Effects of Differing Chirality on the Hydrogen-bonding Behaviour of Di- and Tri-peptide Derivatives

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The self-association of di- and tri-peptide derivatives with a well-defined configuration at their chiral C atoms and 1:1 mixed diastereoisomeric pairs of these tripeptide derivatives have been investigated by means of IR spectroscopy in CH_2Cl_2. In most—but not in all—cases major differences in the association behaviour have been recorded on changing the configuration at only one C atom.

In our investigations on hydrogen bonding one of the major points of interest has been the behaviour of optically active compounds. If such compounds interact by hydrogen bonding with molecules which also possess a chiral centre, then 'associative' diastereoisomers are formed, which have, in principle, different entropies ($-\Delta S$) and enthalpies ($-\Delta H$) of association, in the same way that diastereoisomeric molecules are fundamentally different in their intramolecular energies. The ability of the enantiomers and diastereoisomers to associate intermolecularly in an enantioselective way, is possibly the reason for different biochemical and biological properties of molecules of opposite configuration.

In recent years we have published several papers on the molecular interactions of optically active compounds.[1-3] We have now extended our field of interest to the self-association of a diastereoisomeric pair of di- and tri-peptide derivatives. The substances investigated were *N*-acylated dipeptides and *N*-acylated tripeptide esters synthesized mainly as reference peptides for HPLC research. The results of the spectroscopic investigations and some discussions of these are given.

The formula of the substances investigated are given in the Scheme.

dipeptide derivatives

$PhCH_2OCONH\overset{*}{C}HR^1CONH\overset{*}{C}HR^2CO_2H$

L L or D

	R^1	R^2
(1)	CH_3	CH_2Ph
(2)	CH_3	$CH(CH_3)_2$
(3)	H	CH_2Ph
(4)	$CH(CH_3)_2$	$CH(CH_3)_2$

tripeptide derivatives

$PhCH_2OCONH\overset{*}{C}HR^1CONH\overset{*}{C}HR^2CONH\overset{*}{C}HR^3CO_2CH_3$

L L or D L

	R^1	R^2	R^3
(5)	CH_3	CH_2Ph	$CH(CH_3)_2$
(6)	H	CH_3	CH_2Ph
(7)	CH_3	$CH(CH_3)_2$	$CH(CH_3)_2$
(8)	$CH(CH_3)_2$	$CH(CH_3)_2$	$CH(CH_3)_2$

Scheme. General structure of the di- and tri-peptide derivatives.

All substances have only the L-configuration at the 1 (and 3) positions but one species of every diastereoisomeric pair has the L-configuration at position 2, and the other species have the D-configuration at that position.

Results and Discussion

The region of the IR spectrum corresponding to NH appears to be rather similar for the peptides. There is always a free NH band of approximately the same intensity at precisely 3 420 cm^{-1} and, depending on the temperature and concentration, an associate band which is long-wavelength shifted by up to *ca.* 100 cm^{-1}. A strong steric influence due to the different R groups cannot be detected. We were surprised to find only one free NH band which represented the absorption of all (three or two) NH groups in all substances under investigation. This fact complicates the interpretation of the results, because the different NH groups [even in the case of substance (**8**)] do not possess the same absorption coefficients *a priori*. Therefore it is impossible to distinguish which NH groups take part in the hydrogen bonding and which remain free. But it may be deduced that a decrease of the absorption coefficients of the free NH bands of $> 50\%$ in the case of dipeptide derivatives and $> 33\%$ for that of the tripeptide derivatives means a participation of at least two NH groups in the hydrogen bonds.

In this study we began with an investigation of the *N*-protected dipeptide carboxylic acids. In addition to the broad $-CO_2H$ dimer band there is a sharp NH band at 3 420 cm^{-1}, which is strongly dependent in its intensity on temperature and/or concentration. With decreasing temperature and/or increasing concentration a broad associate band appears, at *ca.* 100 cm^{-1} lower wavenumber due to the formation of hydrogen bonds. Because the intensity relation between free and associate bands is strongly dependent on the concentration, the interaction is evidently an intermolecular one. Only the small asymmetry of the free NH band, as demonstrated in Figure 4(*b*) may be an indication of a very small number of intramolecularly associated molecules.

Although only very small differences have been found in the position of the free NH band and in the carbonyl group region, remarkable differences have appeared in the number of associated NH groups which depend on the configuration. The values of $\Delta\nu_{NH}$ (cm^{-1}) and $-\Delta H$ (kJ mol^{-1}) are summarized in Table 1. The L forms of compounds (**1**) and (**2**) are more inclined to undergo NH association than the D forms and the fact that L-(**2**) exhibits a sharper associate NH band than D-(**2**), which is given in Figure 1, may be taken as an indication of the association to different acceptor sites. The high difference in ΔH probably means that in the case of the associated L molecules other part of the molecules, perhaps other NH bonds, are in better steric positions from which to form additional attractive

Table 1. $\Delta\nu_{NH}$ (cm^{-1}) and $-\Delta H$ (kJ mol^{-1}) for the peptides under investigation.

Compound	$\Delta\nu_{NH}$	$-\Delta H$
D-(**1**)	85	9.5
L-(**1**)	95	13.4
D-(**2**)	70	13.0
L-(**2**)	70	19.7
D-(**3**)	85	11.5
L-(**3**)	85	11.5
D-(**5**)	92	9.5
L-(**5**)	92	9.5
D-(**6**)	82	8.4
L-(**6**)	92	13.0
D-(**7**)	95	6.3
L-(**7**)	105	15.6
D-(**8**)	36	5.5
L-(**8**)	104	16.8

Table 2. Physical properties of the substances under investigation.

Compound	Optical rotation	M.p./°C	HPLC retention time
	$[\alpha]_D^{22}$ (c = 2 m)		
L-(**1**)	+13.1 (C_2H_5OH)	124–125	
D-(**1**)	+3.6 (DMF)	74–75	
L-(**2**)	−18.5 (C_2H_5OH)	143–144	
D-(**2**)	+23.1 (DMF)[a]	102–103	
L-(**3**)	+39.2 (C_2H_5OH)	126–127	
D-(**3**)	−38.9 (C_2H_5OH)	125–126	
L-(**4**)	−32.5 (1 m KOH)	139–141	
D-(**4**)	+46.0 (DMF)[a]	178–180	
	$[\alpha]_D^{25}$ (c = 1 m, CH_3OH)		
L-(**5**)	−5.2	153–154.5	9.70
D-(**5**)	−15.8	59.5	11.57
L-(**7**)	−63.8	181–183	6.78
D-(**7**)	−9.2	161–162	8.35
L-(**8**)	−62.7	214–215	8.27
D-(**8**)	−6.7	157–158	10.42

[a] Measured at 578 nm.

interactions in the associated complexes. As expected, we cannot detect any difference between the association behaviour of the D- and L-forms of compound (**3**), because both species are enantiomers with equal enthalpic and entropic potential. Unfortunately, the L form of compound (**4**) is not sufficiently soluble in CH_2Cl_2. The greatest decrease in intensity of the free NH bands on association takes place in the case of compound L-(**2**). But this decrease amounts only to *ca.* 40%, and therefore we cannot conclude for certain that in any of the compounds (**1**)–(**3**) both NH bonds are engaged in hydrogen bonding.

The influence of configurational changes at the R^2 position on the association behaviour of the tripeptide derivatives are summarized in Table 2. In all cases (**5**)–(**8**) the configurationally homogeneous species is more inclined to take part in the association. Only in the case of the species L-(**8**), at concentrations *ca.* 6 × 10^{-2} mol dm^{-3} at room temperature, was the extinction coefficient of the free NH band $<2/3$ of that in the association-free state. This means that two NH groups of one molecule are involved in the hydrogen bond. The difference in the association behaviour of the diastereoisomers of compound (**8**) is dramatically demonstrated by the difference in the shifts of the NH bands, namely 104 cm^{-1} (L) and 36 cm^{-1} (D). Only in this case are $-\Delta H$ and $\Delta\nu_{NH}$ linearly correlated in accordance with the Badger–Bauer rule.[4] It should be mentioned that in a recent paper concerning the hydrogen bonding between pure enantiomers of chiral alcohols and of sulphoxides[3] we had found practically no difference in the dependence of $-\Delta H$ on the configuration, in contrast to the situation reported here. The reason for the differences in $-\Delta H$ is possibly that different approaches of the interacting molecules, depending on the configuration at C-2, are possible.

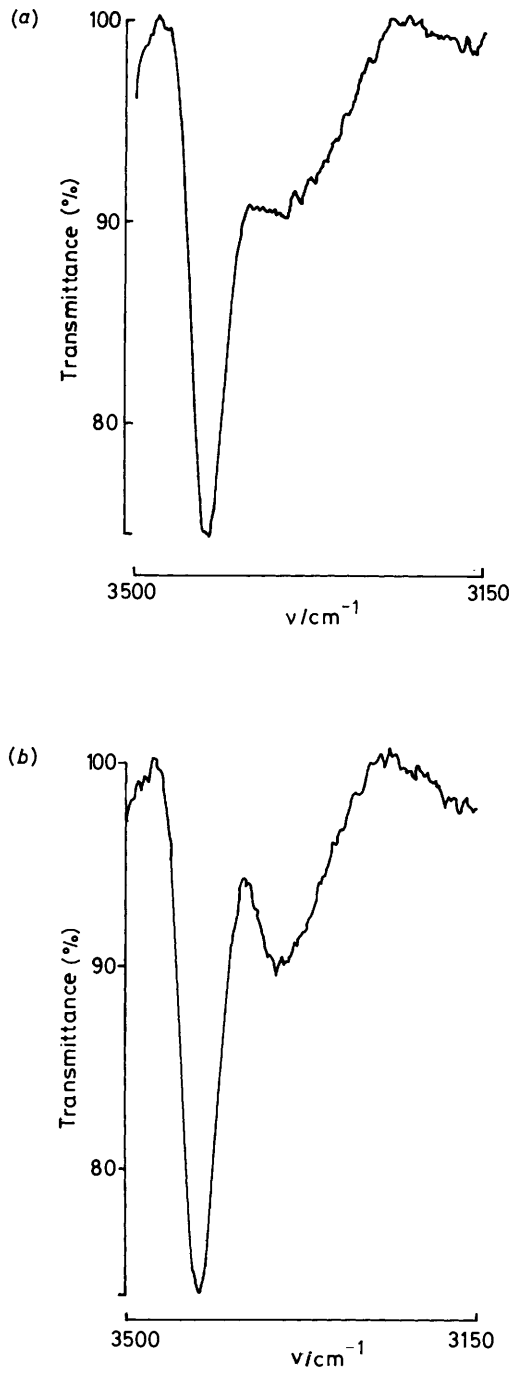

Figure 1. IR spectra of (*a*) D-(**2**) and (*b*) L-(**2**); 3 mg substance in 300 mg CH_2Cl_2, thickness 0.25 mm, 11 °C.

The spectra of the compounds D,L-(**8**) are given in Figures 2–5. The hydrogen bond exists between the NH groups and the amide CO groups. This is demonstrated in the case of L-(**8**) by a partial shift of the band at 1 676 cm^{-1} to *ca.* 1 660 cm^{-1} (Figure 3). In all other cases the shift of the CO band is much smaller and amounts only to *ca.* 3 cm^{-1}.

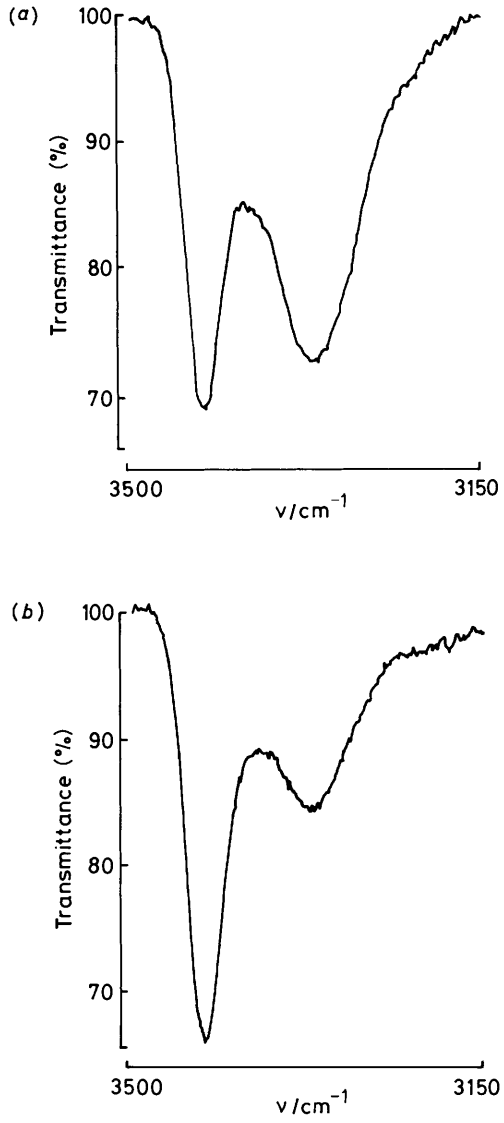

Figure 2. IR spectra of L-(**8**) at (*a*) 11 °C and (*b*) 34 °C; (2.76 mg in 203 mg CH_2Cl_2, thickness 0.25 mm).

Taking into account the large number of atoms in the molecular chain of the tripeptide, the strong influence of the configuration on the association is difficult to understand. It might be expected that, independent of the configuration, appropriate positions which could undergo association would always be adopted by means of internal rotation. But probably the N–C and even the C–C single bonds exhibit partial double-bond character, restricting internal rotation and producing differences in the association ability.

Remarkably, both diastereoisomers of compound (**8**) show the largest difference of all compounds investigated as regards their HPLC retention times. We also studied 1 : 1 mixtures of the diastereoisomers of compounds (**5**), (**7**), and (**8**). The association behaviour of the mixture D,L-(**8**) may be described as an arithmetic average of D- and L-association. In the case of compound (**7**), however, the associate NH band in the spectrum of the mixture deviates to *ca.* 10% higher absorbance than the arithmetic average. In this case the addition of the other component of the diastereoisomeric pair evidently creates new association possibilities. Surprisingly, a small, comparable effect has been observed in the case of the D,L mixture of compound (**5**), although both the pure diastereoisomers behave identically in association.

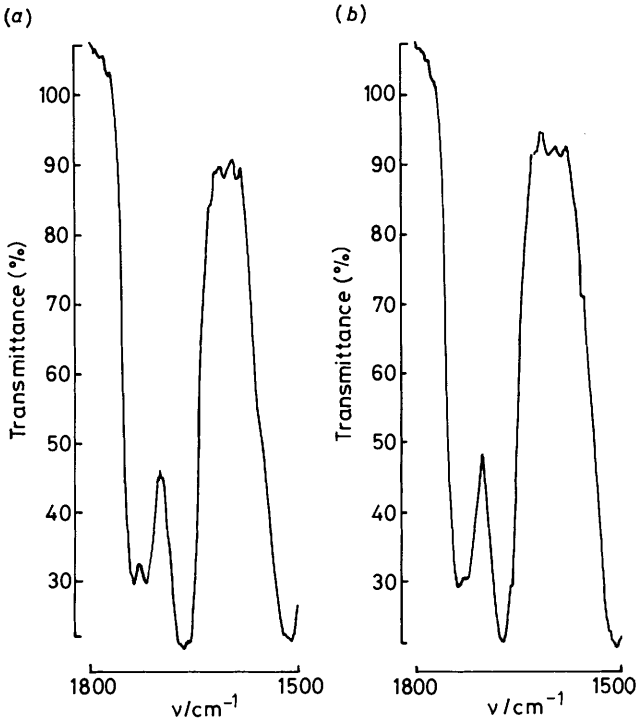

Figure 3. IR spectra of L-(**8**) in the C=O region (same conditions as in Figure 2).

Conclusions

As regards the principal outcome of our study we have to admit that the range of this paper is not sufficient to draw general conclusions as to the correlation of configuration and association ability. On the other hand, we have found a significant ability to form hydrogen bonds among the molecules with the same configuration at their chiral centres for all but one of the compounds investigated. It will be the subject of our work in the future to obtain more results which may be the foundation for the formulation of a general relation for the influence of configuration on molecular interactions.

Experimental

The spectroscopic work was performed using a Zeiss (Jena) M80 IR spectrophotometer. NaCl and CaF_2 cells, normally 0.1 mm, were used. CH_2Cl_2 was used as a solvent. The spectra were measured at 10 and 38 °C, and the temperature was monitored using a water jacket device. The lowest concentrations were *ca.* 0.007 mol dm^{-3} and the highest were up to 0.09 mol dm^{-3}. The enthalpy values were determined by relating the integrated intensities of the free and the associate NH band at 10 °C and at 38 °C. The intensity of the associate band has been taken as a measure of the quantity of the associated species. This procedure is not an unambiguous one, because the temperature dependence of the absorption coefficient of the associate band may differ from that of the free band. If it is higher than that of the free band—and that is what we assume from our experience with such bands—then the ΔH values given here may be too high. Also, different types of associates (linked by one or two hydrogen bonds) may be formed, disturbing the simple linear $\ln K$ *vs.* T^{-1} relation. On the other hand, we checked our procedure for the calculation of ΔH by using only the variation of the free NH band on decreasing the temperature. The corresponding decrease of this band was taken as a measure of half the molar quantity of newly formed associates, which

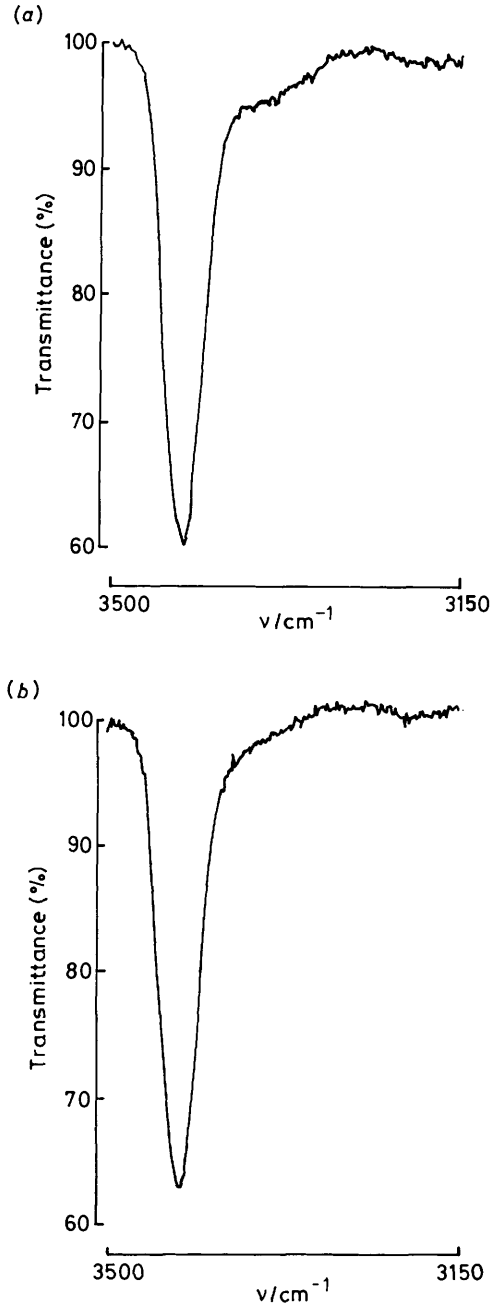

Figure 4. IR spectra of D-(**8**) at (*a*) 11 °C and (*b*) 34 °C (same conditions as in Figure 2).

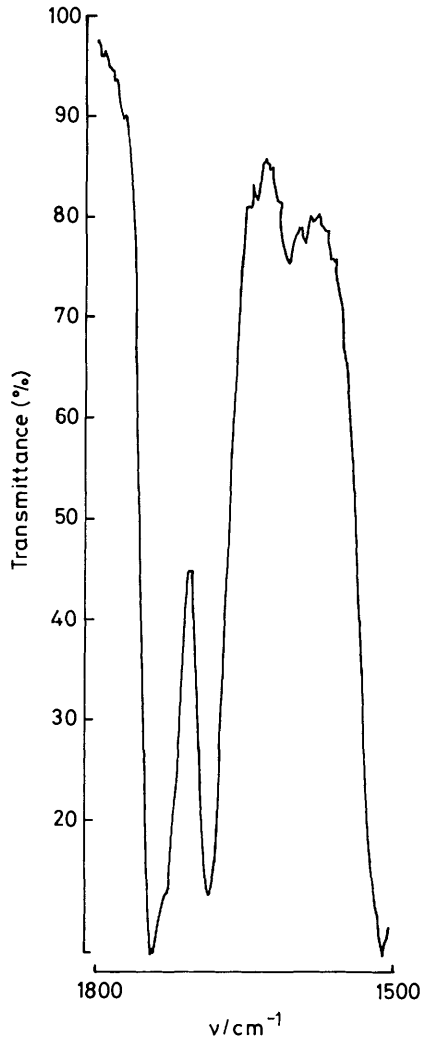

Figure 5. IR spectrum of D-(**8**) in the C=O region at 11 °C (same conditions as in Figure 2).

should be dimers under the conditions used for the calculation. In the case of L-(**8**), which we chose as an example, good agreement between the results of both methods was obtained, *i.e.* 4.0 kcal mol^{-1} by the first method and 4.3 kcal mol^{-1} by the verification procedure. (This last value has not been taken into account in this paper).

The benzyloxycarbonyl dipeptides were obtained from the corresponding benzyloxycarbonyl D- or L-amino acids and amino acid esters using the dicyclohexylcarbodi-imide procedure. For saponification the *N*-protected dipeptide esters were treated with 1 mol dm^{-3} NaOH in dioxane.[5] The tripeptide derivates were prepared by the DCC-additive approach using 1-hydroxybenzotriazole as a suppressor for racemization.[6] The stereochemical purities of the prepared tripeptide esters were estimated by HPLC[7] M.p.s, optical rotation values and HPLC retention times are given in Table 2. HPLC was performed on a Hewlett–Packard HP 1090 with a column packed with Lichrosorb RP 18, particle size 5 μm, column dimensions 4.6 mm × 250 mm; mobile phase, 65% aq. MeOH, flow rate 1.0 cm^3 min^{-1}. Detection was by means of photodiode array, λ 210 nm.

The synthetic work of this paper was performed by C. G. and H. J. and the spectroscopic measurements were performed by U. S. (in part) and by A. K.

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