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

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The DC polarographic reduction of 11 new 5-azacytidine (5-azaCyd) and 5-azauridine (5-azaUrd) derivatives in strictly anhydrous dimethylformamide (DMF) was investigated. The reduction occurred within one two-electron step for all of the substances except 6-amino-3-(β-D-ribofuranosyl)-1,3,5-triazine-2,4(1*H*,3*H*)-dione, which was reduced in two one-electron steps. The 5-azaCyd monomethyl derivatives (6-methyl-5-azaCyd and N^4-methyl-5-azaCyd) gave polarographic maxima of the 1st kind. Substitution in position 6 poses a marked hindrance to the reducibility of the nucleoside analogues. The α-lipoic acid test was applied to all the compounds to obtain the potential carcinogenity index (tg α). The highest tg α value, viz. 0.372, was found for 6-methyl-5-azaCyd; this value even exceeds that of 5-azaCyd (0.295), a compound which has been included in the category of substances probably carcinogenic to humans in the WHO classification. For the majority of the remaining compounds, the tg α values do not suggest any significant carcinogenic activity.

Owing to the successful application in clinical oncology of nucleoside antimetabolites of the pyrimidine group, such as arabinosylcytosine and 5-fluorouracil derivatives, the synthesis and properties of additional, new synthetic analogues of the natural components of nucleic acids of this kind are attracting interest in various fields of science[1]. An interesting group of such new compounds includes derivatives of 5-azacytidine, a compound which has found use, e.g., in the therapy of acute myeloid leukemia[2].

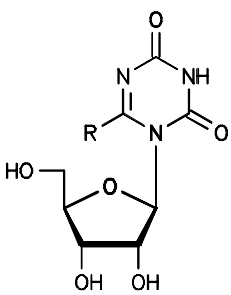
Polarographic reduction of some synthetic 1,3,5-triazine bases and nucleosides in an anhydrous medium in relation to their potential carcinogenity has been studied by us previously[3]. The present paper includes the results of investigation of the polarographic reduction of additional derivatives of 5-azacytidine (5-azaCyd) and 5-azauridine (5-azaUrd), viz. compounds *I–XIII*, with substitution in positions 6 and N^4. Carcinogenic activity of those substances has not been studied as yet and is unknown; therefore, we employed the α-lipoic acid test method[4,5] to determine their potential carcinogenity index (tg α). Moreover, data of the reduction potential ($E_{1/2}$), reversibility or irreversibility of the reduction process, and of the numbers of electrons ac-

cepted by the nucleoside analogues during the reduction were examined. All the data obtained were analyzed in relation to the structure of the compounds.

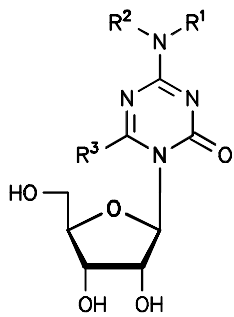
EXPERIMENTAL

The substances were synthesized by published procedures (Table I) and their purity was checked by melting temperature determinations, thin layer chromatography, elemental analysis, and spectrometric measurements.

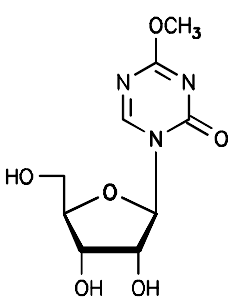
N,*N*′-Dimethylformamide, which served as the solvent in all polarographic measurements, was a commercial product of Fluka (Swiztzerland) and was purified by double vacuum distillation prior its use[6]. Its water content, which was measured titrimetrically by the Karl Fischer method, never exceeded 0.1%. α-Lipoic (D,L-6,8-thioctic) acid was obtained from Koch Light Laboratories (Colnbrook, U. K.).



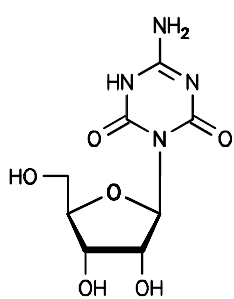
I, R = H
II, R = NH_2



	R^1	R^2	R^3
III	H	H	H
IV	H	H	NH_2
V	H	H	CH_3
VI	H	H	OCH_3
VII	CH_3	H	H
VIII	CH_3	CH_3	H
IX	$CH_2C_6H_5$	H	H
X	–	H	H
XI	C_4H_9	C_4H_9	H



XII



XIII

Polarographic measurements were performed on a PA4 polarographic analyzer interfaced to an XY 4106 two-line recorder (both Laboratorni pristroje, Prague, Czech Republic). The three-electrode connection was applied, using a mercury dropping electrode with a drop time of 3 s and a mass flow rate of 2.27 mg s^{-1} (mercury reservoir height 81 cm) as the indicator electrode, a saturated calomel electrode (SCE) adapted to work in nonaqueous solutions as the reference electrode, and an OH 9377 platinum compensation electrode (Radelkis, Budapest, Hungary) as the auxiliary electrode. The solution analyzed was accommodated in a polarographic cell which was kept at 25 °C under a nitrogen stream. Tetrabutylammonium perchlorate (TBAP) at a concentration of 150 mmol l^{-1} served as the supporting electrolyte. The analyte concentration was invariably 0.5 mmol l^{-1}. When examining the effect of α-lipoic acid on the polarographic reaction, the α-lipoic acid-to-nucleoside molar ratio was varied within the range of 0.1 : 1 to 2.4 : 1. The reversibility/irreversibility of the individual reduction steps was investigated by the switched curve method. The number of electrons involved in the reduction was determined by logarithmic analysis of the polarographic curves as the dependence of the log $(i_d/i_l - i_d)$ value on the voltage E, where i_d is the diffusion current at voltage E and i_l is the limiting current.

RESULTS AND DISCUSSION

5-AzaCyd and 5-azaUrd are synthetic analogues of the natural pyrimidine nucleosides cytidine and uridine, respectively, which are component parts of nucleic acids. In 5-aza (1,3,5-triazine) analogues, the carbon atom in position 5 of the pyrimidine base is isosterically replaced by a nitrogen atom, giving rise to synthetic antimetabolites, some of which have found application in clinical oncology[2,7]. Since it is feasible that any one of the synthetic analogues of this type may exhibit interesting biological activity, we examined their polarographic properties in aprotic conditions (anhydrous DMF) and determined their potential carcinogenity index (tg α).

All the compounds included in Table I are polarographically active. The parent compounds, 5-azaUrd (*I*) and 5-azaCyd (*III*), which have been studied by us previously[3,8], are reduced within a single two-electron irreversible step under the experimental conditions applied and their $E_{1/2}$ values approach each other closely (–1.920 and –1.960 V vs SCE, respectively). Substitution of the hydrogen atom in position 6 of the basic structure of the base by an amino group brings about a marked shift of the $E_{1/2}$ potential to more negative values (–2.710 and –2.750 V vs SCE for *II* and *IV*, respectively). The number of electrons involved in the reduction as well as the irreversibility of the reduction process remains unchanged. Both the observed $E_{1/2}$ values of the 6-amino derivatives and the higher values of this parameter for the cytidine analogues as compared to the uridine analogues are consistent with the theoretical explanation of the generally poorer reducibility, or virtual irreducibility of cyclic amino compounds[9].

Introduction of a methyl group in position 6 (*V*) or replacement of the hydrogen atom by a methyl group in position N^4 (*VII*) of the basic skeleton of the 5-azaCyd molecule is associated with the appearance of 1st kind eddy maxima at $E_{1/2}$ = –2.260 and –2.125 V vs SCE for the two compounds, respectively (Fig. 1). The maxima are sharp in both cases and occur at the beginning of the limiting current. If both hydrogen atoms in the

TABLE I
Half-wave potentials $E_{1/2}$ and the tg α parameter of 5-azaCyd and 5-azaUrd derivatives

Compound	$E_{1/2}$, V (SCE)	tg α	Ref.
5-Azauridine (*I*)	–1.920	0.114	14
6-Amino-5-azauridine (*II*)	–2.710	0.090	16
5-Azacytidine (*III*)	–1.960	0.295	15
6-Amino-5-azacytidine (*IV*)	–2.750	0.200	17
6-Methyl-5-azacytidine (*V*)	–2.260	0.372	18
6-Methoxy-5-azacytidine (*VI*)	–2.540	0.094	17
N^4-Methyl-5-azacytidine (*VII*)	–2.125	0.220	19
N^4,N^4-Dimethyl-5-azacytidine (*VIII*)	–2.130	0.110	19
N^4-Benzyl-5-azacytidine (*IX*)	–2.135	0.000	19
N^4-Furfuryl-5-azacytidine (*X*)	–2.130	0.105	19
N^4,N^4-Dibutyl-5-azacytidine (*XI*)	–2.190	0.050	19
4-Methoxy-1-β-D-ribofuranosyl-1,3,5-triazin-2(1*H*)-one (*XII*)	–1.860	0.187	19
6-Oxo-5-azacytidine (*XIII*)	–2.055 –2.540	0.120	17

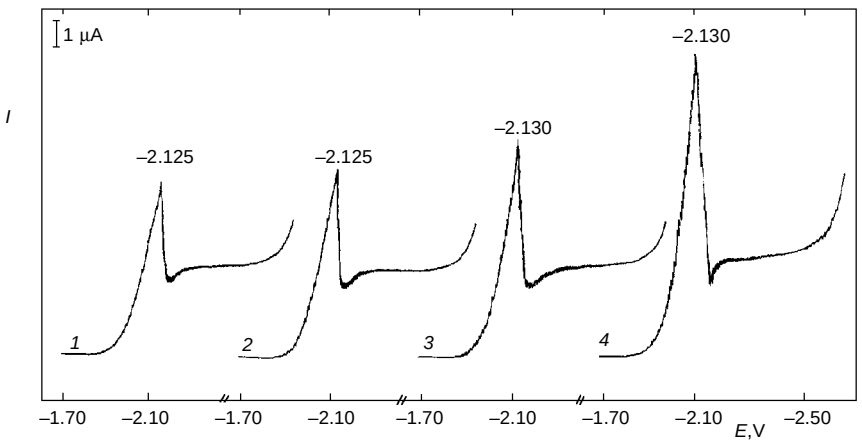


FIG. 1
Effect of α-lipoic acid on the polarographic reduction of N^4-methyl-5-azacytidine (*VII*, c = 0.5 mmol l^{-1}) in anhydrous DMF. Concentration of α-lipoic acid, c (mmol l^{-1}): *1* 0, *2* 0.4, *3* 1.2, *4* 2.0

$-NH_2$ group in position 4 of the basic skeleton of 5-azaCyd (*III*) are substituted by methyl groups to give compound *VIII*, the eddy effect at the indicator electrode is suppressed and the corresponding maximum does not appear. The reduction of this compound proceeds in a single irreversible two-electron step. The eddy maximum does not appear for the compound with a methoxy group in position 6 (*VI*) either, its $E_{1/2}$, however, is shifted markedly to more negative values (Table I). A shift of $E_{1/2}$ to more negative values is also observed if the hydrogen atom of the amino group of 5-azaCyd is substituted by bulkier substituents, viz. the benzyl (*IX*) or furfuryl (*X*) groups, or on double substitution by 1-butyl groups (*XI*); the reduction mechanism remains unchanged.

Replacement of the amino group in position 4 of the basic skeleton of 5-azaCyd by a methoxy group (*XII*) brings about an easier reducibility. Compound *XII* is better amenable to reduction than any other 5-aza analogue possessing an amino group in position 4, whose $E_{1/2}$ values are reported in this article or in the previous paper[3]. The reduction mechanism, however, remains intact.

An interesting change is observed for compound *XIII*, where carbonyl groups are present on both sides of the nucleoside bond. The reduction mechanism converts into a two-step process, where the first step is reversible and both steps are one-electron ones (Table I).

The polarographic reduction of all the compounds was also examined in the presence of α-lipoic acid to obtain their potential carcinogenity parameter (tg α). A survey of functions of α-lipoic acid in the living organism has been presented previously[3,4,10]. This substance contains a disulfidic bond in its molecule, and although on its own giving no polarographic reduction wave in nonaqueous media, affects the polarographic reduction of compounds which exhibit carcinogenic activity, namely so that the diffusion current of the first polarographic wave of the potentially carcinogenic compound increases with increasing concentration of α-lipoic acid in solution. The slope of this linear plot,

$$\text{tg } \alpha = i_d/c_{LA}, \qquad (1)$$

where i_d is the diffusion current in μA and c_{LA} is the α-lipoid acid concentration in μmol l^{-1}, is a measure of potential carcinogenity of the compound in question (Fig. 2) and can serve as a pre-screening parameter.

Table I demonstrates that the tg α value is low for the majority of the compounds studied. Our previous studies[3,4,10] suggest that substances possessing tg α values within the region of 0 to 0.1 will exhibit no carcinogenic activity. From among the nucleosides studied in this work, the compounds *IX*, *XI*, *II*, and *VI* meet this condition. If the tg α value is higher than 0.1 but does not exceed 0.2, its carcinogenic potential can be considered insignificant. This concerns compounds *X*, *VIII*, *I*, *XIII*, *XII*, and *IV*. The highest tg α value, viz. 0.372, is found for compound *V*, which is 6-methyl-5-azaCyd.

This tg α value is really high in view of the fact that 5-azaCyd itself, whose tg α value is 0.295, has been included in the category of compounds probably carcinogenic to humans in the World Health Organization (WHO) classification. This is very serious in the light of the fact that while benz(*a*)anthracene is carcinogenically inactive[11], its methylation in position 10 brings about a medium carcinogenity, and 9,10-dimethylbenz(*a*)anthracene, i.e. the compound emerging from the additional methylation of 10-methyl(*a*)anthracene in position 9, is one of the strongest chemical carcinogens ever. A similar effect of introduction of methyl groups has been found for other compounds as well, for instance for the cholanthrene–3-methylcholanthrene and dibenz(*a*,*c*)anthracene–5-methyldibenz(*a*,*c*)anthracene pairs[12]. Thus, 6-methyl-5-azaCyd (*V*) can be expected to be carcinogenically active, based on the observed tg α values of this compound and the parent 5-azaCyd (*III*). No such increase in the potential carcinogenity index was observed for the methylation of the amino group in position 4 of the 1,3,5-triazine ring; on the contrary, the tg α values of the compounds *VII–XI* are lower than that of the parent 5-azaCyd. The effect of disubstitution is higher than that of monosubstitution (substances *VI* and *VII* or *XI*), which is consistent with the sterically more pronounced influence of the bulkier substituents on the decrease in the carcinogenity index (*VI* vs *X* and *IX*). Our results agree with published data concerning aromatic amines methylated at the amino group[13].

In conclusion, the carcinogenic activity of the majority of the compounds studied will be very low, in accordance with our previous findings[3]. For some of them (*III* and *V*), however, the potential carcinogenity index is very high, which warrants the use of DC polarography in the pre-screening of new biologically active substances; in fact,

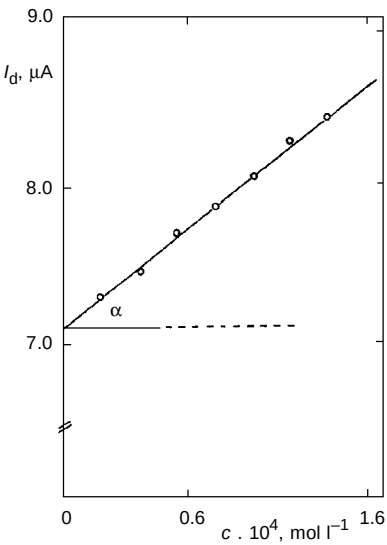


FIG. 2
Dependence of the N^4-methyl-5-azacytidine (*VII*) reduction wave height on the concentration of α-lipoic acid

this method makes it possible to identify compounds that should be tested for carcinogenity and mutagenity as recommended by the WHO.

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