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Investigation on the Electrochemistry and Cytotoxicity of Organic Nitrates and Nitroamines

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Abstract: Laboratory scale quantities of a series of organic nitrates and nitroamines were obtained by nitration with dinitrogen pentoxide in dichloromethane medium. Twenty seven synthesized compounds were explored by voltammetry methods and their cytotoxicity for mice splenocytes was evaluated. N.N'-dinitropiperazine, DINA and hexandiol-1,6-dinitrate were determined as some of the most toxic compounds. Several compounds having non-planar cyclic, bicyclic or cage structures (IHN, TNAD, DINGU, TEX) were found as less toxic, possibly due to poorer penetration through the cell membrane.

Keywords: aliphatic nitrate, nitroester, N-nitroamine, peak potential, cytotoxicity

Introduction

Organic nitrates (R-O-NO_2) and nitroamines (R_1R_2-N-NO_2) are oxygen-rich high energy compounds, used widely as propellants, explosives and rocket fuels [1, 12]. For more than a century organic nitrates have been prescribed for the treatment of stable angina, acute coronary syndromes and congestive heart failure [2]. It was determined that they are potential vasodilators which dilate both normal and abnormal coronary arteries by relaxing vascular smooth muscle [5, 6, 13]. Although organic nitrates continue to be widely used for medical therapy, their therapeutic utility is recognized as problematic one because the

development of tolerance limits their clinical efficacy. Sustained administration of organic nitrates like nitroglycerin (GTN) was found to be associated with adverse effects on vascular function which appear to be mediated by an increase in the nitrate-induced generation of reactive oxygen species (ROS). The same problems were demonstrated with isosorbitol nitrate and dinitrate [3-5].

Another organic nitrate pentaerythritol tetranitrate (PETN) has also been used in the therapy of angina. Investigation on the animals showed that the continuous therapy with PETN does not cause increased free radical generation or hemodynamic tolerance. PETN, in contrast to all other organic nitrates, is able to up-regulate enzymes with a strong antioxidative capacity thereby preventing the tolerance and development of endothelial dysfunction [5-6].

Thus, the investigation of the reductive bioactivation and free radical formation processes of organic nitrates are very important for the understanding of mechanisms of their positive and negative effects on human health [9, 10, 17-20].

On the other hand, organic nitrates and aliphatic N-nitroamines are manufactured in massive quantities as HEMs for military and civilian applications. Production of such large quantities of these compounds causes water and soil pollution in the manufacturing areas and increases occupational hazards [19, 21-24]. During our research of high energy materials [14, 15] we have synthesized a number of mono-, di- or polynitrates and N-nitroamines possessing various aliphatic, alicyclic and cage (isowurtzitane-type) structures.

The aim of this study is a preliminary investigation of the electrochemical and cytotoxic properties of series of representatives from both groups of non-aromatic nitrocompounds, varying in chemical structures. It was attempted to define the relationships between electrochemical parameters, different calculated molecular characteristics and toxicity of selected compounds.

Materials and Methods

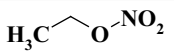
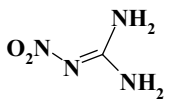
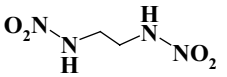
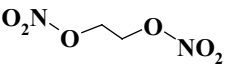
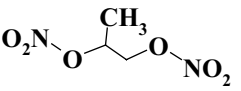
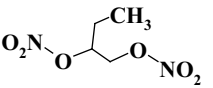
Synthesis of the nitrates and nitroamines was carried out by means of the reaction of starting aliphatic/alicyclic hydroxyl- and aminoderivatives with N_2O_5 in CH_2Cl_2 medium at -15 to +15 °C temperature. The obtained nitrates and nitroamines (1-27) were identified by TLC, IR and NMR spectroscopy and used for electrochemical investigations. Voltammetric experiments were performed using Parstat 2273 (Princeton Applied Research) potentiostat controlled by Power Suite electrochemical software. Glassy carbon (Princeton Applied Research, diameter 2 mm) working electrode, saturated Ag/AgCl (+205 mV vs. NHE) reference electrode, and Pt wire (56 mm^2) auxiliary electrode were used

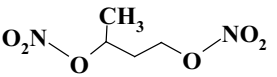
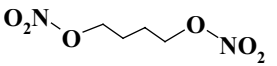
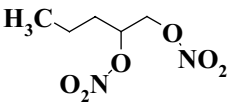
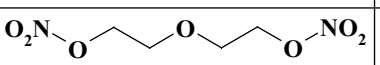
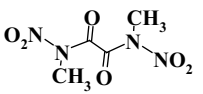
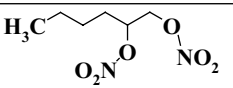
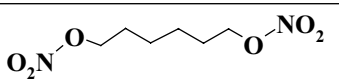
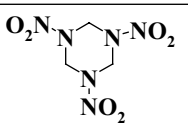
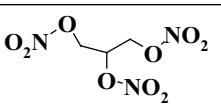
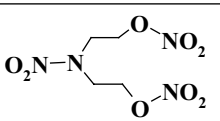
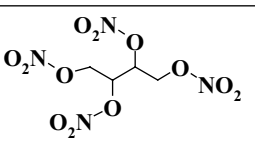
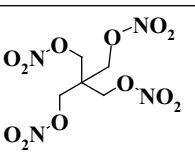
in a standard three-electrode scheme. The glassy carbon electrode was polished with a suspension of alumina powder (1 μm), and then rinsed thoroughly with deionized water. The anaerobic conditions were obtained by purging the solutions (0.05 M K-phosphate + 0.1 M KCl, pH 7.0, 25 °C, compound concentration, 0.4-1.0 mM) with argon for 20 min. Stock solutions of compounds (0.1 M) were prepared in DMSO. Calculation of molecular properties was done using chemical software ACDLabs (Advanced Chemical Development, Toronto).

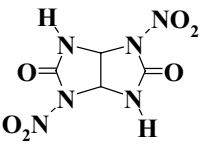
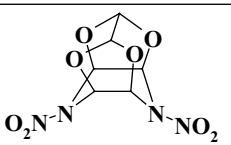
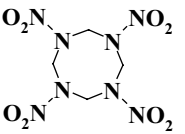
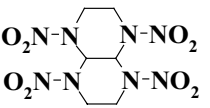
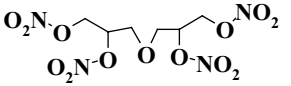
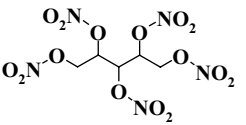
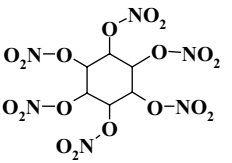
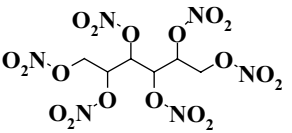
Results and Discussion

Because of the typical electrode fouling during the repetitive scans in the presence of nitrocompounds, only the electrochemical parameters referring to the first scan are presented in this work. Structures and chemical names of synthesized compounds are listed in Table 1. Voltammetric characteristics of nitrates and nitroamines are presented in Table 2.

Table 1. Structures and chemical names of synthesized compounds

No.	Structure of tested compound	Name	Brutto formula	Mw
1.		ethylnitrate	$C_2H_5NO_3$	91.07
2.		nitroguanidine, (NQ)	$CH_4N_4O_2$	104.07
3.		1,2-ethandinitramine, (EDNA)	$C_2H_6N_4O_4$	150.09
4.		1,2-ethanediol dinitrate, (EGDN)	$C_2H_4N_2O_6$	152.06
5.		1,2-propandiol dinitrate	$C_3H_6N_2O_6$	166.09
6.		N,N'-dinitro-piperazine, (DAZIN)	$C_4H_8N_4O_4$	176.13
7.		1,2-butanediol dinitrate	$C_4H_8N_2O_6$	180.12

No.	Structure of tested compound	Name	Brutto formula	Mw
8.		1,3-butandiol dinitrate	$C_4H_8N_2O_6$	180.12
9.		1,4-butanediol dinitrate	$C_4H_8N_2O_6$	180.12
10.		1,2-pentanediol dinitrate	$C_5H_{10}N_2O_6$	194.15
11.		diethylene glycol dinitrate	$C_4H_8N_2O_7$	196.12
12.		N,N'-dinitro-N,N'-dimethyloxamide	$C_4H_6N_4O_6$	206.12
13.		1,2-hexanediol dinitrate	$C_6H_{12}N_2O_6$	208.17
14.		1,6-hexanediol dinitrate	$C_6H_{12}N_2O_6$	208.17
15.		1,3,5-trinitro-perhydro-1,3,5-triazine, (RDX)	$C_3H_6N_6O_6$	222.12
16.		1,2,3-propantriol trinitrate (nitroglycerine)	$C_3H_5N_3O_9$	227.09
17.		dinitroxydiethyl-nitramine, (DINA)	$C_4H_8N_4O_8$	240.13
18.		erythritol tetranitrate, (ETN)	$C_4H_6N_4O_{12}$	302.11
19.		pentaerythritol tetranitrate, (PETN)	$C_5H_8N_4O_{12}$	316.14

No.	Structure of tested compound	Name	Brutto formula	Mw
20.		1,4-dinitro-3,3a,6,6a-tetrahydroimidazo [4,5-d]imidazole-2,5-dione, (DINGU)	$C_4H_4N_6O_6$	232.11
21.		4,10-dinitro-4,10-diaza-2,6,8,12-tetraoxa-isowurtzitane, (TEX)	$C_6H_6N_4O_8$	262.14
22.		octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, (HMX)	$C_4H_8N_8O_8$	296.16
23.		trans-1,4,5,8-tetranitro-1,4,5,8-tetraazadecalin, (TNAD)	$C_6H_{10}N_8O_8$	322.19
24.		diglycerol tetranitrate	$C_6H_{10}N_4O_{13}$	346.17
25.		xylitol pentanitrate	$C_5H_7N_5O_{15}$	377.14
26.		inositol hexanitrate, (IHN)	$C_6H_6N_6O_{18}$	450.14
27.		mannitol hexanitrate, (MHN)	$C_6H_8N_6O_{18}$	452.16

Cytotoxicities of selected organic nitrates and nitroamines were determined for primary mice splenocytes. Stock solutions of compounds were prepared in DMSO. Due to low solubility of several compounds (11-13, 21, 22) after dilution with water only tentative CL_{50} values were determined.

Table 2. Evaluated molecular properties, peak potentials and cytotoxicity for mice splenocytes of synthesized nitrates and nitroamines

No.	Name	Calc. Log P (ACDLabs)	Calc. VdW volume (molecule volume, Å^3)	Calc. polar surface area of the molecule. (PSA)	E_{pred}, mV (Ag/AgCl, 50 mV/s), pH 7.0	Cyto-toxicity for spleno-cytes CL_{50}, (μM)
1.	ethylnitrate	1.32	78.32	55.06	-1075	94
2.	nitroguanidine, (NQ)	-1.19	81.81	110.23	***	250
3.	1,2-ethandinitramine, (EDNA)	-0.60	117.71	115.70	***	62.5
4.	1,2-ethanediol dinitrate, (EGDN)	1.57	110.88	110.12	-634	>250
5.	1,2-propandiol dinitrate	1.84	127.28	110.12	-768	156
6.	N,N'-dinitropiperazine	-0.25	141.24	98.12	-1103	47
7.	1,2-butanediol dinitrate	2.44	144.27	110.12	-1300	85
8.	1,3-butanediol dinitrate	2.44	144.27	110.12	-1280	157
9.	1,4-butanediol dinitrate	2.44	144.27	110.12	-1230	52
10.	1,2-pentanediol dinitrate	2.99	161.07	110.12	-863	175
11.	diethylene glycol dinitrate	1.37	153.46	119.35	-705	>250
12.	N,N'-dinitro-N,N'-dimethyloxamide	-1.47	155.96	132.27	***	>250
13.	1,2-hexanediol dinitrate	3.50	177.87	110.12	-883	>250
14.	1,6-hexanediol dinitrate	3.50	177.87	110.12	-1076	28
15.	1,3,5-trinitroperhydro-1,3,5-triazine, (RDX)	-0.50	160.55	147.19	-976 -554	140
16.	1,2,3-propantriol trinitrate (nitroglycerine)	2.19	160.02	165.14	-1007	187

No.	Name	Calc. Log P (ACDLabs)	Calc. VdW volume (molecule volume, Å^3)	Calc. polar surface area of the molecule. (PSA)	E_{pred}, mV (Ag/AgCl, 50 mV/s), pH 7.0	Cyto-toxicity for spleno-cytes CL_{50}, (μM)
17.	dinitroxydiethyl-nitramine, (DINA)	1.36	180.60	159.18	-835 -1101	18
18.	erythritol tetranitrate, (ETN)	2.81	209.17	220.23	-847	175
19.	pentaerythritol tetranitrate, (PETN)	2.90	225.62	220.23	-770	38
20.	1,4-dinitro-3a,6,6a-tetrahydroimidazo [4,5-d]imidazole-2,5-dione, (DINGU)	-1.14	159.62	156.32	-1104	350
21.	4,10-dinitro-4,10-diaza-2,6,8,12-tetraoxa-isowurtzitane, (TEX)	-0.88	155.43	134.59	-1200	>250
22.	octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine, (HMX)	-0.73	213.47	196.25	-998	>250
23.	trans-1,4,5,8-tetranitro-1,4,5,8-tetraazadecalin, (TNAD)	-0.86	236.29	186.24	***	250
24.	diglycerol tetranitrate	2.79	251.76	229.47	-565 -738	59
25.	xylitol pentanitrate	3.42	258	275.29	-960	148
26.	inositol hexanitrate, (IHN)	3.87	296.67	330.35	-780	200
27.	mannitol hexanitrate, (MHN)	4.038	307.46	330.35	-830	109

*** Peak potential was not determined due to low solubility.

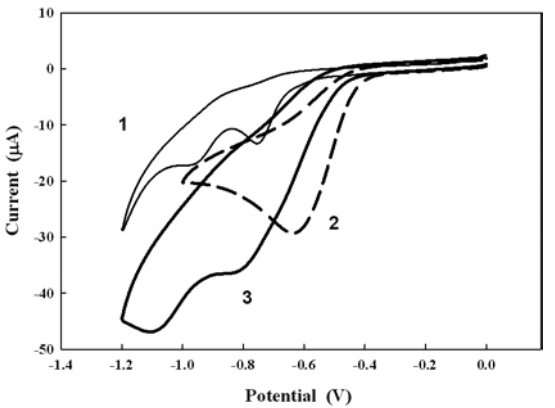


Figure 1. Cyclic voltamperograms of some aliphatic and alicyclic nitrates: 1 – diglycerol tetranitrate (21), 2 – ethyl nitrate (1) and 3 – inositol hexanitrate (26).

In general, some of the tested alicyclic or cage compounds, as (26, IHN) or (20-23), having non-planar structure are less toxic for the cells (200-350 μM), possibly due to poor penetration through the cell membrane, as illustrated by IHN example below (Figure 2).

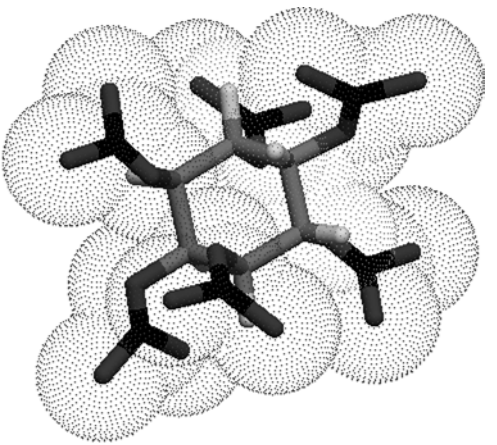


Figure. 2. 3D image of the molecule of inositol hexanitrate (26, INH), demonstrating a non-planar O-nitro groups arrangement, which is very important for biological properties, including toxicity.

Conclusions

In this work we have determined peak potentials of 27 synthesized organic nitrates and nitroamines. Their peak potentials varied from -1300 mV for 1,2-butanediol dinitrate to -554 mV for RDX. A determined cytotoxicity for mice splenocytes varied considerably, from 18 μM (17, DINA) to 350 μM (20, DINGU). In general, some of the tested alicyclic, bicyclic or cage compounds, as (26, IHN) or (20-23), having a non-planar structure and an increased molecule volume are less toxic for the cells. After comparison of all determined characteristics of selected compounds we have found a very slight tendency for cytotoxicity of compounds to increase with an increase in hydrophobicity. Correlations of other molecular parameters were not defined.

Acknowledgement

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