SPECTRA IN THE MIDDLE AND FAR IR REGIONS OF CYCLIC PEPTIDE COMPOUNDS WITH A cis AMIDE GROUP

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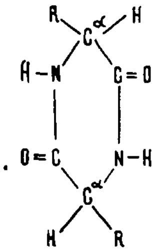
At the present time, the IR spectra of secondary amides [1-2] and of peptides [3-6] with the trans configuration of the amide bond have been studied in fairly great detail. The authors concerned have made assignments of the frequencies of the normal vibrations over a wide region of the spectrum (4000-40 cm^{-1}) and have singled out those particular vibrations that characterize the trans configuration of the amide group in the solid state. It has been found that particular value for the identification of the conformational states of peptide compounds is presented by the frequencies of bands located in the middle and far IR regions (amide I-amide VII) [3, 4]. Thus, from the features of the IR spectra it is possible to determine the trans configuration of an amide bond fairly reliably. At the same time, the second possible configuration of the amide bond – the cis form – has been studied quite inadequately. An assignment has been made of the frequencies of the normal vibrations of the cis configuration for substituted amides [7-8], lactams [9], glycylglycine anhydride, and other piperazinediones [1-12, 36], but the published results are contradictory to some extent and therefore further investigations are necessary. In this communication we give the results of a study of the IR spectra of piperazinediones with various amino acid residues having the general formula



and their deutero analogs (Table 1).

TABLE 1*

Compound No.	Compound	Formula of the compound	R
I	cyclo-(-Gly-)$_2$	$(-CH_2CONH-)_2$	–
II	cyclo-(-Gly-)$_2$	$(-CH_2COND-)_2$	–
III	cyclo-(-Gly-)$_2$	$(-CD_2CONH-)_2$	–
IV	cyclo-(-Ala-D-Ala-)	$(-CHR-CONH-)_2$	CH_3
V	cyclo-(-Ala-)$_2$	$(-CHR-CONH-)_2$	CH_3
VI	cyclo-(-Ala-)$_2$	$(-CHR-COND)_2$	CH_3
VII	cyclo-(-Val-)$_2$	$(-CHR-CONH-)_2$	$CH(CH_3)_2$
VIII	cyclo-(-Val-)$_2$	$(-CHR-COND-)_2$	$CH(CH_3)_2$
IX	cyclo-(-*n*Val-)$_2$	$(-CHR-CONH-)_2$	$CH_2CH_2CH_3$
X	cyclo-(-*n*Val-)$_2$	$(-CHR-COND-)_2$	$CH_2CH_2CH_3$

*Amino acids of the L series except when stated otherwise.

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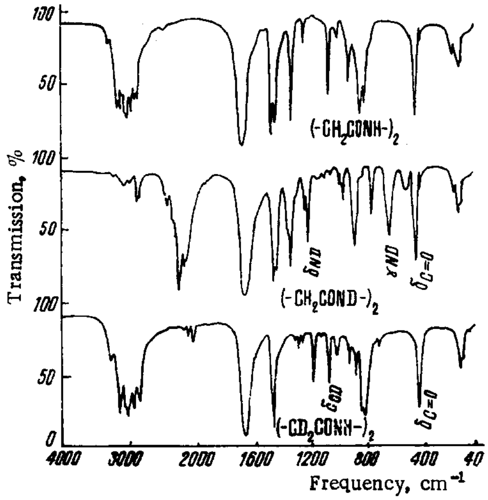

Fig. 1. IR absorption spectra of compounds (I-III) (KBr tablets down to 400 cm^{-1} and mulls with paraffin oil below 400 cm^{-1}).

It is known that such structures can be encountered in natural materials [37, 39]. It appeared of interest to study the sensitivity of the IR spectra to a change in the geometry of the piperazinedione ring, which may be either planar or nonplanar [13-19].

Figure 1 shows the spectra of compounds (I-III) in the 4000-40 cm^{-1} region. Let us first consider the vibrations up to 160 cm^{-1}; the vibrations of the hydrogen bond and of the crystal lattice will be discussed below. As is well known from x-ray structural studies, the molecule of glycylglycine anhydride (I) is planar and belongs to symmetry group C_{2h} [14]. The distribution of the 24 vibrations of the molecule (without taking into account the radicals at C^{α}) according to the types of symmetry has the form Γ_u = 9Ag + 4Au + 8Bu + 3Bg. The 4Au and 8Bu vibrations are active in the IR spectrum and are, respectively, nonplanar and planar in relation to the plane of the ring. Thus, our problem includes the finding of eight planar and four nonplanar vibrations in the observed spectrum of compound (I). The results of the interpretation of the vibrational spectrum of compound (I) are given in Table 2.

The assignment of the nonplanar vibrations is a matter of some complexity. The band at 840 cm^{-1} has been assigned by Karplus and Lifson [12] to the vibration of the CH_2 group and that at 808 cm^{-1} to the nonplanar vibration of the N—H groups. An analysis of the IR spectra of compounds (II) and (III) (see Fig. 1) permits these bands to be assigned more correctly, since in the spectrum of compound (II) the band at 840 cm^{-1} has shifted into the 662-cm^{-1} region and in compound (III) it has shifted into the 847-cm^{-1} region. Furthermore, the band at 808 cm^{-1} can be assigned to the nonplanar deformation vibration of C =O groups: on deuteration it apparently shifts into the 670-cm^{-1} region in the case of compound (I) and to about 740 cm^{-1} in the case of compound (II). This assignment is also confirmed by the fact that the amide VI band shifts considerably when different groups in the amides [2] and diamides [3-5, 21] are deuterated, in contrast to the band of the deformation vibration of C =O groups (amide IV band) which is displaced insignificantly on N-deuteration [2-5]. We have found no bands ascribed to the twisting vibration about the C—N bond (or VII); the only possible band for assignment to this vibration, at 178 cm^{-1}, disappears in the spectrum of compound (III), which should not take place in the deuteration of the CH_2 groups [3], and no other bands are found in the low-frequency region of the spectrum (up to 150 cm^{-1}) of cyclo-(-Gly-)$_2$.

The band in the form of a shoulder at ~780 cm^{-1} must also be considered. Karplus and Lifson [12] assigned it to the nonplanar deformation "antisymmetric" vibration of NH groups. In our opinion, this assignment is unlikely for two reasons: because of its low intensity and because of the presence in the spectrum of compound (II) of a band at 779 cm^{-1} which shows an insignificant shift of this band on N-deuteration. These facts, by analogy with literature information [3, 4, 9] may be considered to favor the assignment of the band at ~780 cm^{-1} to the planar deformation vibration of the $C_{\alpha}CN$ angle.

X-ray structural information for compounds (IV) and (V) shows that the first of them has a planar structure of the ring and the second a nonplanar structure [15, 16]. In view of this, the assignment of the bands in the IR spectrum of compound (IV) (Fig. 2) was performed by analogy with cyclo-(-Gly-)$_2$ (Table 3).

The appearance of bands at 1665, 1330, 697, and 455 cm^{-1} in compound (IV) but not in the spectrum of cyclo-(-Gly-)$_2$ should probably be explained by interaction between neighboring molecules in the crystal [22]. In the spectrum of Ala-D-Ala in DMSO only one band is present, at 1680 cm^{-1}. Thus, our hypothesis proves to be correct.

The nonplanar molecule of cyclo-(Ala-)$_2$, the IR spectrum of which is shown in Fig. 3, belongs to symmetry group C_2. If the side chain of the amino acid radical is disregarded, the distribution of these 24 vibrations according to the types of symmetry has the form Γ_u = 13A +11B. Since all the vibrations are IR-active, a larger number of bands must be expected in the observed spectrum of compound (V) than in the spectrum of (IV). However, their IR spectra scarcely differ in respect of the number of bands, which is due to the small splitting of the bands because of the weak interaction between equivalent groups, which are remote from one another in the cyclo-(-Ala-)$_2$ molecule.

TABLE 2. Frequencies of the Normal Vibrations of Cyclo-(-Gly-)$_2$

Frequency, cm^{-1}	Assignment	Type of symmetry	Notes
3180	N—H stretch	Bu	—
2918	CH$_2$ stretch antisym.		
1690	C=O stretch	Bu	—
1470	CH$_2$ def.		
1442	N—H def.	Bu	—
1375	?		
1340	C—N stretch	Bu	—
1250	?		
1075	N—CH$_2$ stretch + def. angle	Bu	[11]
995	C—C stretch	Bu	[12, 20]
915	CH$_2$ twist		
840	N—H def.	Au	Amide V
808	C=O def.	Au	Amide VI
708 (sh.)	CH$_2$—C—N def. (CCN)	Bu	$C_\alpha CN$
449	C=O def.	Bu	Amide IV
178	?		

Table 4 gives the assignments of the bands in the spectrum of compound (V) on the basis of an analysis of the spectra of compounds (VI) (see Fig. 3), (I), and (IV).

On comparing the spectra of compounds (I) and (IV) with that of compound (V) it is easy to see that with a nonplanar structure of the molecule several absorption bands appear in the 670-600-cm^{-1} region. Making use of information on the IR spectra of lactams [9], we assigned the bands in this region to the vibration of the CNC$^\alpha$ angle, which it is possible to consider as a characteristic index of the nonplanarity of the ring in peptides with a cis-amide group.

Thus, the results of an analysis of the spectra of cyclic peptides with a cis-amide group in the middle and far IR regions enable characteristic vibrations for the cis configuration to be isolated and be compared with those for the trans configuration in linear peptides (Table 5). However, as mentioned previously [6], the use of vibrations in the amide I-III region to indicate the presence of the cis configuration in peptides is possible only when the cis configuration has a considerable predominance, because a number of characteristic amide bands overlap with the bands of other vibrations, such as the deformation vibrations of CH$_2$ and CH$_3$ groups.

No x-ray structural information on compounds (VII) and (IX) is known from literature sources. Nevertheless, the similarity of the spectra of these compounds (Fig. 4) to the spectrum of compound (V), and also an analysis of the spectra of the N-deuterium-substituted analogs permits an assignment to be

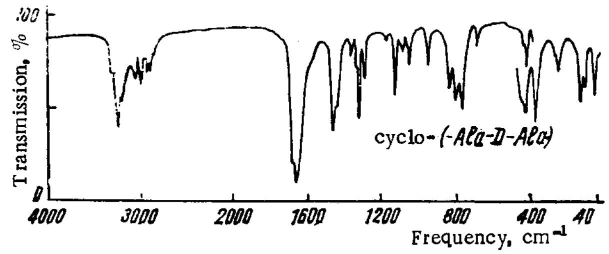

Fig. 2. IR absorption spectra of compound (IV).

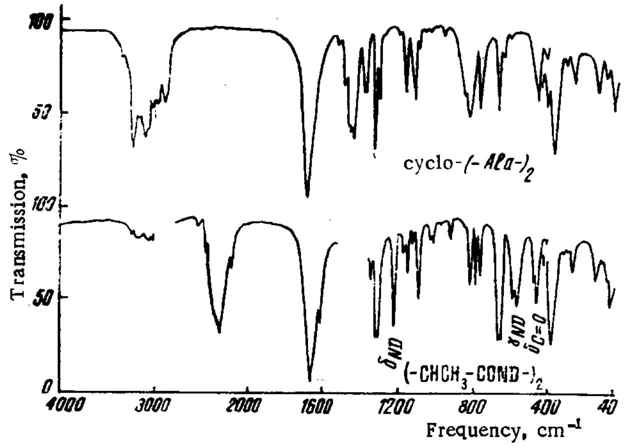

Fig. 3. IR absorption spectra of compounds (V) and (VI) (at the positions of interruption of the spectrum are the absorption bands of paraffin oil).

TABLE 3. Frequencies of the Normal Vibrations of Cyclo-(-Ala-D-Ala-)

Freq., cm^{-1}	Assignment	Type of symmetry
3250	N–H stretch.	Bu
2980	CH_3 stretch.	
1680	C=O stretch.	Bu
1665		
1470	C–CH_3 def. antisym.	
1450	N–H def.	Bu
1370	C–CH_3 def. sym.	
1340	—N stretch.	Bu
1330	C–N stretch.	
1300	?	
1130	N–CH_3stretch.+def.angle	Bu
1095	?	
1060	C–C stretch.	
962	C–C stretch.	Bu
853*		
840	N–H def.	Au
810	C=O def.	Au
780	CH_3–C–N def.	Bu
697	?	
455	C=O def.	Bu
436		
392	Deformation vibrations of the skeleton	
288		
270		

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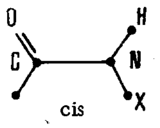
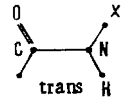
* Band observed only in the spectrum of a mull in paraffin oil

TABLE 4. Frequencies of the Normal Vibrations of Cyclo-(-Ala-)$_2$

Freq., cm^{-1}	Assignment	Type of symmetry
3190	N–H stretch.	A, B
3080		
2980	CH_3 stretch.	
1690	C=O stretch.	A, B
1465	C–CH_3 def. antisym.	
1450	N–H def. planar	A, B
1375	C–CH_3 def. sym.	
1321	C–N stretch.	A, B
1302	?	
1155	N–CH_3 stretch+def. angle	A, B
1120		
960	C–C stretch	A, B
845	NH–def. nonplanar	A, B
826	C=O def. nonplanar	A, B
770	C^{α} CN	A, B
671	CNC_{α}	A, B
658		
611		
484	C=O def.	A, B
477		
426	Deformation vibrations of the skeleton	—
388		
313		
284		

TABLE 5

Amide* group	Vibration, cm^{-1}						
	amide I	amide II	amide III	amide IV	amide V	amide VI	amide VII
	1670–1690	1440–1460	1320–1340	450–500	830–850	790–830	?
	1650–1660	1550–1570	1260–1310	610–700	720–730	520–610	180–250

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* Figures for the trans configuration taken from the literature [1-4, 21].

made of the vibrations and, on this basis, some conclusions to be drawn concerning the structure of cyclo-(-Val-)$_2$ and cyclo-(-nVal)$_2$. All the assignments are given in Table 6.

The absence of bands in the 1550-1570-cm^{-1} region and also the values of the amide I-VI bands show the cis configuration of the amide groups in compounds (VII) and (IX). In addition, in cyclo-(-Val-)$_2$ and cyclo-(-nVal-), there are bands in the 670-600-cm^{-1} region which show a nonplanar structure of the rings. This conclusion is confirmed by the results of calculations and of investigations of a number of piperazinediones in solutions by the NMR method [38]. In the case of compound (IX) the appearance of bands relating to the twisting vibrations of CH_2 and CH_3 groups around a C–C bond in the long side chain in the 770-720-cm^{-1} region is possible [20], which explains some difference of the spectrum in this region from the spectra of the other cyclic peptides.

Results of investigations of the intermolecular vibrations of monomers bound into a dimer by hydrogen bonds have been given in many publications. The authors of these publications have found the values of the frequencies of stretching vibrations proper of the hydrogen bonds for dimers of acetic acid [23], formic acid [24-25], other carboxylic acids [26], and benzene derivatives [27]. A knowledge of the frequencies of the stretching vibrations proper of the hydrogen bond makes it possible to determine to what extent these

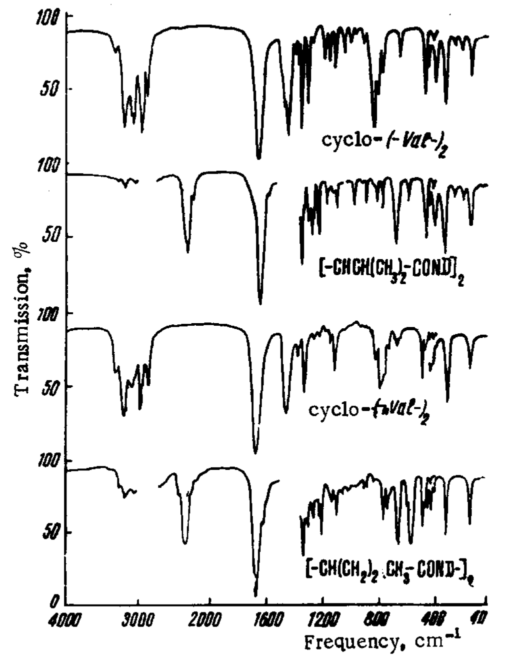
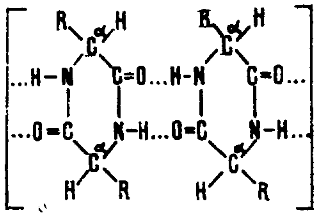


Fig. 4. IR absorption spectra of compounds (VII-X).

vibrations affect the interacting molecules, i.e., whether they are localized in the complex X−H···Y with their frequency independent of the residual mass of the interacting molecules or the opposite situation exists. By considering, on the basis of x-ray structural and electron-diffraction data [14-16, 23], the molecules of compounds (I), (IV), and (V) in the form of dimers, we have calculated the frequencies of the stretching vibrations of the hydrogen bonds proper for cyclo-(-Gly-)$_2$. It was assumed that the vibrational interaction between the dimers is a second-order effect; in this case the frequency can be determined from the formula for the vibrations of a diatomic molecule [26, 27]

$$\nu' = \frac{1}{2\pi c}\sqrt{\frac{mf}{\mu}}, \tag{1}$$

where f is the force constant of one hydrogen bond; m is the number of hydrogen bonds (in our case, m = 2); and μ is the effective reduced mass.



For the calculations of the values of f we used a semiempirical potential function (V) describing the properties of the hydrogen bond which was proposed by Lippincott and Schroeder [29, 30]. Assuming that the X−H···Y system is linear, we find the value of the force constant from the condition

$$f = \left(\frac{\partial^2 V}{\partial R^2}\right)_{r,\,R}$$

Here, r (0.98 Å) and R (2.84 Å) are the X−H and X···Y distances at equilibrium, corresponding to the true values taken from electron-diffraction figures [23]. The expression for f in the developed form is as follows:

$$f = \frac{D_0^* n^*}{2} exp\left[-\frac{n^*}{2}\cdot\frac{(R-r-r_0^*)^2}{(R-r)}\right]\cdot\left\{\frac{2r_0^{*2}}{(R-r)^3} - \frac{n^*}{2}\left[1-\frac{r_0^{*2}}{(R-r)^2}\right]^2 + \left[1-\frac{r_0^*}{(R-r)^2}\right]\cdot\left[\frac{b^2R^2-1}{bR^2-\frac{R}{2}}\right]\right\}.$$

D_0^* is determined from the formula $D_0^* = K_0^* r_0^* / n^*$. The values of r_0^*, n^*, b, and K_0^* were taken from the literature [30] for the linear N−H···O system.

Thus, for compound (I) it was found that $f = 0.941 \cdot 10^4$ dyne/cm. From formula (I) let us determine the values of ν' for two possible reduced masses: 1) μ_1 − the reduced mass of the complex N−H···O; and, 2) μ_2 − the reduced mass of the two interacting molecules. In the first case, $\nu'_{\mu_1} = 197$ cm^{-1}, and in the second case $\nu'_{\mu_2} = 73$ cm^{-1}. In view of the pronounced anharmonicity of the $\vec{X}-\vec{H}\cdots\vec{Y}$ vibrations in dimers [26, 27, 31], let us introduce a correction for the values of ν'_{μ_1} and ν'_{μ_2} obtained. The anharmonicity factor $\chi = 0.045$ was taken to be the same as for carboxylic acids [27], since it is unknown for the cyclic peptides studied. Taking anharmonicity into account, $\nu_\mu = \nu'_\mu(1-2\chi)$. Hence $\nu_{\mu_1} = 170$ cm^{-1}, and

TABLE 6. Frequencies of the Normal Vibrations of Cyclo-(-Val-)$_2$ and Cyclo-(-nVal-)$_2$

Cyclo-(Val-)$_2$		Cyclo-(-nVal-)$_2$	
frequency, cm^{-1}	assignment	frequency, cm^{-1}	assignment
3195 3090	N–H stretch.	3210 3090	N–H stretch.
2960	CH_3 stretch.	2960	CH_3 stretch.
1665	C=O stretch.	1675	C=O stretch.
1463	C–CH_3 def. antisym.		
1450	N–H def. planar	1450	N–H def. planar
1375	C–CH_3 def. sym.	1380	C–CH_3 def. sym.
1350	C–N stretch.	1332	C–N stretch.
1295	?	1297	?
1145 1108	N–CH–R stretch+def. angle	1145 1120	N–CH_2–R stretch+def. angle
1043 1000 970	C–C stretch.	1010 930	C–C stretch.
847	N–H def. nonplanar	827	N–H def. nonplanar
825	C=O def. nonplanar	787	C=O def. nonplanar
790	C^{α} CN	740	C^{α} CN
656	CNC^{α}	670 662 625	CNC^{α}
474 470	C=O def. planar	493 472	C=O def. planar
408 331 269 210	Def. skeleton	440 423 413 325	Def. skeleton

$\nu_{\mu_2} = 66$ cm^{-1}. On comparing the calculated values obtained for ν_{μ_1} and ν_{μ_2} with the absorption bands in the spectra of compounds (I, IV, V, VII, and IX) below 160 cm^{-1} (see Figs. 1-4), it can be seen that in all the compounds there is a strong band in the 150-cm^{-1} region which is closest to the value of ν_{μ_1} and can be assigned to the stretching vibration proper of a hydrogen bond. Consequently, this vibration can be considered as localized in the complex N–H···O: it does not depend on the mass of the molecules forming the dimer. This conclusion confirms the assumption made previously of a weak interaction of the vibrations between the dimers. Our assignment also agrees well with the results of investigations on the long-wave spectra of crystalline imidazole forming a dimer [32, 33] in which a band at 142 cm^{-1} has been assigned to the stretching vibration proper of the hydrogen bond of the complex N–H···N (R = 2.86 Å).

It is interesting to note that in the spectrum of the planar compound (I), instead of one band at 140 cm^{-1}, as was shown in [34], there are two bands, at 149 and 138 cm^{-1}. The same pattern is observed in the spectrum of compound (IV) (bands at 153 and 132 cm^{-1}). The appearance of two bands in the region of the stretching vibrations of the hydrogen bond must probably be explained by Davydovskii splitting through interaction between the N–H···O vibrations, as takes place in the dimer of acetic acid [23]. It may be assumed that the band at 178 cm^{-1} in the spectrum of cyclo-(-Gly-)$_2$ also appears as the result of an interaction of the N–H···O vibrations and is due to the stretching vibration proper of the hydrogen bond. In the spectrum of imidazole [32], in addition to the band at 142 cm^{-1} a band at 179 cm^{-1} has also been assigned to this vibration.

The bands below 130 cm^{-1} in the spectra of compound (I) (84 cm^{-1}), (IV) (82 cm^{-1}), and (V) (112 and 72 cm^{-1}) are assigned, by analogy with imidazole [32], to the deformation vibrations proper of the hydrogen bond. However, such assignment requires additional experimental and computing confirmations and therefore cannot be considered as definitive.

EXPERIMENTAL

The cyclopeptides (I, IV, V, VII, and IX) were obtained by the method proposed by Nitecki et al. [35]. The degree of deuteration of the N-and C-deuterium analogs was checked from the absorption bands of the N–H stretching and CH_2 deformation vibrations.

The IR spectra were recorded on a Perkin-Elmer 257 spectrometer in the 4000-625-cm^{-1} region, a Hilger H-800 spectrometer in the 1000-400-cm^{-1} region, and an FiS-21 instrument in the 500-40-cm^{-1} region. The samples were used in the form of tablets with KBr down to 400 cm^{-1} and in the form of mulls with paraffin oil between polyethylene windows in the 500-40-cm^{-1} region. The accuracy of the measurements below 2000 cm^{-1} was ±2 cm^{-1}, and the calibration of the instruments was performed with respect to the spectra of polystyrene and of water vapor.

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SUMMARY

1. The results of an analysis of the spectra of cyclic peptides in the middle and far IR regions have enabled the characteristic vibrations of the cis configuration of the amide group to be distinguished.

2. A difference has been found in the IR spectra of planar and nonplanar piperazinedione rings.

3. The frequency of the stretching vibration of the hydrogen bond in cyclic peptides has been determined and its characteristic nature has been shown.

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