S 6.80.

6-Ethyl-5,8-dihydroxy-2,3,7-tris(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (XII). Overall yield (after TLC and crystallization) 0.383 g (89%), dark red crystals, R_f 0.33 (A), mp 232–234°C (from MeOH). IR spectrum ($CHCl_3$), ν, cm^{-1}: 1756 (MeCO), 1601 (C=O), 1375, 1248, 1190, 1060, 1045. ^1H NMR spectrum ($CDCl_3$), δ, ppm: 1.15 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 1.97 (Ac), 1.98 (2Ac), 2.00 (4Ac), 2.02 (Ac), 2.03 (Ac), 2.09 (Ac), 2.11 (Ac), 2.12 (12OAc), 3.03 q (2H, $C\mathbf{H}_2CH_3$), 3.62 d.d.d (1H, 5′-H), 3.69 m (1H, 5′-H), 4.03 m (3H, 6′-H), 4.15 m (3H, 6′-H), 5.11 m (6H, 2′-H, 4′-H), 5.26 m (3H, 3′-H), 5.32 d (1H, 1′-H, J = 10.0 Hz), 5.60 d (1H, 1′-H, J = 10.0 Hz), 5.62 d (1H, 1′-H, J = 10.3 Hz), 13.33 s (α-OH), 13.59 s (α-OH). Found, %: C 49.72; H 5.06; S 7.23. $C_{54}H_{64}O_{31}S_3$. Calculated, %: C 49.69; H 4.94; S 7.37.

6,7-Bis(β-D-glucopyranosylsulfanyl)-2,5,8-trihydroxy-1,4-dihydronaphthalene-1,4-dione (XIII). Compound **VIII**, 0.280 g (0.3 mmol), was dispersed in 20 ml of anhydrous methanol, and 0.9 ml of a 1.06 N solution of sodium methoxide in methanol was added under stirring. After addition of MeONa, the mixture turned blue, and the precipitate quickly dissolved. According to the TLC data, the conversion of initial quinone **VIII** was complete in 17 min, and only one colored product was formed (R_f 0.30, B). The mixture was neutralized with KU-2 (H^+) exchanger until it turned bright red, the ion-exchange resin was filtered off, the filtrate was evaporated under reduced pressure with addition of toluene, and the residue was dissolved in anhydrous methanol. The product was precipitated by carefully adding toluene. The filtrate was evaporated, and the residue was subjected to preparative TLC using solvent system B to isolate an additional amount, 0.032 g, of compound **XIII**. Overall yield 0.143 g (80%), dark violet powder. R_f 0.30 (B), mp >360°C. IR spectrum (KBr), ν, cm^{-1}: 3407 (OH), 1595, 1561, 1450, 1402, 1211, 1104, 1045. ^1H NMR spectrum (DMCO-d_6), δ, ppm: 3.07 m (2H, 6′-H), 3.17 m (4H, 2′-H, 3′-H), 3.38 m (4H, 4′-H, 6′-H), 3.47 m (2H, 5′-H), 4.22 br.s (2H, OH), 4.89 br.s (2H, OH), 5.06 br.s (2H, OH), 5.21 d (1H, 1′-H, J = 9.3 Hz), 5.22 br.s (2H, OH), 5.50 d (1H, 1′-H, J = 9.9 Hz), 5.82 s (3-H), 13.14 s (α-OH), 15.30 s (α-OH). Found, %: C 44.30; H 4.53; S 10.64. $C_{22}H_{26}O_{15}S_2$. Calculated, %: C 44.44; H 4.41; S 10.78.

Compounds **XIV** and **XV** were synthesized in a similar way. For reaction conditions (reactant ratio and reaction time), see Table 2.

6,7-Bis(β-D-glucopyranosylsulfanyl)-2,5,6-trihydroxy-3-methyl-1,4-dihydronaphthalene-1,4-dione (XIV). Overall yield 0.150 g (82%), R_f 0.32 (B), mp 193–196°C. IR spectrum (KBr), ν, cm^{-1}: 3419

(OH), 1601 (C=O), 1456, 1384, 1314, 1136, 1042. ^{1}H NMR spectrum (DMCO-d_6), δ, ppm: 2.01 s (3H, 3-CH_3), 3.09 m (2H, 6′-H), 3.14 m (2H, 6′-H), 3.22 m (3H), 3.36 m (2H), 3.50 m (2H), 5.30 d (1H, 1′-H, J = 9.3 Hz), 5.44 d (1H, 1′-H, J = 8.8 Hz), 12.91 s (α-OH), 13.99 s (α-OH). Found, %: C 45.25; H 4.70; S 10.61. $C_{23}H_{28}O_{15}S_2$. Calculated, %: C 45.39; H 4.64; S 10.54.

3-Ethyl-6,7-bis(β-D-glucopyranosylsulfanyl)-2,5,6-trihydroxy-1,4-dihydronaphthalene-1,4-dione (XV). Overall yield 0.140 g (75%), R_f 0.39 (B), mp 193–195°C. IR spectrum (KBr), ν, cm^{-1}: 3405 (OH), 1602 (C=O), 1456, 1385, 1320, 1250, 1138, 1108, 1039. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.07 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.54 q (2H, $C\mathbf{H}_2CH_3$), 3.07 m (2H, 6′-H), 3.17 m (4H, 2′-H, 3′-H), 3.34 m (4H, 4′-H, 6′-H), 3.51 m (2H, 5′-H), 4.22 br.s (2H, OH), 4.93 br.s (2H, OH), 5.09 br.s (2H, OH), 5.29 d (1H, 1′-H, J = 9.1 Hz), 5.37 br.s (2H, OH), 5.43 d (1H, 1′-H, J = 9.3 Hz), 12.90 s (α-OH), 14.09 s (α-OH). Found, %: C 46.18; H 4.91; S 10.42. $C_{24}H_{30}O_{15}S_2$. Calculated, %: C 46.30; H 4.86; S 10.30.

Deacetylation of glycoside XI. Compound **XI**, 0.189 g, was dispersed in 14 ml of anhydrous methanol, and 0.2 ml of a 1.06 N solution of sodium methoxide in methanol was added under stirring at room temperature. The mixture turned blue, and the precipitate quickly dissolved. According to the TLC data, the conversion of **XI** into a mixture of colored products was complete in 40 min. No other products appeared in the mixture on prolonged keeping of the reaction mixture (60 min). The mixture was neutralized with KU-2 (H^+) exchanger and filtered, the filtrate was evaporated under reduced pressure, and the residue was separated by preparative thin-layer chromatography using solvent system C to obtain four colored fractions.

Fraction with R_f 0.83 (C), yield 0.030 g, dark red powder. According to the ^{1}H NMR data, it was a mixture of 6-ethyl-5,8-dihydroxy-3-methoxy-2-(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (**XVIa**), 6-ethyl-5,8-dihydroxy-2-methoxy-3-(tetra-*O*-acetyl-β-D-glucopyranosylsulfanyl)-1,4-dihydronaphthalene-1,4-dione (**XVIb**), and tetra-*O*-acetyl-β-D-thioglucopyranose (**II**) at a ratio of ~1.5:1:0.6. Repeated chromatography gave a mixture of thioglucosides **XVIa** and **XVIb** with the same ratio. Overall yield 0.023 g (20%). IR spectrum ($CHCl_3$), ν, cm^{-1}: 1755 (MeCO), 1603 (C=O), 1559, 1436, 1369, 1228, 1044. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm: major isomer **XVIa**: 1.25 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 1.90–2.20 (8Ac), 2.73 q (2H, $C\mathbf{H}_2CH_3$), 3.72 m (1H, 5′-H), 4.06 m (2H, 6′-H), 4.15 m (2H, 6′-H), 4.24 s (3H, CH_3O), 5.09 m (2H, 2′-H, 4′-H), 5.27 t (1H, 3′-H), 5.59 d (1H, 1′-H, J = 10.3 Hz), 7.08 s (1H, 7-H), 12.71 s (α-OH), 12.85 s (α-OH); minor isomer **XVIb**: 1.26 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 1.90–2.20 (8Ac), 2.74 q (2H, $C\mathbf{H}_2CH_3$), 3.72 m (1H, 5′-H), 4.06 m (2H, 6′-H), 4.15 m (2H, 6′-H), 4.27 s (3H, CH_3O), 5.09 m (2H, 3′-H, 4′-H), 5.27 t (1H, 3′-H), 5.53 d (1H, 1′-H, J = 10.3 Hz), 7.06 s (1H, 7-H), 12.46 s (α-OH), 13.15 s (α-OH).

Fraction with R_f 0.40 (C), yield 0.006 g (7%), red powder. According to the ^{1}H NMR data, it was a mixture of 6-ethyl-2-(β-D-glucopyranosylsulfanyl)-5,8-dihydroxy-3-methoxy-1,4-dihydronaphthalene-1,4-dione (**XVIIIa**) and 6-ethyl-3-(β-D-glucopyranosylsulfanyl)-5,8-dihydroxy-2-methoxy-1,4-dihydronaphthalene-1,4-dione (**XVIIIb**). IR spectrum (KBr), ν, cm^{-1}: 3414 (OH), 1607 (C=O), 1558, 1436, 1384, 1268, 1198, 1033. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: major isomer: 1.20 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.68 q (2H, $C\mathbf{H}_2CH_3$), 3.12 m (2H), 3.25 m (2H), 3.37 m (1H), 3.57 m (1H), 4.17 s (3H, CH_3O), 5.26 d (1H, 1′-H, J = 9.8 Hz), 7.24 s (1H, 7-H), 12.17 s (1H, α-OH), 12.88 s (1H, α-OH); minor isomer: 1.20 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.68 q (2H, $C\mathbf{H}_2CH_3$), 3.12 m (2H), 3.25 m (2H), 3.37 m (1H), 3.57 m (1H), 4.14 s (3H, CH_3O), 5.29 d (1H, 1′-H, J = 9.5 Hz), 7.23 s (1H, 7-H), 12.66 s (1H, α-OH), 12.74 s (1H, α-OH).

Fraction with R_f 0.37 (C), yield 0.038 g (47%), bright red powder. According to the ^{1}H NMR data, it was a mixture of (2*R*,3*R*,4*R*,4a*S*,12a*S*)-8-ethyl-2-hydroxymethyl-3,4,7,10-tetrahydroxy-3,4,4a,12a-tetrahydro-2*H*-1,5-dioxa-12-thianaphthacene-6,11-dione (**XVIIa**) and (2*R*,3*R*,4*R*,4a*S*,12a*S*)-9-ethyl-2-hydroxymethyl-3,4,7,10-tetrahydroxy-3,4,4a,12a-tetrahydro-2*H*-1,5-dioxa-12-thianaphthacene-6,11-dione (**XVIIb**) at a ratio of ~5:4. IR spectrum (KBr), ν, cm^{-1}: 3446 (OH), 1595 (C=O), 1573, 1447, 1385, 1295, 1252, 1217, 1192, 1135, 1101, 1061, 1043, 1012, 911, 889, 771. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: major isomer **XVIIa**: 1.18 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.65 q (2H, $C\mathbf{H}_2CH_3$), 3.32 m (2H, 4-H, 13-H), 3.50 m (1H, 3-H), 3.60 m (1H, 4a-H), 3.61 m (1H, 13-H), 3.75 m (1H, 2-H), 4.73 br.s (1H, OH), 5.02 d (1H, 12a-H, J = 7.7 Hz), 5.41 br.s (1H, OH), 5.68 br.s (1H, OH), 7.26 s (1H, 9-H), 12.23 s (1H, α-OH), 12.46 s (1H, α-OH); minor isomer **XVIIb**: 1.19 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.66 q (2H, $C\mathbf{H}_2CH_3$), 3.32 m (2H, 4-H, 13-H), 3.50 m (1H, 3-H), 3.60 m (1H, 4a-H), 3.61 m (1H, 13-H), 3.75 m (1H, 2-H), 4.73 br.s (1H, OH), 5.02 d (1H, 12a-H, J = 7.7 Hz), 5.41 br.s (1H,

OH), 5.68 br.s (1H, OH), 7.21 s (1H, 8-H), 11.95 s (1H, α-OH), 12.75 s (1H, α-OH).

6-Ethyl-2,3-bis(β-D-glucopyranosylsulfanyl)-5,8-dihydroxy-1,4-dihydronaphthalene-1,4-dione (XIX) was isolated from the fraction with R_f 0.11 (C). Yield 0.010 g (8%), dark red powder, mp 207–209°C (from MeOH). IR spectrum (KBr), ν, cm^{-1}: 3416 (OH), 1632, 1602 (C=O), 1437,1388, 1265, 1223, 1045. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.21 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.69 q (2H, $C\mathbf{H}_2CH_3$), 3.09 m (4H), 3.19 m (4H), 3.30 m (2H), 3.49 m (2H), 4.39 br.s (2H, OH), 4.98 br.s (2H, OH), 5.17 br.s (2H, OH), 5.41 d (1H, 1′-H, J = 9.3 Hz), 5.46 d (1H, 1′-H, J = 9.3 Hz), 5.56 br.s (2H, OH), 12.26 s (α-OH), 12.77 s (α-OH). Found, %: C 47.39; H 5.05; S 10.62. $C_{24}H_{30}O_{14}S_2$. Calculated, %: C 47.52; H 4.98; S 10.57.

6-Ethyl-2,3,7-tris(β-D-glucopyranosylsulfanyl)-5,8-dihydroxy-1,4-dihydronaphthalene-1,4-dione (XX) was synthesized by deacetylation of compound **XII** and was isolated by preparative thin-layer chromatography. Yield 0.105 g (61%), R_f 0.11 (C), dark red powder, mp >360°C. IR spectrum (KBr), ν, cm^{-1}: 3419 (OH), 1618 (C=O), 1386, 1257, 1202, 1045. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm: 1.11 t (3H, $CH_2C\mathbf{H}_3$, J = 7.5 Hz), 2.99 q (2H, $C\mathbf{H}_2CH_3$), 3.06 m (1H), 3.10 m (7H), 3.20 m (6H), 3.51 m (4H), 4.30 br.s (1H, OH), 4.34 br.s (2H, OH), 4.96 br.s (1H, OH), 5.09 d (1H, 1′-H, J = 9.3 Hz), 5.14 br.s (1H, OH), 5.17 br.s (2H, OH), 5.14 br.s (1H, OH), 5.17 br.s (2H, OH), 5.41 d (1H, 1′-H, J = 9.3 Hz), 5.42 d (1H, 1′-H, J = 9.3 Hz), 5.42 br.s (1H, OH), 5.54 br.s (2H, OH), 13.06 s (α-OH), 13.34 s (α-OH). Found, %: C 40.09; H 5.16; S 12.13. $C_{30}H_{40}O_{19}S_3$. Calculated, %: C 40.00; H 5.03 S 12.01.

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