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Application of vibrational spectroscopy to the study of structure and stability of anionic derivatives containing cyano-, carbonyl- and nitro groups. Experimental and theoretical approach.

Extended habilitation summary of scientific contributions

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For participation in the competition for the academic position of "Associate Professor" in the professional field 4.2. Chemical sciences, scientific specialty "Organic Chemistry", for the needs of Lab. "Structural Organic Analysis", Announced in the State Gazette: issue 40 of 16.05.2025

Sofia

A list of 26 scientific papers (cited according to the criteria in the template with an index as follows **1C; 1D**) is submitted for the competition, beyond which are the publications included in the PhD dissertation. The scientific works for the competition have been published in journals indexed in the world databases Scopus and Web of Science as follows: in journals falling into the category Q1 (6 publications); in journals falling into the category Q2 (8 publications), in journals falling into the category Q3 (3 publications); in journals falling into the category Q4 (7 publications); in printed editions without impact factor (2 publications). By June 2025, a total of 79 citations from journals indexed in the global databases Scopus and Web of Science have been noted from the research papers submitted in this competition for "Associate Professor".

All of the scientific papers submitted to this competition are in the field of organic chemistry and, in particular, in the preparation of anionic derivatives of organic compounds representing drugs, potent poisons or new compounds with potential biological activity. We will discuss in more detail the interpretation of vibrational spectra and how they can be simulated. The presentation of the scientific results will be accompanied by a discussion on the place of the research in the relevant scientific field and its contribution to the elucidation of scientific problems in that field.

I. Introduction

Organic anionic derivatives - carba-, aza-, oxyanions are intermediates in a large number of chemical reactions [1-4], including organic syntheses and biochemical reactions in biological media [5,6]. Also, many drugs are taken as salts or they are sufficiently C-H, N-H or O-H acidic to be deprotonated to a significant extent in the body. Therefore, the study of the structure and properties of anions is important for organic chemistry and biochemistry [3-6]. Radical anions are also anionic derivatives, although they possess an open shell. Excepting chemical preparation, they can also be produced under biological conditions under the action of reductases [7,8], leading to a variety of sometimes desirable but often undesirable effects of some drugs. In an aerobic environment, radical anions can be a source of superoxide [7,9], and in an anaerobic environment, the radical anions of nitro compounds can be reduced to nitroso-, hydroxyl amino-, and amino derivatives that are hepatotoxic [7,10-15]. Radical anions in biological media are very reactive and can also bind to anaerobic parasites, bacteria, tumor cells and exhibit significant cytotoxicity [7]. Once in the human circulatory system, they are rapidly oxidized with the formation of superoxide anions, and hydrogen peroxide and hydroxide radicals formed during nitroreduction can have carcinogenic effects [7,9,14,15].

Data on the structure of anionic derivatives are of fundamental importance for elucidating the mechanism and kinetics of chemical reactions or are relevant to elucidating the biological activity of these compounds, in relation to the supposed participation of their anions and radicals in the mechanism of action of drugs in the body. The formation of organic anions and radical anions leads to significant, sometimes fundamental, changes in their vibrational spectra [3,4,16]. This is most often expressed in a clear to strong shift of the functional group bands and in a significant to strong increase in the intensity of the these bands [4,16]. The greater the change in electron density distribution, causes greater vibrational changes. Therefore, vibrational spectroscopy is very sensitive and informative about the changes in the structure accompaning the conversion of organic molecules into anionic derivatives. In most cases the anionic derivatives are very unstable and can only be isolated in rare cases. For their study, a methodology has been developed based on the combined use of experimental IR spectra and quantum chemical calculations, with the help of which the structures of many anions, data for which are absent or limited in the literature, have been correctly interpreted. Due to their solubility and reactivity, non-trivial spectroscopic techniques and solvents are used, the cuvettes are made of more special windows. Techniques for electrochemical generation of radical anions in a special electrolysis cuvette have also been developed [Fig. 1] .

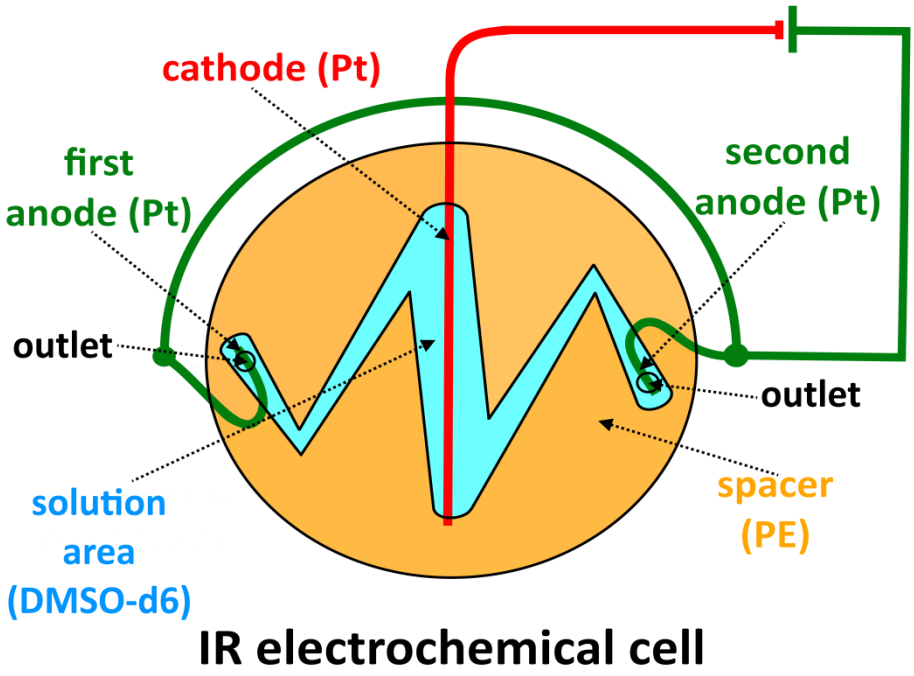


Figure 1. Scheme of IR electrochemical cuvette

II. A combined experimental-theoretical approach to the interpretation of vibrational spectra of anionic derivatives

II.1. Difficulties and specialities in measuring experimental IR spectra of anions and radical anions.

The preparation of carba-, aza-, and oxyanions is usually carried out by the reaction: A-H + MB $\rightarrow$ A:$^-$ M^+ + HB, where MB are alkaline/alkaline earth methylates or hydrides in a vial or ampoule for a few minutes. The reaction can be carried out in some aprotic solvents tetrahydrofuran (THF), hexamethylphosphoric triamide (HMFA), dimethylformamide (DMFA), dimethyl sulfoxide (DMSO). DMSO is the most suitable for the following reasons: 1) it dissolves the maximum number of organic compounds; 2) it dissolves the maximum number of anions; 3) it forms solvent-separated ionic aggregates, which causes further stabilizes the anions and prevents side-reactions; 4) it possesses the least number of bands in the middle IR region; 5) it is relatively non-corrosive to, polymers and CaF_2 cuvettes; 6) the cost of the deuterated derivative is relatively affordable. The technique allows the resulting anionic derivatives to be immediately transferred to a CaF_2 cuvette and measured. A disadvantage is that the region under investigation is limited from 4000 to 1100 cm-^{1}.

Radical anions can be prepared in a solution of THF or HMFA directly with an alkali metal according to the equation: A + M $\rightarrow$ $[A]^{\cdot-}$ + M^+. However, this technique has a very limited application due to the following reasons: 1) these solvents have too many bands in the mid-IR region; 2) they dissolve a limited number of radical anions; 3) they often form contact ion aggregates, which shifts and broadens the most important cyano-, carbonyl-, nitro bands in the analysis. 4) high tendency to undesirable reactions such as hydrogen evolution. For these reasons, a special electrolysis cell (Fig. 1.) has been developed in the SOA lab that allows electrochemical generation of radical anions in a medium of DMSO or DMSO-d_6 and tetrabutylammonium salt. It is designed to separate the cathodic from the anodic products, which allows only the spectrum around the cathode to be observed. The advantages of the method are that the reaction is carried out in a quasi-hermetic (oxygen free) environment; changes in the spectrum can be by the time; solvent-separated ionic aggregates are formed; reversibility, i.e. the polarity of the electrodes can be reversed, so that at the end it can be checked whether the original spectrum is restored. The latter procedure gives indications of the stability of the radical anion in the process of electrochemical generation. A disadvantage

is the incomplete conversion, but this usually does not interfere with the analysis, since it should observes at regular intervals of 5 or 10 min. which intensity of the bands the increases the, which intensity decreases, and which remains unchanged. The main difficulty of the experiment is due to the rigorous requirements for the purity of the solvent and reagents, especially with respect to moisture and oxygen.

II.2. Challenges in interpretion the obtained experimental results.

There are tens of thousands of articles in the literature on IR spectra of organic compounds, while information on anions and radical anions is scarce. The interpretation and assignment of a vibrational spectrum usually involves normal coordinate analysis, comparison of data from the literature, often from tables in textbooks and reference books. However, data on anions (except for carboxylates) are not present in them. The main characteristic intervals for the cyano-, carbonyl and nitro groups of the anions and radical anions are significantly shifted and expanded compared to those observed in the molecules. This complicates the assignment of the spectra and necessitates the application of an experimental-theoretical approach. And if for nitrile anionic derivatives the new bands fall in the region 2200-2000 cm^{-1}, where organic compounds rarely absorb, the characteristic intervals of the functional groups for carbonyl and especially for nitro compounds the characteristic intervals of the functional groups overlaps with those of aromatic, heterocyclic and other functional groups. The assignment of a carbonyl or nitro band by intensity is not always possible. In a number of cases, the bands of aromatic skeletal vibrations compete with or exceed the intensity of those of functional groups. This is the case of phenolates [17,**1D**], azanions of acetanilides [18,**1B**], radical anions of benzophenones [**2B**], nitrobenzenes [**3B**], azanions of benzimidazoles [**3B**]. Isotopic substitution is expensive and time-consuming, so it is necessary to apply a mixed approach - to combine experimental data with theoretical ones.

Density functional theory with the most commonly used hybrid functional B3LYP [19] provides good possibilities for simulation of vibrational spectra [16,**2B,4B**]. However, theoretical calculations of anionic derivatives impose some significant differences from those applied to molecules. First of all, the issue of ionic aggregation should be resolved. It is known from the literature that 2 types of ion pairs are observed in organic compounds – contact and solvent-separated [1,16]. Nonpolar solvents and low temperatures favor the formation of contact, and polar solvents and high temperatures – solvent-separated ionic aggregates [1]. In the simulation of the vibrational spectra of contact ion pairs, it is essential

to describe the influence of the solvent in some way (explicitly or continuum), because the vibrational frequencies depend on both the ionic radius and the polarity of the solvent [16]. Ionic aggregates of different types can coexist in solution, as is the case with the dianion of malononitrile in THF solution [16]. In some cases, the effect can be an increase in the nitrile frequencies up to 30 cm^{-1} (σ-coordination), in others a decrease by up to 20 cm^{-1} (π-coordination) [16,20], and non-stoichiometric aggregates can also be formed, which lead to the appearance of broad multiplet bands of about 100 cm^{-1} [16]. Due to these features, it is preferable to work in DMSO/DMSO-d_6 where the geometry of the solvent-separated anion/radical anion can be calculated most easily (without a counterion), and the results are satisfactory even if the calculations are carried out for the gas phase [16].

It is known that the calculated quantum-chemical vibrational frequencies are higher than the experimentally observed ones by several percent [21,22], and this is due to the set of approximations, the most significant share of which has the harmonic approximation [21,22]. For compounds with several tens of frequencies, deviations of about 100 cm^{-1} greatly complicate the analysis. For this reason, special scaling factors have been derived for various methods and functionals to "bring" the theory closer to the experiment [21,22]. This method of scaling gives good results for organic molecules measured in non-polar solvents, but does not work very well when it comes to charged particles such as anions and radical anions measured in polar DMSO [16,**2B,4B**]. Additional error accumulates when describing the influence of the solvent by PCM [23], since the scaling factors are derived for simple, mainly inorganic molecules for gas phase [16,21,22]. An additional inconvenience is that 2 scaling factors are derived for the frequencies above and below 1800 cm^{-1}, which overestimates the frequencies of the mid-frequency oscillations [16,**4B**]. For these reasons, a different approach has been adopted in our laboratory: the spectrum of the starting molecule is measured in DMSO, the theoretical and experimental frequencies are compared, and a scaling equation is derived by regression analysis, according to which the theoretical frequencies of the anion/radical anion are scaled. In this way, mean absolute deviations (MAD) of 5-10 cm^{-1} are obtained [16].

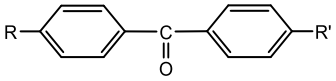
II.3. Prediction of nitrile frequencies and intensities observed in molecules, anions and radicals based on ab-initio and density functional theory.

When assignment of the spectra of polynitrile anion derivatives has been performed [16], it was noticed that even when scaling with a molecule-specific equation, the theory

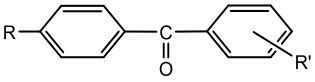
slightly underestimates only the nitrile frequencies compared to those of all other vibrations. This led to the establishment of special scaling factors for the nitrile stretching vibrations due to the availability of sufficiently collected experimental data [**3B**]. The nitrile stretching frequencies were calculated for 45 molecules and the corresponding scaling factors and equations were found for 11 basis sets and the B3LYP functional [**3B**]. The lowest MAD of 8 cm^{-1} was obtained at the B3LYP/6-311++G(d,p) level. For 42 nitrile anions, the corresponding frequencies were calculated at 11 levels of theory and the scaling factors and equations were determined. The lowest MAD of 11 cm^{-1} was obtained at the B3LYP/6-31+G(d) level [**3B**]. However, the results for the other higher basis sets of the "Pople" type were almost as good. From these results it was concluded that the use of basis sets with diffuse functions in the future is mandatory, and the "Dunning" type basis sets are not preferable for simulation of IR spectra from the point of view of accuracy and computational time. The nitrile frequencies of 32 radical anions at 10 levels of theory were calculated and the corresponding scaling factors and equations were determined. It was found that the addition of polarization d-functions leads to an increase in the MAD. This led to the use of basis sets with only added diffuse functions and the lowest MAD was obtained - 9 cm^{-1} at the B3LYP/6-311+G level [**3B**]. It is assumed that nitrile radical anions make some exception to the general dependences and that it is still good to use the B3LYP/6-311++G** level when the entire spectrum is considered and the radical anions contain other functional groups. However, it has been concluded that "triple zeta" basis sets are preferable to "double zeta" when simulating IR spectra of radical anions, while in closed-shell systems "double zeta" may be a good compromise in terms of computational time [**3B**].

The accumulation of theoretical data led to the study of the possibilities of the theory for predicting nitrile intensities, although it is known from the literature that quantitative prediction of different types of vibrations is practically impossible [24]. The nitrile intensities in DMSO solution of 56 compounds were calculated and the corresponding scaling factors, equations and CAOs at 16 levels of the HF and B3LYP theory were determined [**3B**]. The results showed that the basis set expansion does not improve the results at all, and *ab initio* outperforms DFT in this respect. However, the use of the PCM-B3LYP/6-31G level (calculated on Gaussian-03) gave satisfactory results - MAD 15 $km.mol^{-1}$.

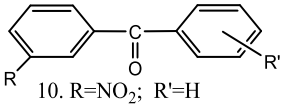
II.4. Application of density functional theory and solvation models to describe the spectral and structural changes induced by the conversion of substituted benzophenones into ketyl radicals.



1. R=H; R'=H
2. R=H; R'=Ph
3. R=CN; R'=H
4. R=CN; R'=CN



5. R=NO_2; R'=H
6. R=NO_2; R'=4-OMe
7. R=NO_2; R'=4-NMe_2
8. R=NO_2; R'= 3-NO_2
9. R=NO_2; R'=4-CN

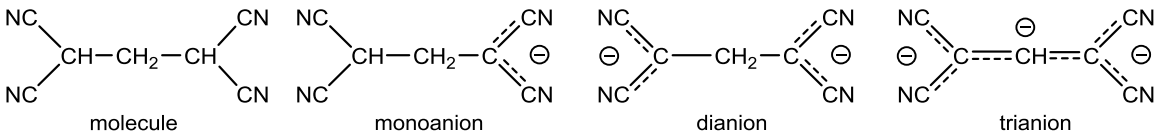


10. R=NO_2; R'=H
11. R=NO_2; R'=4-CN
12. R=NO_2; R'= 4-NMe_2
13. R=NO_2; R'=3-CN
14. R=NO_2; R'=3-NO_2

In the simulation of IR spectra of a series of 14 benzophenones containing substituents, it turned out that the B3LYP/6-311+G(d,p) level is not able to predict satisfactorily the decrease in carbonyl frequencies caused by their conversion into radical anions. This result was unexpected and raised doubts about the possibility of using this functional in the future in our studies, especially for radical anions with non-planar structures such as benzophenones. Additional calculations were carried out with a smaller series and different functionals, from which it was determined that the B3LYP [19], B1LYP [25], BHandHLYP [26], CAM-B3LYP [27] functionals are the most promising, and we proceeded to raise the theoretical level of the calculations [**2B**]. Due to the organic computational resources, a mixed ONIOM - IEF-PCM model was chosen [23,28,29], which is offered by the Gaussian 09 software package. The conclusion that was made was that the B3LYP functional is sufficiently reliable for predicting spectral changes if the influence of the solvent is described, and the ONIOM[B3LYP/6-311+G(2df,p)//6-311+G]-IEFPCM level is a very good compromise in terms of accuracy and computational cost [**2B**]. The MAD between the predicted and observed carbonyl frequencies decreased from 31 to 4-6 cm^{-1} for the different functionals, which gave us the reason for us to assume that the model adequately describes the structure and conjugation in the compounds, as well as the polarizability of the functional groups [**2B**]. At the same theoretical level, an analysis was carried out of the change in bond lengths, valence angles and electron density distribution upon the conversion of molecules into radical anions [**2B**].

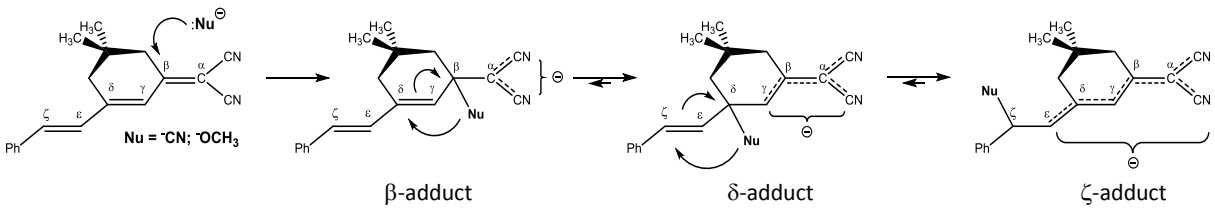
II.5. Vibrational spectra and structure of carbanion derivatives.

II.5.1. IR spectra and structure of the carbanions of 1,1,3,3-tetracyanopropane.



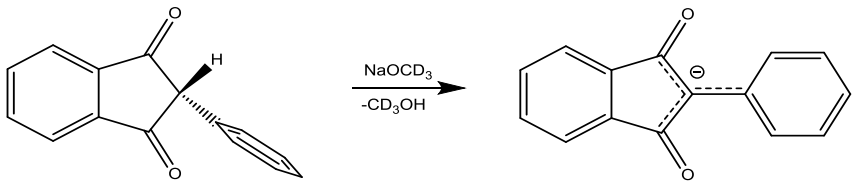
Tetracyanopropane (methylene-bis-malononitrile) was synthesized by a known method and its carbanions were prepared. The aim was to follow the influence of the closely located carbanion centers on the frequency of the nitrile group. The monoion was obtained with $NaOCH_3$ in DMSO solution, the dianion in the same solvent with dimsyl sodium, and the trianion with metalating reagents alkaline naphthalenides in THF solution [**5B**]. The nitrile frequencies of the monoanion turned out to be typical for previously observed dicyanomethanides with the difference that no Fermi resonance was observed as it stands, for example, in the carbanion of malononitrile [16]. The conversion of the monoanion into a dianion led to a pronounced decrease in the nitrile frequencies by an average of about 30 cm^{-1} and thus the induction effect of the vicinal carbanion center can be accounted for. The conversion of the dianion into a trianion led to an additional decrease in the nitrile frequencies by about 100 cm^{-1}, and thus the mesomeric effect of the geminal carbanion center can be accounted for, and the cumulative effect of the three neighboring carbanion centers is a decrease by almost 300 cm^{-1} [**5B**]. At the B3LYP/6-31++G(d.p) level, a conformational analysis of the resulting carbanions was performed, and changes in bond lengths and valence angles were evaluated. The distribution of the NBO-carbanion charge by fragments for each degree of deprotonation was clarified [**5B**].

II.5.2. Studing the preparation and isomerization of the carbanion adducts of 2-{5,5-dimethyl-3-[(2-phenyl)vinyl]cyclohex-2-enylidene}malononitrile through their experimental and theoretical IR spectra.



First solutions of the neutral compound in DMSO/DMSO-d_6 with a certain concentration of the neutral compound were prepared, the integral intensities of the most intense bands were determined and the data were compared with the theoretical ones at the B3LYP/6-31++G(d,p) level [**6B**]. A very good agreement was found between theory and experiment MAD = 5 cm^{-1}. Afterwards, the nucleophiles KCN and NaOCH3 were added to the solutions and the IR spectrum was measured over a certain time period. The data showed practically complete conversion of the β-carbanion adduct via δ- to γ-adduct within 3 hours [**6B**]. The increase in the area of delocalization of the carbanion charge during the isomerization led to a slight but measurable increase in the nitrile frequencies by about 10 cm^{-1}. Based on the calculations, the most significant changes in the bond lengths, bond angles, and electronic charges of the resulting isomers were determined [**6B**].

II.5.3. IR spectra and structure of the phenindione carbanion.

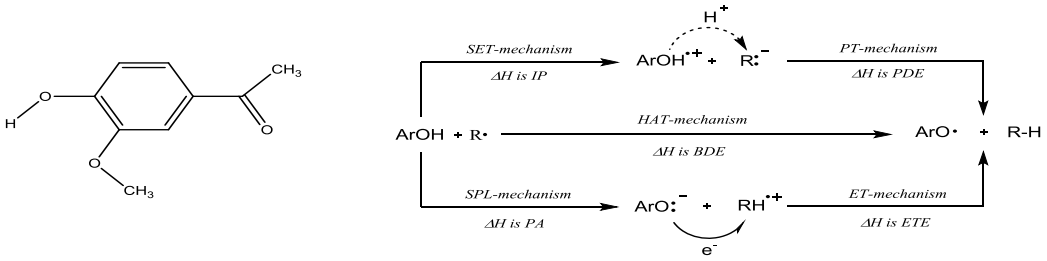


Phenindione is an anticoagulant of pharmaceutical importance. The central carbon atom is strongly CH-acidic and suggests a significant degree of deprotonation in the body. To better understand its biological action, in which it reduces the action of vitamin K and increases blood clotting time, experimental and theoretical studies of the structure and vibrational spectra of its carbanion were carried out [**2D**]. At the IEFPCM B3LYP/6311+G(2df,p) level, an excellent correspondence was found between the theoretical and experimental IR spectra with MAD 3 and 5 cm^{-1} for the neutral compound and the carbanion. The predicted decrease in the carbonyl frequencies is 105 and 168, and the measured 105 and 164 cm^{-1}. The theoretical data for the bond lengths and valence angles were compared with the data from the X-ray structural analysis of the neutral compound and again an excellent correspondence between theory and experiment was achieved [**2D**]. It was found that the transformation of the molecule into a carbanion is accompanied by a complete change in the shape of the compound, since the tetragonal configuration of the central carbon is transformed into a trigonal one, which leads to the planar structure of the carbanion [**2D**]. The calculated Hirshfeld charges showed the high degree of resonance stabilization of the

carbanion charge - only 13% of it remains localized at the carbanion center, the rest is distributed almost equally to the other 3 rings [**2D**].

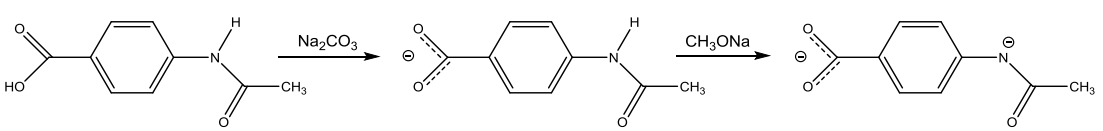
II.6. Vibrational spectra and structure of oxy- and azanion derivatives.

II.6.1. IR spectra and structure of the oxyanion of apocynin.



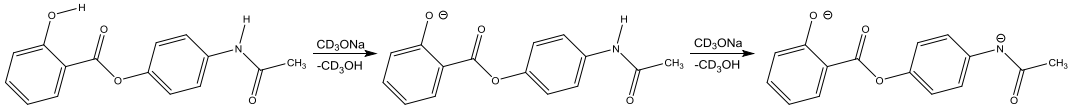
Apocynin is the active substance of the plant *Picrorhiza kurroa*, extracts of which have long been used in folk medicine in India [30,31]. Clinical studies have shown that it exhibits diverse activity against liver, cardiovascular [32] and neurodegenerative diseases such as Alzheimer's and Parkinson's [31]. Its mechanism of action is debatable, but it is assumed that apocynin inhibits NADPH oxidase in leukocytes, and in endothelial and vascular smooth muscle cells it acts primarily as an antioxidant [33]. In order to clarify the mechanism of antioxidant action at the level of M05-2X/ 6-311++G(3df,3dp) [34], the most stable conformers of the molecule and intermediates along the three reaction pathways – radical, radical cation and anion [**1D**] were determined. At the same theoretical level, the dissociation enthalpies of BDE (HAT mechanism) and the enthalpies of the other two mechanisms SET-PT and SPLET were calculated. The calculations were performed in different media: gas, benzene, water (solvation model (IEFPCM)) and were compared with the same values calculated for vanillin [**1D**]. The possibility of scavenging reactive oxygen species from both compounds was assessed by comparing the enthalpies in the three aforementioned media. The theoretical results showed that in a lipophilic medium the HAT mechanism is preferred, and in a hydrophilic one the SPLET mechanism [**1D**]. Since in the latter case the intermediate is an oxyanion, we obtained, measured and assigned its IR spectrum in DMSO solution, using the IEFPCM B3LYP/6-311++G(2df,p) level for the theoretical data [**1D**].

II.6.2. IR spectra and structure of the oxyanion and dianion of acedobene.



Acedobene (4-acetamidobenzoic acid or 4-carboxyacetanilide) and its sodium and potassium salts are found in various pharmaceutical products with antiviral effects [35-36]. It has been found to be a metabolite of para-aminobenzoic acid [35], benzocaine [36], and has found application in coordination chemistry and crystal engineering [37,38]. Despite the large number of studies of acedobene and its metabolites, including ATR-FTIR spectra of human skin [39], detailed spectroscopic studies of its anions are missing in the literature. For this reason, their preparation in DMSO-d_6 solution was started, with the oxyanion obtained with Na_2CO_3 and the dianion with $NaOCD_3$ [**3D**]. At the B3LYP/6-311+G(2df,p) level in DMSO solution (IEFPCM), the deprotonation energies, the different conformers of the molecule and the anions, including the s-*trans*-*cis* conformation, have been calculated [**3D**]. Their IR spectra have also been assigned at the same theoretical level, the changes in the spectra at both degrees of deprotonation have been discussed, the changes in the positions of the carbonyl, amide-II and amide-III bands have been shown [**3D**]. A structural analysis has been carried out, the largest changes in the bond lengths and bond angles of the anions and the distribution of the oxyanion and azanion charge by fragments have been calculated [**3D**].

II.6.3. IR spectra and structure of the azanion and dianion of salophene.



Salofen (Fenetsal, Acetaminosalol, (4-acetamidophenyl)2-hydroxybenzoate) is an antirheumatic, antipyretic, analgesic and intestinal antiseptic agent [14]. Due to its antimicrobial activity, it is used as an ingredient in cosmetic products, pharmaceutical ingredients, surgical materials, etc. [15]. Its IR spectra are included in many databases, but there are no studies of its anions. The oxyanion was obtained in equimolar, and the oxy-aza-dianion in an excess of sodium methylate in DMSO-d_6 solution. At the B3LYP/6-311++G(d,p) (IEFPCM DMSO) level, the geometries and deprotonation energies of the molecule to obtain the two anions were calculated [**7C**]. The bands of the obtained compounds were assigned, the position of the newly obtained amide-I, amide-II, amide-III bands was determined and the MAD for the molecule and the anions were determined to be 8, 6 and 9 cm^{-1}, respectively [**7C**]. A structural analysis was performed, and the data for the

molecule were compared with crystallographic data. By the theoretical approach, the largest changes in the bond lengths, valence angles, NBO-electron charges upon conversion of the molecule into an oxyanion and of the oxyanion into a dianion were determined [**7C**].

II.7. Vibrational spectra and structure of anionic and radicalanionic derivatives of nitroaromatic compounds.

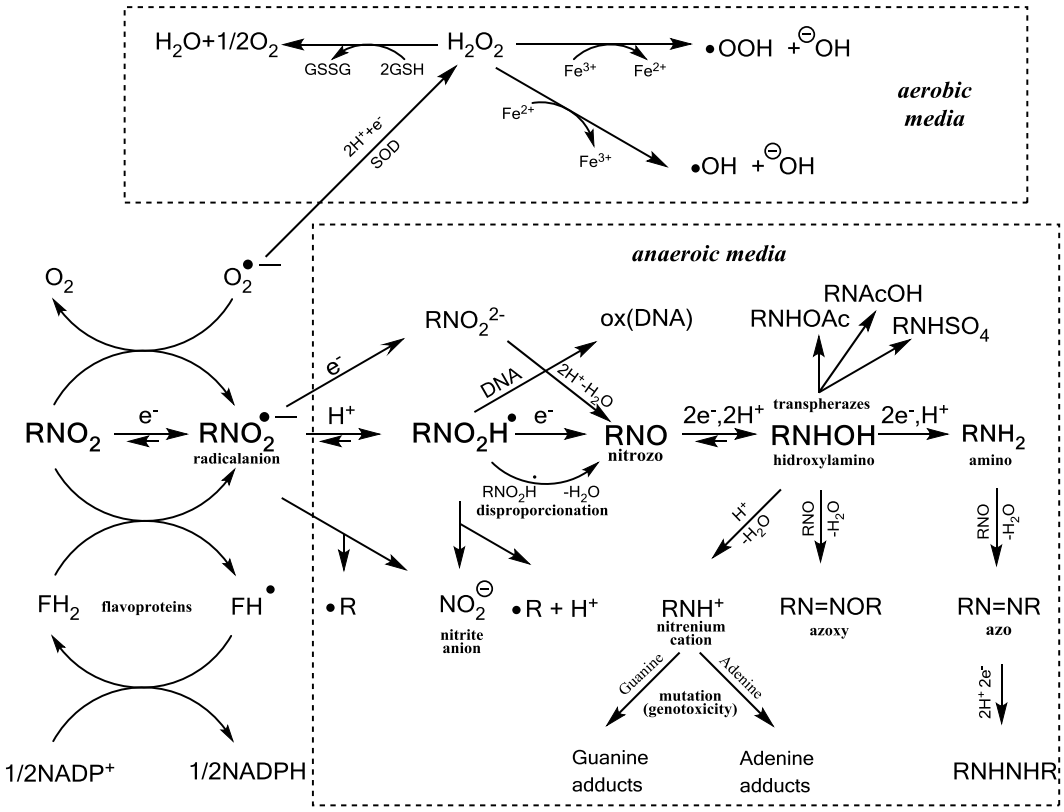
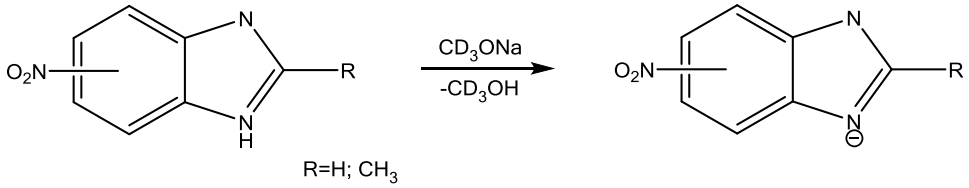


Figure 2. Metabolic conversions of nitrocompounds

Organic compounds containing a nitro group (nitrobenzenes, nitroimidazoles, nitrofurans) are included in the composition of many drugs with diverse applications for the treatment of cardiovascular diseases, bacterial and parasitic infections, and cancer. [10-12] In biological conditions, under the action of enzymes such as Cytochrome P-450 (CYP) reductase, xanthine oxidase, aldehyde oxidase, and quinoline reductase, nitro-containing radical anions are produced, which lead to the generation of superoxide, reactive oxygen species, and ultimately oxidative stress (Fig. 2) [10-12,42]. On the other hand, in hypoxic conditions, which are observed in solid tumors, the nitro group in the resulting radical anions is further reduced, especially in acidic media, to nitroso-, hydroxylamino and amino groups, which leads to proven cytotoxicity [7,43-45] (Fig. 2.) In this way, various nitrogen-containing

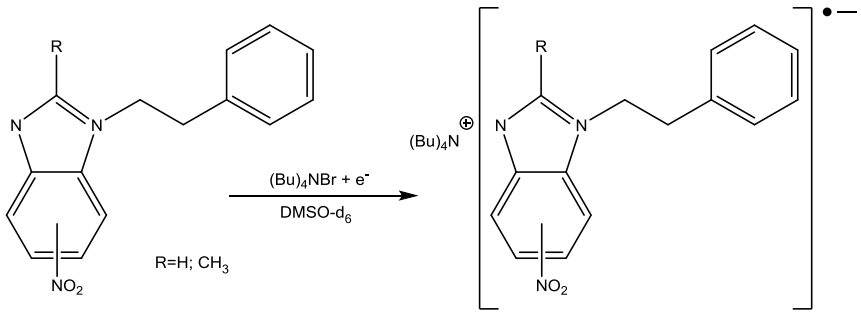
compounds are obtained, one of which is the nitrenium cation, which leads to mutations in the tumor DNA and hinders cell division [10,43]. On this basis, nitro-containing prodrugs have been created, complementing chemo- and radiotherapy [43,46,47]. In addition to the beneficial effects, nitro derivatives also have hepatotoxicity due to oxygen deficiency and reduction of the nitro group in biological conditions [42,48]. Therefore, the development of new nitroaromatic derivatives with reduced toxicity to healthy cells is important for pharmaceutical chemistry.

II.7.1 IR spectra of and structure of the azanions of 5(6)-nitrobenzimidazole and 2-methyl-5(6)-nitrobenzimidazole.



The benzimidazole heterocycle interacts with many enzymes and proteins and is a pharmacophore with diverse medical applications. Benzimidazole derivatives have shown a wide range of biological activities as antineoplastic, anti-inflammatory, antibacterial, antifungal, anthelmintic, antiviral and antioxidant agents. Substituted 5(6)-nitrobenzimidazoles have shown potential as nitroreductase prodrugs in [49,50]. There are fragmentary data in the literature on the reduction potentials of the products of nitroreduction of the radical anion of the unsubstituted derivative by cyclic voltammetry in buffer solutions [51], but we did not find any data (spectral or voltammetric) on the radical anion itself. There are also no data on its azanions. Unfortunately, radical anions of both compounds were not prepared, since electrochemical reduction in the cuvette (Fig. 1) led to the formation of azanions and the evolution of hydrogen. However, the spectra obtained with sodium methylate were cleaner. At the IEFPCM B3LYP/6-311++G(d,p) level, the molecules and azanions were calculated [**3C**]. The frequencies of the nitro group decrease for the asymmetric stretching vibration by 28 and 30 cm^{-1}; for the symmetric one by 25 and 30 cm^{-1}, respectively. However, the largest spectral changes affect the C-N stretching skeletal vibrations of the imidazole ring, the intensity of which increases significantly and these are the most intense bands in the spectra of the azanions [**3C**]. The theory confirmed the spectral changes and showed that the largest changes in the bond lengths are precisely in the imidazole ring, and over half of the azanion charge remains localized in the anion center [**3C**].

II.7.2. IR, Raman spectra and structure of the radical anions of 5(6)-nitro phenylpropyl-1H-benzimidazole and 2-methyl-5(6)-nitro-1-phenylpropyl-1H-benzimidazole.

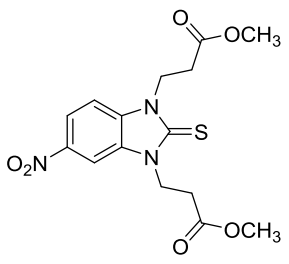


There are data in the literature on cytotoxic studies of 1- and 2-substituted nitro benzimidazoles [52-54], with the activity attributed to the formation of a radical anion in a hypoxic environment, but the only data on the formation of such anion are derived from the cyclic voltammetric study of 2-phenyl-5(6)nitrobenzimidazole [55]. The data are important, since the measurements were carried out in an aprotic environment and the obtained reduction potential corresponds precisely to this intermediate, and not to the further products of its reduction (Fig. 2). However, no data were found in the literature on the formation of other radical anions of benzimidazoles. The electrochemical reduction of both compounds was carried out and the data were compared with the theoretical ones at the IEFPCM B3LYP/6-311++G(d,p) level. The conversion of the molecules into radical anions leads to a strong decrease in $\nu_{as}(NO_2)$ with 278 and 283 cm^{-1}. According to the theory, the decrease of $\nu_s(NO_2)$ is 318 cm^{-1} and 285 cm^{-1} and the band falls below 1100 cm^{-1} and cannot be observed. Raman spectra, however, confirmed the presence of this band at 1067 cm^{-1} [**3C**]. A strong band, typical of IR spectra of nitroaromatic radical anions, appears at 1336 and 1332 cm^{-1} corresponding to the stretching $\nu(C\text{-}NO_2)$ vibration. When the molecules are converted into radical anions the frequency of $\nu(C\text{-}NO_2)$, its frequency increases according to theory above 300 cm^{-1} (and the theoretical intensity also increases strongly) but unfortunately we could not experimentally confirm the frequency in the spectra of neutral compounds (theoretical 1082 and 1079 cm^{-1}) because in infrared spectroscopy the calcium fluoride windows absorb below 1100 cm^{-1} in infrared spectroscopy calcium fluoride glasses absorb, and in Raman spectra such a band could not be registered due to the strong fluorescence and not very high intensity [**3C**]. In general, it can be summarized that the spectral changes are qualitatively similar to

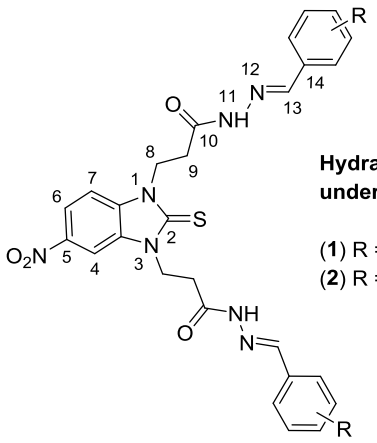
those observed in nitroaromatic compounds, in which the nitro group is not directly conjugated to another electron-withdrawing group. The largest changes affect the benzimidazole cycle and the nitro group, and the bands of the side substituent are almost not affected. In the Raman spectrum of the radical anion, the band for ν(C=N) at 1596 cm^{-1} is particularly intense, although much weaker in the infrared spectrum [**3C**]. The structural data obtained from the theory are consistent with the changes in the vibrational spectra: the N=O bond in the nitro group is lengthened from 1.21 to 1.30 Å, and the C-N bond to the benzene nucleus is shortened from 1.46 to 1.40 Å. 100% of the NBO spin density is located in the nitrobenzimidazole fragment, and over 70% of it - in the nitro group [**3C**].

In order to study the nitroreduction potential of the synthesized compounds under hypoxic conditions and to assess their hepatoprotective activity, additional theoretical and experimental cyclovoltametric studies were carried out [**3C**]. The determined reduction potential of -1.17 V is in very good agreement with the literature data for 1-phenyl-nitrobenzimidazole -1.14 V [55]. At the M06-2X/6-311++G(d,p) level, the spin densities, the energy differences HOMO-HUMO and HOMO-SOMO and the electron affinities of the two compounds were calculated, and the data were compared with other bioactive nitrobenzenes and nitrobenzimidazoles. The results show that the nitroreduction propensity of the two compounds is greater than that observed of 1-methyl-4-nitroimidazole, but lower than that observed of nitrobenzene and misonidazole [**3C**].

II.7.3 IR spectra and structure of radical anions of nitrobenzimidazole thiones.



Ester derivative of 2-nitro-benzimidazole-2-thione



Hydrazone derivatives under study:

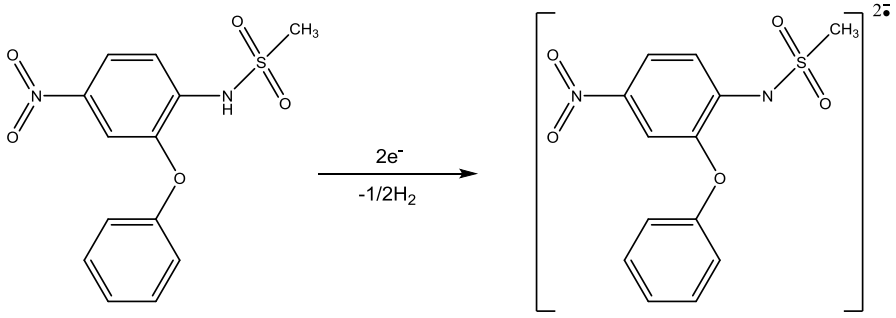
(**1**) R = 2-OCH_3;
(**2**) R = 3-OCH_3;

Benzimidazole-thiones as analogues of melatonin have been synthesized in search of new compounds with antioxidant and hepatoprotective properties [**4D**]. Biochemical studies

with hepatocytes and theoretical studies of possible mechanisms of antioxidant action in lipid media have been carried out for the ester derivative [**4D**]. At the IEFPCM B3LYP/6-311++G(d,p) levels for the ester and IEFPCM B3LYP/6-311+G(d) for the hydrazones, all possible conformers of the molecules and radical anions have been calculated and the most stable forms have been determined [**5D, 8C**]. They were used in the simulation of IR spectra. The changes in the frequencies of the nitro group in the spectra of the resulting radical anions of the thiones are qualitatively similar, but slightly smaller than those observed for nitrobenzimidazoles. $\nu_{as}(NO_2)$ decreases by 230 cm^{-1} for the ester and 266 cm^{-1} for the hydrazones, $\nu s(NO_2)$ decreases by 242 and 240 cm^{-1}, respectively, and the increase in ν(C-NO_2) is 205 and 248 cm^{-1}. The bands of the thiol groups are not significantly shifted and do not change their intensity much. The bands due to the vibrations of the substituents do not undergo a significant change, since the spin density is distributed entirely over the benzimidazolethione fragments [**5D, 8C**]. In order to understand the influence of the substituents, the NBO spin densities and charges of the radical anions were compared, and it was found that more electron density is distributed to the nitro group in the radical anion of the ester. The energy differences between the HOMO-LUMO and HOMO-SOMO orbitals and the electron affinities were calculated, and the data were compared with other nitro-containing aromatic and heterocyclic compounds. Theoretical data show that the SOMO orbital does not correspond to the LUMO+1, but to the LUMO+2 orbital in the hydrazone derivative. This fact, in addition to the energy shift of the SOMO orbital relative to the LUMO, suggests that in the analysis of energies, the HOMO-SOMO value should be preferred in the future, rather than the trivially imposed HOMO-LUMO approach [**5D, 8C**]. From the analysis of the theoretical data, it was concluded that the tendency to form radical anions under hypoxic conditions is almost the same and their stability is quite close, which means that the differences in their hepatotoxicity are due not to the first initial stage of nitroreduction, but to one of the subsequent stages, shown in Fig. 2 [**5D, 8C**].

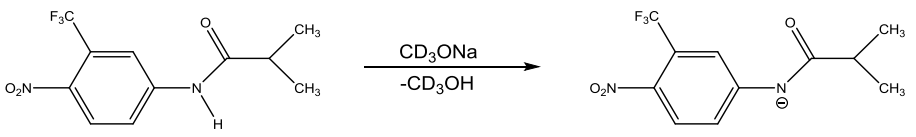
The diazanion of the hydrazone derivative was prepared and its IR spectrum was measured. The largest changes are near the azanion center, the carbonyl frequency decreases by 144 cm^{-1}, ν(C=N) by 44 cm^{-1}, ν(C-NCO) by 58 cm^{-1}, and ν(N-NCO) by 56 cm^{-1}. Theoretical data show that the electron density from the azanion charge is delocalized throughout the hydrazone fragment and conjugated with that of the phenyl fragment, with only about 3% being distributed to the nitrobenzimidazolethione fragment [**5D, 8C**].

II.7.4. IR spectra and structure of the nimesolide radical dianion.



Nimesulide is a nonsteroidal anti-inflammatory drug that has a strong analgesic and antipyretic effect [56]. It causes rare but unpredictable liver reactions, which are due to biochemical nitroreduction in oxygen deficiency [57,58]. The reduction products were studied by cyclic voltammetry in buffer solutions at different pH [59-61], and it was found that the radical anion is stabilized in a strongly basic environment pH=12 [61]. The different conformers of its anionic derivatives were calculated at IEFPCM B3LYP/6-311++G(2df,2dp) level in DMSO solvent [**9C**]. Theoretical spectra show that during the electrochemical reduction in the IR cuvette (Fig. 1) a radical dianion was obtained. This result is not surprising, since hydrogen evolution is observed during the process, but theoretical data show that this is not due to the formation of an azanion, which can also be expected [**9C**]. The conversion of the molecule into a radical dianion leads to significant changes in the IR spectrum. $\nu_{as}(NO_2)$ decreases by 303 cm^{-1}; $\nu_s(NO_2)$ decreases by 283 cm^{-1}; $\nu_{as}(SO_2)$ decreases by 105 cm^{-1}; $\nu_s(SO_2)$ decreases by 75 cm^{-1}; and $\nu(C\text{-}NO_2)$ increases by 248 cm^{-1} [**9C**]. A strong band due to the valence vibration of the $\nu(C\text{-}NSO_2)$ bond appears at 1298 cm^{-1} [**9C**]. The NBO electronic charges and spin densities of the molecule, radical anion, and radical dianion were calculated. It has been found that the conversion of the radical anion into a radical dianion leads to an increase in the spin density and charge of the nitro group, and over 80% of the azanion charge remains localized in the azanion center [**9C**].

II.7.5. IR spectra and structure of anionic derivatives of flutamide.



Flutamide is a nonsteroidal competitive inhibitor of dihydrotestosterone and is used in the treatment of prostate cancer [62,63]. It is assumed that it has hepatotoxicity due to nitroreductive metabolism [64,65]. According to electrochemical studies, it is irreversibly reduced in phosphate buffer [66]. Its IR spectra were measured in DMSO-d_6/CaF_2 and $CDCl_3$/NaCl **[1C]**. The Boltzmann distributions of the possible conformers of the molecule and the anionic derivatives were calculated based on the Gibbs energies at the IEFPCM B3LYP/6-311++G(d,p) in solvent DMSO. The azanion was obtained in a solution of DMSO-d6 and sodium methylate. The carbonyl frequency decreases by 170 cm^{-1}, the frequency of the stretching vibration of the C-N bond increases by 62 cm^{-1}, the frequencies of the asymmetric and symmetric vibration of the nitro group decrease by 87 and 99 cm^{-1}, respectively [**1C**]. The conversion of the molecule into an azanion is accompanied by a strong increase in the intensity of the bands corresponding to the aromatic skeletal vibrations. The NBO and Hirshfeld electronic charges were calculated, which show relatively weak delocalization to the electron-withdrawing groups, with 70% of the azanion charge remaining localized in the amide fragment [**1C**].

The electrochemical reduction carried out in the IR cuvette (Fig. 1) leads to the release of hydrogen, and when comparing the spectra it was found that an azanion is formed, and not a radical dianion, likewise for nimesulide [**9C**]. This is probably due to the low pKa value of flutamide 4.8 [64]. For these reasons, the analysis of the spectral changes was only theoretical [**1C**]. At the M06-2X/6-311++G(2df,2dp) level in solvent water, the HOMO-SOMO energy differences for the radicalanion and radicaldianion were calculated, and in this case, large differences were obtained with those calculated for HOMO-LUMO. According to the data on the reduction potential and NBO spin densities, the nitroreduction propensity of flutamide is lower than that of nimesulide, but higher than that of nitrobenzene [**1C**].

III. Perspectives for future work

1) Preparation of radical anions of nitrofuran and nitroimidazole compounds.

2) Extending the experimental scope of studies by Raman, EPR spectra and cyclic voltammetry.

3) Extending the theoretical scope of the studies by finding a functional suitable for theoretical rescaling of reduction potentials of nitro compounds.

4) Upgrading the equipment - software and computer configurations for quantum chemical calculations, AC voltage and amperage current source needed for future electrochemical studies, by applying for funding under national programs.

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