EFFECT OF SOME PESTICIDE DINITROPHENOLS AND OTHER DINITROPHENYL DERIVATIVES ON WHEAT SEED GERMINATION

EFECTUL UNOR PESTICIDE DINITROFENOLICE ŞI A UNOR DINITROFENIL DERIVAŢI ASUPRA GERMINAŢIEI SEMINŢELOR DE GRÂU

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Abstract. *Dinitrophenols and their ester derivatives proved to be effective pesticides due to their multiple biological actions, being used as insecticides, ovicides, acaricides, fungicides, and herbicides. However, the toxicity mechanism remained unclear so far in spite of the fact that such compounds may have an anticancer activity or may be useful in the neurodegenerative pathologies. Consequently, we synthesized some new as well as well-known dinitrophenyl conjugates with amino acids and peptides, such as dinitrophenyl-glutathione, dinitrophenyl-glycine, dinitrophenyl-glycylglycine and dinitrophenyl-tetraglycine and tested their biological activity compared with simpler dinitrophenols and dinitrophenyl ethers or other chemical compounds. Dinitrophenyl moiety was suspected to induce toxicity, whereas lateral chains and groups modulate both the toxicity and its relationship with the solubility.*

Key words*:* dinitrophenyl conjugates, pesticide toxicity, wheat, germination.

Rezumat*. Dinitrofenolii şi derivaţii lor eterici sunt utilizaţi drept pesticide, având multiple acţiuni biologice. Totuşi, mecanismul lor de toxicitate a rămas nelămurit până în prezent, deşi astfel de compuşi pot fi agenţi anticanceroşi sau pot fi utilizaţi în bolile neurodegenerative. In consecinţă, noi am sintetizat o serie de compuşi dinitrofenil eterici şi i-am testat în experimente biologice, comparative cu dinitrofenolii cunoscuţi. Am folosit şi conjugaţi cu peptidele şi aminoacizii cum ar fi dinitrofenil-glutationul, dinitrofenil-glicina, dinitrofenilglicilglicina şi dinitrofenil-tetraglicina. Concluzia noastră a fost că nucleul dinitrofenilic determină efectul biologic al compuşilor investigaţi, în timp ce catenele laterale doar modulează toxicitatea, precum şi legătura acesteia cu solubilitatea.*

Cuvinte cheie: dinitrofenil conjugaţi, toxicitatea pesticidelor, grâu, germinaţie.

INTRODUCTION

Dinitrophenyl derivatives are substances with high toxicity and multiple biological actions [1]. Their biological activity is partly based on blocking the oxidative phosphorylation. However, they may be involved in disruption of

electron transfer in the two photosystems of the photosynthesis. Since data from the literature are still inconclusive and do not endorse a specific mechanism of action of dinitroderivatives on living organisms, the synthesis of new dinitrophenols, dinitrophenyl conjugates and phenolic dinitroethers in order to study their biological activity is of paramount importance. For example, no one knows precisely the effect of the atom at position 1, which is linked to the dinitrophenyl moiety. It may be oxygen, nitrogen or sulfur. The investigation of the biological activity of some ethers dinitrophenyl compared to that of dinitrophenylthiols and dinitrophenyl-amino acids or amines could help the clarification of the mechanism of action of these dinitroderivatives [2-7].

The purpose of this work is the synthesis of a large number of dinitrophenyl derivatives and the study of their biological activity compared to that of the corresponding dinitrophenols. Comparative experiments using FTIR and fluorescence techniques are also performed. The research of the known dinitrophenols and derivatives or newly synthesized ones on living organisms is carried out using wheat seeds being germinated.

MATERIALS AND METHODS

Synthesis of the investigated compounds and the characterization of their physico-chemical and biological properties were performed with the methods described previously [2,3,6]. Biological material was wheat samples (*Triticum aestivum*), Henika variety.

Reagents were of analytic purity (Merck, Sigma, and Chimopar) and the solution and the water slurries were prepared using redistilled water, acetone or ethyl alcohol. A series of dinitrophenol ethers were synthesized, using dinitrochlorobenzene, with variants of the methods described in literature [9]. Dinitrophenyl-glutathione, dinitrophenyl-glycine, dinitrophenyl-glycylglycine and dinitrophenyl–tetraglycine were also synthesized. The reaction was performed classically or under microwave.

Apparatus. The infrared spectra were taken on a Jasco FT/IR 660 Plus Fourier spectrometer. The melting points and the elemental composition were also determined and ^{1}H-RMN (Bruker Advance DRx400, 400 MHz) spectra and mass spectra were performed using a Vestec 601 mass spectrometer. Mass spectra of dinitrophenyl-conjugates with peptides and amino acids were carried out at Konstanz University, Germany, on a Esquire 3000Plus mass spectrometer (Bremen, Germania). Circular dichroism studies indicated the interaction between these compounds and proteins and were performed on a Jasco-715 spectropolarimeter, in 0.5-mm cuartz cuvettes, in the wavelength range 260 – 180 nm.

Treatment solutions and suspensions. Experiments and germination tests were made in Petri dishes, on Watmann no. 1 double filter paper, at the laboratory temperature (20 °C). Several solutions and suspensions of dinitrophenols, dinitrophenyl ethers and conjugates, with concentrations ranging between 10^{-4} M and 10^{-2} M were used.

Procedure. Germination was determined according to ISTA rules (Seed Science and Technology, 1993). Separately, each lot of 50 seeds was treated for one hour with 5 mL of solution or suspension of treatment. Afterwards, the seeds were uniformly deposited on filter paper in Petri dishes, along with the treatment solutions. The resulted plantlets were cut from the seed 7 days after, weighed and measured

(the height, **H**, in cm and the mass **m** in grams). Radicles mass and length were also measured.

Statistics. The results were processed using the Tukey test [8].

RESULTS AND DISCUSSIONS

Dinitrophenyl-glutathione, dinitrophenyl-glycine, dinitrophenyl - glycylglycine and dinitrophenyl - tetraglycine were synthesized and characterized being used in further biological experiments. For comparison, we have used glutathione (a tripeptide composed of glycine, cysteine and glutamic acid, with a detoxification role in living cell), the corresponding amino acids and peptides, as well as dinitrophenols and dinitrophenyl ethers such as 2,4-dinitroanisole, 2, 4-dinitro-*o*-cresol, 2,4-dinitro-1-(octadecyloxy) benzene, 3-(2,4-dinitrophe-noxy) propane-1,2-diol, named here dinitrophenyl glycerol (DNG) etc. We synthesized the glutathione conjugate knowing the detoxification properties of this tripeptide against dinitroclorobenzene in the body in case of poisoning (Vaidya & Gerke, 2007).

In the mass spectrum, the molecular weight of 472.2 units of dinitrophenyl-glutathione was clearly visible (M+H = 473.2 amu). By fragmentation and elimination of a water molecule, a fragment with a mass of 455.2 units appeared, which denotes the peptidic structure of this conjugate (fig.1).

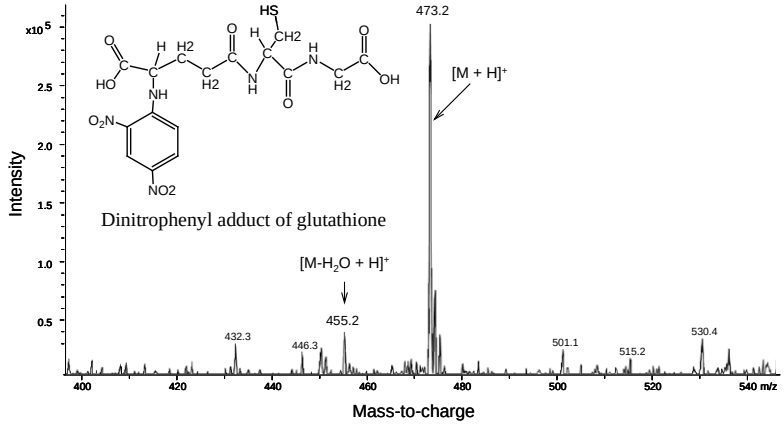

Fig. 1. ESI ion trap mass spectrum of dinitrophenyl-glutathione.

DNOC and 2,5-dinitrophenol showed a strong fluorescence extinction of tryptophan, vitamin B_2 and acyl-coenzyme A dehydrogenase, while compounds such as p-nitrophenol, p-nitrobenzoic acid or even 2.4-dinitrobenzoic acid which are not uncoupling agents did not behave as quenchers of fluorescence. Therefore, a close relationship between the two properties was established (fig. 2).

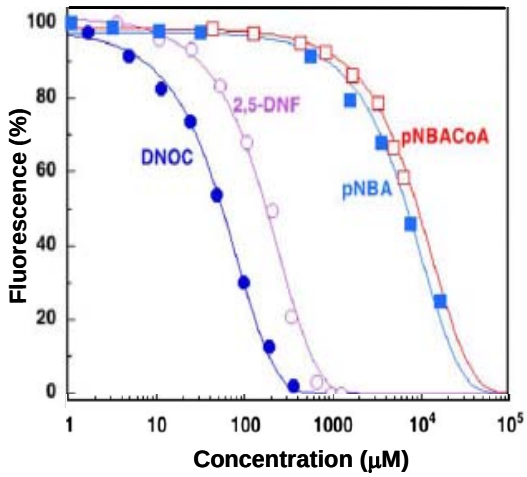

Fig. 2. Fluorescence quenching of E376Q mutant MCAD enzyme by 4,6-dinitro-*o*-cresole (DNOC), 2,5-dinitrophenol (2,5-DNF), p-nitrobenzoyl-acetyl-CoA (pNBA-CoA) and p-nitrobenzoic acid (pNBA) in phosphate buffer, pH 7.

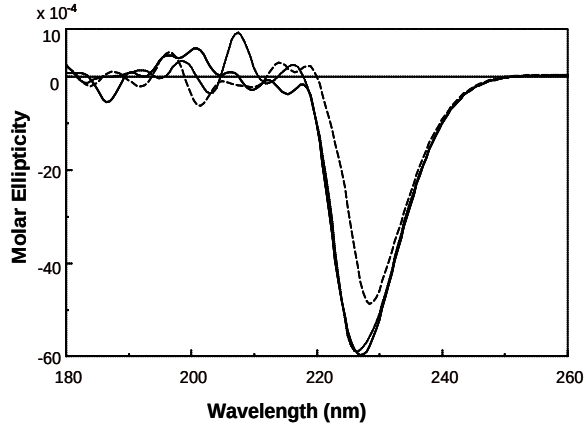

Fig. 3. CD spectra show the effect of 2,6-dinitrophenol on the conformation of the albumin molecule (continuous line: molar ellipticity of albumin, dotted line: ellipticity of 1/1 mole/mole adduct of 2,6-DNP with albumin; segmented line: 5/1 molar ratio adduct of 2,6-DNP with albumin).

2,6-Dinitrophenol influenced more strongly albumin conformation only in a high concentration, corresponding to a 5:1 molar ratio (fig. 3). A different behavior has the adduct of dinitroclorobenzene with glycerin, which has shifted the absorption maximum from 228 nm to about 226 nm. At lower wavelength, the spectrum of 5:1 adduct of DNG has a completely different shape from that of

albumin, while wavelengths close to 180 nm, all spectra had different absorbances.

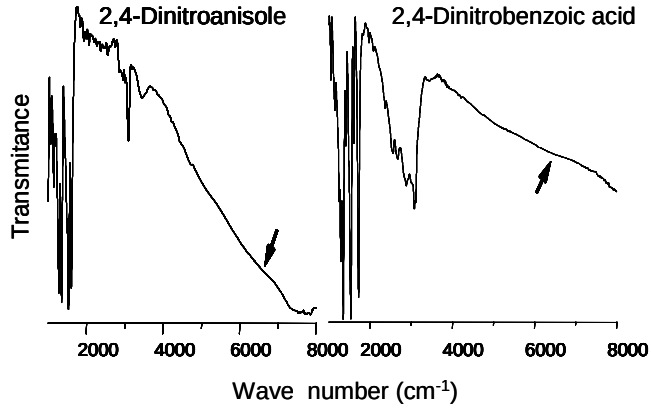

Fig. 4. FTIR spectrum of dinitroanisole (uncoupling agent) and that of dinitrobenzoic acid (with no such action).

Dinitrophenols and dinitrophenyl derivatives absorb mostly in the near infrared, while compounds without uncoupling activity e.g. 2,4-dinitrobenzoic acid, had no such optical properties (fig. 4).

A significant difference between the dinitrophenetole (DNF) action and dinitroanisole (DNA) one was found in spite of literature data which assign similar biological activities to both dinitrophenyl ethers.

It was assumed that, in order to manifest its action, dinitrophenetole hydrolyzes to afford the toxic dinitrophenol. However, on using thin layer chromatography, we demonstrated that dinitrophenetole is as toxic as dinitroanisole; previously, we found that these compounds act without being hydrolyzed [6].

Glutathione had no influence on the toxicity of dinitrophenols, whereas dinitrophenyl-glutathione and dinitrophenyl-conjugates of other peptides displayed specific biological activities, corresponding to their chemical structures.

The mechanism of toxicity is probably related to acido-basic properties of dinitrophenyl derivatives, with their solubility and spectral properties. It was found an interesting relationship between near infrared absorption, the fluorescence extinction, and the biological effect of dinitroderivatives. We suggest here that a more comprehensive approach of their toxicity is needed and not only the reducing the bioenergetics of ATP formation to proton translocation through mitochondrial membranes during the formation of the ATP molecules, as required by Mitchell's hypothesis [4.5].

CONCLUSIONS

The newly synthesized dinitrophenyl derivatives as well as the known ones were characterized by spectral techniques and tested within the wheat germination experiments. Experiments in which fluorescence quenching of dinitrophenyl derivatives have been measured as well as the strong absorption of these compounds at about 6000 cm-1 in the near infrared, at energies approximately equal to those for the formation of the ATP molecule suggest a direct radiation-based energy transfer. Contrary to Macovschi's theory, Peter Mitchell (Nobel laureate) hypothsized that proton translocation through biological membranes is needed to form ATP. Alternative mechanism of toxicity is proposed to allow dinitrophenyl derivatives and pesticide-like compounds to be extensively used in the field as well as in human or veterinary clinic.

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