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The new inhibitors of ribonucleotide reductase—comparison of some physico-chemical properties

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Abstract

Amidox (AX), didox (DX) and trimidox (TX), compounds synthetized as new ribonucleotide reductase inhibitors, have been investigated by ultraviolet (UV) spectrophotometry, polarography and high performance liquid chromatography (HPLC). The experiments have been performed at various pH values. The changes in UV absorption of the compounds studied were recorded and it was demonstrated that these changes are related to the pH and to structural features of the investigated molecules. From the compounds included in our series of experiments, only amidox and trimidox are reduced during polarographic experiments in Britton–Robinson buffer. The reduction of both compounds proceeded in two one-electron steps in acidic pH. One two-electron diffuse irreversible wave was observed at basic pH. The values of the half-wave potential became more negative in accordance with the increasing pH. HPLC assay also showed changes in the retention of compounds investigated, particulary when the pH of the mobile phase was close to the dissociation constant of the particular drug. The changes of physico-chemical properties detected by the all used methods are related to different chemical structures (the most significant changes were observed in alkaline pH). © 1997 Elsevier Science B.V.

Keywords: Ribonucleotide reductase inhibitors; DC polarography; UV spectrophotometry; HPLC

1. Introduction

Amidox (AX), trimidox (TX) and didox (DX) (Fig. 1) are derivatives of polyhydroxy-substituted benzohydroxamates. They have been synthetized as new inhibitors of ribonucleotide reductase (RR) [1,2]. There is a linkage between RR activity and neoplastic proliferation [3]. RR catalyzes the conversion of ribonucleoside diphosphates to deoxyribonucleotide diphosphates, which is a rate-limiting step of de novo DNA synthesis [4,5]. Therefore RR plays an important role in DNA replication, provides a unique metabolic target for chemotherapeutic approaches to cancer treatment [6]. One of the known RR inhibitors, which is clinically used, is hydroxyurea [7].

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AX and TX were synthetized as analogues of DX in a search for superior compounds [8]. Firstly, DX has been investigated for its anticancer activity in a number of animal tumor systems and also in phase I and II clinical trials [9,10]. It also acts synergistically with adriamycin and reduces anthracycline toxicity [11]. Trimidox has also shown antitumor activity in L1210 leukemia and greater effectivity than hydroxyurea [12]. Combination of trimidox with other drugs, e.g. tiazofurin [13] and Pt-containing compounds or cyclophosphamide resulted in synergistic effects [14].

Our study was directed towards an evaluation of the relation of modified chemical structure reflected in DC polarography data, UV spectra and high performance liquid chromatography (HPLC) characteristics under various experimental conditions. It has been shown that pH plays an important role in all experimental systems as it initiates the change of chromophoric groups. These changes were shown in UV spectra, in reductability of polarographed compounds and in chromatographic retention. The obtained information might be of use in the future pre-clinical and possible clinical investigation of these new anticancer drugs.

2. Experimental

2.1. Materials

AX, DX and TX were obtained from one of the authors (H.L.E., Richmond, VA). Methanol, potassium hydroxide were purchased from Merck (Darmstadt, Germany), all compounds for preparation of Britton–Robinson buffer, orthophosphoric acid and triethylamine were purchased from Fluka (Buchs, Switzerland).

2.2. Spectrophotometry

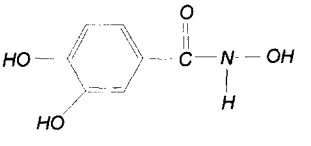
UV spectrum of AX, DX, TX were recorded using a Perkin Elmer 557 double beam wavelength spectrophotometer in the wavelength range of 190–400 nm. The studied compounds were dissolved in 0.01 M KH_2PO_4 to the final concentration of 10^{-4} M. The pH values were adjusted with a few drops of 5 M KOH or H_3PO_4. The UV spectra of all studied compounds were recorded in water and methanol.

2.3. DC polarography

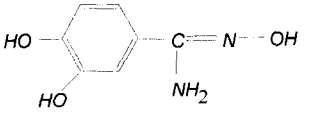
All polarographic experiments were performed in the same way as it was already described [15]. The concentration of all compounds was 10^{-3} M. They were dissolved directly in polarographic cell in Britton–Robinson buffer adjusted to the chosen pH.

2.4. HPLC system and operating conditions

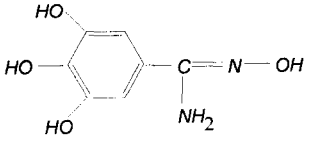
A Pye Unicam HPLC system consists of a PU 4030 controller, PU 4011 gradient pump, PU 4020 variable-wavelength UV detector (Philips, UK) and Rheodyne Model 7125 injector (20 μl sample loop). Peaks were recorded with a Model 4880 Data station, version 1.1 (Ati Unicam, UK). The chromatography in reverse-phase mode (column



Didox (DX)



Amidox (AX)



Trimidox (TX)

Fig. 1. Chemical structure of the compounds.

Table 1
Absorption maxima of amidox, didox and trimidox depending on pH

Didox						
pH	4.0	7.1	7.6	8.6	9.7	
λ_{max}	251, 285	253, 287	255, 291	299	269, 297	
Trimidox						
pH	4.7	6.9	7.6	9.1	10.7	
λ_{max}	256	260	—	242, 317[a]	303, 389[a]	
Amidox						
pH	4.4	5.7	7.2	7.6	8.3	11.3
λ_{max}	255, 287	250, 283	247, 289	240, 272	253, 287	240, 305

[a] Only shoulders were recorded at pH of 9.1 and 10.7.

Supelcosil LC 18, 150 × 4.6 mm i.d., 3 μm, flow rate 0.5 ml min^{-1}) was used. The mobile phase consists of 0.05 M H_3PO_4, 5% methanol and 0.05% triethylamine (v/v). The appropriate pH of the mobile phase was adjusted with H_3PO_4 or 5 M KOH. UV detection was conducted at 255 nm.

3. Results and discussion

The UV spectra of AX, DX and TX were recorded in methanol, water and 0.01 M KH_2PO_4 at various pH values.

Didox has two absorption maxima at 250 and 289 nm in water and methanol. The UV spectra of DX at pH 4.0, 7.1, 7.6, 8.6, 9.7 were recorded. Absorption maximum and the shape of absorption curve of DX at pH 4.0 and 7.1 were the same in water and methanol. Only one maximum at pH 8.6 was observed at 299 nm. At pH 9.7, two absorption maxima are shifted approximately 15 nm to higher absorption wavelength. It can be hypothetized that these changes are caused by structural changes of DX. Absorption maximum of TX is 269 nm in water and in methanol. Absorption maxima of TX at chosen pH are shown in Table 1 and depicted in Fig. 2. The absorption curve was sloping down without defined absorption maxima at pH 7.6. The UV spectrum of TX at pH 9.1 and 10.7 was recorded in the wavelength range of 190–500 nm as the colour of solutions gradually changed from yellow to brown with increasing pH to more basic (from 7 to 10.7). No absorption maximum but two shoulders (see Table 1) were recorded at pH 9.1 and 10.7. The last compound from our series -AX- has two absorption maxima in methanol (259, 310 nm) and in water (253, 283 nm). Only a slight shift of wavelength was observed. On the other hand, there were visible changes in the shape of the spectrum as the first maximum decreased and the second increased with increasing pH values.

The greatest change in UV spectrum of TX occured in the pH range 7.0–7.5. TX became probably completely oxidized at the pH over 7.5 and its structure is different from original structure. The alteration in UV spectrum of AX and DX could be explained by dissociation of molecules.

Polarographic measurements were performed in the pH range from 3.4 to 10.0 in buffer solutions. It was observed that only DX could not be reduced under these conditions. However, AX and TX were polarographically reduced, although their behaviour is different in acidic and basic solutions. The reduction of both AX and TX proceeded in two well defined one-electron steps up to a pH of 4.9. Both polarographic waves have a diffuse character, the first being reversible, while the second one irreversible. There is an appreciable shift of half-wave potentials ($E_{1/2}$) of I and II polarographic waves to more negative values (Table 2). This occured increasingly from pH 4.9 (close to the dissociation constants of AX and TX $pK_{a1} = 5.2$, $pK_{a2} = 8.2$) to pH 7.0. At pH 7.0, another marked $E_{1/2}$ shift was recorded but this shift was not accompanied by qualitative changes

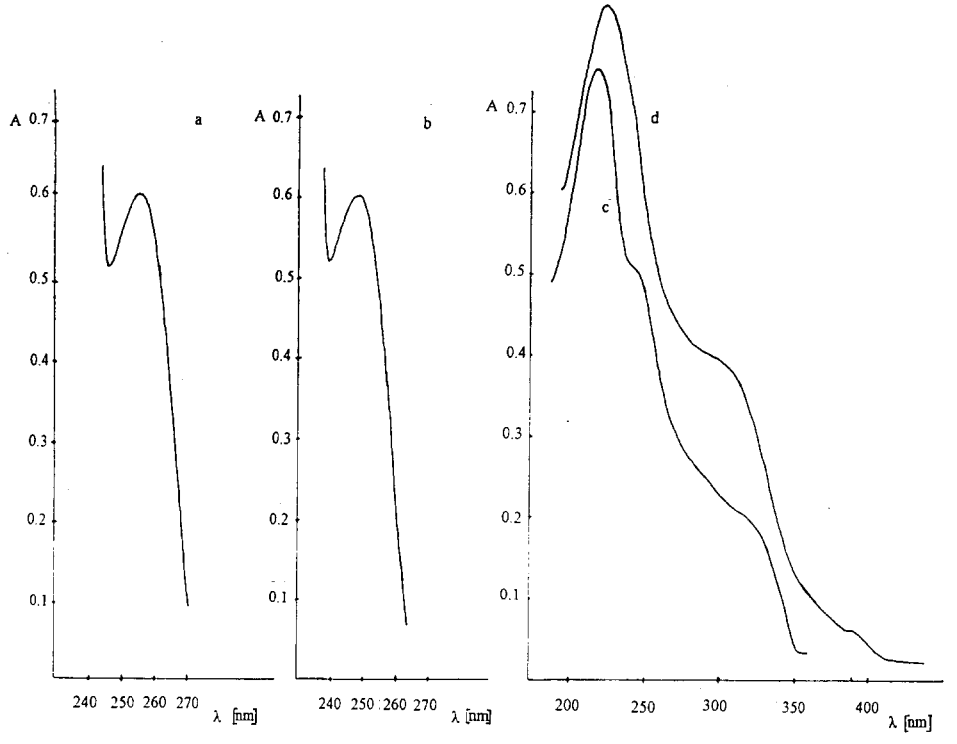


Fig. 2. The UV spectra of trimidox in 0.01 M KH_2PO_4 at different pH. (a) pH 4.7; (b) pH 6.9; (c) pH 9.1; (d) pH 10.7.

in the reduction mechanism. In the basic solution of pH 8, which is close to pK_{a2} of AX and TX, original I and II polarographic waves merged into the one two-electron diffuse irreversible wave with $E_{1/2}$ potential of the original II wave (Table 2). The reduction of AX and TX stayed unchanged in higher pH but the values of $E_{1/2}$ shifted markedly towards more negative potentials. Changes of the polarographic behaviour of TX are depicted in Fig. 3.

Table 2
Half-wave potentials ($E_{1/2}$) of amidox, didox and trimidox depending on pH

pH	Trimidox		Amidox	
	$E_{1/2(I)}$ (V)	$E_{1/2(II)}$ (V)	$E_{1/2(I)}$ (V)	$E_{1/2(II)}$ (V)
3.4	−1.190	−1.420	−1.200	−1.430
4.9	−1.320	−1.540	−1.300	−1.560
7.0	−1.480	−1.720	−1.480	−1.720
7.2	−1.490	−1.730	−1.500	−1.720
8.0		−1.730		−1.720
8.4		−1.770		−1.760
8.9		−1.830		−1.800
10.0		−1.850		−1.840

Hence it followed that between the reduction mechanism of AX and TX (TX is a derivative of gallic acid) are no significant differences. However, significant qualitative differences depending on pH were found in their reduction properties. Reduction of both compounds provided two well defined one-electron waves under acidic and neutral condition. This process proceeded irreversibly in one two-electron step under basic condition. TX is related to gallic acid, however, polarographic reduction of gallic acid has not been documented yet. The only available data provided us with the information about its oxidation at different pH. Some authors [16] have observed that gallic acid gives two oxidative waves in solution up to pH of 6.0, the first of them is more positive, belonging to an adsorption process, the second one to two-electron irreversible process. The oxidation is one-electron single step process

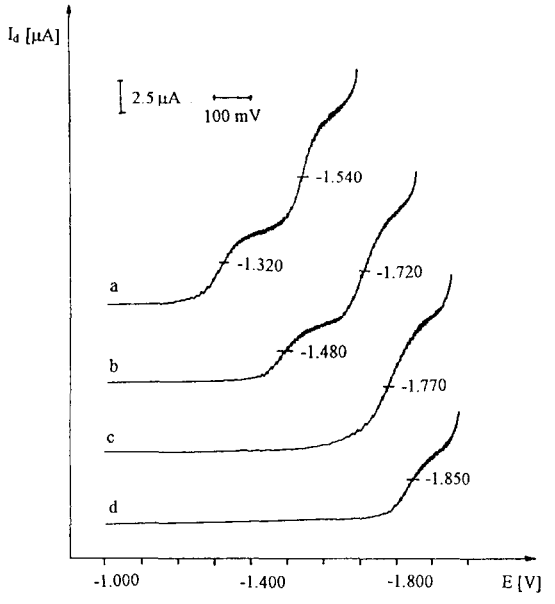


Fig. 3. Polarographic reduction of trimidox in Britton–Robinson buffer at different pH. Curves: (a) pH 4.9; (b) pH 7.0; (c) pH 8.4; (d) pH 10.0. Starting voltage −1.00 V, scan rate 5 mV s^{-1}.

in neutral and basic conditions. The adsorption wave fades out. The $E_{1/2}$ value gradually became more negative with increasing basicity ($E_{1/2,\ pH\ 1.8}$ = +0.493 V to $E_{1/2,\ pH\ 11.2}$ = −0.770 V vs. saturated calomel electrode, SCE). The same authors have also assumed, on the background of these results, the formation of unstable quinoic intermediate, which undergoes further oxidation [17].

The HPLC assay in reverse-phase mode was used to investigate the behaviour of RR inhibitors depending on pH of the mobile phase. With regard to the operating range of the bonded silica columns (3.0–7.5), pH values were chosen between 3.0 and 7.5. The retention of AX and TX increased with increasing pH. The retention of AX was more stronger compared to that of TX. AX and TX were strongly retained when the pH was over 5.0. The retention of both, AX and TX was decreasing at a pH of 7.5 (Fig. 4). These changes are in accordance with the dissociation constants of the compounds investigated. Dissociation changes the polarity of AX and TX during the process of chromatography. In contrast, the retention of DX was decreased when increasing pH. Retention of DX did not change as markedly as the retention of AX and TX.

In conclusion, some structural changes of TX take place under basic condition. We suppose that it could be caused by quinoic structure formation

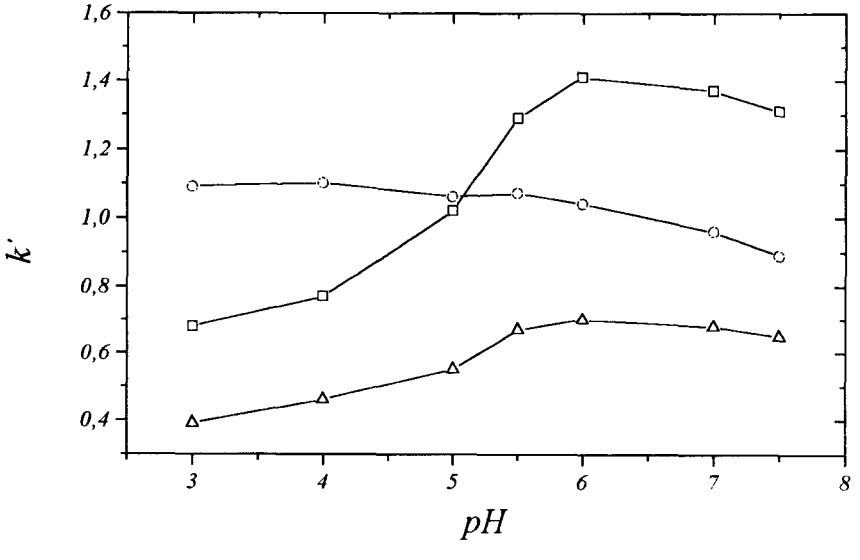


Fig. 4. Effect of pH on capacity factors (k') of AX (□), DX (○) and TX (△). Chromatographic conditions can be seen in Section 2.

which is commonly known for the gallic acid derivatives. Reversibility resp. irreversibility of quinoic structure formation might be dependent on pH (intermediate formation in the pH range 7.0–7.5, further oxidation at the pH over 7.5). These findings are confirmed by the presented spectroscopic and polarographic data. The changes in UV spectrum of AX as well as the changes in chromatographic retention of AX correspond to the dissociation constants pK_{a1} and pK_{a2} of AX. It can be supposed that the same is valid for DX. The obtained information might be of use in elucidation of biochemical mechanism(s) of studied RR inhibitors and in the analytical determination (HPLC assay) of these compounds, e.g. choice of suitable separation conditions, their determination in biological samples and pharmacokinetics study.

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