INVESTIGATION OF THE POLAROGRAPHIC PROPERTIES AND POTENTIAL CARCINOGENITY OF SOME HYDROXYUREA DERIVATIVES BY DC POLAROGRAPHY

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Polarographic reduction was studied for a series of 7 urea derivatives and the results were used to assess their potential carcinogenity. The polarographic reduction was examined in absolutely anhydrous dimethylformamide by DC polarography. In the conditions applied, the majority of the compounds was reduced within a single two-electron step, only biuret and its formyl derivative were reduced in two one-electron steps. The potential carcinogenity of the substances was assessed based on the tg α value of the slope of dependence of the polarographic wave height on the concentration of α-lipoic acid added as a test substance. For hydroxyurea, which is the only substance in this series for which a carcinogenic activity has been demonstrated, the tg α parameter attained a value of 0.290. Still higher values were obtained for the formyl derivatives – formylbiuret (0.362) and 2-carbamoyl-1-formylguanidine (0.510). So high tg α values warn of a significant potential carcinogenity. The other substances studied exhibited considerably lower tg α values, indicating that their potential carcinogenity will be low.

Key words: Urea derivatives; DC polarography; Carcinogenity.

Hydroxyurea (**2**) is a derivative of urea (**1**) exhibiting antineoplastic effects, also with respect to neoplasms of human origin[1–3]. Its routine clinical use, however, is limited to chronic myeloid leukemia[4]. In cells, hydroxyurea blocks the conversion of ribonucleotides to deoxyribonucleotides, a process which is catalyzed by the enzyme ribonucleotide-reductase[4].

In addition to the antineoplastic aspect, hydroxyurea and its derivatives are of interest because they form parts of the structures of considerably more complex biologically active molecules of synthetic 1,3,5-triazine (5-azapyrimidine) analogues of natural components of nucleic acids[5].

Since the polarographic reduction of urea derivatives in the series selected has not been studied extensively as yet, we examined their basic polarographic characteristics in an aprotic medium by DC polarography. Their potential carcinogenity was assessed based on their polarographic reduction in the presence of α-lipoic acid[6–9].

EXPERIMENTAL

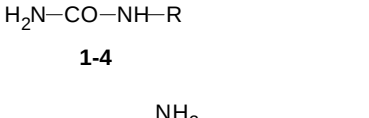
Melting temperatures were determined on a Kofler stage and are not corrected. Optical rotatory power was measured on a Perkin–Elmer 141 MCA polarimeter at 22 °C. The purity of the substances was checked by thin layer chromatography, elemental analysis, melting temperature measurement, and spectroscopically. UV spectra were scanned on a Beckman DU-65 spectrophotometer.

Urea and hydroxyurea were commercial chemicals obtained from Aldrich-Chemie (Steinheim, Germany). Biuret, formylbiuret and 2-carbamoylguanidine were prepared by published procedures (Table I). Dimethylformamide (DMF), which served as the solvent in all polarographic measurements, and tetrabutylammonium perchlorate (TBAP) were products of Fluka (Switzerland). DMF was purified by double vacuum distillation[10] prior to use. The water content of DMF, which was checked periodically by Karl Fischer dead-stop titrations, never exceeded 0.1%. α-Lipoic acid (D,L-6,8-thioctic acid) was obtained from Koch-Light Laboratories (Colnbrook, U.K.).

Polarographic measurements were performed on a PA4 polarographic analyzer equipped with a two-line recorder (Laboratorni pristroje, Prague, Czech Republic). The three-electrode connection was applied, using a dropping mercury electrode as the indicator electrode (drop time 3 s, flow rate 2.27 mg s^{-1}), a saturated calomel reference electrode (SCE), and an OH 9377 standard platinum compensation electrode (Radelkis, Hungary). Polarographic reduction of the compounds was conducted in a thermostatted vessel at 25 °C under a stream of dry nitrogen. The analyte concentration was invariably 0.5 mmol l^{-1}, the concentration of TBAP was 150 mmol l^{-1}. In experiments involving α-lipoic acid, the acid-to-analyte molar ratio was varied over the range of 0.1 : 1 to 2.4 : 1. The reversibility or irreversibility of the reduction steps was studied by the switched curve method[11]. The number of electrons involved in the reduction was obtained by logarithmic analysis of the polarographic curves as the log $i_d/(i_l - i_d)$ vs E plots (i_d is the diffusion current at voltage E, i_l is the limiting current).

2-Carbamoyl-1-formylguanidine (**6**)

2-Carbamoyl-1-formylguanidine was obtained as a minor fraction (8%) from the mother liquors after hydrogenolysis of 1-(2,3,5-tri-*O*-benzyl-β-D-arabinosyl)-5-azacytosine using palladium on activated carbon as the catalyst. The known 1-β-D-arabinufuranosyl-5-azacytosine[12] (ara-AC) is the major product



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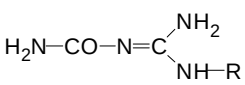

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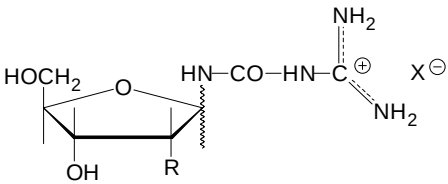

7, 8

	R	X
1	H	
2	OH	
3	CO–NH_2	
4	CO–NH–CHO	
5	H	
6	CHO	
7	H	HCOO
8	OH	CH_3COO

of this reaction, conducted in the presence of hydrogen chloride in alcohol. Compound **6** apparently emerges from the hydrolytic splitting of arabinose from 2-(β-D-arabinofuranosylaminocarbonyl)-1-formylguanidine, which is an intermediate product in the hydrolysis of ara-AC (ref.[13]). This kind of intermediate was first detected during the hydrolysis of 5-azacytidine[14]. Compound **6** failed then to be prepared because the direct formylation of amidinourea led, with a simultaneous cyclization, to 5-azacytosine. Compound **6**: M.p. 214–215 °C (methanol), UV spectrum (water): 234 (4.25). For $C_3H_6N_4O_2$ (130.1) calculated: 27.70% C, 4.65% H, 43.06% N; found: 27.37% C, 4.82% H, 42.81% N.

2-Deoxy-D-erythropentofuranosylaminocarbonylguanidinium Formate (**7**)

This compound was prepared by hydrolysis of 2′-deoxy-5-azacytidine[15] with an aqueous solution of ammonia at a concentration of 1 mol l^{-1}, conducted as with 5-azacytidine[14]. This reaction was accompanied by partial anomerization, so that the product was a mixture of the α and β anomers. Compound **7**: m.p. 152–154 °C (decomp.) (ethanol), $[\alpha]_D$ +36.2° (*c* 0.50, water) (α : β = 1 : 1), UV spectrum: 221 (3.85) at pH 7.0, 223 (4.38) at pH 11.0. For $C_8H_{16}N_4O_6$ (264.2) calculated: 36.36% C, 6.10% H, 21.20% N; found: 36.32 C, 6.07% H, 21.42% N.

D-Ribofuranosylaminocarbonylguanidinium Acetate (**8**)

This compound was obtained by filtering an aqueous solution of β-D-ribofuranosylaminocarbonylguanidinium picrate[14] over a Dowex-1 column in the acetate cycle, evaporating the eluate, and crystallizing the residue from ethanol. This operation was accompanied by anomerization, so that the product was also a mixture of the α and β anomers. Compound **8**: m.p. 135–137 °C (decomp.) (ethanol), $[\alpha]_D$ +92.4° (*c* 0.15, water) (α : β = 9 : 1), UV spectrum 222 (3.84) at pH 7.0, 224 (4.30) at pH 11.0. For $C_9H_{18}N_4O_7$ (294.3) calculated: 36.73% C, 6.16% H, 19.04 N; found: 36.78% C, 6.21% H, 19.30% N.

RESULTS AND DISCUSSION

Except for urea (**1**), all compounds listed in Table I are polarographically active. Urea was not reduced in the experimental setup used, with DMF as the solvent. This compound, containing two amino groups in a molecule, gave no polarographic wave. On the other hand, hydroxyurea (**2**), where one of the hydrogen atoms of the amino group is replaced by a hydroxy group, gave a two-electron diffusion wave, which, however, was ill-developed due to the very negative half-wave potential (Table I). This is consistent with the reducibility data of amino compounds, which do not reduce at all[16] (e.g. urea) or reduce very reluctantly (cf. hydroxyurea). If the hydrogen and hydroxy group (–HOH) in the hydroxyurea molecule are replaced by an amidine group $=C(NH_2)_2$, the reducibility of the product, viz. 2-carbamoylguanidine (**5**), is facilitated and its half-wave potential is nearly 900 mV more positive than that of hydroxyurea (Table I). Reduction of **5** is a one-step two-electron process and the polarographic wave is of diffusion-controlled and irreversible nature.

A substantially different polarographic behaviour was observed for 2-carbamoyl-1-formylguanidine (**6**). The substitution by a formyl group brings about appearance of an adsorption wave with a half-wave potential $E_{1/2}$ = –0.130 V and of a polarographic

maximum of the 1st kind at –1.950 V vs SCE. The adsorption nature of the former was proved based on the dependence of its current on the mercury column height and also based on the fact that its height was independent of analyte concentration.

2-Deoxy-D-erythropentofuranosylaminocarbonylguanidinium formate (**7**) and D-ribofuranosylaminocarbonylguanidinium acetate (**8**) in anhydrous DMF are reduced in single two-electron reversible steps with values of $E_{1/2}$ = –2.120 and –2.270 V vs SCE, respectively.

Biuret (**3**) and formylbiuret (**4**) are the only two compounds in the series that are reduced in two well-defined steps. The first step is a diffusion-controlled one-electron reversible process, the other, also diffusion-controlled and one-electron step, is an irreversible process. Logarithmic analysis gave a slope of 58 mV for both compounds, corresponding to a charge number of 1 (ref.[17]).

Potential carcinogenity of the compounds was examined based on their reduction in anhydrous DMF in the presence of α-lipoic acid[6–9]. The diffusion-controlled wave height increased linearly with increasing concentration of α-lipoic acid (Fig. 1) for all substances except **7**. The slope (tg α) of the corresponding plot (Fig. 2) is a suitable quantity for assessing the potential carcinogenity of the substances at a pre-screening level. As a rule of thumb[6–9], substances whose tg α values do not exceed 0.100 are harmless as carcinogens, substances whose tg α values lies within the region of 0.100–0.200

TABLE I
Half-wave potentials $E_{1/2}$ and the parameter tg α of the urea derivatives

Compound	$E_{1/2}$, V (SCE)	tg α	Ref.
Urea (**1**)	–	not determined	–
Hydroxyurea (**2**)	–2.760	0.290	–
Biuret (**3**)	–2.040 –2.350	0.100	13
Formylbiuret (**4**)	–2.100 –2.395	0.362	22
2-Carbamoylguanidine (**5**)	–1.880	0.060	23
2-Carbamoyl-1-formylguanidine (**6**)	–0.130 –1.950	0.510	this work
2-Deoxy-D-erythropentofuranosylaminocarbonylguanidinium formate (**7**)	–2.120	0.000	this work
D-Ribofuranosylaminocarbonylguanidinium acetate (**8**)	–2.270	0.150	this work

possess a marginal potential carcinogenity, and substances with tg α values in excess of 0.200 warn of potential carcinogenity; the higher this parameter, the higher the probability of a carcinogenic activity of the substance. In the series studied (Table I), tg α values lower than 0.100 (no carcinogenity) were found for compounds **3**, **5**, and **7**, and a tg α value between 0.100 and 0.200 (marginal carcinogenity) was observed for compound **8**. Values higher than 0.200 were measured for hydroxyurea (**2**, 0.29), formylbiuret (**4**, 0.362) and 2-carbamoyl-1-formylguanidine (**6**, 0.510). From among the three compounds, carcinogenic activity has only been investigated for hydroxyurea which, administrated to laboratory mice in doses of 100 mg/kg body weight, induced hepatocarcinomas in 45% animals[18]. Hydroxyurea is also the first known inhibitor of the enzyme ribonucleotidereductase and exhibits an antineoplastic activity[18]. Its clinical application field is not very wide but it is used in the prevention of complications of intracerebral leukostasis in patients with acute myelogenic leukemia where the blastic cell counts in peripheral blood are increased significantly[19,20].

In addition to hydroxyurea, its two formyl derivatives – formylbiuret (**4**) and 2-carbamoyl-1-formylguanidine (**6**) – also exhibit high tg α values. The formylamidino derivative is reduced more readily that formylbiuret (Table I). The reduction mechanism of formylbiuret is identical with that of biuret. The same applies to the 2-carbamoylguanidine (**5**) / 2-carbamoyl-1-formylguanidine (**6**) pair, only the difference in the $E_{1/2}$ values is somewhat higher (70 mV). It is noteworthy that the introduction of the formyl group into the parent molecule brings about a significant increase in the tg α value, viz. from 0.100 to 0.362 for the **3**–**4** pair and from 0.060 to 0.510 for the **5**–**6** pair, which is

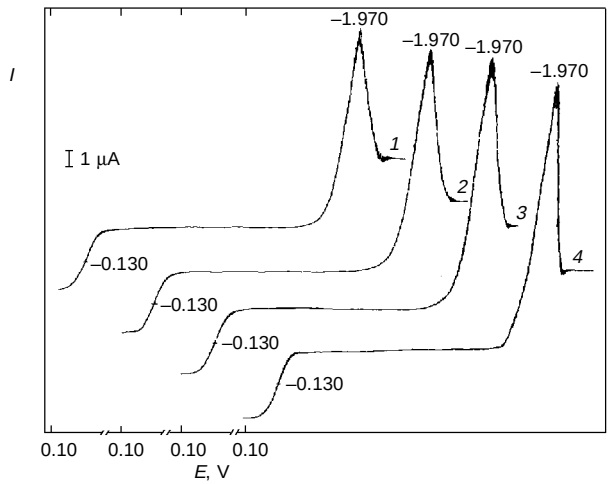

FIG. 1

Effect of α-lipoic acid on the polarographic reduction of 2-carbamoyl-1-formylguanidine (c = 0.5 mmol l^{-1}) in anhydrous DMF. Concentration of α-lipoic acid c_{la} (mmol l^{-1}): *1* 0, *2* 0.8, *3* 1.6, *4* 2.4

a shift from non-carcinogens to potential carcinogens. The data suggest that formylbiuret (**4**) and, in particular, 2-carbamoyl-1-formylguanidine (**6**) will exhibit carcinogenic effects.

The situation is different with 2-deoxy-D-erythropentofuranosylaminocarbonylguanidinium formate (**7**) and D-ribofuranosylaminocarbonylguanidinium acetate (**8**). Involving sugar components in their molecules, these compounds are closely related to nucleosides and serve as intermediates in the synthesis of nucleosides. Compound **7** – the only one in the series examined – exhibits a tg α value of 0.000, that is, its polarographic wave height is unaffected by the presence of α-lipoic acid, in which respect this substance resembles the non-carcinogenic polyaromatic compounds: naphthalene, anthracene, and pyrene[6]. For compound **8** the tg α value is 0.150, which suggests that in the environment, this compound will pose no appreciable carcinogenic risk.

The higher tg α values of the formyl derivatives **4** and **6** as compared to the parent compounds **3** and **5**, respectively, may be explained in terms of their formylation activity, capable of formylating the –SH, –OH, and $-NH_2$ groups in enzymes. This capability is appreciably higher for **6** than for **4** due to the considerably higher rate of hydrolytic splitting of the formyl group from formylamidinoureas than from formylbiurets[13,14]. Since sugar derivatives of formylamidinourea have been proved to be intermediates in the hydrolysis[14] of the less carcinogenic nucleosides of 5-azacytosine, it is conceivable that such substances contribute to cause of carcinogenity of 5-azacytosine nucleosides. Amidinoureas formed by further hydrolysis of the formylamidinoureas do not seem to be carcinogenic. On the other hand, formylbiuret, which is the intermediate in the hydrolysis of 5-azauracil to biuret[13], is slightly less carcinogenic than 5-azauracil,

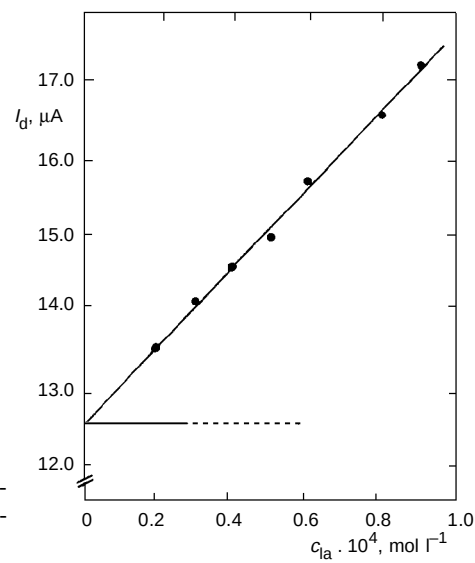

FIG. 2
Dependence of the 2-carbamoyl-1-formylguanidine reduction wave hight on the concentration of α-lipoic acid

perhaps due to the ability of the latter to form rather stable covalent adducts[13] – an ability that 5-azacytosine derivatives are lacking[21].

In conclusion, the results of the polarographic examination suggest that the carcinogenic activity of the compounds **2**, **5**, **7**, and **8** will be low, whereas the compounds **3**, **4**, and **6** are potential carcinogens whose carcinogenity and mutagenity should be additionally tested by procedures recommended by WHO.

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