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ORIGINAL ARTICLE

Tyrammonium 4-nitrophthalate dihydrate: structural and spectroscopic elucidation

Tsonko Kolev · Bojidarka B. Koleva · Rüdiger W. Seidel · Heike Mayer-Figge · Michael Spiteller · William S. Sheldrick

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Abstract Tyrammonium 4-nitrophthalate has been synthesized and its structural and spectroscopic properties elucidated by single-crystal X-ray diffraction, solid-state polarized IR-spectroscopy of oriented colloids in a nematic host, HPLC with tandem mass spectrometry (HPLC ESI-MSMS), and TGV and DSC methods. The compound crystallizes in the monoclinic $P2_1/c$ space group and its structure consists of a 3D network of molecules joined by intermolecular interactions with the participation of cations, anions and two solvent molecules. The tyrammonium cation adopts a *T trans* configuration with corresponding angles of $\phi_1 = 76.0(4)°$, $\phi_2 = 54.8(1)°$ and $\phi_3 = 63.4(1)°$, respectively. In the 4-nitrophthalate anion, the COO^- and COOH groups are turned off the plane of the benzene ring at angles of $\tau_1 = 88.1(5)°$ and $\tau_2 = 22.1(7)°$, respectively.

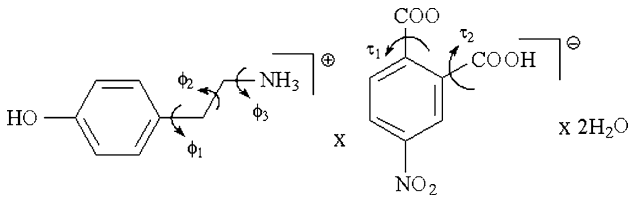
Keywords Tyrammonium 4-nitrophthalate dihydrate · Crystal structure · Solid-state linear polarized IR-spectroscopy · HPLC ESI MS/MS

Introduction

As a part of our systematic structural and spectroscopic studies of biologically active small molecules (Kolev et al. 2007a, b, c) we report herein the crystal structure and spectroscopic properties of tyrammonium 4-nitrophthalate dihydrate (Scheme 1). Tyramine is a metabolic product of tyrosine and an endogenous ligand for mammalian trace amine-associated receptors and may act as a neuromodulator that regulates neuronal activity in the basal ganglia (Shen and Johnson 2005). Clinical assessments of its autonomic function often include tyramine infusion (Holmes et al. 2005). A full understanding of the competitive receptor-binding manner of small molecules requires knowledge of the conformational preference of these molecules, which depend mainly on the protonation processes. Independent of this requirement, experimental studies of correlations of structure-spectroscopic properties are rare. Only the crystal structures of several tyrammonium salts have been previously reported (Ishida and Inoue 1981; Tamura et al. 1974; Ogawa et al. 1980; Zakaria et al. 2002; Koleva et al. 2007a, b) In-depth quantum chemical calculations have been concerned with the conformations of neutral tyramine, and its zwitterion both in the gas phase and in aqueous solution. Tyraminium hexahydrate was studied for this purpose (Melandri et al. 2007; Nagy et al. 2005). It was found that the most stable conformers in the gas phase are the folded ones in which the amino hydrogen atoms interact with the aromatic π-cloud, indicating that the conformational properties of tyramine are mainly determined by non-bonding interactions (Melandri and Maris 2004). The quantum chemical calculations in solution show that the water solvent causes remarkable modifications of the torsion angles relative to the gas phase values (Nagy et al. 2005). So, in this paper, we have focused on the correlation of the structural and IR-spectroscopic properties of tyrammonium 4-nitrophthalate dihydrate, by means of single-crystal X-ray diffraction and solid-state IR-polarized (IR-LD) spectroscopy of oriented colloids as suspensions in a nematic liquid crystal

T. Kolev · M. Spiteller
Institut für Umweltforschung, Universität Dortmund, Otto-Hahn-Strasse 6, 44221 Dortmund, Germany

B. B. Koleva (✉) · R. W. Seidel · H. Mayer-Figge · W. S. Sheldrick
Lehrstuhl für Analytische Chemie, Ruhr-Universität Bochum, Universitätsstraße 150, 44780 Bochum, Germany
e-mail: BKoleva@chem.uni-sofia.bg



Scheme 1 Chemical diagram of tyrammonium 4-nitrophthalate dihydrate

(Ivanova et al. 2004, 2006, 2007; Koleva et al. 2007a, b). Crystal structures containing the 4-nitrophthalate anion are rare and only six structures, namely those of 1,10-Phenanthrolin-1-ium 2-carboxy-4-nitrobenzoate (Guo 2005), Anilinium 2-carboxy-4-nitrobenzoate, 4-Chloroanilinium-, 4-Bromoanilinium—and 4-Iodoanilinium 2-carboxy-4-nitrobenzoate as well as 1-Methylpiperazine-1,4-di-ium 4-nitrophthalate 4-nitrophthalic acid monohydrate have been reported previously (Glidewell et al. 2005).

Experimental

Methods

The X-ray diffraction intensities were measured in the ω scan mode on a Siemens P4 diffractometer equipped with Mo K_α radiation (λ = 0.71073 Å, θ_{max} = 25°). The structure was solved by direct methods and refined against F^2 (Sheldrick 1995, 1997). An ORTEP plot in Fig. 1 illustrates the anion and cation structures at the 50% probability level. The hydrogen atoms were constrained to calculated positions and refined using a riding model in all cases.

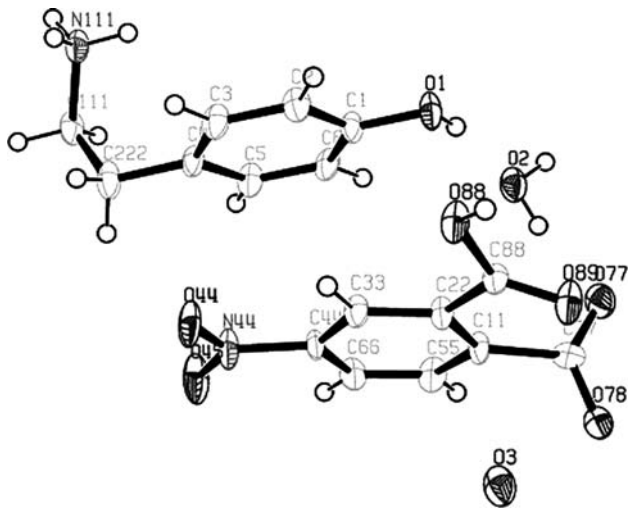


Fig. 1 ORTEP plot of the 50% thermal ellipsoids; hydrogen atoms are shown as spheres of arbitrary radii

Conventional and polarized IR-spectra were measured on a Thermo Nicolet OMNIC FTIR-spectrometer (4,000–400 cm^{-1}, 2 cm^{-1} resolution, 200 scans) equipped with a Specac wire-grid polarizer. Non-polarized solid-state IR spectra were recorded using the KBr disc technique. The oriented samples were obtained as a colloid suspension in a nematic liquid crystal ZLI 1695. The theoretical approach, experimental technique for preparing the samples, procedures for polarized IR-spectra interpretation and the validation of this new linear-dichroic infrared (IR-LD) orientation solid-state method for accuracy and precision, has been presented previously. The influence of the liquid crystal medium on peak positions and integral absorbances of the guest molecule bands, the reological model, the nature and balance of the forces in the nematic liquid crystal suspension system, and the morphology of the suspended particles have also been discussed (Ivanova et al. 2004, 2006, 2007; Koleva et al. 2007a, b).

HPLC-MS/MS analysis

The analyses of the samples were performed with a Thermo Finnigan surveyor LC-Pump. Compounds were separated on a Luna C18 column (150 × 2 mm, 4 µm particle size) from Phenomenex (Torrance, CA, USA). The mobile phase consisted of water + 0.1% HCOOH (A) and acetonitrile + 0.1% HCOOH (B) using a gradient program. The compound was detected via UV and a TSQ 7000 (Thermo Electron Corporation, Dreieich, Germany) mass spectrometer. The spectra were obtained using the TSQ 7000 equipped with an ESI ion source and operated under the following conditions: capillary temperature 180°C; sheath gas 60 psi and spray voltage 4.5 kV. One mg/ml of the sample was dissolved in acetonitrile and injected into the ion source by an auto sampler (Finnigan Surveyor). The Excalibur 1.4 software was employed to process the data.

The elemental analysis was carried out according to the standard procedures for C and H (as CO_2, and H_2O) and N (by the Dumas method). The thermogravimetric study was carried out using a Perkin-Elmer TGS2 instrument. The calorimetric studies were performed on a DSC-2C Perkin Elmer apparatus under argon.

Synthesis

Tyrammonium 4-nitrophthalate dihydrate was synthesized by mixing tyramine (0.5630 g) and 4-nitrophtalic acid (0.2222 g) dissolved in 10 ml water at a 1:1 molar ratio. The resulting solution was stirred continuously for 4 h at 40°C and then left to evaporate slowly over a period of about a week. The colourless crystalline was filtered off

and dried under air at 298 K. The following values were found: C, 50.03; H, 5.23; N, 7.30; [$C_{16}H_{20}N_2O_9$] calcd.: C, 50.00; H, 5.25; N, 7.29%; Yield 92 %. The TGV and DSC data in the temperature range of 300–500 K show a weight loss of 9.37% (enthalpy effect ΔH of 14.21 kcal/mol] at 410 K corresponding to the two water molecules included in the crystal lattice of the compound. The HPLC ESI-MS/MS spectra of tyrammonium 4-nitrophthalate dihydrate as a function of the relative abundancy versus time (*t*) are obtained. The data afford a peak at *m/z* 238.94 (*t* = 16.91) corresponding to the ammonium (NH_4^+) adduct of the $[C_8H_{12}NO]^+$ ion with a molecular weight of 220.20. It is well known that the Na^+ and NH_4^+ adducts are typical for ESI-MS and for this reason, a combination of HPLC MS–MS determination is preferable for the analysis of peptides and their derivatives.

Results and discussion

Crystal structure of tyrammonium 4-nitrophthalate dihydrate

The structure of tyrammonium 4-nitrophthalate dihydrate (Fig. 1) consists of a 3D network (Fig. 2) of molecules joined by intermolecular interactions with participation of cationic and anionic species as well as water molecules. Hydrogen bonds of types $_{(Tyr)}NH_3^+ \ldots COO^-$ (2.914 Å), $_{(Tyr)}NH_3^+ \ldots O = COH$ (2.866 Å), $_{(Tyr)}NH_3^+ \ldots ONO$ (3.039 Å), $_{(Tyr)}NH_3^+ \ldots OH_2$ (2.832 Å), $_{(Tyr)}OH \ldots OH_2$ (2.611 Å), $HOH \ldots OCO$ (2.748 and 2.741 Å) and $_{(phth)}OH \ldots OCO^-$ (2.604 Å) are observed. The tyrammonium cation adopts the *T trans* configuration with ϕ_1–ϕ_3 angle values of 76.0(4)°, 54.8(1)° and 63.4(1)° (Scheme 1, Fig. 2). The effect of the counterion on the configuration of tyrammonium cation can be illustrated comparing the obtained data with those of bis(tyrammonium)sulfate (Kolev et al. 2007c), where differences of 67.37°, 4.5° and 7.37° for ϕ_1–ϕ_3 angles have been obtained. In this case the unit cell contains two non-equivalent cations with *T* and pseudo *T trans* configuration, respectively. The $\tau_1$and τ_2 angles (Scheme 1) in the 4-nitrophthalate anion are 88.1(5)° and 22.1(7)°, respectively. These data are in accordance with previously reported results for 4-nitrophthalates (Sheldrick 1997, Shen and Johnson 2005, Tamura et al. 1974), where the corresponding angles vary within the ranges 88.0(0)–92.1(6) and 11.4(3)–26.7(5)°, respectively.

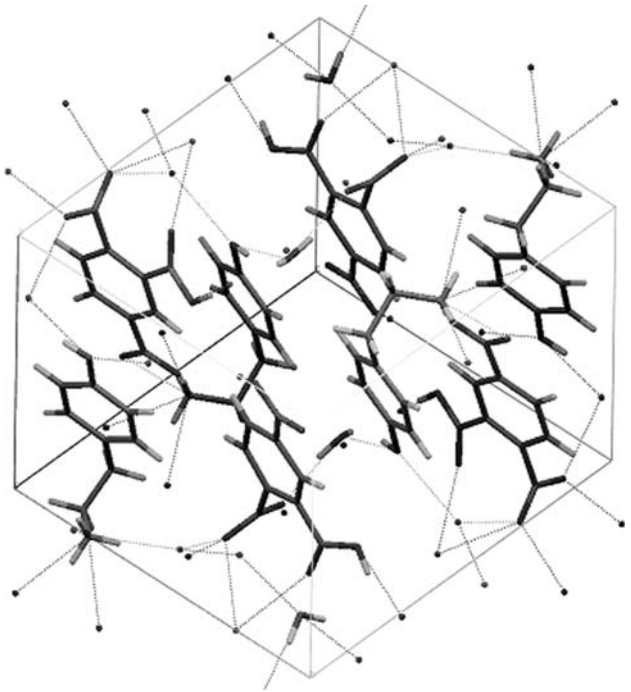


Fig. 2 Hydrogen bonding in the crystal of tyrammonium 4-nitrophthalate dihydrate

Conventional and linear-polarized IR-spectroscopy

Non-polarized IR- and difference IR-LD spectra are depicted in Fig. 3. According to the methodology described in Ivanova at al. (2004, 2006, 2007) and Koleva et al. (2007a, b), a reasonable degree of macro-orientation of the suspended particles is obtained. In the whole 3,500–400 cm^{-1} region, the IR-spectrum (Fig. 3.1) is characterized by a significant overlapping effect due to the absorption bands of both the cationic and anionic moieties as well as the presence of two solvent molecules. In the 3,500–1,700 cm^{-1} range, the broad absorption peak of ν^{as}_{NH3} and ν^{s}_{NH3} stretches overlaps with the aromatic in-plane modes of both the benzene fragments (of tyrammonium cation and 4-nitrophthalate anion) as well as the ν_{OH} of the solvent molecules and ν_{OH} of the COOH group in the 4-nitrophthalate anion. A series of bands are observed between 1,750 and 1,450 cm^{-1} starting with intensive maxima at 1,693 and 1,635 cm^{-1} belonging to δ^{as}_{NH3+} and $\delta^{as'}_{NH3+}$. The band at 1,711 cm^{-1} belongs to the $\nu_{C=O}$ stretch of COOH in the 4-nitrophthalate anion. The **8a** and **8b** bands of the differently substituted benzenes are at 1,614 cm^{-1} (**8a**, 4-nitrophthalate) and 1,609 cm^{-1} (**8a**, tyrammonium cation), while the bands at 1,595 and 1,585 cm^{-1} are assigned to **8b** modes of the corresponding species. The intensive bands at 1,518 and 1,345 cm^{-1}, correspond to ν^{as}_{NO2} and to ν^{s}_{NO2} frequencies and those at 1,527 and 1,401 cm^{-1}—to ν^{as}_{COO-} and ν^{s}_{COO-}. Direct confirmation of this assignment follows from the reduced IR-LD spectra where the ν^{s}_{NO2} and ν^{s}_{COO-} stretches are eliminated at an equal dichroic ratio (Fig. 4), due to the co-linear orientation of the corresponding transition moments

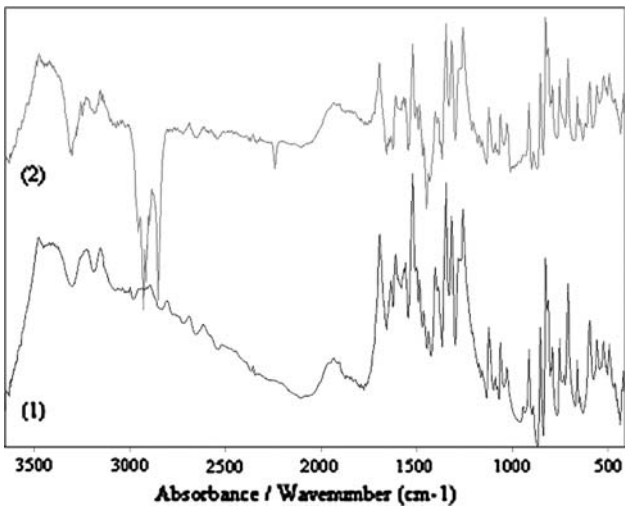


Fig. 3 Non-polarized IR-(1) and difference IR-LD (2) spectra of tyrammonium 4-nitrophthalate dihydrate

in the 4-nitrophthalate anion (Fig. 4), where all of the molecules are close to coplanar in the unit cell. This is also valid for the mutual disposition of the corresponding out-of plane ($\mathbf{B_1}$) modes of the different substituted benzenes in the tyrammonium cation and 4-nitrophthalate anion (Fig. 5), which leads to the simultaneous disappearance of the bands at 850 cm^{-1} ($\mathbf{11}\gamma_{CH}$, 4-nitrophthalate) and 825 cm^{-1} ($\mathbf{11}\gamma_{CH}$, tyrammonium cation) at an equal dichroic ratio (see Fig. 5).

It could be concluded that the new salt of tyramine with 4-nitrophthalic acid has been synthesized, isolated and

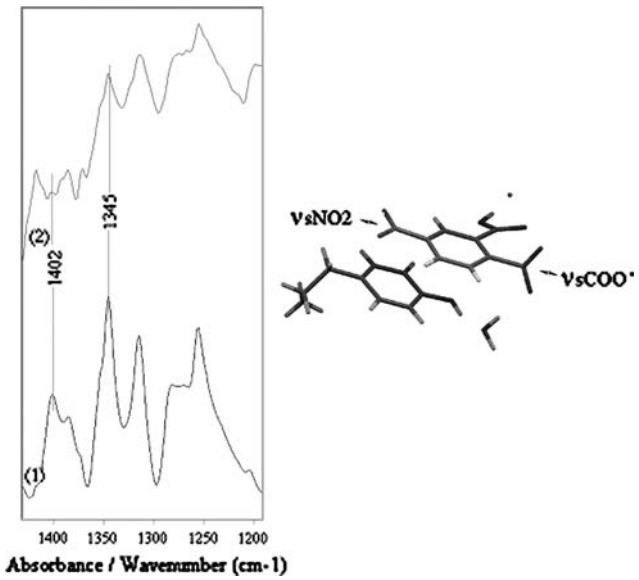


Fig. 4 Non-polarized IR-(1) and reduced IR-LD (2) spectra of tyrammonium 4-nitrophthalate dihydrate after elimination of the band at 1,345 cm^{-1}

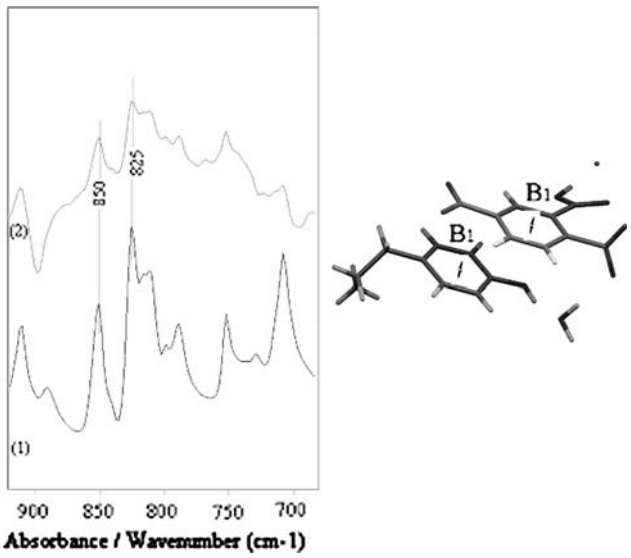


Fig. 5 Non-polarized IR-(1) and reduced IR-LD (2) spectra of tyrammonium 4-nitrophthalate dihydrate after elimination of the band at 850 cm^{-1}

structurally determinated by single crystal X-ray diffraction. The spectroscopic properties have been elucidated by means of solid-state polarized IR-spectroscopy of oriented colloids in a nematic host, HPLC with tandem mass spectrometry (HPLC ESI-MSMS), TGV and DSC methods. A monoclinic $P2_1/c$ space group is obtained of the compound studied. The crystal structure consists of a 3D network of molecules joined by intermolecular interactions with participation of cations, anions and two solvent molecules. The tyrammonium cation adopts the *T trans* configuration with corresponding angles of $\phi_1 = 76.0(4)°$, $\phi_2 = 54.8(1)°$ and $\phi_3 = 63.4(1)°$, respectively. In the 4-nitrophthalate anion, the COO^- and COOH groups are turned off the plane of the benzene ring at angles of $\tau_1 = 88.1(5)°$ and $\tau_2 = 22.1(7)°$, respectively.

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