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A facile synthesis and IR-LD spectral elucidation of *N*-acetyl amino acid derivatives

A.G. Chapkanov[1*], T.A. Dzimbova[2], B.B. Ivanova[3]

[1]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, SWU"N. Rilski'', 66 "I. Mihailov" Str., 2700 Blagoevgrad, Bulgaria*

[2]*Institute of Molecular Biology "R. Tsanev", Bulgarian Academy of Sciences, 21 Acad. G.Bonchev str., 1113 Sofia, Bulgaria*

[3]*Faculty of Chemistry, Sofia University, 1 J. Boucher Boulevard, 1164 Sofia, Bulgaria*

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Abstract: The synthesis of N-acetyl derivatives of α-amino acids (L–Gly, L–Val, L–Phe, L–Ile, L–Pro and L–Cys) using a simple and efficient method for preparation was carried out. The N-acetyl derivatives were obtained using acetic anhydride in basic conditions at room temperature. The methyl amides of N–acetyl amino acid were synthesized by mixed anhydride procedure using a Piv–Cl as a reagent. The conventional and IR–LD spectral analysis were applied for elucidation and characterization of typical IR-bands of the starting and intermediate compounds. Spectral investigation includes IR-characteristic bands assignment and elucidation of amino acids as zwitterions structures – L-Valine (L–Val), L–Proline (L–Pro) L–Cystein (L–Cys) and their intermediates by linear-dichroic infrared (IR–LD) spectroscopy of oriented solid sample as a nematic liquid crystal suspension. The experimental IR-characteristic bands obtained, were accompanied with spectral elucidation and structural prediction of the investigated compounds.

Key words: α-amino acids, N-acetyl-derivatives, IR –LD spectral analysis

INTRODUCTION

The profound significance of biological compounds in our daily lives has prompted a lot of research into their analysis and identification. The determination of peptide and protein structures constitutes one of the pillars of current investigation in molecular biology and biochemistry.

A great interest to N-acetyl derivatives of α-amino acids is because of their wide spectrum of actions [1]. There are many examples for N–acetylated amino acids in living organisms. N–acetyl derivatives of L–proline, L-glutamine and 4–hydroxyl–L–proline are particular importance, because of their uses as medicines and biologically active supplements. N–Acetyl–4–hydroxy–L–proline has found practical use in producing of the medicines which has anti-inflammatory and analgesic actions [2, 3]. N-Acetylaspartic acid is a derivative of aspartic acid, which is the second most concentrated molecule in the brain after the amino acid glutamate. It is synthesized in neurons from the amino acid aspartate and acetyl coenzyme A. N–Acetyl-L-glutamine is used in sport medicine as a component for increasing training intensity, muscle growth and strength. N–Acetyl cysteine is derived from cysteine found in food and synthesized in the body. It helps the body to synthesize glutathione, which is used as a mucolytic agent to reduce the viscosity of mucous secretions [4].

The development of an effective synthetic method of N-acethyl derivatives of amino acids has a practical importance, as well as the development of fast and simple analytical methods for structure determination. Powder X–ray diffraction is one of the powerful and routine method for determination of solids in pharmaceutical industry. However, the method is relatively expensive, difficult for operation and requiring a preliminary samples treatment.

Infrared (IR-) and Raman spectroscopy are other also wide used in the practice and pharmaceutical industry. However, in many cases the choice of suitable method for analysis often is difficult on account of the typical for crystals effects of Fermi-resonance [5–7]. Linear-polarized IR-spectroscopy of oriented colloids in nematic host could resolve a lot of these problems. The method is unique for experimental assignment of the characteristic bands and local structural elucidation independently of the crystalline or amorphous character of the samples [8–11]. Different N-acetyl amino acid derivatives were synthesized, characterized and reported earlier

* To whom all correspondence should be sent:
E-mail: chapkanov@swu.bg

as well as and the corresponding spectral study [12–19].

EXPERIMENTAL

Materials and methods

Amino acids and solvents were purchased from Fluka and used without further purification. Melting points were measured on Büchi (Switzerland) model 535. Optical rotation was determined on Polarimeter 141, Perkin Elmer (USA). For TLC silicagel plates (Merck, $60F_{254}$) were used and following systems: A) $CHCl_3$: MeOH : H_2O (80:30:5); B) CH_3CN : H_2O (4:1).

SYNTHESIS

N-Acetyl amino acids Amino acid (0.1 g) was dissolved in 5N NaOH (3 ml) and acetic anhydride (2 × 0.2 ml) was added over 15 min. period of time. Reaction mixture was stirred at room temperature for 1 hour and the water was evaporated under reduced pressure. The residue obtained was dried and used without further purification in the next reaction stage.

N-Acetyl amino acid methyl amides. To the solution of N–acetyl amino acid (3 mM) in the solvents (2 ml DMF and 2 ml THF) mixture, NMM (0.33 ml, 3 mM) was added and the reaction mixture was chilled to –10ºC. The reagent Piv-Cl (0.37 ml, 3 mM) was added dropwise and after 10 minutes solution of methyl amine hydrochloride (1g, 15 mM) and Et_3N (0.415 ml, 15 mM) in water (2 ml) was added. The reaction process completed after 1.5 hour at –10ºC and the solvents were evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (10 ml) and washed with water (2 × 10 ml). Organic layer was dried over anhydrous Na_2SO_4 and $CHCl_3$ was evaporated. The pure product was obtained after column purification (silicagel) and eluent: CH_3CN : H_2O (4 : 1, v/v).

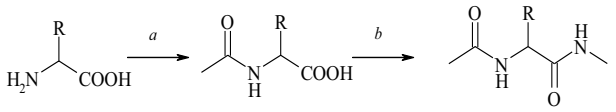
METHODS

The IR-spectra were measured using a Bomem-Michelson 100 FT-IR-spectrometer (4000 – 400 cm^{-1}, 2 cm^{-1} resolution, 150 scans) equipped with a Perkin Elmer wire-grid 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 guest-compound 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 of the prepared mull in the rubbed direction promotes in addition the sample orientation [20, 21]. For polarized IR-spectra interpretation, the IR–LD spectroscopy use stepwise reduction procedure [22–25]. The IR-LD data interpretations are consisted in subtraction of the perpendicular spectrum (IRs – a result of the 90º 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 absorbance into positive and negative bands. Positive bands originate from transition moments which form average angles with the orientation direction (n) between 0º and 54.7º (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.

RESULTS AND DISCUSSION

This work is a part of systematic synthetic, spectroscopic and structural investigations on amino acids, their derivatives and small peptides about the possibilities for application as potential medicines [26–28]. The aim was to develop simple and efficient methods for preparation N-acetyl-α-amino acids derivatives using a mixed anhydride procedure – the action of acetic anhydride using water as the reaction medium. The synthesis of the desired N-acetyl amino acid methyl amides was done according to the Scheme 1.

The first step in our scheme is the synthesis of N-acetyl amino acid derivatives using acetic anhydride at basic conditions (5N NaOH) in aqueous solution and room temperature for 1 hour.



a) 5N NaOH, $(CH_3CO)_2O$; *b*) NMM, PivCl, CH_3NH_2. HCl, Et_3N, THF+DMF

Scheme1. Synthesis of N–acetyl amino acid methyl amides

N–Acetyl amino acids were used on the next step after evaporation of the solvent, drying of the crude product without further purification. N–Acetyl amino acid methyl amides were obtained in mixed anhydride procedure – Piv–Cl was used as a reagent. The reaction was carried out at -10°C in mixture of THF and DMF as solvents. The reaction process continued for 1.5 hour and the desired N-acetyl amino acid methyl amide was obtained after column purification (silicagel, CH_3CN **:** H_2O, 4:1, v/v). Some physical constants and chemical characteristics of the synthesized compounds are presented in Table 1.

Table 1. Some physical data and characteristics of amino acid derivatives

Amino acid derivatives	Melting point (mp), °C	Optical rotation $[\alpha]_D^{20}$	Yield (%)
Ac–Pro	115–117	-86 (C=1, C_2H_5)	77
Ac–Val	124–125	+7,4 (water)	82
Ac–Gly	207–209	–	80
Ac–Cys	109–112	–35 (water)	81
Ac–Phe	168–169	+47 (water)	78
Ac–Ile	oil	+42 (water)	81
Ac–Pro-NH–CH_3	32	–210°(C=1, water)	25
Ac–Gly-$NHCH_3$	oil		27
Ac–Cys-$NHCH_3$	81	-24 (MeOH)	31
Ac–Phe-$NHCH_3$	196	+2.9 (MeOH)	33
Ac–Ile–$NHCH_3$	220	–1.5 (MeOH)	26

IR-SPECTRAL ANALYSIS

The spectral results of the investigated compounds are assigned on the basis of known IR-data about similar systems [29-32]. The characteristic IR-bands of the pure amino acids L-Val, L-Pro and L-Cys are listed in Table (2). Using the statement, that pure amino acids stabilize zwitterionic (H_3N^+-R-COO- (*L*val and L-Cys)) and H_2N^+-R-COO^- (*L*-Pro) structure with characteristic IR-spectral bands of -NH_3^+ and -COO^-groups, the comparison and assignment of the solid-state IR-spectra (Figs. 2.1) of the systems studied was carried out. The method of polarized IR-spectroscopy is appeared to be unique for the experimental proving of the vibrational bands to corresponding modes. In all cases a preliminary deconvolution and curve-fitting procedure for the peak positions obtained and corresponding integral absorbance are in the 1750 – 1500 cm^{-1} region according to [18].

Table 2. IR-characteristic bands of some amino acids in 1700 – 1350 cm^{-1} region

Assignment ν[cm^{-1}]	L–Val	L–Cys	L–Pro
νNH_3^+	3200 – 2700	3320–1800	
$\nu^{as}NH_3^+$		3170	
νNH_2^+	-		3000 – 2400
$\delta^{as}NH_3^+$	1629	1654	
$\delta'^{as}NH_3^+$	1612	1610	
$\delta^{s}NH_3^+$	1567	1530	
δNH_2^+			1626, 1612
ν^{as}COO-	1585	1589,	1567, 1552
ν^{s}COO-	1508	1397,	1407, 1402
νSH		2551	
$\delta^{as}NH_3^+ + tNH_3^+$		2068	
νC–N		1349	

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

The comparative spectral analysis shows the presence of bands in the 3320 – 1800 cm^{-1} region assigned to the ν^{as}_{NH3+} and ν^{s}_{NH3+} stretching vibrations of the protonated amino group -NH_3^+ for amino acid L-Val and L-Cys and ν_{NH2+} stretching vibrations for L-Pro. The band with frequency at 3170 cm^{-1} belongs namely to ν^{as}_{NH3+} of L-Cys. In the spectra of L-Val and L-Cys are observed two caple of bands at 1629, 1612 cm^{-1} and at 1654, 1610 cm^{-1} characterizing asymmetric banding vibrations (δ^{as}_{NH3+}). The corresponding bands for protonated –NH_2 group in L-Pro are at 1626 and 1612 cm^{-1}. (see Table 2). The bands at 1567 cm^{-1} (L-Val) and at 1530 cm^{-1} (L-Cys) are assigned to the symmetric banding vibrations (δ^{s}_{NH3}+). The typical -COO^- maxima are in the 1600 – 1400 cm^{-1} spectral range. The character of the band at 1585 cm^{-1} and 1508 cm^{-1} are assigned and belonging to the asymmetric and symmetric stretching modes of COO-fragment (ν^{as}_{COO-} and ν^{s}_{COO-}) in the molecule of L-Val. The bands at 1589 and 1397 cm^{-1} correspond to ν^{as}_{COO-} and ν^{s}_{COO-} stretching vibrations of COO-fragment of L-Cys. For L-Pro are definited two pair of bands corresponding at 1567, 1552 cm^{-1}(ν^{as}_{COO-}) and 1407, 1402 cm^{-1} (ν^{s}COO-) probably as a result of different molecul interactions (see Fig.1). The observed other bands for L-Cys at 2068 cm^{-1} corresponds to δ^{a}_{NH3+} + τ_{NH3+} combination mode, while the intensive band at 2551 cm^{-1} corresponds to ν_{SH} stretching vibration of the SH-group.The band at 1349 cm^{-1} belongs to ν_{C-N} stretching mode.

Using stepwise reduction procedure of IR–LD method, the obtained reduced spectrum for L-Pro

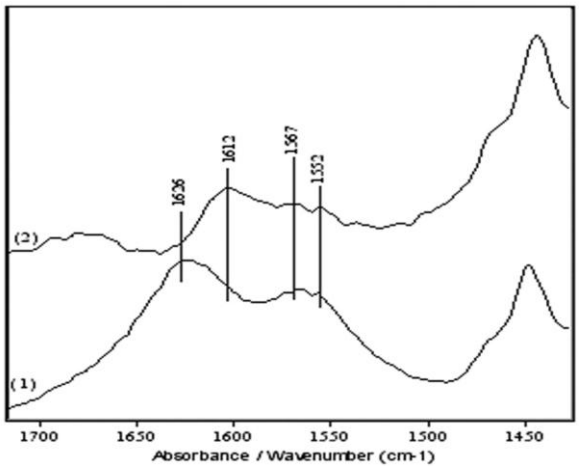


Fig. 1. Non-polarized IR-(1) and reduced IR-LD (2) spectra of L-Pro after elimination of 1626 cm^{-1} peak

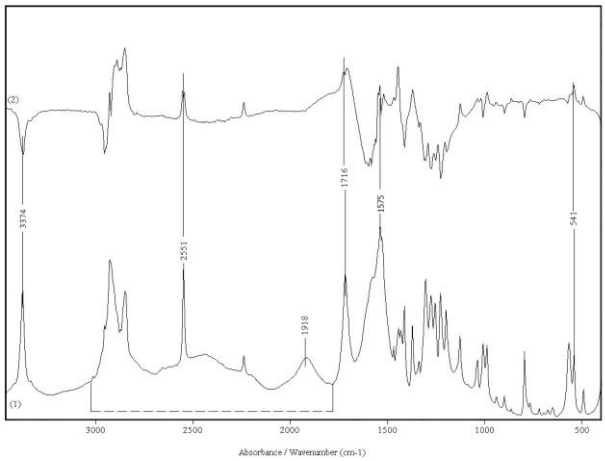


Fig. 2. Non-polarized IR-(1) and difference IR-LD (2) spectra of N-acetyl-L-cysteine

illustrated the strong reduction of 1567 cm^{-1} peak with the 1626 cm^{-1} one (see Fig.1.2 and Table 2). Due to the last procedure second pairs of observed maxima at 1612 cm^{-1} and 1552 cm^{-1} probably correspond to δ_{NH2+} and ν^{as}COO- modes of other second molecule which is very likely to be oriented differently in the frame unit cell of *L*-Pro. This assumption is confirmed by single crystal X–ray data of *L*–Pro [33], where the unit cell contains four molecules which are similarly mutual oriented by pairs. Similar behaviour is observed in L–Val, where the simultaneously elimination of pairs of peaks at 1629 cm^{-1} and 1612 cm^{-1} confirmed their character as δ^{as}_{NH3+} and $\delta^{'as}_{NH3+}$ (Tab. 2) due to their possession of the same symmetry class.

It is necessary to underline, that the non-polarized IR- and IR–LD spectra of N-acetyl-L-cysteine (Fig.2) are characterized with significant degree of particles orientation in nematic liquid crystal [10, 11], allowing the precise assignment of corresponding IR-bands. The observed broad absorption band in the 3100 – 1800 cm^{-1} range belongs to ν_{OH} stretching vibration of the intermolecular interaction OH-group of the COOH, typical for carboxylic acid. The obtained maximum with low intensity at 1918 cm^{-1} is typical too for systems with stronger intermolecular hydrogen bonding. The intensive band at 3374 cm^{-1} belongs to ν_{NH} stretching vibration of the amide fragment, while the maximum at 1716 cm^{-1} to $\nu_{C=O}$ stretching vibration of COOH group (see Fig.2).

The reduction of last band in the difference IR–LD spectrum (Fig.2) indicates, that the transition moment of corresponding C=O vibration is oriented towards the orientation direction (**n**) of the liquid crystal at angle of 54.7º (magic angle). The intensive band about 1575 cm^{-1} belongs to δ_{NH} bending vibration. This maximum is strong reduced with the elimination of the $\nu_{C=O}$ band from –COOH group (Fig.2) which is in accordance with the assumption, that both transition moments are approximately equal oriented. The same procedure leads to elimination of the band at 541 cm^{-1}, indicating probably its belongings to bending vibration ($\delta_{C=O}$) of carbonyl group. On the other hand, the elimination of the $\nu_{C=O}$ band leads to reduction of the maximum at 2551 cm^{-1} characterizing stretching vibration of ν_{SH}, which shows, that both transition moments are also near to co-linear oriented.

CONCLUSION

The N-acetyl- derivatives of α-amino acids (L–Gly, L–Val, L–Phe, L–Ile, L–Pro and L–Cys) were synthesized by efficient and simple method using acetic anhydride in basic conditions and mixed anhydride procedure following using Piv-Cl as a reagent. The IR - spectral investigation includes determination of the characteristic bands of some strating compounds (as zwitterionic structure) and N-acetyl- derivatives (N-acetyl-L-cysteine). The method of linear polarized vibrational IR-spectroscopy of oriented colloids in nematic host is applied on L–Val and L–Cys, with a view to obtain experimental bands assignment and local structural elucidation in solid-state. The obtained experimental IR-LD results confirm the applicability of the used spectral method for structural determination.

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СИНТЕЗ И ИЧ-ЛД СПЕКТРАЛНО ОПРЕДЕЛЯНЕ НА *N*-АЦЕТИЛ АМИНОКИСЕЛИННИ ПРОИЗВОДНИ

А. Г. Чапкънов[1*], Т. А. Дзимбова[2], Б. Б. Иванова[3]

[1]*Катедра "Химия", ПМФ, Югозападен университет „Н. Рилски", 2700 Благоевград*
[2]*Институт по молекулярна биология „Акад. Р. Цанев", БАН, 1113 София*
[3]*Химичен факултет, СУ „Св. Климент Охридски", 1164 София*

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(Резюме)

В настоящата работа е представен лесен и ефикасен метод за синтез на N-ацетилни производни на α-аминокиселините (L-Gly, L-Val, L-Phe, L-Ile, L-Pro и L-Cys). N-Ацетилните производни са получени с помощта на оцетен анхидрид в алкална среда при стайна температура. Метиламидите на N-ацетил аминокиселините са синтезирани с използването на метода на смесените анхидриди и Piv-Cl като реагент. За определяне на характеристичните ИЧ-ивици на изходните и междинни съединения е приложен конвенционален и ИЧ-ЛД спектрален анализ. Спектралните изследвания включват определяне на характеристичните ИЧ-ивици на аминокиселините като цвитер-йонни структури - L-валин (L-Val), L-пролин (L-Pro) L-цистеин (L-Cys) и техните производни с ИЧ-ЛД спектроскопия в нематичен течен кристал. Експерименталните характеристични ИЧ-ивици са използвани за спектрално охарактеризиране и предсказване на структурата на изследваните съединения.