A STUDY OF THE STRUCTURE OF NITROARGINYLPROLINE BY IR SPECTROSCOPY

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In plasma kinins [1], the amino acid sequence -arginylprolylprolyl- is frequently found and takes a leading role in the formation of the secondary signal at the level of the receptor [2]. The actual tripeptide arginylprolylproline is also biologically active [3]. A necessary prerequisite for the study of the fine mechanism of the action of this fragment is the determination of its conformation. The spatial structure of peptides containing proline has been the subject of voluminous experimental [4] and theoretical [5] investigations in recent years. Furthermore, the most probable states of the side chain of arginine have been calculated [6, 7], but the interaction of these two amino acid residues has not been studied. It is known that proline bends a peptide chain and decreases the conformational freedom of a preceding amino acid residue [8, 9].

The conformations of peptides depend strongly on the properties of the surrounding medium, i.e., on the solvent [10]. From the biological point of view, the conformations of peptides not only in water but in nonpolar solvents representing lipid media are of interest. To determine the possibility of the formation of an intramolecular hydrogen bond by IR spectroscopy in chloroform solution, we have studied di- and tripeptides containing nitroarginine and proline residues and also a nitroarginine derivative (Table 1).

Compounds (I-VII) were synthesized by the mixed-anhydride method (see Experimental). The IR spectra of compounds (I-V) (Fig. 1) each have an absorption band at above 3400 cm^{-1} which may be assigned to the vibrations of a free NH group [11]. In addition to this, the compounds containing nitroguanidine groups (I-III, V) have absorption bands in the region of NH stretching vibrations below 3400 cm^{-1}, which shows the presence of a hydrogen bond in them. The results of a comparison of the spectra of compounds (III) and (IV) show that of the two potential proton donors – the single amide group of the main

TABLE 1. Assignment of the Vibrational Frequencies of the NH and CO Groups in the IR Spectra of Compounds (I-V) in Chloroform at a Concentration of $1 \cdot 10^{-2}$ M, Layer Thickness 0.4 mm

Compound	Formula	ν NH, cm^{-1}		ν CO, cm^{-1}		
		free	bound*	*a*	*b*, *c*	*d*
I	Boc-Arg(NO_2)ProOBzl	3400, 3430	(3250–3350)	1695	1643	1742
II	BocArg(NO_2)ProNEt_2	3448	3346	1691	1635	—
III	BocArg(NO_2)ProProOBzl	3455	3352	1706	1635	1742
VI	BocAlaProProOBzl	3422	—	1708	1646	1742
V	BocArg(NO_2)NEt_2	3400, 3430	(3250–3350)	1690	1638	—

* The frequencies in parentheses correspond to the boundaries of the flat part of the spectral curve where there is appreciable absorption but no definite peak can be isolated.

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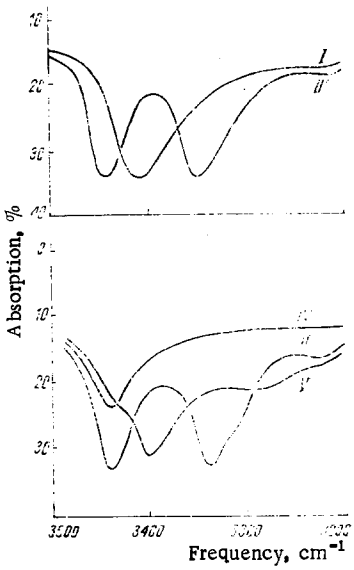
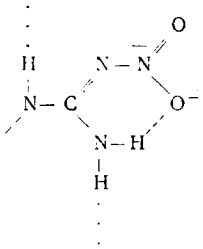


Fig. 1. IR absorption spectra in the region of NH stretching vibrations of dilute solutions of compounds (I-V) in chloroform at a concentration of $1 \cdot 10^{-1}$ M and a layer thickness of 0.4 mm.

TABLE 2. Frequencies of the NH (and NH_2) Stretching Vibrations of Tripeptide (III) in Chloroform at Various Dilutions

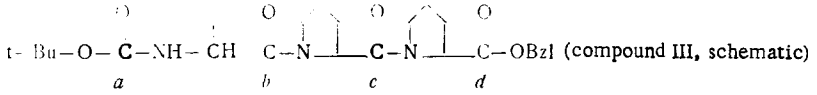
Concentration, %	ν NH, cm^{-1}	
	free	bound
$1 \cdot 10^{-1}$	3450	3350
$1 \cdot 10^{-2}$	3455	3352
$5 \cdot 10^{-3}$	3453	3350

chain and the nitroguanidine group – it is the latter which forms the hydrogen bond. According to the literature [12], nitroguanidine gives an "intragroup" hydrogen bond, i.e., one intramolecular within the nitroguanidine group; nevertheless another two hydrogen atoms may form a hydrogen bond:



The results of a comparison of the bands of the stretching vibrations of bound NH groups show that their integral intensities for compounds (II) and (III) are greater than for compounds (I) and (V). This can be explained by the formation in compounds (II) and (III) of another hydrogen bond in addition to the intragroup bond. When a solution of the tripeptide (III) was diluted, the band of the bound NH groups did not shift in the direction of higher frequencies (Table 2), which shows [13] the existence of an intramolecular hydrogen bond in (II) and (III).

Since in compounds (I) and (V) there is no such bond, and in compounds (II) and (III) there is one, the proton acceptor in compound (III) may be either of the carbonyl groups c or d.



In an analysis of the region of the spectra corresponding to the stretching vibrations of carbonyl groups, bands were assigned to the t-butoxycarbonyl group (*a* ~ 1700 cm^{-1}), the amide groups (b, c ~ 1640 cm^{-1}), and the ester group (d ~ 1740 cm^{-1}). The vibrational frequencies of the carbonyl group d are constant in all the compounds studied. Thus, the absence of a shift in the direction of lower frequencies shows the absence of the involvement of this group in a hydrogen bond. Conversely, the vibrational frequencies of the carbonyl groups b and c in the tripeptide (III) and in the dipeptide (II) as compared with the corresponding frequencies of compound (IV) are shifted in the direction of lower frequencies by 11 cm^{-1}. In a comparison of the results obtained in an analysis of the vibrational spectra of the NH and CO groups it can be seen that only carbonyl group c can act as proton acceptor in compounds (II) and (III). In the UV spectra, λ_{max} of compounds (I) and (III) coincided with λ_{max} of 271 nm for nitroarginine [14], which shows the similar electronic environment of the nitro group in both peptides and nitroarginine.

In view of the formation of an intramolecular hydrogen bond between the nitroguanidine group and the carbonyl group c, and on the basis of literature information on the conformational analysis of peptide fragments [4-9], we have attempted to model the conformation of the dipeptide unit nitroarginylproline with space-filling atomic models (Courtauld). Two values were taken for the torsional angle* ψ of arginine – 300 ± 15°, corresponding to the β conformation, and 130 ± 15° corresponding to a right-handed α-helix. The angles of the side chain of the arginine residue χ_{1-3} were varied in the models, χ_4 ($C^{\delta} - N^{\varepsilon}$) was taken as 180° [16], and χ_5($N^{\varepsilon} - C^{\zeta}$) as 0 or 180°. Since the CO···N angle of the hydrogen bond can be taken as about 35° according to calculation [17], the choice of the values of $\chi_{4,5}$ is not critical because of the possibility of a compensating rotation of the angles χ_{1-3} of the side chain. In the proline residue, both the trans and the cis isomers of the peptide chain were considered. The angle ψ of proline was used in the trans' posi-

*All the values of the torsional angles used in this paper are denoted by the nomenclature of Edsall et al. [15].

tion (330°, convoluted form) or the cis' position (130°, extended form). With the selected values of the torsional angles in the dipeptide unit of nitroarginylproline an intramolecular hydrogen bond can form between the nitroguanidine and carbonyl groups c in the following cases: 1) with ψ of arginine 300° and the proline residue in the trans-trans' state, and 2) with ψ of arginine 300° or 130° and the proline residue in the cis-cis' state. The first case is more likely: the proline residue is present in a more stable conformation [18]. Depending on the side-chain angles χ_{1-3} selected, the hydrogen bond can be formed both by the NH and by the NH_2 of the nitroguanidine group. But it is more likely that an intramolecular hydrogen bond is formed by the NH group, since it is not involved in an intragroup bond. Then the angles χ_{1-4} assume values of 150, 180, 180, and 180°, respectively. The values obtained are in agreement with the results of an x-ray structural analysis of arginine salts [19], giving values of about 180° for the angles $\chi_{2,3}$.

EXPERIMENTAL

The work was performed with amino acids of the L series. The melting points were determined in open capillaries without correction and the optical rotations on a Perkin-Elmer 141 spectropolarimeter at 20°C. The IR spectra were measured on a UR-20 instrument with NaCl and LiF prisms at a slit width of 4 cm^{-1} using solutions in absolute chloroform. The UV spectra were recorded in 95% ethanol on a Specord UV VIS instrument. In the isolation of the protected peptides, their solutions in ethyl acetate were washed with 0.5 N hydrochloric acid at 0°C, with water, with 5% sodium hydrogen carbonate solution, and with water again at room temperature. The purity of the peptides was checked by thin-layer chromatography on Silufol plates in the chloroform–methanol (8:2) (1) and ethyl acetate–hexane (5:1) (2) systems. The analyses of all the compounds corresponded to the calculated figures.

Benzyl Ester of t-Butoxycarbonylnitroarginylproline (I). To a solution of 2.6 g (8.0 mmoles) of t-butoxycarbonylnitroarginine in 15 ml of dimethylformamide were added 1.1 ml (8.0 mmoles) of triethylamine and, at −15°C, 1.0 ml (8.0 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and a suspension of 1.9 g (7.9 mmoles) of the hydrochloride of the benzyl ester of proline and 1.1 ml (8.0 mmoles) of triethylamine in 10 ml of dimethylformamide was added. Then the mixture was stirred at −5°C for another 4 h, the precipitate of triethylamine hydrochloride was filtered off, and the solvent was evaporated in vacuum (50°C). The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, and evaporated to small volume, and the dipeptide was precipitated with petroleum ether. After reprecipitation from ethyl acetate–petroleum ether and maintenance in a vacuum of 1 mm (20°C), the yield of the dipeptide (I) was 2.8 g (70%), mp 79-84°C, $[\alpha]_D^{20}$ −53.5° (c 1.0; ethanol), R_f 0.81 (1), 0.23 (2), $C_{23}H_{34}N_6O_7$.

t-Butoxycarbonylnitroarginylproline Hemi(ethyl acetate) Solvate (VII). Over 1 h with stirring 10 ml of a 1.0 N solution of sodium hydroxide was added to a solution of 5.1 g (10.1 mmoles) of the benzyl ester (I) in 10 ml of methanol. Then the mixture was stirred at room temperature for another 2 h, after which 50 ml of ethyl acetate and 50 ml of water were added. The aqueous layer was washed twice more with ethyl acetate and then it was acidified at 0°C with citric acid (pH 3.0), 8.0 g of sodium chloride was added, and the oil that separated out was extracted with ethyl acetate. The ethyl acetate layer was washed with water four times and was dried with sodium sulfate, and the solvent was evaporated off in vacuum. After two reprecipitations from ethyl acetate with petroleum ether, the yield of the acid (VII) was 2.9 g (62%), mp 105-115°C, $[\alpha]_D^{20}$ −39.0° (c 0.6; ethanol), R_f 0.40 (1), $C_{16}H_{28}N_6O_7 \cdot C_4H_8O_2$.

Hydrochloride of the Benzyl Ester of Prolylproline (VI). To a solution of 10 g (25 mmoles) of the benzyl ester of t-butoxycarbonylprolylproline (oil) [20] in 20 ml of acetic acid was added 120 ml of a 1 N solution of hydrogen chloride in glacial acetic acid. The mixture was kept at room temperature for 1 h, and then the solvent was evaporated off in vacuum and the residue was triturated several times with ether. After recrystallization from methanol with ether, the yield of the hydrochloride (VI) was 7.0 g (83%), mp 181-183°C, $[\alpha]_D^{20}$ −140° (c 0.7; ethanol), $C_{17}H_{22}N_2O_3 \cdot HCl$.

Benzyl Ester of t-Butoxycarbonylnitroarginylprolylproline (III). To a solution of 2.5 g (7.8 mmoles) of t-butoxycarbonylnitroarginine in 15 ml of dimethylformamide were added 1.1 ml (7.8 mmoles) of triethylamine and, at −15°C, 1.0 ml (7.8 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and then 2.5 g (7.4 mmoles) of a suspension of the hydrochloride (VI) and 1.0 ml (7.4 mmoles) of triethylamine in 10 ml of dimethylformamide was added. After this, the mixture was stirred at −5°C for another 4 h, the precipitate of triethylamine hydrochloride was filtered off, and the solvent was evaporated off in vacuum (50°C). The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, evaporated to small volume, and caused to crystallize by the addition

of ether. The yield of the tripeptide (III) was 2.9 g (65%), mp 109-113°C. After recrystallization from chloroform–carbon tetrachloride, mp 115-117°C, $[\alpha]_D^{20}$ −143.6° (c 0.5; ethanol), R_f 0.62 (1), $C_{28}H_{41}N_7O_8$.

Diethylamide of t-Butoxycarbonylnitroarginylproline (II). To a solution of 0.46 g (1.0 mmole) of the acid (VII) in 4 ml of tetrahydrofuran were added 0.14 ml (1.0 mmole) of triethylamine and, at −15°C, 0.13 ml (1.0 mmole) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and then 0.12 ml (1.2 mmole) of diethylamine was added. After a further 4 hours' stirring at −5°C, the precipitate of triethylamine hydrochloride was filtered off, and the solvent was evaporated in vacuum. The residue was dissolved in ethyl acetate, the solution was washed, dried with sodium sulfate, and evaporated to small volume, and petroleum ether was added. The precipitate that deposited was filtered off and was kept in a vacuum over phosphorus pentoxide. The yield of the diethylamide (II) was 0.30 g (64%), decomp. p. above 95°C, $[\alpha]_D^{20}$ −29.7° (c 1.0; ethanol), R_f 0.73 (1), $C_{20}H_{37}N_7O_6$.

Benzyl Ester of t-Butoxycarbonylalanylprolylproline (IV). To a solution of 0.95 g (5.0 mmoles) of t-butoxycarbonylalanine in 6 ml of dimethylformamide were added 0.69 ml (5.0 mmoles) of triethylamine and, at −15°C, 0.64 ml (5.0 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min, and a solution of 1.7 g (5.0 mmoles) of the hydrochloride (VI) and 0.69 ml (5.0 mmoles) of triethylamine in 3 ml of dimethylformamide was added. The mixture was stirred for another 4 h at −5°C, the precipitate of triethylamine hydrochloride was filtered off, and the solvent was evaporated in vacuum. The residue was dissolved in ethyl acetate, the solution was washed, dried with sodium sulfate, and evaporated to small volume, and the tripeptide was precipitated with petroleum ether. After reprecipitation from diethyl ether with petroleum ether, the yield of the tripeptide (IV) was 1.2 g (51%), decomp. p. above 100°C, R_f 0.78 (1), 0.21 (2), $C_{25}H_{35}N_3O_6$.

Diethylamide of t-Butoxycarbonylnitroarginine (V). To a solution of 1.6 g (5 mmoles) of t-butoxycarbonylnitroarginine in 7 ml of dimethylformamide were added 0.69 ml (5.0 mmoles) of triethylamine and, at −15°C, 0.64 ml (5.0 mmoles) of n-butyl chloroformate. The mixture was stirred at −5°C for 20 min and then 0.62 ml (6.0 mmoles) of diethylamine was added. Then the mixture was stirred for another 4 h at −5°C. The precipitate of triethylamine hydrochloride was filtered off, and the solvent was evaporated in vacuum. The residue was dissolved in ethyl acetate and the solution was washed, dried with sodium sulfate, evaporated to small volume, and treated with petroleum ether. The oil that deposited was reprecipitated from ethyl acetate with petroleum ether. The yield of amorphous substance (V) was 0.7 g (37%), $[\alpha]_D^{20}$ −24.1° (c 1.0; ethanol), R_f 0.31 (1), 0.21 (2), $C_{15}H_{30}N_6O_5$.

SUMMARY

A number of protected di- and tripeptides containing nitroarginine and proline residues, and also some derivatives of nitroarginine, have been synthesized and their IR spectra have been recorded in dilute chloroform solution. The results of the IR spectroscopy have been interpreted in the sense of the formation of an intermolecular hydrogen bond between the imino group of the side chain of nitroarginine and the carbonyl group of the following proline residue.

LITERATURE CITED

1. E. Schroeder and K. Lübke, The Peptides, Academic Press (1965).
2. G. I. Chipens, in: The Chemistry and Biology of Peptides [in Russian], Riga (1971), p. 23.
3. R. É. Vegner, G. I. Chipens, V. E. Klusha, and Z. P. Auna, Khim. Prirodn. Soedin., 516 (1973).
4. J. P. Carver and E. R. Blout, in: Treatise on Collagen, Vol. 1, edited by G. N. Ramachandran, Academic Press (1967).
5. B. Maigret, D. Perahia, and B. Pullman, J. Mol. Biol., 29, 275 (1970).
6. B. Pullman, J. L. Coubeils, P. Courriere, and D. Perahia, Theor. Chim. Acta, 22, 11 (1971).
7. G. V. Nikiforovich, I. P. Buevich, and S. G. Galaktionov, Izv. Akad. Nauk BelorussSSR, Ser. Biol., 53 (1971).
8. A. Damiani, P. De Santis, and A. Pizzi, Nature, 226, 542 (1970).
9. M. Maigret, B. Pullman, and J. Caillet, Biochem. Biophys. Res. Commun., 40, 808 (1970).
10. J. A. Schellman and C. Schellman, Proteins, 2, 45 (1964).
11. L. Bellamy, Infrared Spectra of Complex Molecules, 1st ed., Methuen, London (1954).
12. J. H. Bryden, L. A. Burkardt, E. W. Hughes, and J. Donohue, Acta Cryst., 9, 573 (1956).
13. M. Tichy, Advan. Org. Chem., 5, 115 (1965).
14. R. Schwyzer and H. Kappeler, Helv. Chim. Acta, 46, 1550 (1963).

15. J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, Biopolymers, 4, 121 (1966); J. Biol. Chem., 241, 1004 (1966); J. Mol. Biol., 15, 399 (1966).
16. P. K. Ponnuswamy, A. V. Lakshminarayanan, and V. Sasisekharan, Biochim. Biophys. Acta, 229, 596 (1971).
17. C. Ramakrishnan and N. Prasad, Int. J. Prot. Res., 3, 209 (1971).
18. P. J. Flory, Statistical Mechanics of Chain Molecules, Interscience (1969), p. 284.
19. S. K. Mazumdar, K. Venkatesan, and A. V. Lakshminarayanan, J. Mol. Biol., 15, 232 (1966).
20. C. M. Deber, F. A. Bovey, J. P. Carver, and E. R. Blout, J. Amer. Chem. Soc., 92, 6191 (1970).