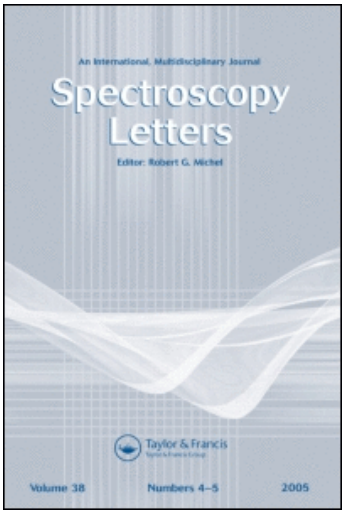

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Bojidarka B. Ivanova[a]
[a] Department of Chemistry, University of Sofia, Sofia, Bulgaria

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N_1 Protonated Salt of Adenine: Solid-State Linear Dichroic Infrared Spectral Analysis

Bojidarka B. Ivanova
Department of Chemistry, University of Sofia, Sofia, Bulgaria

Abstract: An illustration of the possibilities of solid-state linear-dichroic infrared (IR-LD) spectral analysis of a suspension of a sample in a nematic liquid crystal for supramolecular stereostructural characterization is demonstrated comparing the IR-LD spectral results of solid adenine (Ade) and its new N_1 protonated salt (AdeH). In addition, a detailed IR-spectral characterization of the AdeH derivative that explains the corresponding frequencies that change as a result of the protonation process was also done.

Keywords: Adenine, N_1-protonated form, solid-state IR-LD

INTRODUCTION

Of the naturally occurring nucleobases, the adenine moiety exhibits the most flexible binding-site behavior with metal ions and intermolecular hydrogen bond formation. The ring nitrogens N_1 and N_7 are the predominant binding sites.[1] In aqueous solution, the distribution of the metal ions or protons between these sites depends on the pH, the metal, and the other ligands coordinated to the metal, respectively.[2] The tautomerism of the N_1 and N_7 protonated forms of adenine has been studied in a series of papers, including variation of the pH values and obtaining the N_1, N_7, or double site protonated forms of the adenine, and study of the structures[3,4] or spectroscopic characteristics.[5–9] As far as the important biological activity of adenine as a cell structural fragment, this depends on both the neutral and protonated forms.

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Address correspondence to Bojidarka B. Ivanova, Department of Chemistry, University of Sofia, 1164 Sofia, Bulgaria. E-mail: ahbi@chem.uni-sofia.bg

The current work deals with the linear-dichroic spectral analysis of the solid adenine, and its protonated N_1 form, using a nematic liquid crystal suspension methodology. It illustrates a method for orientation of solid-state samples with a view to the prediction of protonation or coordination position in purine derivatives. An IR-LD spectral analysis of amino-purine derivatives was demonstrated using the known polyvinyl chloride (PVC) film technique.[10]

EXPERIMENTAL

Materials and Methods

The adenine was purchased from Bachem Organics (Germany).The polarized and nonpolarized IR spectra in $4000-400\,cm^{-1}$ range were obtained on a Bomem Michelson100 FT-IR Spectrometer equipped with a Perkin-Elmer (Germany) wire-grad polarizer. One hundred fifty scans were performed for each spectrum with a resolution of $2\,cm^{-1}$. The solid-state sample was recorded as KBr pellets. A suspension in 4-cyano-4′-alcylbicyclohexyl mixture (ZLI-1695, Merck, Germany), was used for orientation of compounds. The IR-LD measurements and technique for orientation of solid-state samples as a suspension in nematic liquid crystal have been described in Refs.[11,12,14] An effective orientation was obtained through the following procedure: about 5 mg of the solid sample is added to the appropriate liquid crystal substance until a slightly viscous mixture is produced. The suspension thus prepared is pressed between two KBr-plates also rubbed out in advance in one direction by means of fine sandpaper. The grinding of the prepared mull in the rubbing direction promotes an additional orientation of the sample. The poor IR-spectrum of the used nematic liquid crystal (ZLI 1695), compared to those of Nujol, makes it possible to record the guest compound bands in the whole $4000-400\,cm^{-1}$ range. The presence of the isolated nitrile stretching IR-band at $2233\,cm^{-1}$ additionally serves as an orientation indicator. The corresponding absorbtion ranges of the nematic mesophase are given in Figs. 1 and 2.

The principle and opportunities of IR-LD spectroscopy were described first in Ref.[13]. The procedure used for the IR-LD data interpretation is grounded in subtraction of the perpendicular spectrum, (IRs, resulting from 90° angle between the light beam electric vector and the orientation of the sample) from the parallel one (IRp) obtained with a colinear mutual orientation. The recorded *difference* (IRp − IRs) spectrum (Fig. 1),[1] divides the corresponding parallel (Ap, Fig. 1),[2] and perpendicular (As, Fig. 1),[3] integrated absorbancies of each band into positives originating from transition moments that form an average angle with the orientation direction (**n**) between 0° and 54.7° (magic angle) and negative ones corresponding to transition moments between 54.7° and 90°. A positive development of this method is published in Refs.[15,16] The perpendicular spectrum, multiplied by the

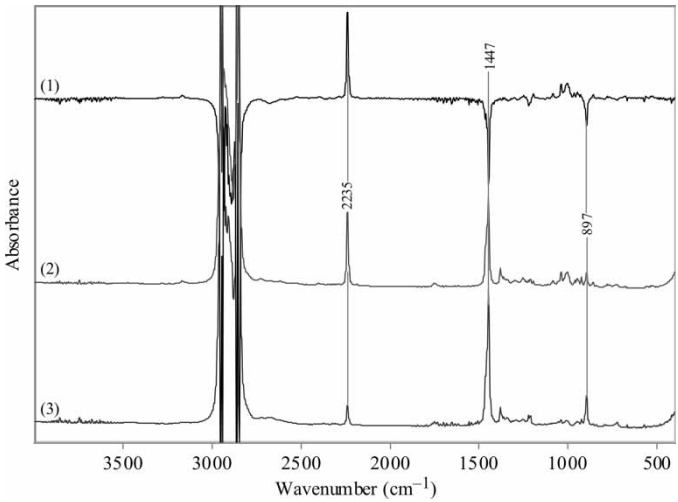


Figure 1. The 4000–400 cm^{-1} region IR-LD spectra of ZLI 1695: difference (1), parallel (2), and perpendicular (3) ones.

parameter **c**, is subtracted from the parallel one and **c** has to vary until a band or set of bands are eliminated. The simultaneous disappearance of bands in the resultant (IRp − cIRs) *reduced* IR-LD spectrum indicates a colinearity of the corresponding transition moments and the attribution of vibrations to an equal

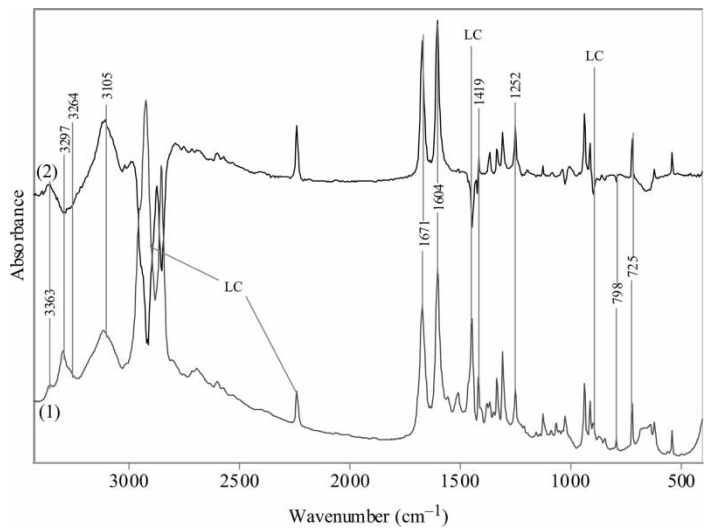


Figure 2. Nonpolarized (1) and difference IR-LD (2) solid-state spectra of adenine as suspension in ZLI 1695.

symmetry class, giving rise to information regarding the mutual disposition of the molecular fragments. This elimination method is graphically carried out using a subtraction procedure attached to the program for processing of IR spectra. The so-called stepwise reduction procedure for interpretation of the polarized IR-LD spectra is described in Refs.[12,14,15].

The Fast Atom Bombardment (FAB) mass spectra were measured on a Fisons VG (Germany) Autospect instrument employing 3-nitronenzyl alcohol as a matrix. The elemental analysis was performed according to classical methods: C and H were determined as CO_2 and H_2O, Nitrogen-through the Duma method.

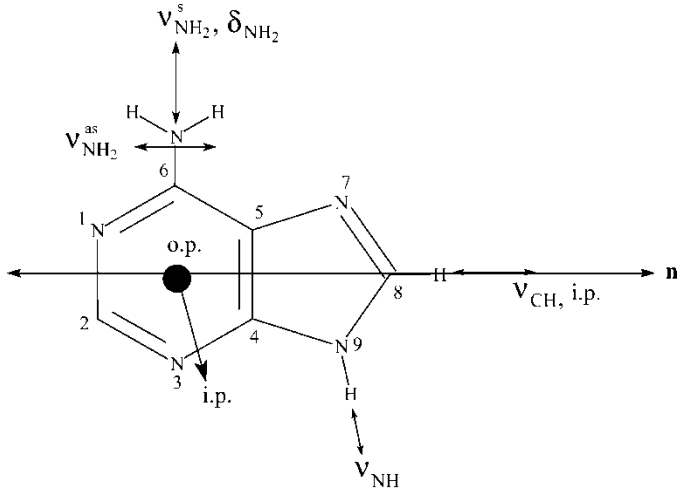
Synthesis

The protonated form of adenine as $[C_5H_6N_5]_2 \cdot SO_4$ was obtained as follows: 0.9865 g of Ade was dissolved in 20 mL methanol; 5 mL H_2SO_4 acid (1 M, Merck) was added. After 12 days, the resultant white polycrystalline solid phase was filtered, washed with methanol, and dried over P_2O_5 in air at room temperature. Yield: 45%. Found: C, 32.63%; H, 3.30%; N, 38.00%. Calculated for $[C_5H_6N_5]_2 \cdot SO_4$: C, 32.61%; H, 3.28%; N, 38.03%. The most intense peak in the mass spectrum of[1] is at m/z 136.14, corresponding to the singly charged $[C_5H_6N_5]^+$ ion with an m/z value 136.14. The mass spectral and elemental analysis data indicate the protonated adenine form.

RESULTS AND DISCUSSION

IR-LD Analysis of Adenine

The difference IR-LD spectrum of Ade (Fig. 2) shows positive bands at 3365 cm^{-1} and 3120 cm^{-1}. The first one may be assigned to ν^{as}_{NH2} and the second band to ν_{C8H} in-plane (i.p.), mode,[17,18] respectively. The negative ν^{as}_{NH2} and ν_{NH} peaks at 3297 cm^{-1} and 3264 cm^{-1} are also observed. In 1750–1500 cm^{-1} region, two intensive positive maxima at 1671 cm^{-1} and 1604 cm^{-1} corresponding to A_1 i.p. purine-stretching vibrations are at hand, thus indicating the average solid macro-orientation of adenine molecules toward the liquid crystal direction (**n**) shown in Scheme 1. The presence of negative out-of-plane (o.p.) purine absorption maximum at 894 cm^{-1} also confirmed the above-mentioned stereostructural result. As far as the other i.p. transition moments of the guest molecule are oriented nearly to 54.7° (magic angle), the disappearance of the band at 1419 cm^{-1} is expected and observed. The observed positive 1252 cm^{-1} and 725 cm^{-1} maxima in difference spectrum may belong to deformation β_{NH}[17,18] and rocking ρ_{NH2} i.p. modes, whereas the negative one at 794 cm^{-1} clearly shows its assignment to the o.p vibration of purine ring. The simultaneous elimination of the



Scheme 1. Adenine structure.

$3299\,cm^{-1}$ and $3259\,cm^{-1}$ (Fig. 3),[2] clearly indicates the real assignment of the discussed maxima to ν^{s}_{NH2} and ν_{NH}; moreover, their transition moments are collinear (Scheme 1). At the same time, the low intensive $1693\,cm^{-1}$ band also disappeared, supposing its assignment to $\delta_{NH2.}$ The disappearance

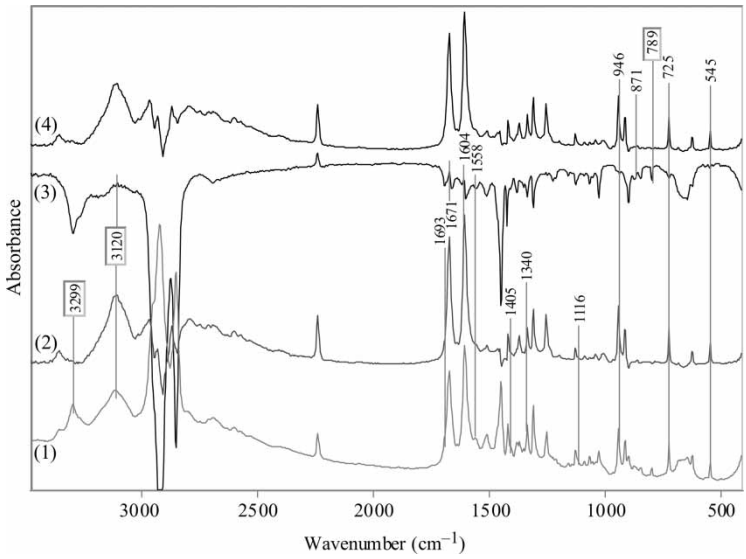


Figure 3. Nonpolarized (1) and reduced IR-LD spectra of adenine after elimination of $3299\,cm^{-1}$ (2), $3120\,cm^{-1}$ (3), and $789\,cm^{-1}$ (4) peaks.

of the 1405 cm^{-1}, 1382 cm^{-1}, 1349 cm^{-1}, all corresponding to i.p. bending purine vibrations[17,18] and 1116 cm^{-1} (i.p.) is also observed.

The elimination of the 3120 cm^{-1} peak (Fig. 3),[3] leads to the disappearance of series of maxima at 3455 cm^{-1}, 1671 cm^{-1}, 1606 cm^{-1}, 1558 cm^{-1}, 1313 cm^{-1}, 1253 cm^{-1}, 1124 cm^{-1}, 946 cm^{-1}, 914 cm^{-1}, 725 cm^{-1}, 545 cm^{-1}. The elimination of the ν^{as}_{NH2}, and i.p. ν_{C9H} peaks with the δ_{NH} and ρ_{NH2} ones indicated additionally their correct assignment, as far as their disappearance with i.p. purine-fragment modes are established. The vanishing of the o.p. mode at 789 cm^{-1} leads (Fig. 3),[4] just to disappearance of the 871 cm^{-1} peak, thus also confirming its o.p. purine mode character.

The IR-LD spectral data about the stereostructural predictions of the neutral compound in solid-state correlated well with the known crystallographic ones.[19] According to Ref.[19], the adenine molecules are disposed in infinite layers in parallel one to another, thus supposing the mutual collinear orientation of corresponding ν^{s}_{NH2}, ν^{as}_{NH2}, δ_{NH2}, ν_{CH}, and o.p. adenine skeleton vibrations.

IR-LD Study of N_1 Protonated Form of Adenine

The nonpolarized and difference IR-LD spectra of N_1 protonated adenine are shown in Fig. 4. In the 3400 cm^{-1}–3200 cm^{-1}, region an intensive band at 3272 cm^{-1}, and a multiple maximum between 2200 and 2280 cm^{-1}, are

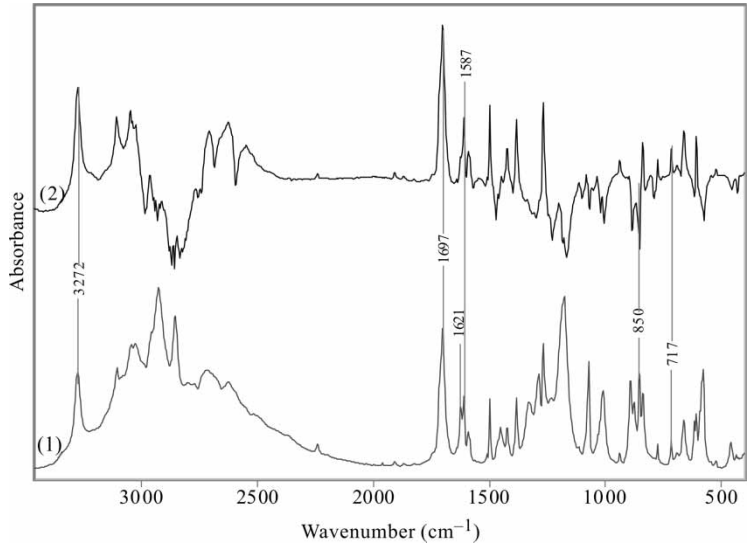
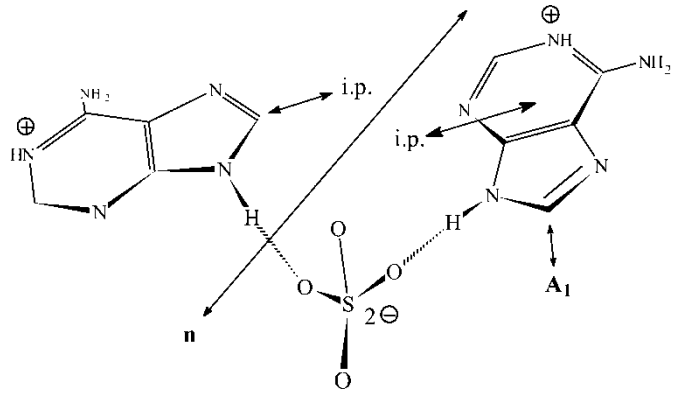


Figure 4. Nonpolarized (1) and difference IR-LD (2) solid-state spectra of N_1 protonated adenine salt as suspension in ZLI 1695.

shown. The first one is assigned to ν_{NH} and the second band to ν_{N+H2}. The result indicated the protonation of the N_1 atoms in pyridine fragments, as far as the profile and behavior of the spectral curve is untypical for imidazole and 2-aminopiridine protonated nitrogens.[13,20] Additional confirmation of this assumption follows from the observing of intensive band about 1697 cm^{-1} of the δ_{N+H2} and disappearance of the characteristics for purine i.p. modes at 1671 cm^{-1} and 1606 cm^{-1}, accompanied with the observation of the imidazole characteristic mode at 1621 cm^{-1}, 1587 cm^{-1}, and 1497 cm^{-1}. The 700–1000 cm^{-1} region is also typical for the imidazole fragment as far as bands at 889 cm^{-1}, 873 cm^{-1}, 850 cm^{-1}, and 836 cm^{-1} are observed, while the purine ones at 939 cm^{-1} and 912 cm^{-1} disappeared. A low-frequency shifting of the ρ_{NH2} mode to 717 cm^{-1} is also at hand, thus indicating the charge transfer in NH_2 group. The 3272 cm^{-1} band is positive in difference spectrum of the protonated form, in contrast with a second multiple one between 2200 and 2600 cm^{-1}, which is negative. These results indicated an orientation of the molecules of AdeH toward liquid crystal direction (**n**) as shown in Scheme 2.

The elimination of the 3272 cm^{-1} peak (Fig. 5),[2] leads to disappearance of the bands at 1714 cm^{-1} (δ_{N+H2}), 1698 cm^{-1} (i.p. purine mode), and i.p. imidazole bands at 1623 cm^{-1}, 1589 cm^{-1}, 1500 cm^{-1}, 1419 cm^{-1}, 1021 cm^{-1}, 1018 cm^{-1}, and 1378 cm^{-1}. The reduction of the 836 cm^{-1}, 871 cm^{-1}, 633 cm^{-1}, and 603 cm^{-1} peaks are also observed. The simultaneous disappearance of the i.p. mode and the ν_{NH} i.p. one indicates the presence of the two molecule protonated adenine oriented as shown in Scheme 2. The elimination of the 854 cm^{-1} o.p. mode leads to disappearance of the 1178 cm^{-1} (Fig. 5)[3]. The last, new peak in the IR spectrum of protonated form belongs to SO_4^{2-} anion included in the salt structure. The result leads to additional information about the space disposition also of the discussed



Scheme 2. Structure of adenine protonated salt.

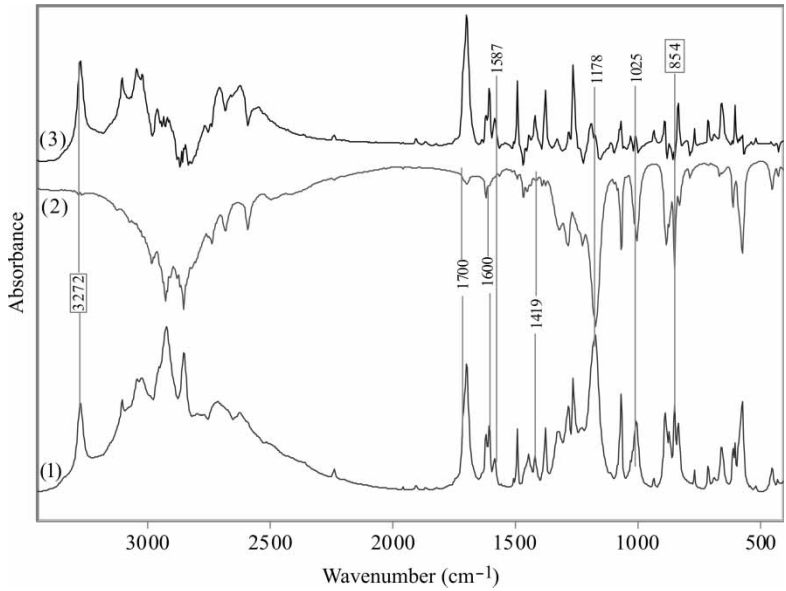


Figure 5. Nonpolarized (1) and reduced IR-LD spectra of $[C_5H_6N_5]_2SO_2$ after elimination of 3272 cm^{-1} (2) and 854 cm^{-1} (3) peaks.

anionic fragment (Scheme 2). The obtained stereostructure correlates well with the similar adenine N_1 or N_9 salts characterized by x-ray diffraction.[3,4]

CONCLUSIONS

By means of the solid-state IR-LD spectral analysis of a suspension in nematic liquid crystal, a supramolecular stereostructure of N_1 protonated salt of adenine was predicted, and a detailed vibrational assignment was done. The IR-LD results were compared with the analogous ones of solid adenine, whose structure is known and experimentally determined by single-crystal X-ray diffraction.

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