L-Valine and L-Proline - Solid-State IR-LD Spectroscopic Study

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Abstract: Spectral investigation including IR-characteristic bands assignment of the amino acids zwitterions $_L$ -Valine ($_L$ -Val) and $_L$ -Proline ($_L$ -Pro) was carried out by linear-dichroic infrared (IR-LD) spectroscopy of oriented solid sample as a nematic liquid crystal suspension. The obtained experimental IR-LD results (transition moment directions) were compared with known crystal X-ray data for molecules orientation in the unit cells of the studied compounds, confirming the applicability of the used spectral method for structural determination. The influence of the protonation on the IR-spectroscopic patterns of the both amino acids is discussed.

Keywords: *L*-Valine, *L*-Proline, IR-LD spectroscopy.

INTRODUCTION

The interaction of Au (III), Pt (II), Ru (III) complexes with peptides and proteins has been widely investigated, due to the potential antitumor effect of the corresponding coordination compounds [1-3]. In order to understand the in vivo manner and coordination mechanism of these metal ions with DNA, a systematic examination of their coordination ability with small peptides and and proteins is necessary [4-7]. From this point of view the particular interest has originated from the donor ability of hetero atoms in valine and proline side chains. Moreover, the peptides based of the last amino acids possessed significant biological activity as for example tripeptide L-Valyl-L-Prolyl-L-Proline has anti hypertensive ACE inhibitor activity [8, 9]. The detailed assignation of IR- characteristic bands for these systems is relatively with difficulty for conventional IR-spectral interpretation, as a result of overlapping IR-peaks due to of the presence of much more one amide fragment. The possibilities of the linear-polarized IR-spectroscopy (IR-LD), however allow to investigate the IR-spectral characteristics of the protonation and coordination processes in amino acids and small peptides.

The structure of the amino acids zwitterions $_L$ -Valine ($_L$ -Val) and $_L$ -Proline ($_L$ -Pro) are shown in Scheme 1 and this paper is focused on their spectral analysis.

Synthesis

 $_L$ -Val and $_L$ -Pro hydrochloride salts were synthesized according to the procedures described in [10, 11]. To the 10 ml methanol solutions of $_L$ -Val (0.5316 g, Sigma) and $_L$ -Pro (0.5124g, Sigma) was added 0.5 ml 10⁻¹M HCl. After about a week the obtained white polycrystalline salts were filtered, washed with methanol and dried over P₂O₅ at room temperature. Yields: 64% for $_L$ -Val and 77% for $_L$ -Pro respectively.

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Scheme 1. Zwitterions chemical structure of the studied amino acids.

For L-Val hydrochloride: Found: C, 39.9; H, 7.5; N, 9.0; $C_5H_{12}NO_2Cl$: Calc: C, 39.1; H, 7.8; N, 9.1; For L-Pro hydrochloride: Found: C, 39.3; H, 6.6; N, 9.4; $C_5H_{10}NO_2Cl$: Calc. C, 39.6; H, 6.7; N, 9.3. The most intensive signals in the mass spectra of both hydrochlorides are: MS(m/z): 118.20 (M^+) for $[C_5H_{12}NO_2]^+$ and MS(m/z):116.32 (M^+) for $[C_5H_{10}NO_2]^+$ with molecule weights 118.16 and 116.14, respectively.

EXPERIMENTAL PART

Methods

The IR-spectra were measured using a Bomem-Michelson 100 FT-IR-spectrometer $(4000 - 400 \text{ cm}^{-1}, 2 \text{ cm}^{-1})$ resolution, 150 scans) equipped with a Perkin Elmer wiregrid polarizer. Non-polarized solid-state IR spectra were recorded, using the KBr disk technique. The oriented samples were obtained as a colloidal suspension in a nematic 4'cyano-4'-alkylbicyclohexyl mixture (ZLI-1695, Merck), whose poor IR-spectrum allows the recording of the guestcompound bands in the whole 4000 - 400 cm⁻¹ range. The presence of the isolated nitrile stretching IR-band at about 2230 cm⁻¹ additionally serves as an orientation indicator. The effective orientation of the solid samples was achieved by the next procedure: the investigated compound (5 mg) was mixed with the liquid crystal substance until obtaining of a slightly viscous suspension. The phase prepared thereby was pressed between two KBr-plates, which in advance were rubbed out in one direction by fine sand-paper. The grinding

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of the prepared mull in the rubbed direction promotes in addition the sample orientation [12-15].

For polarized IR-spectra interpretation, the IR-LD spectroscopy use stepwise reduction procedure [16-19]. The IR-LD data interpretation are consisted in subtraction of the perpendicular spectrum (IRs - a result of the 90° angle between the polarized light beam electric vector and the orientation of the sample) from the parallel spectrum (IRp) obtained with a co-linear mutual orientation. The recorded difference (IRp-IRs) spectrum divides the integrated absorbances into positive and negative bands. Positive bands originate from transition moments which form average angles with the orientation direction (**n**) between 0° and 54.7° (magic angle) and negative bands corresponding to transition moments, which are directed between 54.7° and 90°. In the reducing-deference procedure, the perpendicular spectrum, multiplied by the variable parameter c, is subtracted from the parallel spectrum and parameter c is varied until at least one band or set of bands is eliminated in the obtained (IRp cIRs) reduced IR-LD spectrum. The simultaneous disappearance of these bands in the reduced spectrum provides information about the mutual disposition of the molecular fragments. This elimination method is carried out graphically using the attached subtracting procedure for processing of IR spectra.

The *elemental analysis* was performed by standard procedures for C, and H (as CO_2 , and H_2O) and N - by the Duma's method. The *FAB mass spectra* utilized for the molecule weight determination were recorded on a Fisons VG Autospect instrument employing 3-nitrobenzyl alcohol as a matrix.

RESULTS AND DISCUSSION

This work can be considered as a part of systematic spectroscopic and structural investigations on amino acids and their derivatives, small peptides and their possibilities to form metal complexes and application as potential anticancer medications [20-24].

The characteristic IR-bands of the pure amino acids $_L$ -Val and $_L$ -Pro are listed and assigned in (Table 1). The included

data about the last compounds are assigned on the basis of known IR-data about similar systems [25- 32]. The comparison and assignment of the solid-state IR-spectra (Figs. **2.1** and **4.1**) of the all systems studied is done, using the statement that pure amino acids stabilize H_3N^+ -R-COO⁻ ($_L$ -Val) and H_2N^+ -R-COO⁻ ($_L$ -Pro) zwitterions with characteristic IR-spectral bands of -NH₃⁺, -NH₂⁺ and -COO⁻ groups.

In all cases a preliminary deconvolution and curve-fitting procedure for the peak positions obtained and corresponding integral absorbance are applied as is shown in (Fig. 1) for $_{L^{-}}$ Pro in the 1750 – 1500 cm⁻¹ region.



Figure 1. 1750 - 1500 cm⁻¹ curve-fitted IR-spectrum of _L-Pro.

The protonation in each case leads to disappearance of the typical -COO⁻ maxima in 1630 – 1550 cm⁻¹ spectral range as well as about 1405 cm⁻¹. The character of the 1565 cm⁻¹ and 1407 cm⁻¹ bands as v^{as}_{COO} and v^{s}_{COO} in _L-Pro is confirmed in addition by the obtained IR-spectrum of protonated _L-Pro as hydrochloride salt. It is characterized with the disappearance of the maxima at 1565 cm⁻¹ and 1407 cm⁻¹ and a new peak at 1735 cm⁻¹ is observed, which correspond to $v_{C=O}$ stretching peak of the restored COOH group in the salt.

The simultaneously elimination of pairs of peaks at 1629 cm⁻¹ and 1612 cm⁻¹ (Fig. **2.2**) confirmed their character as

 Table 1.
 IR-Characteristic Bands in 1700 – 1350 cm⁻¹ Region of L-Val and L-Pro

Assignment	v [cm ⁻¹]	
	_L -Val	_L -Pro
v _{NH3+} stretching	3200 - 2700	-
$v_{\rm NH2+}$ stretching	-	3000 - 2400
$\delta^{as}_{\rm NH3+}$	1629	-
$\delta^{'as}_{NH3+}$	1612	-
$\delta^{s}{}_{\rm NH3+}$	1567	-
$\delta_{\rm NH2+}$	-	1626, 1612
V ^{as} coo-	1585	1567, 1552
V ^s _{COO} .	1508	1407, 1402

*IR-data obtained after applied deconvolution and curve-fitting procedures

 $\delta^{a_{s}}_{NH3+}$ and $\delta^{a_{s}}_{NH3+}$ (Table 1) due to their possession of the same symmetry class and irreducible representation of the allowed transition according to [33] (direction of the transition moments). The elimination of the maximum at 1585 cm⁻¹ strongly reduced the 1508 cm⁻¹ peak (Fig. 2.3). In the frame of the unit cell of _L-Val (Fig. 3(1)), the four presented molecules are oriented by the way supposed the relatively co linearity of corresponding v^{as}_{COO-} and v^{s}_{COO-} transition moments between neighboring molecules [10].



Figure 2. Non-polarized IR-(1) and reduced IR-LD spectra of $_L$ -Val after elimination of 1629 cm⁻¹ (2) and 1585 cm⁻¹ (3) peaks.

Thus could be explained and assigned as the belonging of last discussed peaks to asymmetric and symmetric stretching modes of COO⁻ fragment in the molecule of $_L$ -Val. Moreover, the last peaks disappear in the obtained spectrum of protonated form of $_L$ -Val hydrochloride, accompanied with an observation of new peak at 1720 cm⁻¹, corresponding to v_{C=O} stretching mode of -COOH group. According to single crys-



tal X-ray data of _L-Pro [11] the unit cell contains four molecules which are similarly mutual oriented by pairs, thus assumed the near to collinear orientation of δ_{NH2+} and v^{as}_{COO-} in the frame of these neighboring pairs of molecules (Fig. **3** (**2**)).

For that reason the obtained IR-LD reduced spectrum in Fig. (4.2) illustrated the strong reduction of 1567 cm⁻¹ peak with the 1626 cm⁻¹ one (Table 1). Due to the last procedure second pairs of observed maxima at 1612 cm⁻¹ and 1552 cm⁻¹ correspond to δ_{NH2+} and ν^{as}_{COO-} of second different oriented molecule of $_L$ -Pro in the frame of the unit cell. The multiple character of 1750 – 1500 cm⁻¹ region is determined as well with deconvolution and curve-fitting procedure applied, which leads to series of peaks shown in Fig. (1).



Figure 4. Non-polarized IR-(1) and reduced IR-LD (2) spectra of $_L$ -Pro after elimination of 1626 cm⁻¹ peak.

CONCLUSION

Linear-dichroic infrared (IR-LD) spectral analysis of oriented solid sample as a nematic liquid crystal suspension



Figure 3. Unit cells of L-Val [10] and L-Pro [11] respectively (1) and (2).

of amino acids $_L$ -Valine ($_L$ -Val) and $_L$ -Proline ($_L$ -Pro) was carried out. On the base of the obtained spectroscopic data was assigned the IR-characteristic bands of the zwitterions of both amino acids accompanied with structural prediction. The IR-spectral results were compared with some data from X-ray analysis of $_L$ -Val and $_L$ -Pro, confirming the benefit of the used specific method for spectral assignments and structure determination. The protonation influence on the IRspectroscopic patterns of the both amino acids is discussed.

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