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Aromatic hydrazones derived from nicotinic acid hydrazide as fluorimetric pH sensing molecules: Structural analysis by computational and spectroscopic methods in solid phase and in solution

T. Benković [a], A. Kenđel [a], J. Parlov-Vuković [b], D. Kontrec [c], V. Chiş [d], S. Miljanić [a], N. Galić [a,*]

[a] *Division of Analytical Chemistry, Department of Chemistry, Faculty of Science, University of Zagreb, Horvatovac 102a, HR-10000 Zagreb, Croatia*
[b] *INA — Industrija nafte, d.d., HR-10000 Zagreb, Croatia*
[c] *Department of Organic Chemistry and Biochemistry, Ruđer Bošković Institute, PO Box 180, HR-10002 Zagreb, Croatia*
[d] *Faculty of Physics, Babeş-Bolyai University, Kogălniceanu 1, RO-400084 Cluj-Napoca, Romania*

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ABSTRACT

Structural analyses of aroylhydrazones were performed by computational and spectroscopic methods (solid state NMR, 1 and 2D NMR spectroscopy, FT-IR (ATR) spectroscopy, Raman spectroscopy, UV–Vis spectrometry and spectrofluorimetry) in solid state and in solution. The studied compounds were *N*′-(2,3-dihydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**1**), *N*′-(2,5-dihydroxyphenylmethylidene)-3-pyridinecarbohydrazide (**2**), *N*′-(3-chloro-2-hydroxy-phenylmethylidene)-3-pyridinecarbohydrazide (**3**), and *N*′-(2-hydroxy-4-methoxyphenyl-methylidene)-3-pyridinecarbohydrazide (**4**). Both in solid state and in solution, all compounds were in ketoamine form (form I, —CO—NH—N=C—), stabilized by intramolecular H-bond between hydroxyl proton and nitrogen atom of the C=N group. In solid state, the C=O group of **1**–**4** were involved in additional intermolecular H-bond between closely packed molecules. Among hydrazones studied, the chloro- and methoxy-derivatives have shown pH dependent and reversible fluorescence emission connected to deprotonation/protonation of salicylidene part of the molecules. All findings acquired by experimental methods (NMR, IR, Raman, and UV–Vis spectra) were in excellent agreement with those obtained by computational methods.

1. Introduction

Although the chemistry of aryl (Ar—NH—N=CH—R; R=alkyl or aryl) and aroyl (Ar—CO—NH—N=CH—R; R=alkyl or aryl) hydrazones has been extensively studied for over a century [1], they still attract great attention due to their versatile properties and applications. Many compounds of this type are biologically active and have found their use in medicine [2–4]. Synthetic flexibility, simple preparation procedure and the chelating abilities towards metal ions have made them attractive as analytical reagents [5–7]. While most of the older work on hydrazones focused on their applicability in classical analysis [8], nowadays these systems are acknowledged as multitopic ligands for the targeted construction of extended metal-organic architectures. Due to unique structural properties, hydrazones were used as molecular switches and sensors for detecting metal cations, anions and neutral molecules [9–15]. They have also been described as low molecular weight gelators [16], and as molecules bearing robotic arms [17,18].

Aroylhydrazones can be involved in keto-enol tautomeric interconversion (Scheme 1). Since different forms can have diverse properties, the detailed study of such equilibria is relevant not only from theoretical point of view, but also for successful application of these compounds. Ketoamino tautomer (—CO—NH—N=CH—, form I, Scheme 1) is usually the most stable form in solution and in the solid state, since it is stabilized by strong intermolecular or solute-solvent hydrogen bonds [19–23]. In the continuation of our research [7,19,22–27] on aromatic hydrazones in this work we have used spectroscopic techniques (solid state NMR, FT-IR, Raman, UV–Vis, fluorescence), and computational study for structural investigation of hydrazone derivatives (Scheme 2) in solid state and in solution. The influence of solvent polarity, pH value or irradiation on tautomeric equilibria was investigated as well. The deprotonation and protonation of —Cl and —OCH_3 derivatives caused the fluorescence to turn “on” and “off”, making these compounds pH sensors. Furthermore, for —OCH_3 derivative such reversible

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[*] Corresponding author.
E-mail address: ngalic@chem.pmf.hr (N. Galić).

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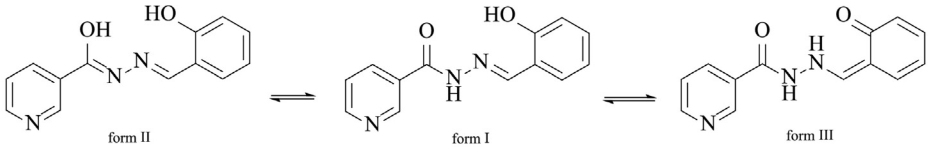


Scheme 1. Possible tautomeric forms of aroylhydrazones.

reaction and two corresponding structural forms of hydrazone molecule can be distinguished by the IR spectroscopy as well.

2. Material and Methods

2.1. Reagents

Compounds **1**–**4** were prepared according to previously reported method [27], by mixing equimolar amounts of nicotinic acid hydrazide (Fluka) with aldehyde (2,3-dihydroxybenzaldehyde, 2,5-dihydroxybenzaldehyde, 2-hydroxy-3-chlorobenzaldehyde and 2-hydroxy-4-methoxybenzaldehyde purchased from Sigma). The reactions were carried out in dry ethanol under argon atmosphere at 85 °C for 20 h. The solvent were evaporated from reaction mixtures and the solids were suspended in CH_2Cl_2 (EtOH was used for **2**), filtered and dried at 50 °C for 24 h. Hydrazones were characterized by standard analytical methods [27]. Deionized water and organic solvents of p.a. purity grade (Kemika) were used for spectroscopic measurements.

2.2. Apparatus

FT-infrared spectra were acquired by means of attenuated total reflectance (ATR) technique on a Bruker Equinox 55 interferometer, using the PIKE MIRacle ATR sampling accessory with a diamond/ZnSe crystal plate. A pressure clamp was used to press the powder substance during the measurement of the solid samples, whereas a drop of the solution was deposited on the crystal plate prior to the measurement of the liquid samples. A spectrum of the respective solvent was used as a background while measuring the ATR spectra of the hydrazone solutions. The ATR spectra were recorded in the single reflection configuration, over the $4000-600$ cm^{-1} spectral range at the resolution of 4 cm^{-1}. A total of 32 scans were averaged for a spectrum. The measured spectra were corrected using the extended ATR correction within the OPUS 6.0 program.

FT-Raman spectra were taken on a Bruker Equinox 55 interferometer equipped with the FRA 106/S Raman module, using Nd-YAG laser excitation at 1064 nm and the laser power of 100 mW and 500 mW for the measurement of the solid substances and solutions, respectively. The solid samples were placed on an aluminium holder, while glass vials were used for handling liquid samples. Spectra were taken in the spectral range between 3500 and 100 cm^{-1}. To obtain a good spectral definition, 128 scans at the spectral resolution of 4 cm^{-1} were averaged for a spectrum.

The NMR spectra were recorded on a Bruker Avance III HD 400 MHz/54 mm Ascend and Bruker Avance 300 NMR spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C using a C/H dual 5 mm probe. Proton spectra with spectral width of 6200 Hz and a digital resolution of 0.09 Hz per point were measured with 64 scans. APT spectra with spectral widths of 18,000 Hz were collected with 256 scans. Digital resolution was 0.27 Hz per point. In the gCOSY experiment 2048 points in the $f2$ dimension and 256 increments in the $f1$ dimension were used. For each increment 16 scans and the spectral width of 4006 Hz were applied. Digital resolution was 1.96 and 15.65 Hz per point in $f2$ and $f1$ dimensions, respectively. Typical spectral conditions for gHMQC and gHMBC spectra were as follows. For gHMQC spectra, spectral width was 3086 and 11,655 Hz in $f2$ and $f1$ dimensions, respectively. 1 K data points were used in the time domain and 256 increments were collected for each data set. The resulting digital resolution was 3.01 and 45.5 Hz per point in $f2$ and $f1$ dimensions, respectively. Spectral width for gHMBC spectra was 3906 and 16,779 Hz in $f2$ and $f1$ dimensions, respectively. In the time domain, 4 K data points were applied. The number of increments for each data set was 256. The acquired spectra had digital resolution of 0.95 and 65.5 Hz per point in $f2$ and $f1$ dimensions, respectively.

Solid state NMR spectra were recorded on Bruker Avance 300 spectrometer equipped with a 4 mm broad band magic angle spinning (MAS) probe. The samples for ^{13}C CP-MAS spectra were spun at the magic angle with 15 kHz. External references were adamantane and glycine. The spectra were acquired with 8000 scans and repetition delay of 7 s.

UV–Vis measurements were carried out using Varian Cary 3 spectrometer. The absorption spectra were recorded in the spectral range from 200 nm to 500 nm. Conventional quartz cells ($l = 1$ cm) were used throughout.

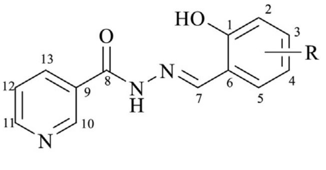
Excitation and emission spectra were recorded on a Perkin Elmer LS55 spectrofluorimeter using standard quartz cell ($l = 1$ cm). Excitation and emission slit widths were 10 nm.

2.3. Samples Preparation

Samples for the measurement of ATR and Raman spectra were prepared by dissolution of the solid substances **1**–**4** in DMSO, resulting in the final hydrazone concentration of 0.20 M. For the measurement of pH dependent ATR spectra, compounds **3** and **4** were dissolved in methanol in concentration of 0.05 M. To obtain alkaline conditions, 1 M NaOH was added into the methanolic solution of hydrazone, which was afterwards neutralized by addition of 1 M HCl. The alternate base/acid additions were repeated twice.

The NMR spectra were recorded in deuterated solvents (DMSO d_6 and MeOD) in sample concentration range from 2 to 30 mg/mL with TMS as the internal standard. For measurements including the addition of base (trimethylamine, $w = 99\%$; $\rho = 0.73$ kg/L) the 0.05 M solution of hydrazones were used.

Stock solutions of compounds **1**–**4** ($c = 1 \times 10^{-3}$ M) were prepared in chloroform, dioxane, acetonitrile (MeCN), dimethylsulphoxide (DMSO), and methanol (MeOH). The working solutions for UV–Vis measurements were prepared in a 10.0 mL flask in pure organic solvents as well as in MeCN/water, DMSO/water and MeOH/water mixtures by adding stock solution of **1**–**4** and an appropriate amount of organic solvent or water. Concentration of the hydrazones in the working samples was 5×10^{-5} M. For fluorescence measurements more diluted solutions were used, concentrations varied from 5×10^{-7} to 2×10^{-5} M. For measurements in acidic/basic media the mixture



R = 2-OH (**1**); 4-OH (**2**); 2-Cl (**3**); 3-OCH_3 (**4**)

Scheme 2. Structure of aroylhydrazones **1**–**4**.