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HETEROCYCLIC ANALOGS OF 5,12-NAPHTHACENEQUINONE. 1. SYNTHESIS OF HETEROCYCLIC ANALOGS STARTING FROM 2,3-DIAMINOQUINIZARINE

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The amination of 2-nitroquinizarine using hydroxylamine gives 2-amino-3-nitroquinizarine, which, upon reduction, gives previously unreported 2,3-diaminoquinizarine, a key intermediate in the synthesis of heterocyclic analogs of 5,12-naphthacenequinone, namely, 4,11-dihydroxyanthra[2,3-d]imidazole-5,10-dione (imidazoloquinizarine), 4,11-dihydroxyanthra[2,3-d][1,2,3]triazole-5,10-dione (triazoloquinizarine), and 5,12-dihydroxynaphtho[2,3-g]quinoxaline-6,11-dione (pyrazinoquinizarine).

Keywords: 2,3-diaminoquinizarine, 4,11-dihydroxyanthra[2,3-*d*]imidazole-5,10-dione, 4,11-dihydroxyanthra[2,3-*d*][1,2,3]triazole-5,10-dione, 5,12-dihydroxynaphtho[2,3-*g*]quinoxaline-6,11-dione, imidazoloquinizarine, pyrazinoquinizarine, triazoloquinizarine, amination, synthesis.

Quite a few derivatives of 5,12-naphthacenequinone have been isolated, synthesized and studied [1, 2]. Derivatives of 5,12-naphthaquinone glycosides, which are anthracycline antibiotics with antitumor activity, have also been synthesized and studied [3]. The basis of the high biological activity of these compounds is the intercalation of the 5,12-naphthacenequinone chromophore into cellular DNA, leading to inhibition of the matrix functions of nucleic acids and, as a consequence, inhibition of cell proliferation [3].

Compounds containing imidazole and pyrazine fragments have high biological activity [4-12] and some of these compounds are capable of intercalation into DNA and have antitumor activity [13-19].

Thus, in the search for new biologically active compounds, considerable interest is found in the synthesis of condensed systems, in which the anthraquinone fragment is fused to heterocycles such as imidazoloanthraquinone, triazoloanthraquinone, and pyrazinoanthraquinone, which have planar structure and, undoubtedly, are suitable as chromophores for intercalators.

Several methods have been described for the synthesis of imidazoloanthraquinone, triazoloanthraquinone, and pyrazinoanthraquinone [20], but possessing the antitumor activity heterocyclic analogs of 5,12-naphthacenequinones with linear structure and electron-donor substituents in the benzene ring fused to the heterocyclic fragment, have not yet been reported. Electron-donor substituents (hydroxy groups) in the α-position relative to the carbonyl groups in the chromophore part of anthracycline antibiotics (5,12-naphthacenequinones) giving rise to intramolecular hydrogen bonding cause an effective electron density distribution and this increases their affinity to DNA [21-23]. The heterocyclic fragments have to lead to a useful charge redistribution in the chromophore relative to 5,12-naphthacenequinone derivatives and change in the

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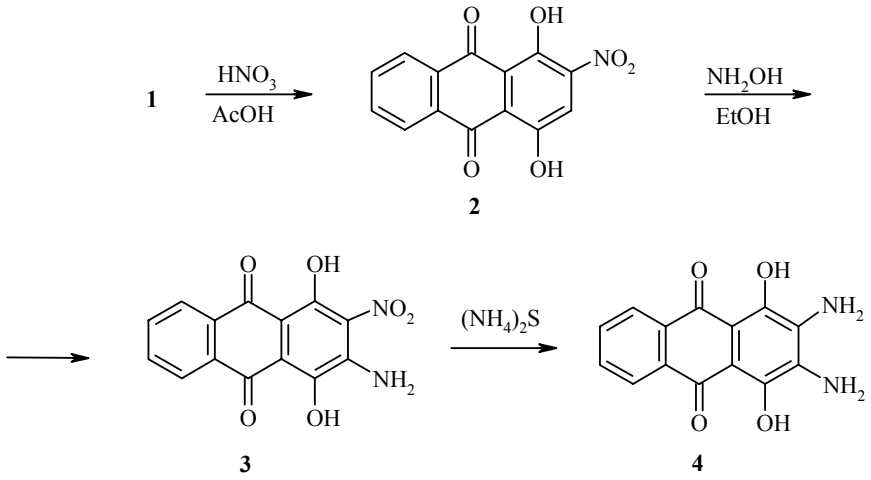
stacking interaction for intercalation into DNA. Furthermore, the synthesis of imidazoloanthraquinones provides a pathway for the preparation of nucleosides of these compounds, which may be considered as anthracycline analogs.

We have already developed methods for the synthesis of 4,11-dihydroxynaphtho[2,3-*f*]indole-5,10-dione (pyrroloquinizarine) [24] and also a dimethyl derivative of 4,11-dihydroxy[2,3-*f*]indazole-5,10-dione (pyrazoloquinizarine) [25]. In the present work, we have developed methods for the synthesis of previously unreported linear 4,11-dihydroxyanthra[2,3-*d*]imidazole-5,10-dione (imidazoloquinizarine), 4,11-dihydroxy-anthra[2,3-*d*][1,2,3]triazole-5,10-dione (triazoloquinizarine), and 5,12-dihydroxynaphtho[2,3-*g*]quinoxaline-6,11-dione (pyrazinoquinizarine), which are heterocyclic analogs of aglycones of anthracycline antibiotics (5,12-naphthacenequinone) and chromophores for the preparation of new classes of intercalators.

The synthesis of condensed systems containing imidazole, pyrazine, and triazole fragments is usually accomplished by the cyclization of *o*-phenylenediamines with derivatives of carboxylic acids, dicarbonyl compounds or nitrous acid. Thus, the key compound for the synthesis of such heterocyclic quinizarine compounds is 2,3-diamino-1,4-dihydroxy-9,10-anthracenedione (2,3-diaminoquinizarine), which has not yet been reported. Therefore, the first step in our work was the development of a new preparative synthesis for 2,3-diaminoquinizarine.

The most common method for the synthesis of *o*-phenylenediamines is the reduction of *o*-nitroanilines obtained by the nitration of aniline derivatives. However, it proved difficult to use this approach for the synthesis of 2,3-diaminoquinizarine since the nitration of 2-aminoquinizarine or its acetyl derivatives by various methods was unsuccessful. Thus, we used the amination of 2-nitroquinizarine by the hydroxylamine. This method, reported by Meisenheimer [26], involves the nucleophilic replacement of a hydrogen atom by hydroxylamine and gives good yields of the desired amines when electron-withdrawing substituents and heterocyclic fragments are found in the aromatic system. Marschalk [26] used this method for the first time for the synthesis of 2-aminoquinizarine [27]. In order to use this method, quinizarine **1** was converted by nitration [28] in a mixture of acetic and nitric acids into 1,4-dihydroxy-2-nitro-9,10-anthracenedione (**2**) in 72% yield. Amination of nitroquinizarine **2** by the action of hydroxylamine in ethanol led to 2-amino-1,4-dihydroxy-3-nitro-9,10-anthracenedione (**3**) in 86% yield. We should note that the introduction of a nitro group into quinizarine **1** markedly activates the system for nucleophilic replacement of a hydrogen atom. Thus, the reaction of hydroxylamine with quinizarine **1** proceeds at reflux in water over 2-3 h, while the amination of its nitro derivative **2** is complete in ethanol after only 1 h at room temperature.



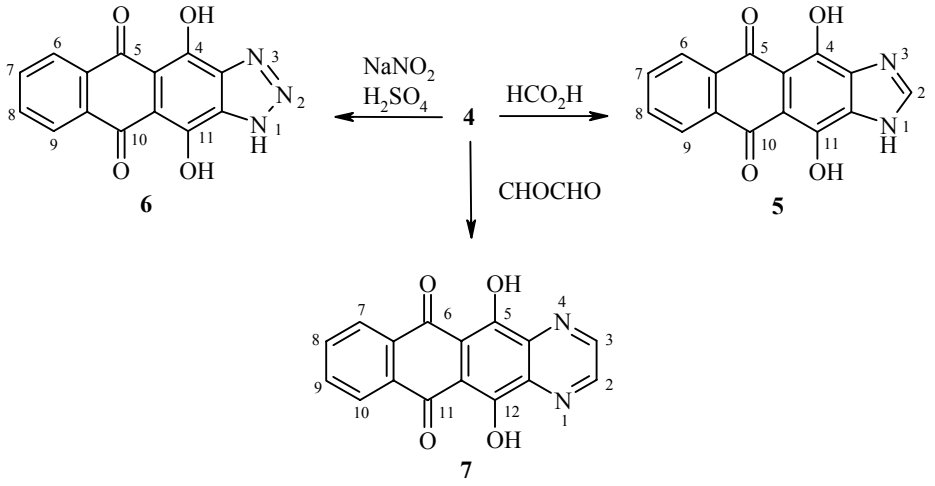
The best method for reducing nitroanthraquinone **3** is heating at reflux in aqueous ammonium sulfide, which leads to previously unreported 2,3-diamino-1,4-dihydroxyanthracene-9,10-dione (2,3-diaminoquinizarine **4**) in 67% yield.

The ^{1}H NMR spectrum of anthraquinone **3** lacks a signal for H-3 in the aromatic ring and a broad singlet appears for the amino group at 8.27 ppm. The ^{1}H NMR spectrum of diamine **4** shows broad singlets for the protons of the amino and hydroxy groups at 5.6 and 14.26 ppm, respectively. The mass spectra of anthraquinones **3** and **4** show molecular ion peaks $[M]^+$ 300 and 270, corresponding to the molecular masses of these compounds. The electronic absorption spectra show that the introduction of an amino group in 2-nitroquinizarine leads to a slight increase in intensity and bathochromic shift of the long-wavelength band by 40 nm in the spectrum of anthraquinone **3** (Fig. 1). The reduction of the nitro group in anthraquinone **3** has no significant effect on the position of the maximum of the long-wavelength band in the spectrum of diaminoquinizarine **4** though it leads to the appearance of a long "tail" up to 660 nm, which imparts an intensive brown color to this compound.

The synthesis of benzimidazole and its analogs is most often accomplished by cyclization of *o*-phenylenediamines with formic acid or triethyl orthoformate [29]. We have found that diaminoquinizarine **4** reacts with formic acid or triethyl orthoformate upon heating at reflux to give 4,11-dihydroxyanthra[2,3-*d*]-imidazole-5,10-dione (imidazoloquinizarine **5**) in 70-75% yield.

Diazotization of diaminoquinizarine **4** by nitrososulfuric acid at 5°C gave 4,11-dihydroxyanthra[2,3-*d*]-[1,2,3]triazole-5,10-dione (triazoloquinizarine **6**) in 72% yield.

Cyclization of **4** by the action of an aqueous solution of glyoxal in boiling dioxane leads to 5,12-dihydroxynaphtho[2,3-*g*]quinoxaline-6,11-dione (pyrazinoquinizarine **7**) in 69% yield.



The ^{1}H NMR spectra of these compounds lack the amino group signals of starting **4**. Singlets for the CH groups of the heterocyclic fragments appear in the spectra of imidazoquinizarine **5** and pyrazinoquinizarine **7** at 8.47 and 9.20 ppm, respectively. The signals of the protons of the unsubstituted aromatic ring are shifted only slightly in comparison with the analogous signals for H-5, H-6, H-7, and H-8 in starting diamine **4**.

The mass spectra of **5-7** show strong molecular ion peaks $[M]^+$ at 280, 281, and 292, respectively, corresponding to their molecular masses. The IR spectra of **5-7** show bands for the OH and NH groups in the vicinity of 3450 cm^{-1}, while the bands for the carbonyl groups of the anthraquinone fragment are found at 1625-1620 cm^{-1} and virtually overlap with the band for the CO groups in quinizarine at 1625 cm^{-1} [30].

The condensation of the heterocycle leads to a slight hypsochromic shift and increase in intensity of the long-wavelength band in the electronic absorption spectra of **5-7** in comparison with the spectrum of starting diaminoquinizarine **4** (Fig. 2). Furthermore, a considerable increase is observed in the intensity of the short-wavelength band at 260 nm. In contrast to the absorption spectrum of quinizarine and pyrazinoquinizarine, the spectra of imidazoloquinizarine **5** and triazoloquinizarine **6** show three bands in the visible region, appearing as two maxima and a short-wavelength inflection. Such "two-peaked" absorption is not found in the spectrum of

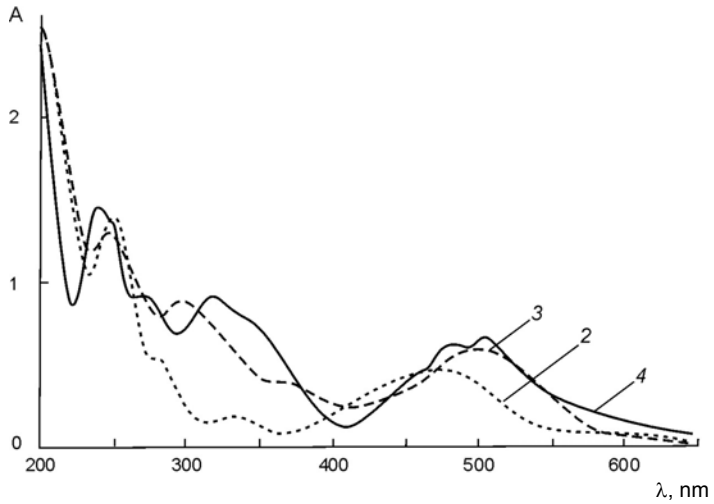


Fig. 1. Electronic absorption spectra of anthraquinones **2-4** (*2-4*, respectively) in ethanol.

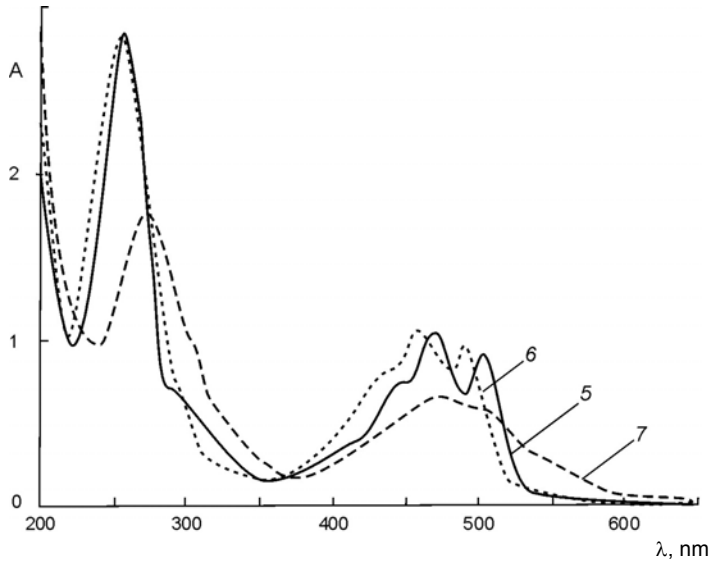
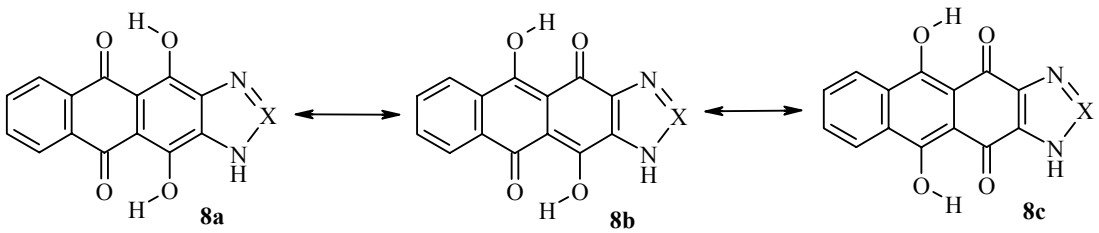


Fig. 2. Electronic absorption spectra of **5-7** (*5-7*, respectively) in ethanol.

quinizarine, but is characteristic for derivatives of 1,4-diamino- and 1-amino-4-hydroxyanthraquinones and attributed to the existence of three excited states for these compounds, differing in the contribution of the 9,10-, 1,10-, and 1,4-anthraquinoid structures [31]. Thus, the nature of the long-wavelength band for azole derivatives **5** and **6** is attributed to the existence of three excited states for these compounds, differing in the contribution of tautomeric 5,10- (**8a**), 4,9- (**8b**), and 4,11-quinoid structures (**8c**).



EXPERIMENTAL

The ^{1}H NMR spectra were taken on a Bruker WP-200 (Varian) spectrometer at 200 MHz in DMSO-d_6 using TMS as the internal standard. The mass spectra were taken on a Varian MAT-112 GC/MS at 250°C. The ionizing voltage was 70 eV. The cathodic emission current was 1 mA. The accelerating voltage was 3 kV. The IR spectra were taken on a Perkin–Elmer-599 spectrometer for KBr pellets. The course of the reactions and purity of the compounds were monitored by thin-layer chromatography on Silufol UV-254 plates.

1,4-Dihydroxy-2-nitro-9,10-anthracenedione (2). Quinizarine **1** (6.0 g, 25.0 mmol) was added to a mixture of 5.0 ml nitric acid (d = 1.38 g/cm^3) and acetic acid (90 ml) and stirred for 3.5 h at 55°C. The reaction mixture was then filtered. The precipitate was washed with acetic acid and water until the wash water was neutral and then dried to give 4.7 g (73%) of compound **2** as fine red crystals; mp 277-279°C.

2-Amino-1,4-dihydroxy-3-nitro-9,10-anthracenedione (3). A solution of hydroxylamine hydrochloride (3.2 g, 57 mmol) in a minimal amount of hot ethanol and a solution of KOH (3.0 g, 44 mmol) in a minimal amount of hot ethanol were cooled and combined. The precipitate of KCl was filtered off after 10 min. The solution obtained was added to a stirred suspension of **2** (5.0 g, 18 mmol) in ethanol (100 ml) and stirred at about 20°C for 1 h. The precipitate formed was filtered off, washed with water, and dried. The crude product was recrystallized from DMF and dried at 100°C to give 4.6 g (86%) of compound **3** as dark-crimson needles; mp 232-233°C. IR spectrum, ν, cm^{-1}: 3400 (NH_2, OH), 3100 (OH), 1620 (C=O), 1510 (NO_2), 1310 (NO_2). Mass spectrum, *m/z* (I_{rel}, %): 300 (100), 283 (5), 270 (6), 253 (6), 252 (28). ^{1}H NMR spectrum, δ, ppm: 15.60 (1H, s, 4-OH); 14.19 (1H, s, 1-OH); 8.40 (2H, m, H-5, H-8); 8.27 (2H, s, 2-NH_2); 7.86 (2H, m, H-6, H-7). Found, %: C 56.19; H 2.79; N 9.12. $C_{14}H_8N_2O_6$. Calculated, %: C 56.01; H 2.69; N 9.33.

2,3-Diamino-1,4-dihydroxy-9,10-anthracenedione (4). Aqueous $(NH_4)_2S$ (100 ml), obtained by saturation of concentrated aqueous ammonium hydroxide (20 ml) by a stream of hydrogen sulfide, was added to a suspension of **3** (3.0 g, 10 mmol) in ethanol (50 ml). The mixture was heated at reflux for 1 h and cooled. The precipitate formed was filtered off, washed with ethanol, and dried. The crude product was recrystallized from DMF and dried to give 1.9 g (67%) 2,3-diaminoquinizarine **4** as dark-brown needles; mp >370°C (subl.). IR spectrum, ν, cm^{-1}: 3400 (OH), 3320 (NH_2), 3100 (OH), 3340, 1642 (C=O). Mass spectrum, *m/z* (I_{rel}, %): 270 (100), 242 (17), 187 (4), 170 (5). ^{1}H NMR spectrum, δ, ppm: 14.26 (2H, s, OH); 8.21 (2H, m, H-5, H-8); 7.73 (2H, m, H-6, H-7); 5.57 (4H, s, NH_2). Found, %: C 62.05; H 3.62; N 10.14. $C_{14}H_{10}N_2O_4$. Calculated, %: C 62.22; H 3.73; N 10.37.

4,11-Dihydroxyanthra[2,3-*d*]imidazole-5,10-dione (5). A solution of **4** (3.0 g, 11 mmol) in formic acid (20 ml) was heated at reflux for 3 h and poured into water (100 ml). Then, 20% aq. NaOH was added until the reaction was slightly basic (universal indicator). The precipitate obtained was filtered off, washed with water (3 × 20 ml), and dried to constant mass. The crude product was recrystallized from DMF and dried to give 2.3 g (75%) of compound **5** as dark-yellow crystals; mp >350°C (subl.). IR spectrum, ν, cm^{-1}: 3450 (NH, OH), 3100 (OH), 1625 (C=O). Mass spectrum, *m/z* (I_{rel}, %): 280 (100), 252 (3), 240 (7). ^{1}H NMR spectrum, δ, ppm: 14.56 (2H, s, OH); 8.47 (1H, s, H-2); 8.29 (2H, m, H-6, H-9); 7.91 (2H, m, H-7, H-8). Found, %: C 64.01; H 2.52; N 9.82. $C_{15}H_8N_2O_4$. Calculated, %: C 64.29; H 2.88; N 10.00.

4,11-Dihydroxyanthra[2,3-*d*][1,2,3]triazole-5,10-dione (6). Compound **4** (0.5 g, 1.8 mmol) was dissolved with stirring in warm sulfuric acid (40 ml). A solution of $NaNO_2$ (0.16 g, 2.5 mmol) in hot sulfuric acid (10 ml) was prepared separately. The solutions were cooled to 5°C and the nitrososulfuric acid solution was then added with continuous stirring and cooling to the solution of diaminoquinizarine, maintaining the temperature not above 10°C. The reaction mixture was maintained with cooling for 20 min and, then, the mixture was heated to 60°C. The mixture was maintained at 60°C for 20 min, cooled, and poured into water (50 ml) with ice (100 g). Then, 20% aq. NaOH was added until neutral (universal indicator). The precipitate obtained was filtered off, washed with water, and dried. The crude product was recrystallized from DMF and dried to give 0.37 g (72%) of triazoloquinizarine **6** as yellow crystals; mp 308-310°C (dec.). IR spectrum,

ν, cm^{-1}: 3450 (NH, OH), 3100 (OH), 1620 (C=O). Mass spectrum, *m/z* (I_{rel}, %): 281 (100), 254 (12), 253 (9), 184 (25). ^{1}H NMR spectrum, δ, ppm: 14.64 (2H, s, OH); 8.30 (2H, m, H-6, H-9); 7.92 (2H, m, H-7, H-8). Found, %: C 59.38; H 2.67; N 14.60. $C_{14}H_7N_3O_4$. Calculated, %: C 59.79; H 2.51; N 14.94.

5,12-Dihydroxynaphtho[2,3-*g*]quinoxaline-6,11-dione (7). Compound **4** (1.0 g, 3.6 mmol) in a mixture of dioxane (20 ml) and 40% aq. glyoxal (3 ml) was heated at reflux for 2 h. The mixture was cooled and poured into water (50 ml). The precipitate formed was filtered off, dried, and recrystallized from DMF to give 0.75 g (69%) of pyrazinoquinizarine **7** as dark-yellow crystals; mp 344-346°C (subl.). IR spectrum, ν, cm^{-1}: 3450 (HN, OH), 1620 (C=O). Mass spectrum, *m/z* (I_{rel}, %): 292 (100), 280 (5), 264 (5). ^{1}H NMR spectrum, δ, ppm (in DMSO-d_6): 14.93 (2H, s, OH); 9.20 (2H, s, H-2, H-3); 8.26 (2H, m, H-7, H-10); 7.92 (2H, m, H-8, H-9). Found, %: C 65.43; H 2.61; N 9.42. $C_{16}H_8N_2O_4$. Calculated, %: C 65.76; H 2.76; N 9.59.

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