CONFORMATION OF DIASTEREOISOMERIC CYCLO(NEOPENTYL-GLYCYL-PROLYLS): NMR, X-RAY, AND CD STUDIES*

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Conformation of diastereoisomeric cyclo(L-neopentylglycyl-L-prolyl) (*IV*) and cyclo(D-neopentylglycyl-L-prolyl) (*VIII*) in solution was studied by ^1H and ^{13}C NMR spectroscopy. Both compounds have approximately the same conformation of the bicyclic moiety in which the 2,5-piperazinedione ring exists in the boat form with pseudoaxial proline H(α) atom, and the proline ring is in the C(γ)-*exo* conformation. Rotation of the neopentyl side chain is markedly hindered. In the *trans*-derivative *VIII* the side chain occupies a pseudoaxial position with staggered rotamer about the C(α)—C(β) bond, the tert-butyl group pointing from the ring in the direction of the nitrogen atom. The preferred rotamer of the *cis*-isomer markedly deviates from this staggered conformation in the *exo*-direction relative to the piperazine ring. X-Ray diffraction analysis shows that crystal conformation of the *trans*-isomer *VIII* is very similar to that in solution. According to CD measurements, steric interactions of the acyclic side chain with the dioxopiperazine ring lead to twisted boat conformations of this ring with non-planar amide groups. From comparison with other proline-containing cyclodipeptides it follows that the effect of these interactions depends on the side chain structure. The neopentyl side chain flattens the boat conformation and reduces the deviation of the amide groups from planarity.

Cyclodipeptides (3,6-disubstituted 2,5-piperazinediones) are used very often as model systems for investigation of the peptide (amide) group. Their relatively rigid spatial arrangement allows detailed studies by physical methods such as NMR spectroscopy or X-ray diffraction. The knowledge of cyclodipeptide geometry enables then interpretation of their properties, *inter alia* also chiroptical ones. This aspect has been studied within the framework of our investigations of chiroptical properties of the amide grouping[1-3]. In our previous papers we described the preparation, crystal structure, and spectral properties of a seies of cyclodipeptides[4-16]. Using suitable starting amino acids, we tried to prepare such model compounds in which geometric parameters are markedly different from those in the parent system, *e.g.* by annelation of 4, 5 or 6-membered ring[6,8-10] or by introduction of a spiro-system[15]. To study the effect of side chains, we synthesized compounds containing the tert-leucine

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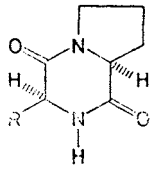
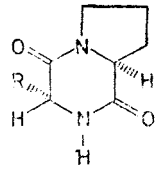
(2-amino-3,3-dimethylbutanoic acid) moiety with the extremely bulky tert-butyl substituent[17,18]. Also another amino acid – neopentylglycine (2-amino-4,4-dimethylpentanoic acid[19]) contains the tert-butyl group, in this case, however, separated by one methylene group from the peptide backbone. Thus, the structural relation between tert-leucine and neopentylglycine resembles that between the coded amino acids valine (with pronounced steric effect of the side chain) and leucine (with markedly hydrophobic side chain).





cis - series		*trans* - series	
I, R = $CH(CH_3)_2$	cyclo(L-Val-L-Pro)	*V*, R = $CH(CH_3)_2$	cyclo(D-Val-L-Pro)
II, R = $C(CH_3)_3$	cyclo(L-Tle-L-Pro)	*VI*, R = $C(CH_3)_3$	cyclo(D-Tle-L-Pro)
III, R = $CH_2CH(CH_3)_2$	cyclo(L-Leu-L-Pro)	*VII*, R = $CH_2CH(CH_3)_2$	cyclo(D-Leu-L-Pro)
IV, R = $CH_2C(CH_3)_3$	cyclo(L-Neo-L-Pro)	*VIII*, R = $CH_2C(CH_3)_3$	cyclo(D-Neo-L-Pro)

The aim of this study is to determine conformation of two diastereoisomeric cyclodipeptides* cyclo(L-neopentylglycyl-L-prolyl) (*IV*) and cyclo(D-neopentylglycyl-L--prolyl) (*VIII*) prepared in our previous work[19], and to study their chiroptical properties.

EXPERIMENTAL

The ^1H and ^{13}C NMR spectra (200 MHz or 50·3 MHz, respectively) were measured on a Varian XL-200 instrument in deuteriochloroform, using tetramethylsilane as internal reference. Chemical shifts and proton coupling constants were obtained by first order analysis from the expanded spectra (2 Hz/cm) using exponential multiplication with gaussian apodization function for resolution enhancement. CD spectra of analytically pure cyclodipeptides were measured with Roussel-Jouan Dichrographe model CD 185/II in cells of optical path 1 cm to 0·01 cm at room temperature (22–25°C). Concentration of the samples was approximately $5 \cdot 10^{-3}$ mol l^{-1}. The data are given in the molar ellipticity units (deg cm^2 $dmol^{-1}$) and are not corrected for the refractive index of the solvent. Spectra of valyl (*I* and *V*) and leucyl (*III* and *VII*) derivatives are essentially in accord with those published by Madison *et al.*[21].

The X-ray diffraction analysis for the *trans*-diastereoisomer *VIII*: $P2_12_12_1$, $Z = 4$, $a =$ = 10·006(2) Å, $b = 19{\cdot}696(6)$ Å, $c = 6{\cdot}365(1)$ Å, CuK_α, $\mu = 6{\cdot}19$ cm^{-1}, $R = 0{\cdot}065$ for 1 177 symmetry independent reflections. Details of the analysis are described elsewhere[16].

* Nomenclature and symbols of the amino acids and peptides obey the published IUPAC–-IUB recommendations[20]. Neo is used for neopentylglycine, Tle for tert-leucine.

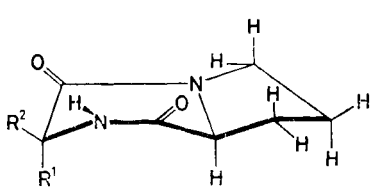
RESULTS AND DISCUSSION

NMR *Measurements*

Structure of the cyclodipeptides *IV* and *VIII* was confirmed and their conformation studied using the ^{13}C and ^{1}H NMR spectroscopy.

The proton decoupled ^{13}C NMR spectra of both compounds *IV* and *VIII* exhibit 10 signals of which the strongest belongs to three equivalent methyl groups of the neopentyl side chain. The signals were ascribed to individual carbon atoms (Table I) on the basis of chemical shifts, number of directly bonded protons ("attached proton test" spectrum[22]), and comparison with ^{13}C NMR data for other proline-containing peptides. Comparison of ^{13}C NMR shifts of analogous carbon atom signals in compounds *IV* and *VIII* shows marked differences (greater than 1 ppm) only for the neopentylglycine C(α) (3·1 ppm) and C(β) atoms (2·65 ppm). This is in accord with the fact that both compounds have approximately the same conformation of the bicyclic moiety and differ in orientation of the neopentyl side chain, this difference being most sensitively reflected by the C(α) and C(β) atoms. The different chemical shifts are obviously due to different steric effects and magnetic anisotropy of the peptide groups in *IV* and *VIII*.

Although the ^{1}H NMR 200 MHz spectra of the cyclodipeptides *IV* and *VIII* do not afford all the NMR parameters (the four proline β- and γ-protons appear as a complex multiplet in the region δ 1·8–2·4), they afford enough information on the configuration and conformational features of both compounds. The obtained ^{1}H NMR parameters (Table I) for protons in the bicyclic part of the molecule are very close to those described[9] for cyclo(L-Leu-L-Pro) (*III*) and cyclo(D-Leu-L-Pro) (*VII*). Important for the configurational assignment are the extremely different values of $J(\alpha, NH)$ (4·7 Hz and 0·7 Hz for the *trans*-derivative *VIII* and *cis*-derivative *IV*, respectively). They are consistent with a boat form of the 2,5-piperazinedione ring and with pseudoaxial proline H(α) proton in both compounds which differ from each other by orientation of the neopentyl substituent: pseudoaxial in the *trans*-derivative *VIII* and pseudoequatorial in the *cis*-derivative *IV* (see formulae *IVa*, *VIIIa*). These conclusions are confirmed by very similar chemical shifts of all the



IV a, R^1 = H ; R^2 = $CH_2C(CH_3)_3$
VIII a, R^1 = $CH_2C(CH_3)_3$; R^2 = H

proline proton signals in *IV* and *VIII*. Further proofs are provided by the allyl- and homoallyl-type long-range interactions of the H(α) atoms in segments including the C(O)—N group of partially double bond character. These coupling constants are very sensitive to the geometric arrangement and achieve markedly high values when the angle between the C(α)—H bond and the plane of the peptide group is close to 90° (maximum π-contribution). In our case, such arrangement can be approximately achieved only with pseudoaxial H(α) atoms. Therefore, we find non-zero

TABLE I

^{1}H and ^{13}C NMR parameters of cyclodipeptides *IV* and *VIII* in deuteriochloroform

Comp.	Residue	NH[b]	^{1}H NMR chemical shifts and coupling constants[a]				
			H(α)	H(β)		H(γ)	H(δ)
VIII	Neo	6·38 b	3·95 ddd $J(\alpha, \beta) = 9·6; 3·3$ $J(\alpha, NH) = 4·6$	1·82 dd $J(\beta, \alpha) = 3·3$ $J(\beta, \beta) = 14·2$	1·62 dd $J(\beta, \alpha) = 9·6$	—	1·01 s (9 H)
	Pro	—	4·09 bdd $J(\alpha, \beta) = 9·5; 6·7$ $J(\alpha, NH') = 0·5$	2·40 m (1 H)		1·81—2·15 m (3 H)	3·57 m (2 H)
IV	Neo	5·88 b	3·98 dm $J(\alpha, \beta) = 8·4; 2·2$ $J(\alpha, NH) = 0·7$ $J(\alpha, \alpha') = 1·5$ $J(\alpha, \delta') = 0·7; 0·6$	2·56 dd $J(\beta, \alpha) = 2·2$ $J(\beta, \beta) = 15·2$	1·28 dd $J(\beta, \alpha) = 8·4$	—	1·00 s (9 H)
	Pro	—	4·13 tm $J(\alpha, \beta) = 9·0; 7·5$ $J(\alpha, NH') = 0·6$ $J(\alpha, \alpha') = 1·5$		1·77—2·42 m (4 H)		3·57 m (2 H)

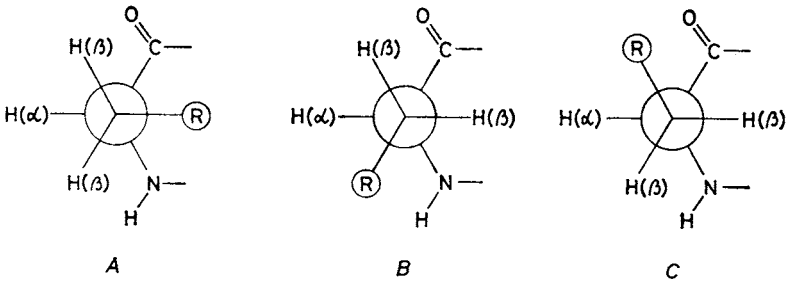
Compound	Residue	^{13}C NMR chemical shifts				
		C=O	C(α)	C(β)	C(γ)	C(δ)
VIII	Neo	166·87	55·98	45·67	30·68	29·72
	Pro	169·35	58·02	28·81	22·32	44·72
IV	Neo	166·11	52·88	43·02	30·17	29·60
	Pro	170·06	59·02	27·94	22·83	45·72

[a] The absolute values of coupling constants are given; α′, β′, δ′, and NH′ indicate the corresponding protons of second amino acid residue; [b] chemical shifts of NH protons are strongly concentration dependent.

values (0·5 Hz) of $^4J(\alpha, NH')$ for the proline H(α) protons in both isomers *IV* and *VIII* whereas the neopentylglycine H(α) proton has non-zero coupling constants $J(\alpha, \alpha')$ (1·5 Hz) and $J(\alpha, \delta')$ (0·7 Hz) (with proline protons) only in the *cis*-derivative *IVa* in which it occupies a pseudoaxial position.

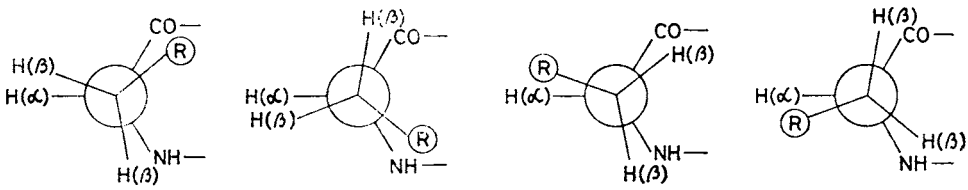
A detailed description of the proline ring conformation requires a complete set of its vicinal *J* constants; however, not all the values were obtained. The available data, first of all the considerably high values of $J(\alpha, \beta)$ ($\cong$ 9 and 7 Hz) and very similar chemical shifts of both the H(δ) protons, indicate preference of a C(γ)-exo conformation with torsion angles between the C(α)—H(α) and C(β)—H(β) bonds of about 150° and 30° and with both H(δ) atoms situated symmetrically to the plane of the neighbouring peptide group.

As concerns the neopentyl side chain, the non-equivalence of chemical shifts of H(β) atoms, and particularly the markedly different pairs of constants $J(\alpha, \beta)$ indicate a hindered rotation about the C(α)—C(β) bond. Usually, analysis of side chain conformation in peptides concerns only three staggered rotamers *A*, *B*, and *C*,



whose approximate population can be calculated from the observed constants $J(\alpha, \beta)$ and from the reference data for $J(\alpha, \beta)$ in the gauche and *trans* arrangement of the hydrogen atoms (see refs[23,24]). The distinction of rotamers *B* and *C* requires a structural assignment of both H(β) signals. Application of this approach to the *trans*-derivative *VIII* with $J(\alpha, \beta)$ = 3·3 and 9·6 Hz, using the values J_g = 3·25 Hz and J_t = 12·4 Hz (ref.[23]), leads to the following rotamer populations: *A* = 0·30, *B* = 0·70, and *C* = 0. The rotamer *C* is destabilized by steric interactions between the bulky substituent and lone electron pairs of the carbonyl oxygen atom. Steric interactions can obviously destabilize also the folded rotamer *A*, in which the substituent points over the piperazinedione ring. No such interaction occurs in the rotamer *B* which therefore should be preferred, in accord with the observation. Application of the same approach to the *cis*-derivative *IV* cannot lead to reasonable results because one of the $J(\alpha, \beta)$ constants is extremely low (2·2 and 8·4 Hz), lower than usual[23,24] values for J_g. This observation might be explained by existence of a preferred rotamer with torsion angles markedly different from the classical staggered arrangement (60°, 180°). According to the relationship $J(\alpha, \beta) = 11{\cdot}0 \cos^2 \Theta - 1{\cdot}4$

$\cos \Theta + 1{\cdot}6 \sin^2 \Theta$ (ref.[23]), the given pair of constants $J(\alpha, \beta) = 2{\cdot}2$ and $8{\cdot}4$ Hz can correspond to torsion angles of 100° and 20°, or 100° and 140°. These are represented by four rotamers *D*, *E*, *F*, and *G*. The forms *D* and *E* are very unlikely



+100°; −20° −100°; +20° +100°; −140° −100°; +140°

D *E* *F* *G*

because the neopentyl substituent in them interacts unfavourably with one or another peptide group, the rotation about the C(β)—C(γ) bond being hindered. These interactions are suppressed in rotamers *F* and *G*. These two can be distinguished by chemical shifts of both H(β) protons which are markedly nonequivalent ($\Delta\delta = 1{\cdot}28$ ppm). The signal of the proton, synperiplanar to carbonyl, should be shifted significantly downfield. Since this lowfield H(β) atom has $J(\alpha, \beta) = 2{\cdot}2$ Hz, corresponding to a torsion angle 100°, the requirements are met only by rotamer *G* in which the neopentyl substituent is somewhat nearer to the less sterically demanding N-end of the peptide group. We can thus assume that, in solution, the *cis*-derivative *IV* exists preferably with the neopentyl substituent in the arrangement *G*.

Geometry of Cyclopeptide VIII in Crystal

The 2,5-piperazinedione ring assumes a twist-boat conformation with the following torsion angles: Φ(Neo) = −29(1)°, Ψ(Neo) = 20(1)°, ω(Neo) = 8(1)°, Φ(Pro) = = −30(1)°, Ψ(Pro) = 21(1)°, ω(Pro) = 7(1)°. The proline ring, described analogously as in the interpretation of NMR spectra, has torsion angles: (H^1(β)—C(β)—C(α)—H(α)) = −167(4)°, (H^2(β)—C(β)—C(α)—H(α)) = −51(4)°, (CO—N—C(δ)—H^1(δ)) = −71(1)°, (CO—N—C(δ)—H^2(δ)) = 49(1)°. It exists thus in an approximately symmetrical conformation describable by a C_2-axis leading through the C(δ) atom and bisecting the C(α)—C(β) bond. The torsion angles of segments without hydrogen atoms confirm this symmetry: (C(δ)—N—C(α)—C(β)) = 31·2(5)° and (C(δ)—C(γ)—C(β)—C(α)) = 29·3(6)°.

The bulky neopentyl pseudoaxial substituent is oriented away from the 2,5-piperazinedione ring, the torsion angles being (NH—C(α)—C(β)—C(γ)) = 80(1)° and (CO—C(α)—C(β)—C(γ)) = −154(0)°. As follows from the NMR spectra, this rotamer also predominates in solution. Conformation of the whole skeleton agrees with that derived from the NMR spectra.

The nonplanarity of the amide (peptide) groups[25] is described by X_N(Neo) = = −2(4)°, X_C(Pro) = 0·1(7)°, X_N(Pro) = −5·2(8)°, X_C(Neo) = 1·0(7)°. Parameters of torsion about the amide C—N bond[26] are τ'(C—NH) = 12(4)° and τ'(C—N) = = 9·8(8)°. The nonplanar arrangement of both the amide groups is thus mainly represented by torsion about the C—N bond.

In the studied crystal (obtained by slow evaporation from ethanolic solution) the molecules of *VIII* are fixed by a system of hydrogen bonds. The significant hydrogen bond N—H(Neo)···O(Neo)′ links the molecules into two helices (antiparallel) along the axis *a*. The weak C—H···O bonds fix the neopentyl moiety.

Chiroptical Measurements

The CD spectra of cyclo(L-Neo-L-Pro) (*IV*) and cyclo(D-Neo-L-Pro) (*VIII*) (Figs 1 and 2 and Table II) exhibit generally four dichroic bands. The positive band at 205 nm and the negative one at the shortest wavelengths are due to the π–π^* transitions of the amide groups, the positive low-intensity band above 230 nm and the negative band between 220 and 224 nm can be ascribed to the amide n–π^* transitions. Similar shape was found also for other cyclodipeptides of the cyclo(X-L-Pro) type[13,21,27]. For comparison, spectra of similar cyclodipeptides, containing a bulky group in the side chain of the moiety X, are also given in Fig. 2.

The similarity of CD spectra of cyclo(X-L-Pro) cyclodipeptides reflects the common structural fragment consisting of 2,5-piperazinedione ring stabilized in a boat conformation by annelated five-membered proline ring[13,21]. On the other hand, even relatively small structural changes of the acyclic substituent in X (the side chain) change significantly the CD spectra (Fig. 2, Table II) in the region of both the amide electron transitions. It has been already pointed out[21,28] that spectra of this type of compounds have nonconservative character in the region of π–π^* bands. The

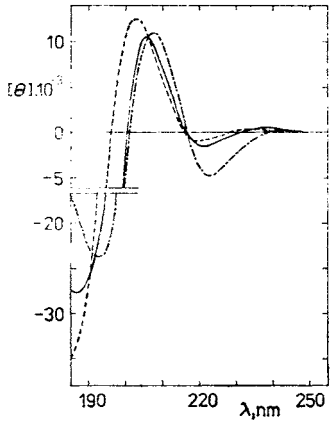


FIG. 1

CD spectra of cyclo(D-neopentylglycyl-L--prolyl) in water (———), 2,2,2-trifluoroethanol (– – – –), and acetonitrile (–·–·–)

short-wavelength negative band is invariably much stronger then the positive one (Figs 1 and 2, Table II). The ratio of apparent intensities of amide π–π^* bands, R (Table III; for definition of R see footnote to Table III), equals at least 2, however, it may achieve values an order of magnitude (or more) higher. In some cases of bulky substituents the positive π–π^* band is not present at all (*e.g.* in the spectra of cyclo-

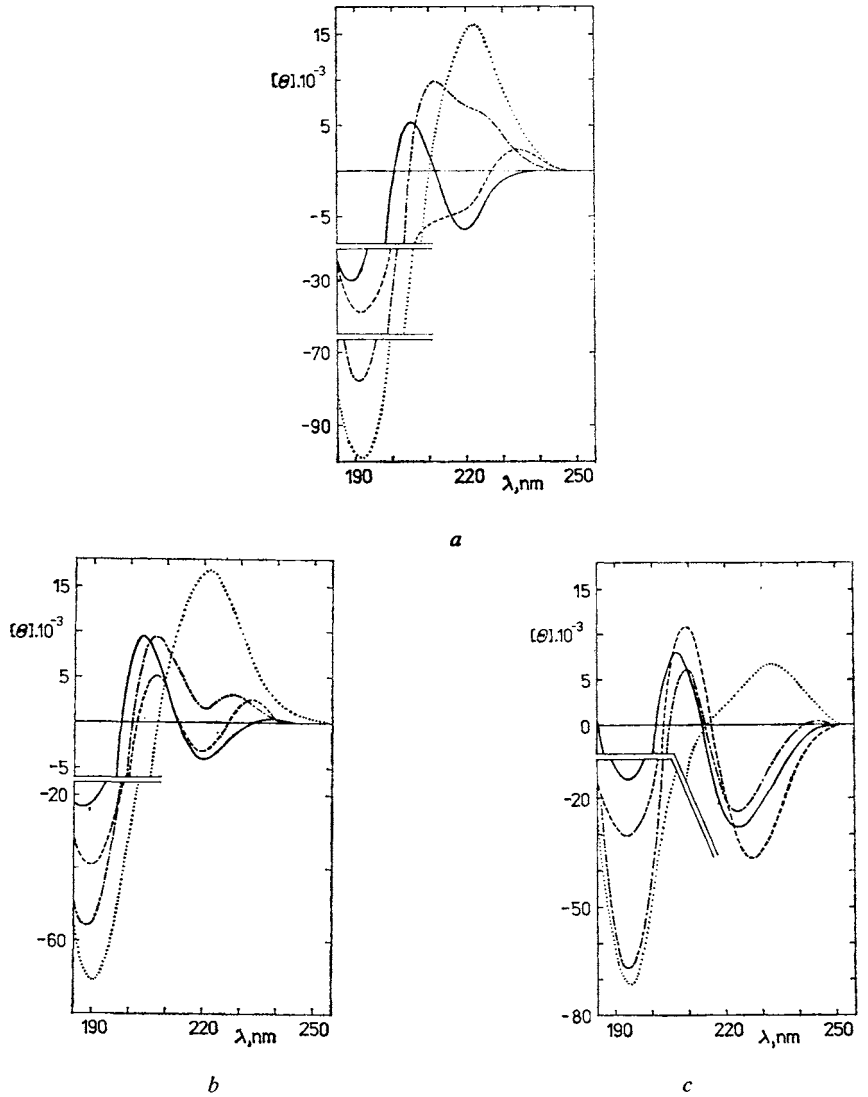


FIG. 2

CD spectra of cyclodipeptides cyclo(L-X-L-prolyl) in *a* water, *b* 2,2,2-trifluoroethanol and *c* acetonitrile. X: neopentylglycyl (———), leucyl (–·–·–·–), tert-leucyl (– – – – –), and valyl (·····)

TABLE II

Circular dichroism of cyclo(L-Ne-L-Pro) and cyclo(D-Neo-L-Pro)

Solvent	λ, nm ([Θ], deg cm^2 dmol^{-1})			
	Amide n–π^* bands		Amide π–π^* bands	
	cyclo(L-Ne-L-Pro) (*IV*)			
TFE[a]	237·5 (+110)	220 (−4 050)	203·5 (+9 500)	188 (−23 000)
Acetonitrile	—	223 (−11 300)	206·5 (+7 900)	194 (−15 000)
Water	—	220 (−6 500)	205 (+5 400)	189 (−30 000)
	cyclo(D-Neo-L-Pro) (*VIII*)			
TFE[a]	233·5 (+300)	220 (−960)	203 (+12 500)	185[b] (−35 000)
Acetonitrile	242 (+310)	222·5 (−4 560)	207·5 (+12 100)	193 (−24 000)
Water	235·5 (+510)	221 (−1 400)	205·5 (+10 400)	187 (−28 000)

[a] 2,2,2-Trifluoroethanol; [b] value of ellipticity at the shortest attainable wavelength.

TABLE III

The amplitude A and the ratio R of the apparent amide π–π^*bands for several cyclodipeptides cyclo(X-L-Pro)

X	TFE[a]		Acetonitrile		Water	
	$A \cdot 10^{-3}$[b]	R[c]	$A \cdot 10^{-3}$[b]	R[c]	$A \cdot 10^{-3}$[b]	R[c]
	trans-disubstituted					
D-Neo	48	2·8[d]	36	2·0	38	2·7
D-Leu	94	3·7[d]	111	4·1[d]	87	3·8
D-Tle	100	3·5	102	4·4	124	4·6
D-Val	105	3·9	87	4·3	101	3·7
	cis-disubstituted					
L-Neo	33	2·4	23	1·9	35	5·6
L-Leu	66	5·8	73	10·8	88	7·9
L-Tle	44	7·3	42	2·8	(25)	*e*
L-Val	(68)	*e*	71	32	103	27·5

[a] 2,2,2-Trifluoroethanol; [b] $A = [\Theta]_{max}$ (positive band) − $[\Theta]_{max}$ (negative band); [c] $R = -[\Theta]_{max}$ (negative band)/$[\Theta]_{max}$ (positive band); [d] for $[\Theta]_{max}$ (negative band) the ellipticity value at 185 nm has been taken; [e] the spectrum shows no positive π–π^* band, see Figs 2*a* and 2*b*, respectively.

(L-Tle-L-Pro) (*II*) in water or cyclo(L-Val-L-Pro) (*I*) in trifluoroethanol, Fig. 2). The origin of the nonconservative behaviour has not been so far satisfactorily explained[21,28–30].

This problem was studied by Sathyanaryana and Applequist[30]. Using the theory of dipole interactions, which involves borrowing the rotational strength between the chromophore and nonchromophoric parts of the molecule and thus also contributions of the side chains, the authors calculated nonconservative spectra of π–π^* transitions with the correct sense for some proline-containing cyclodipeptides. However, the accord with the experimental data is often insufficient (*e.g.* for cyclo(Gly-L-Pro) or cyclo(D-Val-L-Pro) (*V*)) or only apparently good, as in the case of cyclo(L-Val-L-Pro) (*I*) because the positive band in the compared experimental spectrum has entirely an n–π^* character[13]. For the studied type of compounds, theoretical studies[28–30] do not explain even their spectral properties in the region of n–π^* transitions, particularly the presence of the long-wavelength positive band which can be considerably strong and sometimes is the only observable positive band at all (as in the case of cyclo(L-Tle-L-Pro) (*II*) in water or cyclo(L-Val-L-Pro) (*I*) in trifluoroethanol; Fig. 2).

Table III summarizes the parameters of the amide π–π^* bands for cyclodipeptides containing bulky isopropyl or tert-butyl group in the side chain: the sum of apparent, intensities of the π–π^* bands, A (amplitude), and their ratio, R. According to the theory[28–30], the amplitude A reflects the approximate deepness of the boat conformation (A increases with increasing folding of the boat). The parameter R is a measure of the nonconservative character, it cannot be correlated with A and probably is related to another structural parameter of the 2,5-piperazinedione ring. As seen from Table III, in the series of *trans*-disubstituted 2,5-piperazinediones the parameter R depends only little on the side chain whereas in the *cis*-series this dependence is much more pronounced and exhibits a certain regularity. Similar situation, save minor exceptions, exists for the parameter A. Conformation of the side chains in the *cis*-series, as determined from the NMR spectra, is much the same (the so-called "extended to N" conformation prevails) and does not differ dramatically even from the conformation of the *trans*-compounds, at least for the leucine and neopentylglycine dipeptide. We can assume that the differences in the CD spectra of the studied cyclodipeptides reflect different conformations of the 2,5-piperazinedione ring rather than of the side chain. The pseudoequatorial substituent (side chain) in the *cis*-disubstituted 2,5-piperazinediones interacts sterically more strongly with the 2,5-piperazinedione ring and affects thus more effectively its conformation than the pseudoaxial substituent in the *trans*-diastereoisomers. One of conformational parameters, affected by these interactions, is necessarily the conformation of the amide groups (their nonplanar deformation is associated with formation of a twisted boat form). As shown by energy calculations[31], the occurence of twisted conformations of piperazinedione ring is probable, and with growing deepness of the boat[32]

these conformations become more stable than classical boat forms. X-Ray diffraction affords evidence on nonplanar amide groups in crystalline cyclodipeptides cyclo(Gly-L-Pro)[33], cyclo(L-Leu-L-Pro)[34], cyclo(D-Tle-L-Pro)[35], and cyclo(D-Neo-L-Pro)[16] (studied in this work), all with the positive torsion angle ω. As follows from investigation of rigid polycyclic lactams[1-3,36], a nonplanar amide group with positive ω gives rise to a positive bathochromically shifted n–π^* band and a negative π–π^* band. A positive n–π^* band at longer wavelengths in the CD spectra of cyclodipeptides of the cyclo(X-L-Pro) type and the pronounced nonconservative character of the π–π^* bands (parameter R) could thus indicate the presence of twisted conformations (their population and/or the degree of nonplanar deformation of the amide groups). Sathyanarayana and Applequist[30] tentatively included a nonplanar amide group in the calculation of CD spectrum of cyclo(Gly-L-Pro) with promising results.

According to Table III, *trans*-disubstituted 2,5-piperazinediones exhibit higher A values and thus deeper boat forms of the 2,5-piperazinedione ring than the *cis*--isomers. The extraordinarily low value of A for cyclo(D-Neo-L-Pro) (*VIII*) indicates a more shallow conformation of the ring. Most probably, this flattening results from the interactions between the side chain, ending by the tert-butyl group rotor, and the pseudoaxial α-hydrogen atom of the proline moiety (the interactions are more significant than for the equally long chain with leucine isopropyl group). The generally lower values of A for the *cis*- than for the *trans*-diastereoisomers can be interpreted by relative flattening of the 2,5-piperazinedione boat due to the steric interactions of the pseudoequatorial substituent with the vicinal groups on the 2,5--piperazinedione ring, *i.e.* with the carbonyl oxygen and the amino hydrogen atoms (*cf.* ref.[13]). These interactions can be diminished by flattening of the boat form and, of course, also by out-of-plane deformation of the amide groups. This applies particularly to the amide group whose carbonyl is closer to the acyclic substituent[37]. In the series of *cis*-disubstituted compounds we observe a clear dependence of the spectral parameters A and R on the side chain structure. Cyclodipeptides with isopropyl group in the side chain have similar amplitudes which are higher than those of cyclodipeptides with tert-butyl group. Similarly, the R value is higher for the isopropyl cyclodipeptides (except for the pair of the valine and tert-leucine derivatives in water) but it also depends on the side chain length (on the distance between the bulky group and the 2,5-piperazinedione ring). The value of R varies with solvent, particularly in the case of tert-leucine cyclodipeptide.

Thus, cyclodipeptides with the isopropyl group in the side chain exist in a deeper (higher A values) and presumably more twisted (higher R values) piperazinedione boat than cyclodipeptides containing tert-butyl group. The reason of this difference is a different kind of interaction of these two types of side chains with the 2,5--piperazinedione segment. The leucine and valine side chains can assume (and, according to NMR spectra, actually do[38]) the position extended to N, unsymmetrical relative to the ring part of the molecule, in which the contact of the side chain with

the carbonyl oxygen atom is minimized. Under such circumstances, the interaction with the side chain does not prevent the 2,5-piperazinedione ring from assuming the energetically advantageous deeper and twisted boat conformation. Such situation cannot exist in cyclodipeptides containing the tert-butyl group which has a rotor character. In cyclo(L-Tle-L-Pro) (*II*) the methyl groups must be engaged in more or less symmetrical interaction with both vicinal groups which leads to flattening of the boat, at least in this part of the 2,5-piperazinedione ring. Similar, but weaker, symmetrical interactions affect the 2,5-piperazinedione ring also in cyclo(L-Neo-L-Pro) (*IV*). The almost eclipsed conformation of the β-hydrogen atoms, found by NMR spectroscopy, is consistent with this geometry.

The interactions of the side chain with the 2,5-piperazinedione ring manifest themselves also by the dependence of *R* on the chain length and on the degree of solvation. If the bulky group is closer to the piperazinedione ring, its interactions lead to larger nonplanar deformations of the amide groups. The chain–ring interactions are stronger if the effective volume of the vicinal groups is increased by the bonded solvent molecules. In this way we can explain the steepest solvent dependence for the derivative with the bulkier and shorter tert-leucine chain. The largest effect of this type can be expected in water which can be bonded by hydrogen bridges to both solvation sites of the amide group (*cf.* ref.[13]).

In many respects, the dependence of the amide n–π^* bands on structure of the acyclic side chain resembles that of the π–π^* bands. With *trans*-disubstituted 2,5--piperazinediones only small dependence of n–π^* bands on structure was observed (highest dependence was observed in water, *cf.*[13]). Spectra of the D-neopentylglycine cyclodipeptide *VIII*, which are particularly little affected by the solvent, exhibit a predominant negative n–π^* band (Fig. 1). Much more pronounced structure and solvent effects are observed with *cis*-disubstituted 2,5-piperazinediones (Fig. 2*a*–*c*). The n–π^* bands of the neopentylglycine peptide *IV* again are least dependent on the solvent; only one, negative n–π^* band was observed, as predicted for a regular boat conformation of the 2,5-piperazinedione ring[28]. Similarly to π–π^* bands, also the n–π^* bands indicate a regular rather than twist boat form for both the neopentylglycine dipeptides. In the *cis*-series, the dependence of the n–π^* bands on the structure of the side chain is strongly influenced by the solvent. The influence is particularly strong in water (Fig. 2*a*): the spectra differ so much that the close structural similarity of the compounds is not apparent. Thus also the n–π^* bands indicate participation of the solvent in the interaction of the bulky side chain with the 2,5-piperazine ring. As seen from Fig. 2*a*–*c*, the valine cyclodipeptide *I* occupies a special position in the series: In all the solvents its CD spectra exhibit only one, strong positive n–π^* band. This corresponds to its extreme properties in the π–π^* region where the negative band is invariably stronger than in other compounds and, on the contrary, the positive π–π^* band is weak and appears as a shoulder (barely discernible in water) or cannot be detected at all (in trifluoroethanol). Thus, according to our assumptions,

both the n–π^* and π–π^* bands indicate that the highest extent of nonplanar deformations of the amide groups occurs in cyclo(L-Val-L-Pro) (*I*).

CONCLUSIONS

The conformation of the piperazinedione ring in compounds of the type (X-L-Pro) appears as the result of several factors. The basic conformational type, given by annelation of the pyrrolidine ring of the proline moiety, is modified by interactions of the other, acyclic, side chain. The extent and conformational effect of these interactions depend on the chain length, its bulkiness and the type of branching. The conformational role of the solvent, solvating the amide groups, increases with increasing bulk of the side chain.

Characteristic of the neopentylglycine side chain are the interactions of its tert-butyl group, flattening the 2,5-piperazinedione boat conformation and suppressing nonplanar distorsions of the amide groups. The properties of the neopentylglycine side chain resemble thus the shorter tert-leucine, rather than the equally long leucine, chain.

REFERENCES

1. Bláha K., Maloň P.: Acta Univ. Palacki. Olomuc., Fac. Rerum Nat. *93*, 81 (1980).
2. Tichý M., Farag A. M., Maloň P., Kálal P., Bláha K.: Collect. Czech. Chem. Commun. *49*, 834 (1984).
3. Bláha K., Farag A. M., van der Helm D., Hossain M. B., Buděšínský M., Maloň P., Smolíková J., Tichý M.: Collect. Czech. Chem. Commun. *49*, 712 (1984).
4. Bláha K.: Collect. Czech. Chem. Commun. *34*, 4001 (1969).
5. Bláha K., Frič J.: Collect. Czech. Chem. Commun. *35*, 619 (1970).
6. Bláha K., Buděšínský M., Frič I., Smolíková J., Vičar J.: Tetrahedron Lett. *1972*, 1437.
7. Buděšínský M., Bláha K.: An. Quim. C *68*, 743 (1972).
8. Vičar J., Smolíková J., Bláha K.: Collect. Czech. Chem. Commun. *37*, 4060 (1972).
9. Vičar J., Buděšínský M., Bláha K.: Collect. Czech. Chem. Commun. *38*, 1940 (1973).
10. Vičar J., Smolíková J., Bláha K.: Collect. Czech. Chem. Commun. *38*, 1957 (1973).
11. Vičar J., Bláha K.: Collect. Czech. Chem. Commun. *38*, 3307 (1973).
12. Justová V., Bláha K.: Collect. Czech. Chem. Commun. *40*, 662 (1975).
13. Pančoška P., Frič I., Bláha K.: Collect. Czech. Chem. Commun. *44*, 1296 (1979).
14. Vičar J., Bláha K., Piriou F., Juy M., Hung Lam-Thanh, Fermandjian S.: Biopolymers *21*, 2317 (1982).
15. Symerský J., Bláha K., Langer V.: Acta Crystallogr., C *43*, 303 (1987).
16. Symerský J., Bláha K., Langer V.: Acta Crystallogr., C *43*, 490 (1987).
17. Pospíšek J., Bláha K. in the book: *Peptides 1976, Proc. 14th European Peptide Symp., Wepion 1976* (A. Loffet, Ed.), p. 95. Editions de l'Université de Bruxelles, Brussels 1976.
18. Pospíšek J., Bláha K.: Collect. Czech. Chem. Commun. *42*, 1069 (1977).
19. Pospíšek J., Bláha K.: Collect. Czech. Chem. Commun. *52*, 514 (1987).
20. *Nomenclature and Symbolism for Amino Acids and Peptides. Recommendations 1983.* Eur. J. Biochem. *138*, 9 (1986).
21. Madison V., Young P. E., Blout E. R.: J. Am. Chem. Soc. *98*, 5358 (1976).

22. Le Cocq C., Lallemand J.-Y.: J. Chem. Soc., Chem. Commun. *1981*, 150.
23. Kopple K. D., Wiley G. R., Tauke R.: Biopolymers *12*, 627 (1973).
24. Bystrov V. F.: Prog. Nucl. Magn. Reson. Spectrosc. *10*, 41 (1976).
25. Warshel A., Levitt M., Lifson S.: J. Mol. Spectrosc. *33*, 84 (1970).
26. Winkler F. K., Dunitz J. D.: J. Mol. Biol. *59*, 169 (1971).
27. Kubo A., Takahashi K., Arai T.: Experientia *33*, 12 (1977).
28. Hooker T. M. Jr., Bayley P. M., Radding W., Schellman J. A.: Biopolymers *13*, 549 (1974).
29. Bowman R. L., Kellerman M., Johnson W. C.: Biopolymers *22*, 1045 (1983).
30. Sathyanaryana B. K., Applequist J.: Int. J. Peptide Protein Res. *26*, 518 (1985).
31. Kolaskar A. S., Sarathy K. P.: Biopolymers *19*, 1345 (1980).
32. Ciarkowski J.: Biopolymers *23*, 397 (1984).
33. Dreele R. B. von: Acta Crystallogr., B *31*, 966 (1975).
34. Karle I. L.: J. Am. Chem. Soc. *94*, 81 (1971).
35. Sklenář I., Ječný J.: Acta Crystallogr., B *35*, 513 (1979).
36. Tichý M., Maloň P., Frič I., Bláha K.: Collect. Czech. Chem. Commun. *44*, 2653 (1979).
37. Maloň P., Pospíšek J., Bláha K. in the book: *Peptides 1984, Proc. 18th European Peptide Symp., Djuronaset 1984* (U. Ragnarsson, Ed.), p. 591. Almquist & Wiksell, Stockholm 1984.
38. Madison V., Young P. E., Blout E. R.: J. Am. Chem. Soc. *98*, 5356 (1976).

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