ELECTROCHEMICAL REDUCTION PATHWAYS OF ANTHRACYCLINE ANTIBIOTICS

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(Received for publication February 23, 1982)

The preparative electrochemical reduction of a series of anthracycline antibiotics was performed using the technique of large scale electrolysis and d. c. polarography for recording different reaction pathways. The reduction products were identified by mass spectrometric and chromatographic analyses. Only anthracyclines with sugar residues in the C-10 position (iremycin, roseorubicin A) were reduced reversibly by $2e^-$ to the corresponding hydroquinones. All others with sugar residues in the C-7 position (daunomycin, 5-iminodaunomycin, adriamycin, carminomycin, β-rhodomycin II, aclacinomycin A, 1-deoxypyrromycin) showed an irreversible behavior because of the reductive splitting of the glycosidic bond under formation of 7-deoxy compounds or — if the C-11 OH-group was not present — of dimers, respectively.

From the polarographic behavior[1~3] of some anthracyclines it has been concluded that the quinone group of the anthracycline chromophore is reduced leading to a reductive splitting of the glycosidic bond as is known for chemical and enzymatic reductions[4~9]. However, from our studies including preparative electrolysis, thin-layer chromatography and mass spectrometry different reduction pathways are possible depending on aglycon structure and the position of the sugar residues. These findings together with the results of reoxidation of the chromophores have some relation with biological activity.

Materials and Methods

Anthracyclines

Anthracyclines were obtained as follows:

Daunomycin and adriamycin:	Farmitalia (Milano)
Carminomycin:	Institute of Antibiotics (Moscow)
Iremycin and β-rhodomycin II:	ZIMET (Jena)
Aclacinomycin A, roseorubicin A and 1-deoxypyrromycin:	Sanraku-Ocean Co. and Institute of Microbial Chemistry (Tokyo)
5-Iminodaunomycin:	NIH-NCI (Bethesda)

Electrolysis

The electrolysis on a large mercury pool cathode was carried out in a coulometric cell combined with a dropping mercury electrode for recording d.c. polarograms during large scale reduction and reoxidation. The potential (against a saturated calomel electrode) of the mercury pool was fixed on the diffusion current by the Universal Analyzer OH-104 (Radelkis). Concentration of anthracyclines: 1.9×10^{-5} M; buffer solution: 0.066 M phosphate, pH 7, 10% or 20% ethanol, stirred at 30°C and deaerated by argon.

Isolation of Reduction Products

The yellow-red pigments in the reoxidized reaction mixtures, in a total volume of 5 ml, were extracted

with chloroform. After washing with water the chloroform solutions were dried over Na_2SO_4 and evaporated under reduced pressure. The oily residues were used directly in the TLC and mass spectrometric analysis.

TLC: Silica gel (HP-TLC-Alufolien Kieselgel 60, nano DC, E. Merck); the solvent systems: S_1, $CHCl_3$ - MeOH - H_2O - acetic acid (80: 20: 6: 14); and S_2, $CHCl_3$ - MeOH - acetone (20: 1: 1) were used.

Mass spectrometry: The mass spectra were recorded on a JEOL JMS-D 100 spectrometer (75 eV, temperature of ion source 200°C, temperature of direct inlet system 150~190°C, high resolution measurements were performed using the peak matching technique with PFK as standard).

Results and Discussion

The data of the reduction products of anthracyclines are shown in Table 1.

The large scale electrolysis with coulometric equipment and analyses by d.c. polarography using a dropping mercury electrode in the same vessel shows either reversible behavior for the redox-cycle (Fig. 1a) or an irreversible one (Fig. 1b).

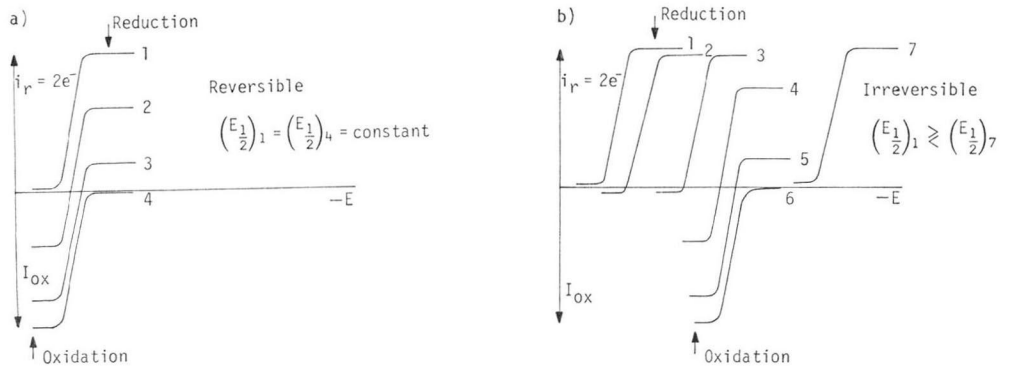
The reversible case (1a) was recorded only for iremycin and roseorubicin A; that means the hydroquinone has been formed immediately without changing the half wave potential (compare Fig. 1b). For all other derivatives we found the splitting pathway, namely at first only a little displacement of the quinone reduction steps until 2 electrons per molecule are involved. Afterwards the hydroquinone is formed exhibiting a more negative half wave potential ($E_{1/2}$, Fig. 1b). Therefore the sum of $4e^-$ are needed starting with the reductive splitting of the glycosidic bond in the C-7 position.

The reduction products (Table 1) were identified by comparison of their mass spectra (high resolution measurements of $(M)^+$ and characteristic fragment ions) and Rf values on thin-layer chromatography (TLC) compared with authentic samples. The mass spectra (MS) of 7,7′-bis-(7-deoxyaklavinon) and of 7-deoxyaklavinon was the same but their Rf values on TLC were quite different. According to the electrochemical behavior we found two reduction types (Fig. 1):

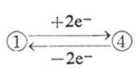
1) reversible exchange of $2e^-$, $E_{1/2}$=const.: iremycin, roseorubicin A,

2) irreversible uptake of $2e^-$, $E_{1/2}$=shifts to negative values: all others.

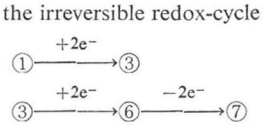
Fig. 1. The two preparative electrolysis types recorded by polarography.



a) the reversible redox-cycle:



b) the irreversible redox-cycle:



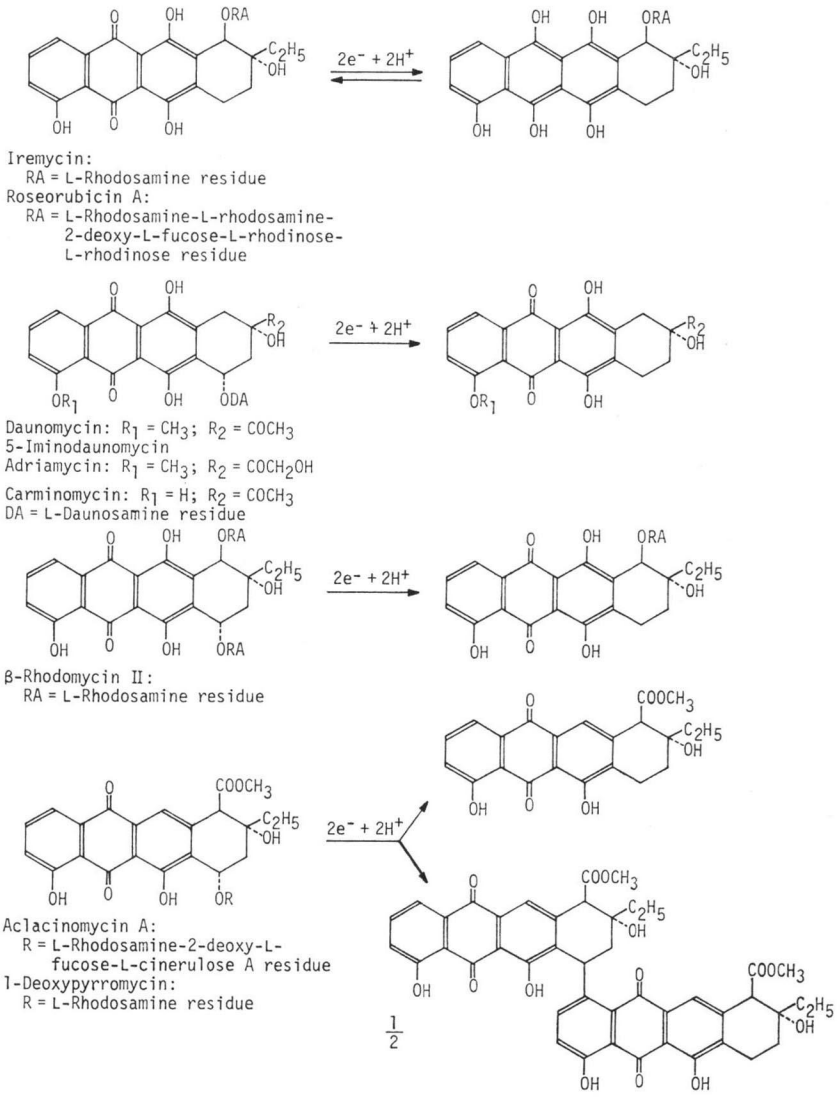
(Step ⑥ of 5-iminodaunomycin undergoes a further chemical reaction.)

Table 1. Two electron reduction of anthracycline glycosides by large scale electrolysis.

Anthracycline glycoside		Reduction product				
	$E_{1/2}$ [V]		Mass spectrum [m/z]	Rf value S_1	Rf value S_2	$E_{1/2}$ [V]
Roseorubicin A	−0.77	none	—	0.28	—	−0.77
Iremycin	−0.70	〃	527 $(M)^{\dagger}$, 370 $(M-C_8H_{15}NO_2)^+$, 353 $(M-C_8H_{16}NO_3)^+$, 352 $(370-H_2O)^+$, 174 $(C_8H_{16}NO_3)^{\dagger}$.	0.49	—	−0.70
β-Rhodomycin II	−0.66	Iremycin	〃	0.49	—	−0.70
Daunomycin	−0.64	7-Deoxydaunomycinone	382 $(M)^{\dagger}$, 364 $(M-H_2O)^{\dagger}$, 339 $(M-CH_3CO)^+$, 321 $(364-CH_3CO)^+$.	—	0.54	−0.67
5-Iminodaunomycin	−0.67	7-Deoxy-5-iminodaunomycinone	381 $(M)^{\dagger}$, 363 $(M-H_2O)^{\dagger}$, 338 $(M-CH_3CO)^+$, 320 $(338-H_2O)^+$.	—	0.44	−0.71
Adriamycin	−0.64	7-Deoxyadriamycinone	398 $(M)^{\dagger}$, 380 $(M-H_2O)^{\dagger}$, 349 $(380-CH_2OH)^+$, 339 $(M-COCH_2OH)^+$.	—	0.41	−0.67
Carminomycin	−0.70	7-Deoxycarminomycinone	368 $(M)^+$, 350 $(M-H_2O)^{\dagger}$, 325 $(M-CH_3CO)^+$, 307 $(350-CH_3CO)^{\dagger}$.	—	0.60	−0.73
Aclacinomycin A	−0.50	7-Deoxyaklavinone	396 $(M)^{\dagger}$, 378 $(M-H_2O)^{\dagger}$, 376 $(M-H_2O-H_2)^{\dagger}$,	—	0.63	−0.52
		7,7′-Bis(7-deoxyaklavinone)	319 $(378-CH_3COO)^+$, 307 $(M-H_2O-HCOOCH_3)^{\dagger}$.	—	0.37	
1-Deoxypyrromycin	−0.50	7-Deoxyaklavinone	〃	—	0.63	−0.54
		7,7′-Bis(7-deoxyaklavinone)		—	0.37	

$E_{1/2}$=half wave potential against S. C. E., pH 7, 20% methanol.

Fig. 2. The four types of pathways of the first two electrons resulting in hydroquinone formation (compare Fig. 1a) or splitting of the antibiotic (compare Fig. 1b).



Regarding the positions of sugar residues and OH-groups we found four types by TLC and MS (Fig. 2):

1) no splitting of the glycosidic bond at the C-10 position,
2) splitting of the glycosidic bond at the C-7 position forming 7-deoxyanthracyclinone the same product as after chemical[4] and enzymatic[8,9] reductions,
3) sugars at C-7 and C-10: only splitting of the C-7 glycosidic bond, as found for hydrogenation,[6]
4) sugar at C-7 (no OH at C-11): the main product is the 7-deoxy dimer besides a small amount of monomer as shown by OKI[7,9] in chemical and enzymatic reductions.

Comparing these results one can draw the following preliminary general conclusion for the two pathways:

a) Sugar in C-10 position there is no splitting of the antibiotics.

b) Sugar in C-7 position causes splitting and yields either deoxyanthracycline or — if no OH is present in C-11 position — yields a dimer.

Up to now aclacinomycin A is the only derivative which undergoes dimerization upon anaerobic reduction[10], however, not all known combinations of substituents have been checked. Another exception exhibits the 5-iminodaunomycin concerning further reduction products[14].

From a therapeutic point of view[10] the sugars are necessary for high binding to nucleic acids on the one hand but on the other hand the rate of splitting of the glycosidic bond is to be taken into account. According to the comprehensive structure-activity investigations[13] on 92 anthracyclines and furthermore our preliminary results[14] about antitumor rhodomycins against P-388 leukemia in mice it is not precluded that the cytotoxicity (antileukemic effectivity) of anthracyclines can be correlated to the reductive splitting tendency of the glycosides at position C-7 and (or) position C-10.

Furthermore we showed polarographically[3] that during reoxidation of the hydroquinones different amounts[11] of H_2O_2 are generated, which may induce DNA strand-scission[12] and degradation of membrane constituents, too.

Hence the polarograms indicate not only the main pathways of reduction but exhibit also the products of reoxidation.

Moreover it is possible to reduce separately other groups of ring D at more negative potentials[3]. Comparing the electrolysis of anthracyclines with the results of chemical and enzymatic reduction it is obvious that this electrochemical method can be used to model metabolic reactions.

Acknowledgements

Basic suggestions regarding a generalized reduction scheme by Prof. HAMAO UMEZAWA and Dr. TOMOHISA TAKITA from the Institute of Microbial Chemistry, Tokyo, their helpful discussions, and the gifts of anthracyclines by Prof. TOSHIKAZU OKI from the Sanraku-Ocean Co., Fujisawa, are gratefully acknowledged. Dr. ROBER GLAZER from NCI, Bethesda, Md, kindly placed 5-iminodaunomycin at our disposal.

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