

PhD THESIS

**STRUCTURE ELUCIDATION OF MOLECULES
WITH BIOLOGICAL IMPORTANCE BY
VIBRATIONAL AND NMR SPECTROSCOPY**

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INTRODUCTION

Based on many years of spectroscopic research, this Thesis provides a selection of my papers, published in the last decade. The aim of the work was to give a general view about the potentials of vibrational and NMR spectroscopy, applied for structure determination of molecules with biological importance.

During the years I had the possibility to participate in the work of several research groups at home and abroad. Chapters 4.2, 5.2 and 6.1 are the results of cooperation with my colleagues working at the Chemical Research Center of the Hungarian Academy of Sciences. Chapter 4.1 originates from the one year period scholarship I spent at the University of East Anglia in Norwich. Chapter 5.1 demonstrates the results of a joint project made with colleagues at the University of Perugia.

I would like to thank all of my coworkers for providing interesting new compounds for structure elucidation. While reviewing here our common publications, I am obviously emphasizing my own contribution, trying to separate it from the results of the coauthors.

OBJECTIVES

Infrared and Raman spectroscopy is conventionally applied for studying the products of organic syntheses, however they can be also used for tracking changes during biological processes.

In Chapter 4.2 quantum chemical calculations and normal coordinate analysis has been performed for the interpretation of the unusual spectral behavior of intermediates produced during the synthesis of ergoline derivatives. Chapter 4.1 deals with the labeling of proteins by organometallic tags which has recently attracted biological interest. The aim of this research was to determine whether the label was capable of tracking the local changes near the surface of the enzymes.

In Chapter 5.1 the unexpected side products of Diels-Alder cycloadditions have been studied, their structure having been described purely by NMR techniques.

Chapters 5.2 and 6.1 have common origin: experiments have been carried out in order to investigate the addition reactions of nitrogen containing heteroaromatics. Further to the characterization of the reaction products, the structural parameters of the reacting salts were correlated with the appropriate NMR data to predict the exact site of the nucleophilic attack.

APPLIED METHODS

The majority of infrared and Raman spectroscopic investigations were performed in the Optical Spectroscopy Department of the Chemical Research Center of the Hungarian Academy of Sciences, on a NICOLET Magna 750 Fourier transform spectrometer and a NICOLET 950 FT-Raman spectrometer using NIR excitation. During my stay in Norwich the IR spectra were taken on a Perkin Elmer 1720X Fourier transform spectrometer.

Quantum chemical calculations have been performed with the Gaussian program package, while for normal coordinate analysis the MOLVIB program written by Sundius (University of Helsinki) has been used.

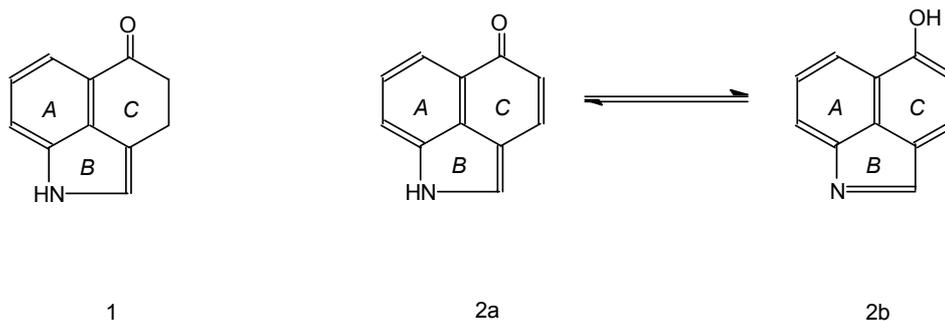
NMR spectra were run on a Varian Unity Inova spectrometer (1H 400 MHz), equipped with a broad band and an inverse 1H{13C} PFG probe. The following methods were applied routinely: 1D NMR: selective CW decoupling, NOE-difference, DPGSE-NOE, 2D NMR: PFG-HSQC, PFG-HMBC. The above mentioned 2D experiments were used for mapping the partners of coupling through one or more bonds, while NOE measurements were applied for the detection of nuclei being in dipolar coupling, i.e. for the determination of spatial proximity.

NEW SCIENTIFIC RESULTS

1. Enzymes of known structure, α -chymotrypsin, ribonuclease, triacylglycerol lipase, and alkaline phosphatase have been labeled by organometallic carbonyl compounds on their surface. By comparison with the spectroscopic data related to the interaction of the organometallic tag with model compounds like amino acids, ethylammonium chloride, sodium propionate, poly-amino acids and N-protected amino acids, the binding sites on the enzyme surface have been determined. I have found that reaction of the four investigated enzymes with the organometallic tag occurred predominantly by formation of adducts with lysine side chains at the enzyme surface with slightly different mechanism in each case. The protein – organometallic tag interaction could be differentiated according to the direction of the frequency shifts observed in the IR spectrum.

I proved that the above mentioned organometallic tags were acting as real bioprobes, i.e. they responded to the pH changes near the enzyme surface by reversible IR frequency shifts. For the first time in the literature, I applied these organometallic tags in real biological systems. I have recorded the IR spectra of irontricarbonyl labeled flavonoids, attached to the protein fractions of plant extracts.

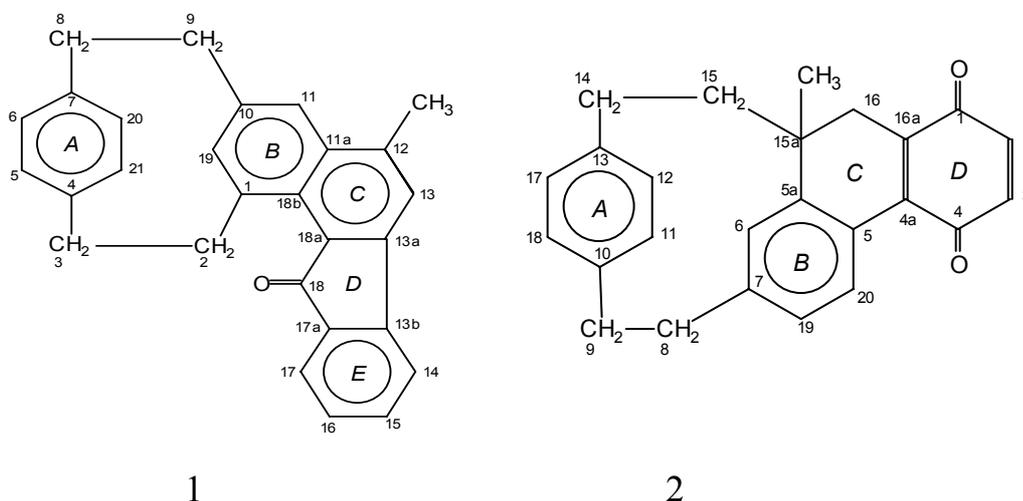
2. Uhle's ketone is the starting material of several organic syntheses leading to ergoline type compounds. Infrared spectroscopy was applied as a first control measurement to check the reaction products. Two types of the investigated molecules have been distinguished: molecules with a skeleton similar to Uhle's ketone (1), and oxidized (dehydrogenated) molecules in two different tautomeric forms: keto (2a) and enolic (2b).



In the case of the latter compound the usually strong, characteristic carbonyl band appeared with unusually low frequency and intensity. In order to find theoretical support or justification to this phenomenon, the scaled quantum mechanical force field method was invoked using semiempirical AM1, ab initio RHF/3-21G, and density functional B3LYP/6-31G* calculations followed by full normal coordinate analysis. The fairly good agreement between the observed frequencies and those calculated at the DFT level permitted to make reliable band assignments. There was a looser correlation between the

observed and DFT calculated relative absorption intensities. It was stated that the expected $\nu\text{C}=\text{O}$ band intensity is divided between two coupled vibrations making the carbonyl group unrecognizable in the IR spectrum of 2a.

3. NMR measurements were applied for the structure determination of two new compounds, obtained in rather low yields, as side products in Diels-Alder cycloaddition reactions.



The formation of the metaparacyclophane skeleton was undoubtedly confirmed for both molecules. The spatial arrangement, and the configuration of the asymmetry center have been proved on the basis of NOE experiments.

The minimized energy conformations of the investigated compounds have been determined by semiempirical AM1 calculations. They reliably explained the effects influencing the

proton shifts arising from the magnetic anisotropy of aromatic rings.

4. The mechanism of nucleophilic addition reactions of polycyclic heteroaromatic compounds with a bridgehead nitrogen atom has been studied. Connection between the reaction pathway and the structure of the reacting salts has been found, supported by NMR data. The nucleophilic attack is mainly influenced by the charge distribution along the molecule while the site of a possible ring opening is determined by the bond orders. The ^{13}C chemical shift was considered as a diagnostic NMR parameter, being also influenced by the atomic charges near the carbon nuclei and the bond orders.

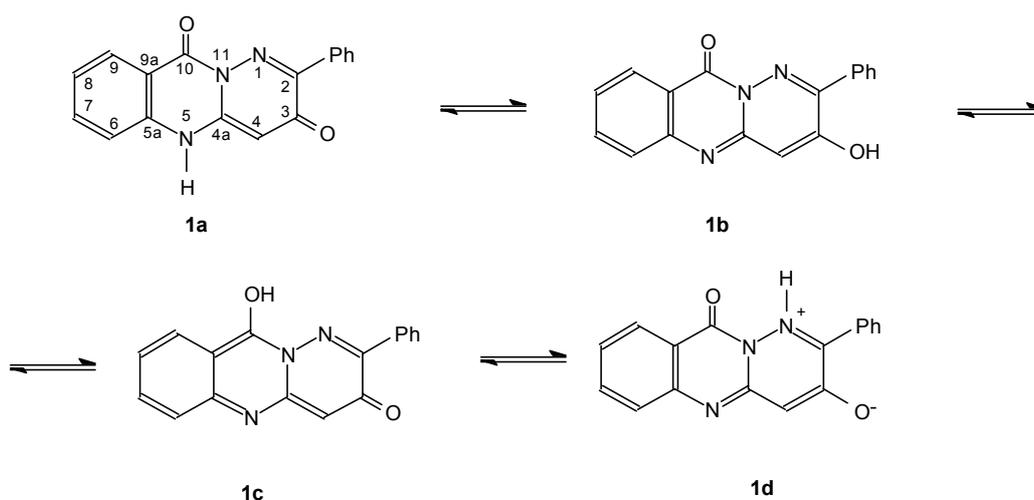
The introduction of electron withdrawing groups into the triazinium salts did not cause a substantial change in the charge distribution, so the carbon chemical shifts as well as the reaction pathways remained unchanged.

The alteration of the anellation of the studied thiazoloazinium salts involved important changes in the electronic distribution, i.e. influenced the atomic charges on the corresponding atoms together with the bond orders, while the rest of the thiazole ring remained unchanged. Both, the atomic charges and the bond orders were calculated by quantum chemical methods. The observed differences of the calculated parameters were also reflected in the measured carbon chemical shifts. So far two out

of the four theoretically possible products of the nucleophilic addition have been identified by NMR methods.

5. Vibrational spectroscopy and NMR methods have been combined, while studying the regioselective alkylation reactions of the linearly fused tricyclic heteroaromatic compound depicted in the next figure.

The tautomeric forms existing in solid or liquid phase have been studied separately; their vibrational spectra were assigned on the basis of theoretical calculations.



The studied tricyclic heteroaromatic compound could be attacked by the alkylation agent at four positions (N1 or N5 and O3 or O10) depending on the reaction conditions. The NMR measurements carried out on the product of the alkylation under anhydrous conditions revealed its structure as a

mixture of N5 and O3 alkylated derivatives. Their NMR data were completely assigned.

The alkylation in aqueous base resulted in an unexpected ring opening followed by the formation of two N-alkylated products, one of them having a zwitterionic structure.

APPLICATION OF THE RESULTS

The results presented here are stimulating further research activities in the fields covered by this Thesis.

Tagging the enzyme surface by organometallics proved unambiguously that the applied irontricarbonyl complexes are excellent biosensors, being able to monitor the changes nearby the surface. Several possibilities are arising to develop them, either by applying new organometallics, or by using new biological media.

Structure elucidation of the starting materials of some organic syntheses – ergoline and cyclophane derivatives – has been performed. The presented work provides a firm basis for answering the questions that may arise in further investigations of new derivatives.

Regioselective alkylations and nucleophilic addition reactions were studied applying vibrational spectroscopy and NMR methods. Based on NMR data, correlation has been found between the

nucleophilic reaction pathway and the structure of the reacting salts.

The observed rules may help in planning further synthetic work.

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