Interproton Coupling Across the *trans*-Peptide Bond ($^5J_{\alpha\alpha'}$) in Chelated Dipeptides

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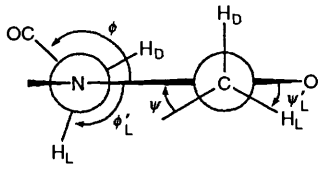
Interproton coupling over five bonds of the peptide moiety of chelated dipeptides in a series of mono-(dipeptidato)cobalt(III) complexes was studied in order to establish whether the coupling is analogous to homoallylic coupling. The rather rigid conformation of chelated dipeptides provided the reference structure for calibrating the coupling across the *trans*-peptide bond. The coupling constants over *cis* and *trans* pathways are both positive. Calibration of the homoallylic type expression afforded results close to those obtained for linear peptides in water but which disagree on the effect of replacement of water with DMSO. Sensitivity of the coupling on changes in electronic π-bond character of the peptide bond was probed by protonation of the peptide oxygen. The magnitude of the coupling was scaled up 40% upon peptide oxygen protonation in strongly acidic media (pD < 0.5), reaching 70% of the homoallylic coupling in butenes. By considering the coupling in chelated dipeptides, in rigid cyclic dipeptides, and in rigid butenes, it has been established that homoallylic coupling conformational dependence of peptides and butenes is essentially the same.

Interproton coupling over five bonds of peptide moiety ^5J[H–C_α–C(O)–N–$C_{\alpha'}$–H] had been used for the elucidation of solution conformation of peptides.[1,2] In these studies, the angular dependence of $^5J_{\alpha\alpha'}$ coupling on the peptide backbone dihedral angles ψ' and φ' (Scheme 1) was assumed to follow



Scheme 1

the valence bond (VB) expression for π-electronic contribution to homoallylic coupling [eqn. (1)].[3] The expression was tested

$$^5J_{\alpha\alpha'} = A \sin^2 \psi' \sin^2 \varphi' \qquad (1)$$

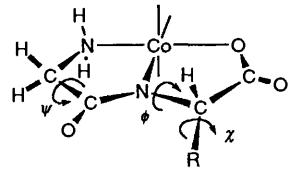
successfully and calibrated against crystal structure data on cyclic dipeptides.[1] It has been found, however, that the magnitude of A is somewhat solvent dependent.[1]

An extension of the same approach to the more biologically relevant peptides containing a *trans*-peptide bond conformation had proved difficult: not only were observed $^5J_{\alpha\alpha'}$ coupling constants small (below 0.5 Hz) but also calibration of eqn. (1) was hampered by conformational nonrigidity of the considered peptides.[2]

The applicability of eqn. (1) in elucidation of peptide conformation has been especially questioned by the theoretical study by Barfield *et al.*[4] In contrast to eqn. (1), the study predicted a negative coupling constant for most out-of-plane orientations of the C_α–H bond and an overall different angular dependence on the dihedal angles. However, in the same study,[4] experimental $^5J_{\alpha\alpha'}$ values across *cis*-peptide bonds of cyclic dipeptides was positive. The experimental finding was reproduced after inclusion of solvent (water) molecules in MO calculations. On this basis, it has been concluded that this type of long-range H–H coupling is dominated by the solvent and that applicability of eqn. (1) appears fortuitous.[4]

The issue has not been pursued since, most probably because a case that would allow new insight into the nature of the coupling was difficult to find. In this respect, we consider metal chelated dipeptides important. The interproton coupling $^5J_{\alpha\alpha'}$ of about 1 Hz has been observed in tripeptides chelated to cobalt(III),[5] but never studied. We undertook a study of $^5J_{\alpha\alpha'}$ interproton coupling across the *trans*-peptide bond in dipeptides chelated to cobalt(III), because these complexes are well characterized.

Chelation of dipeptides to cobalt(III) establishes a rigid backbone of a dipeptide due to formation of two five-membered chelate rings (Scheme 2). Peptide backbone dihedral angles ψ



Scheme 2

and φ are then in the regions 0 ± 20 and 180 ± 20°, respectively.[6–10] The limited conformational space of five-membered chelate rings[11] is further restricted by the peptide bond planarity which requires concerted changes of conformations in the two chelate rings. Specifically, changes of the peptide backbone angles ψ and φ are found mainly opposite to each other.

The well-defined and rather rigid conformation of chelated dipeptides provides an excellent reference structure for study of coupling across the *trans*-peptide bond. There is no dual coupling pathway across the peptide bond, which is a complicating factor in interpretation of $^5J_{\alpha\alpha'}$ of cyclic dipeptides. The most important aspect, however, is in the possibility of a controlled probing of $^5J_{\alpha\alpha'}$ coupling sensitivity on changes in the electronic character of the peptide bond. Namely, peptide nitrogen is directly bonded to cobalt(III) and thus shielded from solvent. Some influence on the peptide bond *via* nitrogen may be possible by replacement of the *trans*-oriented ligand. Peptide oxygen, in contrast, is fully exposed to solvent. Because this oxygen is also the most favourable proton acceptor of a chelated dipeptide, it may be fully protonated.[10]

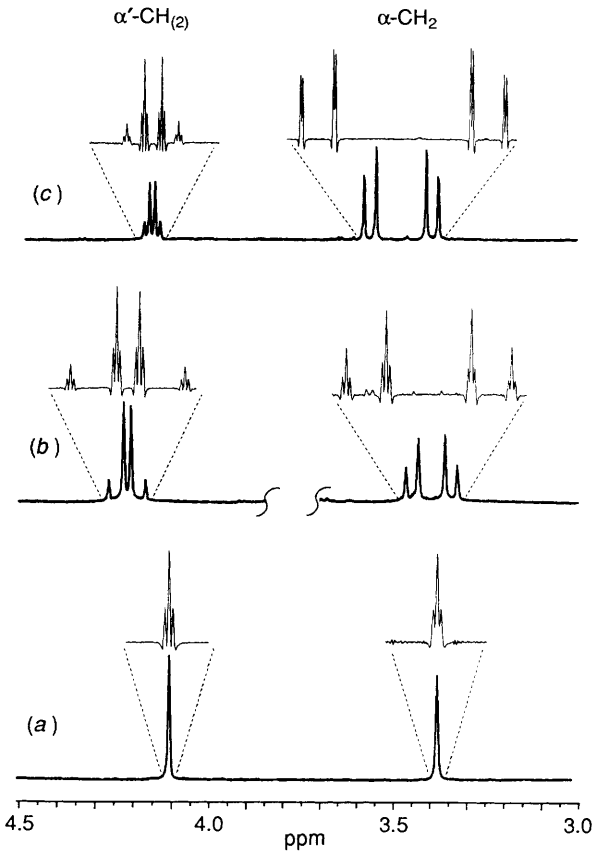


Fig. 1 ^{1}H NMR spectra (500 MHz) in the region of α-C–H resonances of chelated dipeptides in [Co(gly-gly)$(NH_3)_3$] (*a*), Ba[Co(NO_2)(gly-gly)(*R*-ala)]Cl (*b*), and Ba[Co$(NO_2)_3$(gly-gly)] (*c*) complexes dissolved in D_2O. Expanded and resolution-enhanced portions of the spectra display $^5J_{\alpha\alpha'}$ long-range couplings. In (*b*) the α-C–H resonance of *R*-ala is omitted for clarity.

Therefore, a double-bond character of the peptide N–C_α bond may be significantly influenced while retaining the same conformation around the peptide bond. To avoid ambiguity in data interpretation, the mono(dipeptidato) classes of cobalt (III) complexes were selected for the study.

Results and Discussion

Mono(dipeptidato) Complexes.—Complexes studied here were mainly of the type Co(dipep)L_3, where L = NO_2^-; dipepH_2 = glycylglycine, glycyl-L-alanine, glycyl-L-valine, glycyl-L-norvaline,* glycyl-L-leucine, β-alanylglycine; or L = NH_3, and dipepH_2 = glycylglycine. This series of complexes enabled insight into the influence of introduction of a chelated ring side chain and enlargement of the chelated ring on the long-range coupling constant. Most of the complexes from the series were synthesized for the first time. In addition, two glycylglycinato complexes, Λ-*trans*-(NO_2,NH_2)-Ba[Co(NO_2)(gly-gly)(*R*-ala)]-Cl and [Co(gly-gly)NCS(en)] [*R*-alaH = (*R*)-alanine, en = 1,2-diaminoethane], for which crystal structures have been reported,[6-8] were studied. They are of particular interest because of a relatively puckered backbone conformation of the chelated dipeptide. Backbone torsional angles were reported to be $\psi = 16.7°$ and $\varphi = -10.3°$ in the first complex and $\psi = -12.9°$ and $\varphi = 16.4°$ in the Λ-enantiomer of the second complex, thus covering the extreme regions of a limited conformational space allowed to chelated dipeptides.[6-10]

* In this paper, norvaline = 2-aminopentanoic acid.

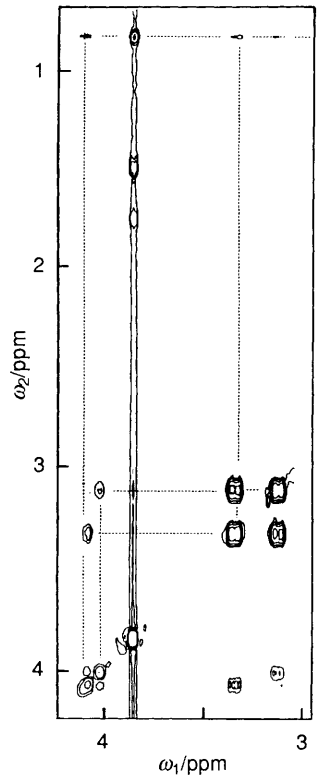


Fig. 2 ^{1}H 2D ROESY spectrum (500 MHz) of Ba[Co$(NO_2)_3$(gly-L-leu)] dissolved in 1:3 D_2O–DMSO mixture. The weak NOE is observed between the leu side chain Me groups (0.76 ppm) and 1 H of the coordinated amine group (4.07 ppm) and 1 H of the chelate ring CH_2 group (3.28 ppm).

Stereospecific Assignment of Peptide α-C–Hs.—Chemical environments of α-carbons in the two chelated rings (Scheme 2) are quite different because one of them (α-carbon) is part of amino-peptidato while the other (α'-carbon) is part of peptidato-carboxylato chelate ring. As expected, α'-C–Hs are always found more deshielded than α-C–Hs. In glycylglycinato complexes, which do not possess any chirality, two protons residing on the same α or α' carbon exhibit the same chemical shift [Fig. 1(*a*)]. This signifies that glycylclycinato chelate rings are planar in these complexes; otherwise, the cobalt(III) magnetic anisotropy or peptide and carboxy group magnetic anisotropy would produce magnetic nonequivalence of CH_2 protons. In complexes that possess configurational asymmetry, as well as in complexes possessing vicinal asymmetry, CH_2 protons became inequivalent [Fig. 1(*b*) and (*c*)]. To assign inequivalent protons stereospecifically may be possible only if they are brought into spatial relationship to the chiral part of a complex. In the series of $[Co(NO_2)_3(gly\text{-}L\text{-}am)]^{2-}$ complexes this has been possible for the complex containing glycyl-L-leucinato chelate. A weak NOE was observed between the leucinato side chain methyl group and one of the amine protons (Fig. 2). From that amine proton, NOE rates were measured to α-CH_2 protons, which allowed identification of a more distant, axially oriented proton as the more shielded one. Measured vicinal coupling constants in the NH_2CH_2 moiety were in agreement with that assignment and corresponded to torsion of *ca.* 10–20° around the H_2N–C_α bond, giving rise to a δ-conformation of the chelate ring. For other complexes in the series, the coupling between protons in the NH_2–CH_2 moiety was essentially the same. Therefore, in that series, the more shielded proton of the α-CH_2 group has been assigned H_D and the less shielded, H_L, according to their prochirality.

In the configurationally asymmetric complexes containing glycylglycinato chelate, the axially oriented protons of the CH_2

Table 1 Chemical shifts and coupling constants of α-C–Hs in the amino-peptidato chelate ring (δ_α, $^5J_{\alpha\alpha'}$, $^2J_{\alpha\alpha}$) and in the peptidato-carboxylato chelate ring [$\delta_{\alpha'}$, $^5J_{\alpha'\alpha}$, $^2J_{\alpha'\alpha}$, $^3J(C_\alpha\text{–N–}C_{\alpha'}\text{–H})$] of the investigated complexes dissolved in D_2O

No.	Complex	Prochirality	δ_α	$^5J_{\alpha\alpha'}$/Hz	$^2J_{\alpha\alpha}$/Hz	$\delta_{\alpha'}$	$^5J_{\alpha'\alpha}$/Hz	$^2J_{\alpha'\alpha}$/Hz	$^3J(C_\alpha\text{–N–}C_{\alpha'}\text{–H})$/Hz
1	[Co(gly-gly)$(NH_3)_3$]Cl		3.38	1.44		4.10	1.44		2.3
2	Ba[Co$(NO_2)_3$(gly-gly)]		3.41	1.41		4.0	1.41		
3	Ba[Co$(NO_2)_3$(β-ala-gly)]		2.35	0.94		4.12	0.94		
4	Ba[Co$(NO_2)_3$(gly-L-ala)]	L	3.58	1.07					
		D	3.42	1.18	16.57	4.18	1.13		2.5
5	Ba[Co$(NO_2)_3$(gly-L-val)]	L	3.72	1.04					
		D	3.26	0.91	16.57	3.93	0.98		
6	Ba[Co$(NO_2)_3$(gly-L-val)]	L	3.73	1.21					
		D	3.32	1.12	16.59	4.19	1.17		
7	Ba[Co$(NO_2)_3$(gly-L-leu)]	L	3.68	1.10					
		D	3.35	1.08	16.52	4.17	1.09		2.6
8	Λ-*trans*-(NO_2,NH_2)Ba-[(Co(NO_2)(gly-gly)(*R*-ala)]Cl	D	3.44	1.54		4.26	1.38		
		L	3.37	1.37	16.71	4.18	1.48	19.20	
9	[Co(gly-gly)(NCS)(en)	L	3.30	1.48		4.02	1.35		
		D	3.21	1.40	16.84	4.06	1.55	18.82	

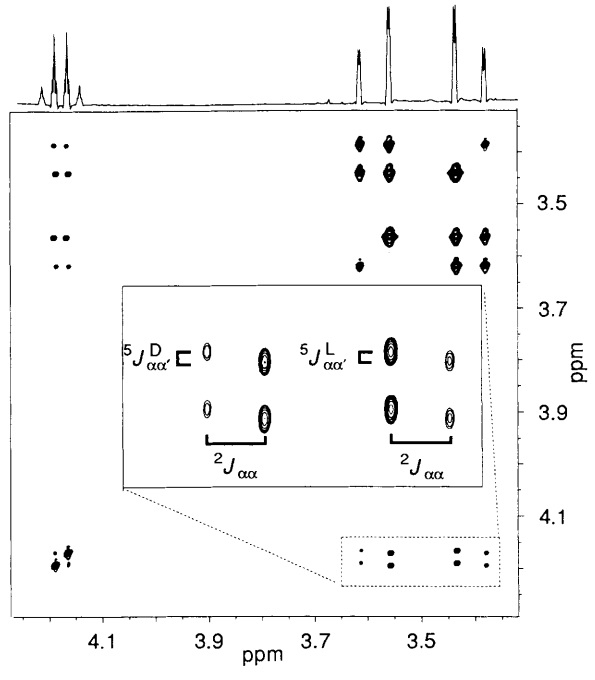


Fig. 3 2D COSY45 spectrum of Ba[Co$(NO_2)_3$(gly-L-ala)] in D_2O. The E-COSY pattern of cross peaks exhibits slope which corresponds to opposite sign of $^5J_{\alpha\alpha'}$ and $^2J_{\alpha\alpha}$ coupling constants.

groups were identified by NOE observed between them and amine or ammine ligand groups in the *cis* position relative to glycylglycinato chelate rings. This information is then considered within the Λ-absolute configuration of the complexes to afford assignment of L and D oriented methine protons.

$^5J_{\alpha\alpha'}$ of Chelated Dipeptides.—Values of $^5J_{\alpha\alpha'}$ as measured in D_2O solution of the investigated complexes are given in Table 1. For glycyl-L-am chelates (am = ala, val, val, leu), the coupling constant measured at right-oriented (D) and left-oriented (L) α-CH_2 protons corresponds to *cis* ($^5J^c_{\alpha\alpha'}$) and *trans* ($^5J^t_{\alpha\alpha'}$) coupling[4] across the peptide bond, respectively. For glycylglycinato chelates, however, only the mean value ($\frac{1}{2}{}^5J^c_{\alpha\alpha'} + \frac{1}{2}{}^5J^t_{\alpha\alpha'}$) has been determined from a deceptively simple splitting pattern [A_2X-type, Fig. 1(*a*)] of α-CH_2 signals.

The sign of the $^5J_{\alpha\alpha'}$ coupling constant relative to α-CH_2 geminal coupling constant (negative) has been determined by applying the small flip angle chemical shift correlation through homonuclear scalar coupling (Fig. 3).[12–13] The sign of both *cis* and *trans* coupling constants was opposite to that of the geminal coupling constant, that is, positive. Previously, it was found that $^5J_{\alpha\alpha'}$ across a *cis*-peptide bond was positive,[4] and the same is established here for the coupling across a *trans*-peptide bond. The result supports VB eqn. (1) for homoallylic type coupling and disagrees with the INDO–MO calculations that predicted the negative values of the coupling.[4] In view of the suggested large sensitivity of the coupling constant on association effects,[4] it may be argued that peptide coordination to cobalt(III) has caused a change of the coupling constant sign. However, the coupling constant seems to be rather insensitive to the nature of the peptide nitrogen bond to cobalt(III) because the influence of the *trans*-oriented ligand is rather weak. Namely, the replacement of NH_3 by NO_2^- ligand in the position *trans* to peptide bond nitrogen causes a small change of the coupling constant (Table 1, complexes **1** and **2**).

Considering the values of $^5J_{\alpha\alpha'}$ coupling constants presented in Table 1, it may be seen that they are always larger in glycylglycinato chelate than in gly-L-am chelates. It may be expected that introduction of a side chain in the peptidato-carboxylato chelate ring makes the ring more puckered due to a spatial requirement of a side chain. To understand the puckering, vicinal coupling between the α-carbon and the $C_{\alpha'}$–H proton was measured in a few complexes. Obtained $^3J(C_\alpha\text{–N–}C_{\alpha'}\text{–H})$ coupling constants are larger in gly-L-am chelates than in glycylglycinato chelate (Table 1) indicating a more equatorial orientation of C_α–H bond.[14] Consequently, the smaller $^5J_{\alpha\alpha'}$ coupling due to smaller dihedral angle φ' in gly-L-am chelates is in accordance with homoallylic type eqn. (1). Enlargement of the amino-peptidato chelate ring from a five- to a six-membered one produced a considerable diminishing of $^5J_{\alpha\alpha'}$ as well (Table 1, complexes **2** and **3**). The effect may be ascribed again to a more pronounced puckering of the chelate ring. The six-membered amino-peptidato chelate ring is flexible and an average value for the geminal protons' chemical shifts and the coupling is observed.

It is known that eqn. (1) does not account for the difference between *syn* and *anti* arrangements about a double bond in the homoallylic coupling.[15] The asymmetry of coupling not covered by eqn. (1) is noted in the $^5J_{\alpha\alpha'}$ coupling constant of gly-L-am chelates. For the $[Co(NO_2)_3(\text{gly-L-am})]^{2-}$ type complexes, $^5J_{\alpha}{}^{D}{}_{\alpha'}$ and $^5J_{\alpha}{}^{L}{}_{\alpha'}$ are of the close values. Predominantly, $^5J_{\alpha}{}^{D}{}_{\alpha'} < {}^5J_{\alpha}{}^{L}{}_{\alpha'}$ cases are found, although a δ-conformation of the amino-peptidato chelate ring would require $^5J_{\alpha}{}^{D}{}_{\alpha'} > {}^5J_{\alpha}{}^{L}{}_{\alpha'}$ according to eqn. (1). The asymmetry is, therefore, of the same sense as in the homoallylic coupling and corresponds to a negative contribution of σ-electron mechanism to *cis*

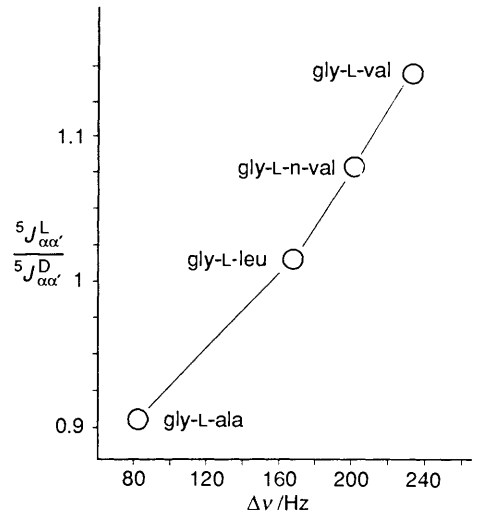


Fig. 4 Correlation of the coupling constant *trans*/*cis* ratio, ${}^5J_{\alpha'\alpha}{}^L/{}^5J_{\alpha'\alpha}{}^D$, and α-carbon gem-H chemical shift difference in the series of $Ba[Co(NO_2)_2(gly\text{-}L\text{-}am)]$ complexes dissolved in D_2O

Table 2 Effect of solvent on the long-range coupling across the peptide bond, ${}^5J_{\alpha\alpha'}$, in chelated dipeptides of selected complexes.

D_2O/DMSO % v/v	$[Co(gly\text{-}gly)(NH_3)_3]Cl$ ${}^5J_{\alpha\alpha'}$/Hz	$Ba[Co(NO_2)_3(gly\text{-}L\text{-}ala)]$ ${}^5J_{\alpha\alpha'}$/Hz	
		L	D
100	1.46	1.07	1.18
50	1.39	0.96	1.07
10	1.34	0.94	1.04
0	1.30	0.87	0.98

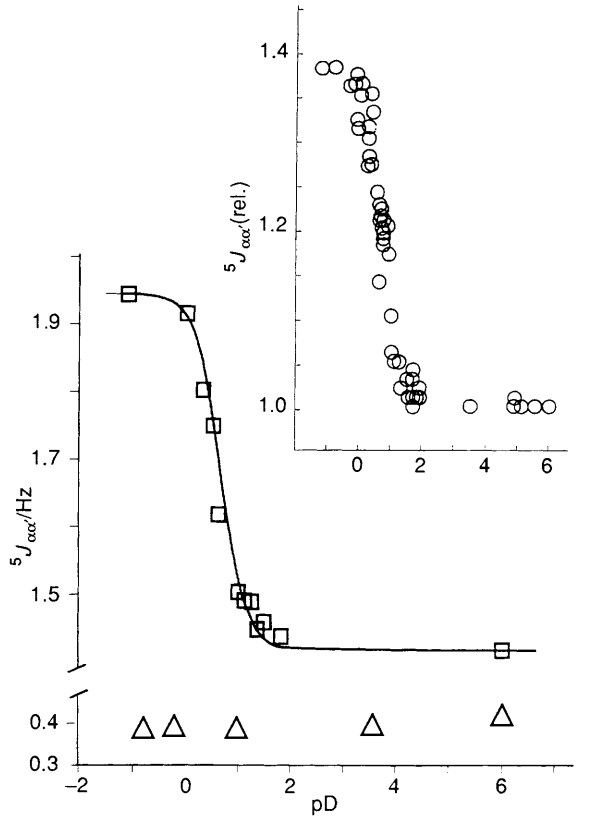


Fig. 5 ${}^5J_{\alpha\alpha'}$ coupling constant pD dependence of chelated glycylglycine in $[Co(gly\text{-}gly)(NH_3)_3]Cl$ (○) and of free glycylglycine (△) dissolved in D_2O. The enlargement of the coupling constant, relative to ${}^5J_{\alpha\alpha'}$ at pD = 6, in the studied complexes (trinitro complexes were not measured below pD = 0 because of degradation) is presented in the upper diagram.

coupling.[15] A correlation exists between the coupling constants asymmetry and α-carbon geminal protons chemical shift difference (Fig. 4). One is tempted to ascribe that correlation to a change in the amino-peptidato chelate ring puckering. However, pronounced sensitivity of the homoallylic *cis* and *trans* coupling difference on substitution at C-1 and C-4 [in the moiety H–C(1)–C(2)=C(3)–C(4)–H] had been established.[15]

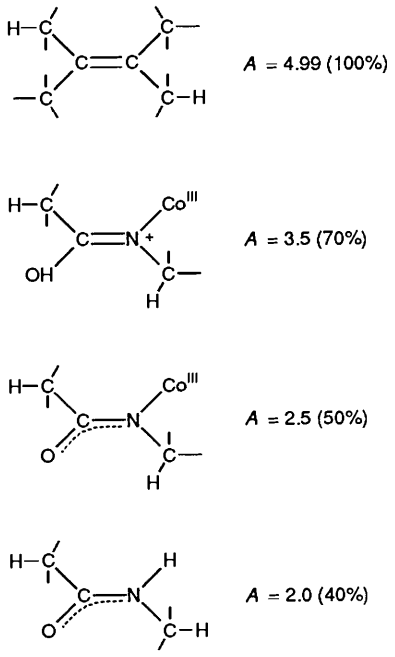
Eqn. (1) has been calibrated for the *trans*-peptide bond in *N*-methylacetamide and *N*-methylformamide, yielding $A = 2.0$ Hz in D_2O.[2] If one disregards the asymmetry of the coupling, then the coupling constant determined from a deceptively simple spectrum of the planar glycylglycinato chelate (1.44 for triamino and 1.41 Hz for trinitro complexes dissolved in D_2O) would yield $A = 2.5$ Hz. The calibration has been reported to be sensitive to solvent.[2] Thus, for *N*-methylacetamide and *N*-methylformamide in DMSO, the large reduction of the coupling was measured, yielding $A = 0.8$ Hz.[2] In contrast, the ${}^5J_{\alpha\alpha'}$ coupling constant of chelated dipeptides is only slightly reduced in DMSO solution (Table 2). This may indicate that conformational changes in less rigid molecules are the major cause for a large reduction of the coupling in DMSO.

pD Dependence of ${}^5J_{\alpha\alpha'}$.—Reversible protonation of peptides chelated to cobalt(III) has been reported,[16,17] and spectrophotometric studies indicated equilibrium in the strongly acidic region (H^+ concentration ~ 1 mol dm^{-3}). Crystal structure analysis of $[Co(gly\text{-}gly)_2]^-$ and $[Co(gly\text{-}glyH)_2]^+$ ions revealed a shortening of the carbon–nitrogen peptide bond from 1.30 to 1.25 Å on protonation of the peptide bond oxygen.[18] This considerable change of the double-bond character of the carbon–nitrogen bond is exploited here in characterization of ${}^5J_{\alpha\alpha'}$ coupling.

pD Dependence of the ${}^5J_{\alpha\alpha'}$ coupling constant in complexes studied is presented in Fig. 4. A sharp increase of the coupling constant in the region $0 < pD < 1$ is observed. A titration curve calculated to fit data obtained for glycylglycinato chelate (Fig. 5) gives $pK = 0.65$. Uncoordinated glycylglycine in the same pD region does not exhibit any significant change of ${}^5J_{\alpha\alpha'}$ (observed value of *ca.* 0.4 Hz corresponds well to a free rotation of CH_2 groups). The relative coupling constant enlargement is essentially the same in all measured complexes and amounts to *ca.* 40%. This brought the coupling constant values much closer to the homoallylic coupling constants measured across the C=C bond.[15] For instance, calibration of the coupling constant according to eqn. (1) indicates that 70% of the homoallylic coupling of butenes has been reached after protonation of the peptide oxygen (Scheme 3). It is also known that homoallylic coupling across the carbon–nitrogen double bond is practically the same as across the caron–carbon double bond.[15] All this strongly suggests that ${}^5J_{\alpha\alpha'}$ coupling across the peptide bond is of the homoallylic type but scaled down due to a lower π-bond character.

Conformational Dependence of ${}^5J_{\alpha\alpha'}$ *Coupling Across the Peptide Bond.*—This conformational dependence had been assumed to be of the homoallylic type,[1,2] but the assumption was later disputed.[4] Consideration of the coupling constants obtained for a rigid conformation of cyclic dipeptides[1,4] and coupling constants for chelated dipeptides obtained here may provide an answer to whether the dependence is of the homoallylic type.

Comparison of the coupling could be made the most general in terms of the relationship between the ${}^5J_{\alpha}{}^D{}_{\alpha'}/{}^5J_{\alpha}{}^L{}_{\alpha'}$ ratio and



Scheme 3

peptide backbone torsional angle. Namely, eqn. (1) allows calculation of the relationships in eqns. (2).

$$\frac{{}^5J^{D}_{\alpha\alpha'}}{{}^5J^{L}_{\alpha\alpha'}} = \frac{\sin^2\psi_{D'}}{\sin^2\psi_{L'}} = \frac{\sin^2(\psi + 300)}{\sin^2(\psi + 60)}$$

$$\frac{{}^5J^{D}_{\alpha'\alpha}}{{}^5J^{L}_{\alpha'\alpha}} = \frac{\sin^2\varphi_{D'}}{\sin^2\varphi_{L'}} = \frac{\sin^2(480 - \varphi)}{\sin^2(240 - \varphi)} \qquad (2)$$

The obtained relationships do not contain the scaling parameter A and therefore do not suffer from the bond order changes (solvent, pH, bond type). Besides, the impact of the difference in the coupling for *syn* and *anti* arranged C_α and $C_{\alpha'}$ carbons with respect to a double bond is largely eliminated.

The corresponding comparison of eqn. (2) with experimental data (reported crystal structure) on cyclic and chelated dipeptides is presented in Fig. 6. The comparison included also rigid allyl compounds,[15] with ${}^5J^{D}$ corresponding to ${}^5J^{c}$. The general dependence of the VB expression for π-electron contribution to the homoallylic coupling is followed well. There is no indication of a different behaviour of peptides with respect to allyl compounds. The asymmetry of the coupling due to the contribution from a σ-electron mechanism[15] seems to flatten the angular dependence, especially in the region of a planar peptide backbone (ψ, φ = 0°, 180°). However, the angular dependence of the coupling constant ratio is so pronounced that for up to 20% deviation from eqns. (2), the torsion angle is reproduced with accuracy better than ± 10°.

The invariance of the ${}^5J_{\alpha}{}^{D}{}_{\alpha'}/{}^5J_{\alpha}{}^{L}{}_{\alpha'}$ ratio to medium effects makes eqns. (2) potentially applicable in the determination of glycine and sarcosine residues' backbone conformation in peptides. Therefore, we shall rewrite eqns. (2) for an n-th residue in a peptide [eqns. (3)]. The important result is that ψ_n and φ_n may be determined independently.

$$\frac{{}^5J^{D}(\alpha_n\alpha_{n+1})}{{}^5J^{L}(\alpha_n\alpha_{n+1})} = \frac{\sin^2(\psi_n + 300)}{\sin^2(\psi + 60)}$$

$$\frac{{}^5J^{D}(\alpha_n\alpha_{n-1})}{{}^5J^{L}(\alpha_n\alpha_{n-1})} = \frac{\sin^2(480 - \varphi_n)}{\sin^2(240 - \varphi_n)} \qquad (3)$$

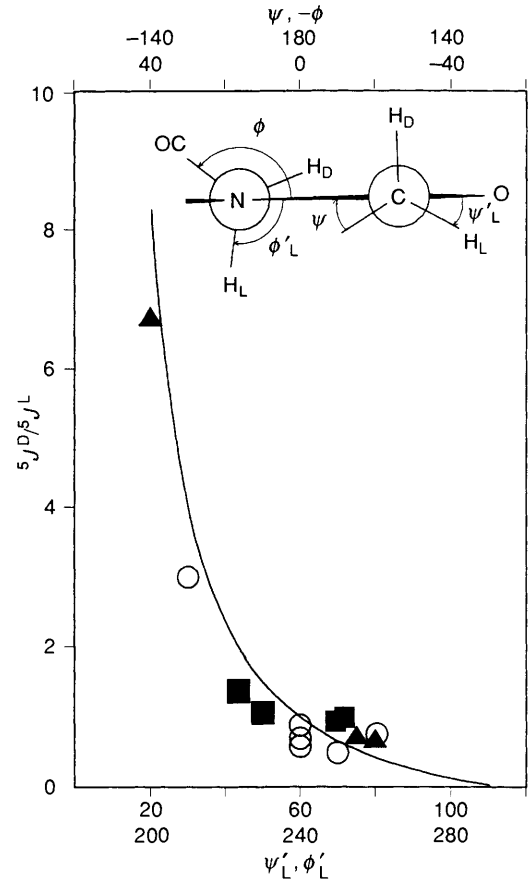


Fig. 6 Conformational dependence of ${}^5J_{\alpha}{}^{D}{}_{\alpha'}/{}^5J_{\alpha}{}^{L}{}_{\alpha'}$, or ${}^5J_{\alpha'}{}^{D}{}_{\alpha}/{}^5J_{\alpha'}{}^{L}{}_{\alpha}$ in chelated dipeptides (■) (complexes **8** and **9** in Table 1) and in cyclic dipeptides (▲) (data for gly-L-pro, ser-L-val and gly-L-tyr from refs. 1 and 4), and of ${}^5J^{c}/{}^5J^{t}$ in rigid butenes (○) (data for substances **4**, **5**, **6a**, **11**, **14** and **17b** from ref. 15) compared with the dependence predicted by eqn. (2)

Conclusions

The study of interproton coupling over five bonds of peptide moiety in chelated dipeptides afforded important results for understanding the nature of that coupling. The coupling constants were positive, their magnitude scaled up with the increase of carbon–nitrogen bond π-character, and their conformational dependence was similar to that of homoallylic coupling.

The result of the study as well as a recent development of NMR techniques that enable measurement of small coupling constants in peptides (< 1 Hz)[22] encourages the use of ${}^5J_{\alpha\alpha'}$ interproton coupling across the peptide bond for elucidation of peptide conformation. In particular, the backbone conformation of glycine and sarcosine residues may be directly determined by applying eqns. (3) [with the degeneracy of orientations inherent to eqns. (3)].

The established sensitivity of scalar coupling across the peptide bond on peptide carbonyl hydrogen bonding may provide a means for monitoring peptide hydrogen bonding. Currently, we are examining the carbonyl hydrogen bonding effect on cross-peptide bond heteronuclear couplings (hydrogen–carbon and carbon–nitrogen) in order to devise a more focused approach to this important application.

Experimental

NMR Measurements.—NMR results were obtained at 500 MHz on a Bruker AMX-500 spectrometer. Digital resolution of 1D spectra was 0.025 Hz. The long-range coupling was

measured directly from the spectra after the resolution enhancement (Gaussian apodization, $F(t) = \exp\{\pi LB[-t + t^2/(2GB*AQ)]\}$, was performed with LB = −2.5, GB = 0.25, and acquisition time AQ = 4.5 s). Chemical shifts are referenced to TSP ($Me_3SiCH_2CH_2CO_2Me$) for spectra recorded in D_2O and to Me_4Si for those recorded in [$^2H_6$]-DMSO. The phase-sensitive ROESY spectrum was obtained with τ = 200 ms and B_1 = 3 kHz. Transients (16) were digitized with 2048 data points for each of 512 increments. Uncorrected pD of the samples was measured directly in a NMR tube (5 mm).

Preparation of Ba[Co(NO$_2$)$_3$(dipep)] Series of Complexes (dipepH$_2$ = glycyl-L-alanine, β-alanylglycine, glycyl-L-valine, glycyl-L-norvaline, glycyl-L-leucine).—The complexes have been synthesized following the procedure reported for preparation of the corresponding glycylglycinato complex.[19] However, the isolation procedure was modified. After completion of the direct synthesis reaction,[19] the reaction mixture was evaporated to 20 cm^3 on a rotary vacuum evaporator, filtered, and poured on a QAE-Sephadex A-25 (35 cm length and 2.5 cm o.d.) prepared in chloride form. The column was first washed with water and then elution was done with a 0.01 mol dm^{-3} barium chloride solution. The second eluted fraction, of an orange colour, contained the desired complex. It was evaporated on a rotary vacuum evaporator to 20 cm^3 (barium chloride, which forms a precipitate, was filtered off a few times during the process) and left overnight in a refrigerator. After filtration of precipitated barium chloride, the filtrate was poured onto a Sephadex G-15 molecular sieve and the adsorbed orange zone eluted with water. The collected solution of the complex was evaporated to 5 cm^3 on a rotary vacuum evaporator, 1 cm^3 of methanol was added, and the solution was left overnight in a refrigerator. The separated crystals of the desired complex were filtered off and washed with ethanol and ether. The yield varies from 50% for the glycyl-L-alaninato complex to about 10% for the glycyl-L-valinato complex. Satisfactory elemental analyses were obtained.

Syntheses of other complexes studied were done according to the reported procedures (complex **1** in Table 1, ref. 20; **8**, ref. 21; **9**, ref. 8).

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