POLAROGRAPHIC REDUCTION AND POTENTIAL CARCINOGENITY OF SYNTHETIC 1,3,5-TRIAZINE BASES AND NUCLEOSIDES

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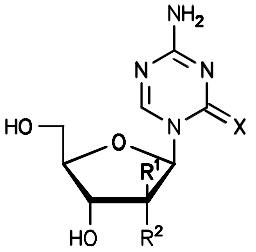
Received September 17, 1993
Accepted February 13, 1994

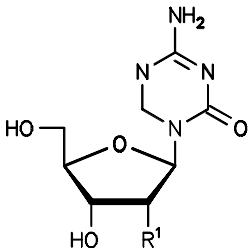
DC polarographic parameters were measured for a series of 15 synthetic 5-aza compounds derived from cytosine, cytidine, uracil and uridine in nonaqueous (dimethylformamide) solutions. The substances in aprotic media are reduced in a single two-electron step at the mercury drop electrode, except for 5,6-dihydro derivatives of 5-azauracil and 5-azauridine which are reduced in two steps. α-Lipoic acid was added to the solutions of the substances, and the slopes tg α of the plots of diffusion current of the substances versus α-lipoic acid concentration, which can serve as an index of potential carcinogenic activity of the substances measured, were determined. The tg α values of all the compounds studied are low as compared to related substances whose carcinogenic activity has been proved. 5-Azacytidine and 5-azauracil are exceptions exhibiting tg α values of 0.295 and 0.400, respectively. For the former compound, this is consistent with the WHO classification as "probably carcinogenic to humans".

The success of arabinosylcytosine in the chemotherapy of leukemia stimulated interest in the investigation of properties of additional synthetic analogues of natural components of nucleic acids, particularly those where activation with deoxycytidine kinase is not necessary[1]. The preparation of synthetic antimetabolites with isosteric replacement of carbon by nitrogen was a straightforward step. From among substances so obtained, 5-azacytidine[2] (5-ACyd) has found application in clinical oncology, in the therapy of acute myeloid leukemia[3]. Another nucleoside from this group, viz. arabinosyl-5-azacytosine[4], is currently under clinical testing.

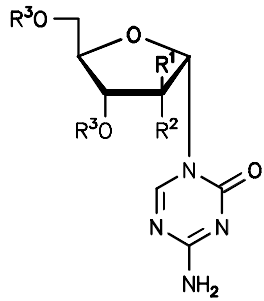
In this work, we examined the polarographic reduction of a series of 5-aza nucleosides (1,3,5-triazine nucleosides) *I – XVII* with regard to their high biological activity[1]; within the polarographic study, we assessed their potential carcinogenity[5] and compared their parameters with those of pyrimidine nucleosides[6–8] and bases[9] and also of the clinically used arabinosylcytosine[6]. Electrochemical reduction of pyrimidine nucleosides has been examined in the past, with differences in some data[10–15]. The mechanism of cytosine reduction in aqueous solutions was described in 1972 (ref.[12]). The



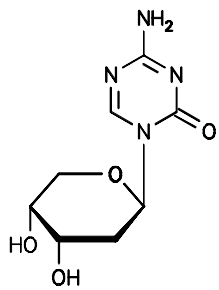
	R^1	R^2	X
I	H	OH	O
II	H	H	O
VIII	OH	H	O
XI	H	OH	S



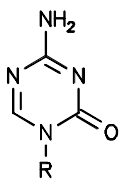
III, R^1 = OH
IV, R^1 = H

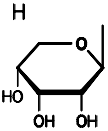


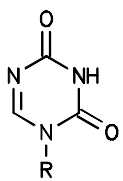
	R^1	R^2	R^3
V	H	OH	H
VI	H	H	H
IX	OAc	H	Ac
X	OH	H	H



VII



XII, R = H
XIII, R = 



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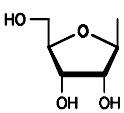
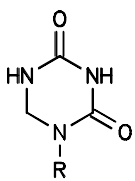
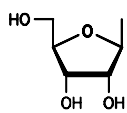
XVI, R = 



XV, R = H
XVII, R = 

polarographic reduction of 1,3,5-triazine nucleosides has not yet been studied on a sufficiently large set of compounds. This was therefore the goal of the present work. In addition to the determination of basic parameters of electrochemical reduction, our aim was to identify the similarity or dissimilarity of the 1,3-5-triazine nucleosides to nucleosides of the pyrimidine series.

The series included 5-azacytidine derivatives, α-D-anomers of 5-aza- and 2′-deoxy-5-azacytidine, 2-thio-5-azacytidine, and 5-aza analogues of uracil and uridine. The reduction of the compounds was also investigated in the presence of α-lipoic acid, which plays the role of the biological growth factor and is directly involved in the decarboxylation of pyruvic acid. Moreover, this compound serves as the electron acceptor in the conversion of the energy of light into thermal energy. With respect to the polarographic reduction, the fact that α-lipoic is a co-ferment transferring hydrogen atoms during some biochemical reactions seems to be most important. In aqueous solutions at pH 7 and 37 °C, α-lipoic acid reduces at a mercury drop electrode at a potential of –0.320 V (ref.[16]) vs SCE. In nonaqueous solutions, this substance gives no polarographic wave in the reduction region even if present in a concentration of 10^{-2} mol l^{-1} but it affects the polarographic behaviour of substances exhibiting carcinogenic activity[6 – 9]. This applies to carcinogenic compounds where enzymatic activation is necessary for the carcinogenity to become manifest (aromatic hydrocarbons) as well as to directly acting, ultimate carcinogens (nitrosamines). In the polarographic reduction, the presence of α-lipoic acid increases the diffusion current of the first polarographic wave of such carcinogenic compounds. The increase is linearly dependent on the α-lipoic acid concentration[6]. The slope tg α of the corresponding plot (where the diffusion current is conventionally in μA and the α-lipoic concentration in μmol l^{-1}) is an index of potential carcinogenity of the compound. For noncarcinogens, such effect is absent.

EXPERIMENTAL

Compounds *I – IV, VI – X* and *XII – XVII* were synthesized following published procedures (references are given in Table I). Compounds *V* and *XI* were obtained from the corresponding tribenzoates[17,18] by methanolysis, as in the case of 5-azacytidine tribenzoate[19]. Compound *V*: m.p. 124 – 128 °C (decomp.) (acetone–water), $[\alpha]_D^{22}$ –74.5° (*c* 0.50, water) (ref.[20]); compound *XI*: m.p. 230 – 231 °C (decomp.) (methanol), $[\alpha]_D^{22}$ +20.8° (*c* 0.15, DMF).

Melting points were determined on a Kofler stage and have not been corrected. Optical rotatory power was measured on a Perkin–Elmer 141 MCA polarimeter. The purity of the compounds was checked by thin layer chromatography, elemental analysis, melting point and spectral methods. *N*,*N*′-Dimethylformamide (DMF), which served as the polarographic solvent, and tetrabutylammonium perchlorate (TBAP), which was used as the supporting electrolyte, were obtained from Fluka (Switzerland). α-Lipoic (D,L-6,8-thioctoic) acid was a product of Koch Light Laboratories (Colnbrook, U.K.). DMF was purified by double vacuum distillation[21] and its water content after treatment did not exceed 0.1 wt.%.

Polarographic measurements were performed on a PA 4 polarographic analyzer which was interfaced to a two-line XY 4106 plotter (both Laboratorni pristroje, Prague, The Czech Republic); the

three-electrode connection with IR compensation was used. A mercury dropping electrode with a drop time of 3 s and a mercury flow rate of 2.27 mg s^{-1} (mercury column height 81 cm) served as the indicating electrode, a saturated calomel electrode (SCE) adapted for measurement in nonaqueous solutions served as the reference electrode. The auxiliary electrode was a commercial OH 9377 platinum electrode (Radelkis, Budapest, Hungary). All polarographic measurements were conducted at room temperature under argon. The concentration of the TBAP supporting electrolyte was 150 mmol l^{-1}, that of the analytes was 0.5 mmol l^{-1}. When examining the effect of α-lipoic acid on the polarographic reduction, the α-lipoic acid-to-analyte molar ratio was varied within the limits of 1 : 0.1 to 1 : 2.4.

TABLE I
Half-wave potentials $E_{1/2}$ and the parameter of potential carcinogenity tg α (in μA μmol^{-1} l) of 1,3,5-triazine bases and nucleosides

Compound	$E_{1/2}$, V (SCE)	tg α	Ref.
5-Azacytidine (5-ACyd) (*I*)	–1.960	0.295	19
2′-Deoxy-5-azacytidine (2′-d-5-ACyd) (*II*)	–2.000	0.210	22
5,6-Dihydro-5-azacytidine (*III*)	–1.965	0.265	23
2′-Deoxy-5,6-dihydro-5-azacytidine (*IV*)	–1.980	0.205	23
4-Amino-1-α-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (α-ACyd) (*V*)	–2.250	0.100	this work
4-Amino-1-(2-deoxy-α-D-erythropentofuranosyl)-1,3,5-triazin-2(1*H*)-one (α-2′-d-5-ACyd) (*VI*)	–2.180	0.170	22
4-Amino-1-(2-deoxy-β-D-erythropentopyranosyl)-1,3,5-triazin-2(1*H*)-one (*VII*)	–2.130	0.100	24
4-Amino-1-β-D-arabinofuranosyl-1,3,5-triazin-2(1*H*)-one (araAC) (*VIII*)	–2.060	0.275	25
4-Amino-1-(2,3,5-tri-*O*-acetyl-α-D-arabinofuranosyl)-1,3,5-triazin-2(1*H*)-one (Ac_3-α-araC) (*IX*)	–2.130	0.100	26
4-Amino-1-α-D-arabinofuranosyl-1,3,5-triazin-2(1*H*)-one (α-araC) (*X*)	–2.170	0.130	25
2-Thio-5-azacytidine (2-S-5-ACyd) (*XI*)	–1.940	0.087	this work
5-Azacytosine (*XII*)	–1.950	0.120	27
4-Amino-1-β-D-ribopyranosyl-1,3,5-triazin-2(1*H*)-one (*XIII*)	–2.155	0.166	28
5-Azauracil (*XIV*)	–1.740	0.400	29
5,6-Dihydro-5-azauracil (*XV*)	–1.970	0.133	30
	–2.350		
5-Azauridine (5-AUrd) (*XVI*)	–1.920	0.114	31
5,6-Dihydro-5-azauridine (*XVII*)	–2.015	0.090	23
	–2.360		

RESULTS AND DISCUSSION

The 1,3,5-triazine nucleobases 5-azacytosine and 5-azauracil as well as all of the nucleosides examined are polarographically active in the aprotic medium (Table I). In nonaqueous DMF under the conditions applied, all of them except 5,6-dihydro-5-azauracil and 5,6-dihydro-5-azauridine are reduced in a single two-electron step. The two compounds which are exceptions are reduced in two one-electron steps, the first step being a reversible process, the second, an irreversible process. The half-wave potentials are given in Table I; the most positive value, –1.740 V vs SCE, is found for 5-azauracil, the most negative value, –2.250 V vs SCE, for the α-D-anomer of 5-ACyd. Introduction of a nitrogen atom into the nucleobase/nucleoside structure results in a 340 – 620 mV shift of $E_{1/2}$ to the positive values (Table II). The two limiting shift values belong to the Urd–5-AUrd and uracil–5-azauracil pairs, respectively.

As compared to 5-ACyd, the half-wave potential of reduction of 2′-d-5-ACyd is 40 mV more negative; the difference reduces to 15 mV for the pair of their 5,6-dihydro derivatives. A minimal difference was also observed for the pairs of 2′-d-5-ACyd and 5-ACyd with the 5,6-dihydro analogues derived from them (20 and 5 mV, respectively). The α-D-anomers of 5-ACyd and 2′-d-5-ACyd are reduced at half-wave potentials which are appreciably more negative (the difference is 290 and 220 mV, respectively). The $E_{1/2}$ value is nearly unaffected by the replacement of the oxygen atom in position 2 by a sulfur atom. Substitution of the ribofuranosyl system by the

TABLE II

Half-wave potentials $E_{1/2}$ and the parameter of potential carcinogenity tg α (in μA μmol^{-1} l) of synthetic 1,3,5-triazine bases and nucleosides in comparison with those of natural pyrimidine and arabinosylcytosine nucleosides

Compound	$E_{1/2}$, V (SCE)	tg α	Ref.
Cytosine	–2.410	0.060	6
5-Azacytosine	–1.950	0.120	this work
Uracil	–2.360	0.060	7
5-Azauracil	–1.740	0.400	this work
Cytidine	–2.470	0.043	6
5-ACyd	–1.960	0.295	5
Uridine	–2.260	0.040	7
5-AUrd	–1.920	0.114	this work
araC	–2.500	0.162	6
araAC	–2.060	0.275	5

ribopyranosyl system in 2′-d-5-ACyd and araAC causes a shift of the half-wave potential to more negative values by 130 and 95 mV, respectively. The $E_{1/2}$ value of 5,6-dihydro-5-azauracil is 230 mV more negative than that of 5-azauracil; this is in contrast to 5-ACyd, where the corresponding difference is small. Moreover, the reduction of 5,6-dihydro-5-azauracil proceeds in two steps, the $E_{1/2}$ value of the second wave being nearly identical with that of uracil (Tables I and II). The results obtained for 5-AUrd and its 5,6-dihydro derivative are similar.

For all of the compounds studied, the diffusion current of the first polarographic wave increases in the presence of α-lipoic acid[6]. The wave height vs lipoic acid concentration plot is linear and can be characterized by its slope tg α, which can serve as a prescreening criterion of potential carcinogenity. For most of the substances, the tg α value is low, particularly in comparison with some structurally related compounds whose carcinogenic activity has been demonstrated[6]. 5-ACyd, a substance which in the past was applied in clinical oncology to the treatment of acute myeloblastic leukemias[3], and 5-azauracil are exceptions, exhibiting tg α values of 0. 295 and 0.400, respectively. The former substance has been classed by the World Health Organization (WHO) in category 2A (ref.[22]) as "probably carcinogenic to humans", based on carcinogenic activity tests performed on animals and on mutagenesis and cytotoxicity tests. In view of this, 5-ACyd was regarded as a positive control in this work. The very high tg α value of 5-azauracil (Figs 1, 2) suggests that this compound may possess some carcinogenic properties. It is noteworthy that the potential carcinogenity decreases appreciably if the double bond between positions 5 and 6 is eliminated; this was not been observed for 5-ACyd and 2′-d-ACyd.

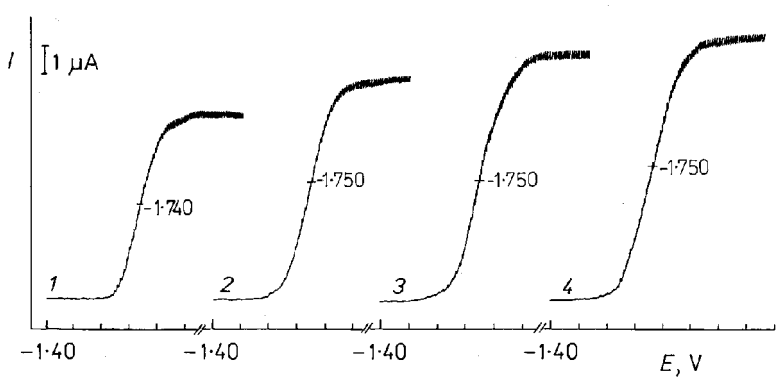


FIG. 1
Effect of α-lipoic acid on the polarographic reduction of 5-azauracil (c = 0.5 mmol l^{-1}) in non-aqueous DMF. Concentration of α-lipoic acid, c_{la} (mmol l^{-1}): *1* 0, *2* 0.2, *3* 0.5, *4* 1

Overall, the results suggest that the potential carcinogenity of the majority of the 5-aza nucleosides examined is relatively low. The use of DC polarography as a prescreening method for identifying potential carcinogenic risk is convenient as compared to the conventional in vivo tests, the Ames test with various strains of *Salmonella typhimurium*, and chromosomal aberration tests, which are all expensive and time consuming. The method which is based on the effect of α-lipoic acid on the polarographic reduction is well suited to the identification of substances that might pose a risk if used in practice. The technique should not replace conventional carcinogenity and mutagenity tests as recommended by WHO (ref.[22]), but it can supplement the tests as an indicator of substances to which attention should be paid in this respect. With regard to their low tg α values and high biological activity – cytotoxic and antivirus activity in particular – 5-aza nucleosides deserve a more extensive and more detailed investigation.

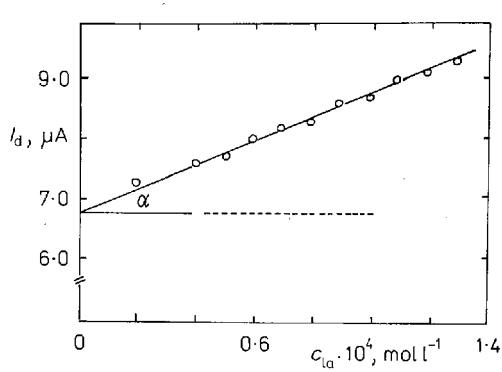


FIG. 2
Dependence of the 5-azauracil reduction wave height on the concentration of α-lipoic acid

The authors wish to express their gratitude to the Central Tax Office – Bratislava, The Slovak Republic and especially to Mr Vladimir Benicek for the support of this research project.

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Translated by P. Adamek.