Co-ordination of Nickel(II) Ions by Angiotensin II† and its Peptide Fragments. A Potentiometric, Proton Nuclear Magnetic Resonance and Circular Dichroism Spectroscopic Study

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A potentiometric and spectroscopic (n.m.r., circular dichroism, and u.v.–visible) study of the complexes of angiotensin II and two of its peptide fragments, Asp-Arg-Val-Tyr and MeCO-Tyr-Ile-His, with Ni^{II} has been undertaken. The results show that, above pH 8, Ni^{II} co-ordinates to four nitrogen donors starting at the terminal amino nitrogen of the aspartyl residue to form a diamagnetic, planar complex. Co-ordination through the imidazole N of the histidyl residue may be present below pH 8—9 but is absent at high pH. The high-resolution ^1H n.m.r. spectrum of angiotensin II and of its nickel(II) complex at pH 11 have been recorded and the side-chain resonances assigned.

Angiotensin II is a linear octapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) and a potent hypertensive hormone which stimulates the muscles of blood vessels. It also mediates the transport of manganese(II) ions across lipid bilayers[1] and its biological functions have been linked with other metal ions, mainly Li^+, Na^+, Ca^{2+}, and Mg^{2+}.[2–5] An earlier claim that metal-ion binding induces conformational changes in the hormone molecule leading to a more physiologically active structure has not been substantiated by a later study which has shown that only minor modifications in the angiotensin conformation take place on metal-ion co-ordination.[6] As a ligand, angiotensin represents a very interesting molecule. It has two potential nitrogen donor centres able to initiate complexation: the NH_2 terminal and the N_{im} of the histidyl (His-) side chain. Manganese(II) ions have been shown to interact with the angiotensin molecule through the His side chain[7] and copper(II) ions also begin co-ordination at this imidazole nitrogen donor centre.[8] At intermediate pH this metal ion forms CuL which, according to spectroscopic evidence, is a 3N species. In this complex the metal ion binds to the imidazole nitrogen of the histidyl residue together with two neighbouring deprotonated peptide nitrogens. Hence the central part of the angiotensin molecule is probably bent around the metal ion while the two ends are unco-ordinated. Above pH 8, however, the co-ordination centres appear to move to the N-terminal part of the peptide and the Cu^{II} is bound through the terminal amino nitrogen and the three neighbouring peptide nitrogens in a 4N complex.[8]

In this paper we present the results of a potentiometric and spectroscopic [n.m.r., circular dichroism (c.d.), and u.v.–visible] study of the interaction of Ni^{II} with angiotensin II and two of its fragments: the N-terminal tetrapeptide Asp-Arg-Val-Tyr and the central tripeptide MeCO-Tyr-Ile-His.

Experimental

Peptide Syntheses.—The angiotensin peptide fragments were synthesised by standard liquid-phase methods and purified as described earlier.[8] Angiotensin II was purchased from a Bachem Company.

Potentiometric Studies.—Stability constants for H^+ and Cu^{2+} complexes were calculated from titration curves carried out at 25 °C using total volumes of 1—2 cm^3. Alkali was added from a micrometer syringe (0.1 cm^3) which had been calibrated by both weight titration and the titration of standardised materials. Changes in pH were followed using a glass electrode calibrated in hydrogen-ion concentrations with $HClO_4$.[9] All solutions were of ionic strength 0.10 mol dm^{-3} (KNO_3) and peptide concentrations of 0.003 mol dm^{-3}. Calculations were made with the aid of the SUPERQUAD computer program.[10] In all cases titrations were carried out at Ni:L ratios of 1:4 to 1:9. The standard deviations quoted were computed by SUPERQUAD and refer to random errors only. They give, however, a good indication of the importance of the particular species in the equilibrium.

Spectroscopic Studies.—The metal source was $Ni(ClO_4)_2 \cdot 2H_2O$ (Fluka) and solutions were 0.001 mol dm^{-3}, with 1.25:1 ligand:nickel ratios. Absorption spectra were recorded on a Cary 219 spectrometer, c.d. spectra on a Mark III Jobin-Yvon dichrographe in the 200—600 nm region. All c.d. spectra are expressed in terms of $\Delta\varepsilon(\varepsilon_l - \varepsilon_r)$. Proton n.m.r. spectra were recorded on a 400-MHz Bruker spectrometer with peptide concentrations of 0.005 mol dm^{-3} at 300 ± 2 K; Ni:L ratios of 1:1.25 and 1:1 were studied. Analysis and simulation of the proton ABC spectra were carried out on a Hewlett-Packard 9826 computer.

Results and Discussion

Protonation constants of all the ligands studied were published in our earlier work.[8] Nickel(II) complex-formation constants calculated using these values are given in Table 1. The kinetics of formation of nickel(II) complexes is comparatively slow, particularly at higher pH values when diamagnetic complexes are formed. Hence pH-metric titrations can be very time-consuming (up to 20 min per point) with concomitant drifts in

† Aspartylarginylvalyltyrosylisoleucylhistidylprolylphenylalanine (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe). Abbreviations used for the amino acids are in accord with the I.U.P.A.C.–I.U.B. recommendations (*Pure Appl. Chem.*, 1984, **56**, 595).
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Table 1. Stability constants (log β_{xyz}) for H^+ and Ni^{2+} complexes $Ni_xL_yH_z$ at 25 °C and I = 0.10 mol dm^{-3} (KNO_3)

	H^+ complexes				Ni^{2+} complexes				
	β_{011}	β_{012}	β_{013}	β_{014}	β_{122}	β_{121}	β_{111}	β_{11-2}	β_{11-3}
Asp-Arg-Val-Tyr (H_3L)[a]	10.27	18.04	21.87	25.01	29.06(4)	19.96(8)	14.5(1)	−12.16(8)	
MeCO-Tyr-Ile-His (H_2L)[a,b]	9.78	16.80	19.74			17.30(4)	12.53(8)		−23.32(4)
Angiotensin II (H_3L)[a]	10.14	17.89	24.46	29.13				−17.9(1)	−27.8(1)

[a] Ref. 8. [b] Log β_{120} = 7.8(1).

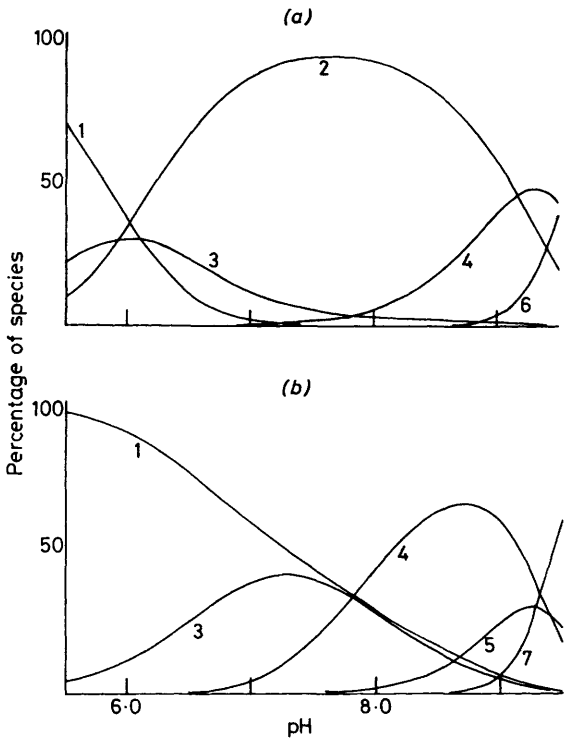

Figure 1. Species distribution diagram for 1:6 mixtures of Ni^{II} (0.001 mol dm^{-3}) with (*a*) Asp-Arg-Val-Tyr and (*b*) MeCO-Tyr-Ile-His. Curves: 1, Ni^{2+}; 2, $[Ni(HL)_2]$; 3, [Ni(HL)]; 4, $[NiHL_2]$; 5, $[NiL_2]$; 6, $[NiH_{-2}L]$; 7, $[NiH_{-3}L]$

pH and standardisation and resulting in values of a lower precision than is possible with metal ions such as Cu^{II}. On the n.m.r. time-scale this results in two sets of resonances, one for free and another for complexed peptide (see later). With angiotensin the situation was further complicated by low solubility of the complexes below pH 8, making it virtually impossible to get reliable formation constants in this region. As a result stability constants for angiotensin–nickel(II) complexes are reported to a lower precision than with the other ligands.

Asp-Arg-Val-Tyr:Ni^{II}.—Potentiometric results show that, up to pH 9, two major octahedral complexes are formed, [Ni(HL)] rapidly followed by the bis complex $[Ni(HL)_2]$ as shown in Figure 1(*a*). The first complex to form, [Ni(HL)], would be a 1N complex and its stability constant should be comparable with that of the 1N complex of tetra-alanine ([NiL]) if the mode of bonding is the same. Since this [Ni(HL)] complex possesses a protonated tyrosyl residue, the protonation constant of this tyrosyl oxygen must be subtracted from the overall formation constant before comparison with the respective tetra-alanine complex can be made, *i.e.* $\log K_{corr} = 14.59 - 10.27 = 4.32$.

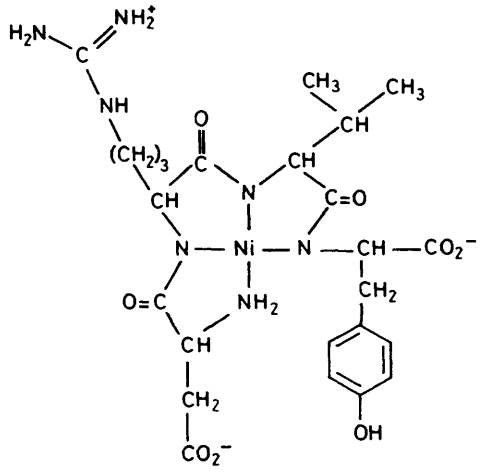

Figure 2. Proposed structure for the $[NiH_{-2}L]$ complex with Asp-Arg-Val-Tyr

This value is significantly higher than that found[11] for the corresponding species with tetra-alanine ($\log K$ = 3.06) and suggests the involvement of the carboxylate of the aspartic acid residue in the metal-ion binding in a similar fashion to that found for copper(II).[8] The bis species $[Ni(HL)_2]$, which is the major complex below pH 9, probably has the same mode of ligand–nickel(II) bonding, *i.e.* the ligands are bound to the Ni^{II} through the terminal amine, the vicinal carbonyl oxygen, and the carboxylate of the aspartic acid residue. At higher pH Ni^{II} can deprotonate amide nitrogen atoms forming N^-–Ni bonds. Deprotonation of this nitrogen in the bis complex would lead to the formation of species such as $[NiHL_2]$ or $[NiL_2]$. With Asp-Arg-Val-Tyr this does not take place until above pH 8, a pH unit higher than the comparable reaction with the tetra-alanine complex due to the extra stability afforded the 1N complex by side chain co-ordination.

Absorption spectra in the pH range 6—9 show a *d–d* transition at 392 nm typical of octahedral nickel(II) complexes.[3] At pH 9 a new *d–d* band appears at 418 nm (ε = 236 dm^3 mol^{-1} cm^{-1}) indicating that the geometry of the complex has changed from octahedral to planar. From the species distribution diagram (Figure 1) this complex must correspond to the $[NiH_{-2}L]$ species found by potentiometry; bis complexes are not expected with planar co-ordination. C.d. spectra also indicate the formation of a planar complex (Table 2) with amine and amide nitrogens involved in co-ordination. The $[NiH_{-2}L]$ complex is presumably a 4N complex comparable to the $[NiH_{-3}L]$ species found with tetra-alanine but with the Tyr residue protonated, as shown in Figure 2. Unfortunately potentiometric titrations were only taken to pH 9.5 with the result that deprotonation to give the $[NiH_{-3}L]$ complex was not confirmed reliably. The change from paramagnetic to diamagnetic Ni^{II} would account for the apparent absence of an $[NiL_2]$ complex; even the complex $[NiHL_2]$ is of only low importance.

N.m.r. spectra. Since the high-pH complex is diamagnetic it

Table 2. Spectroscopic data for nickel(II) complexes with angiotensin and its peptide fragments at high pH

Ligand (complex)	Absorption spectrum λ/nm (ε/dm³ mol⁻¹ cm⁻¹)	C.d. spectrum λ/nm (Δε/dm³ mol⁻¹ cm⁻¹)
Asp-Arg-Val-Tyr		
$[NiH_{-2}L]$	418[a] (236)	458[b] (−2.75)
or $[NiH_{-3}L]$	480(sh)	268[c] (+6.54)
MeCO-Tyr-Ile-His		
$[NiH_{-3}L]$	420[a] (26)	556[d] (+0.07)
		430[e] (−0.36)
		245[c] (+2.24)
Angiotensin II		
$[NiH_{-3}L]$	412[a] (320)	476[b] (−3.10)
		266[c] (+2.24)

[a] The planar *d–d* transition. [b] *A* + *E*(*d–d*) transitions observed in c.d. [c] Intraligand + N^-–Ni^{II} charge-transfer transition. [d] *A* (*d–d*) transition. [e] *E* (*d–d*) transition.

Table 3. Proton n.m.r. chemical shifts, coupling constants (Hz), and rotamer populations for the Asp and Tyr residues of Asp-Arg-Val-Tyr and its complex with Ni^{II} at pH 11; *J* values in Hz and ν values in p.p.m. from $SiMe_4$ with HDO at 4.8 p.p.m.

		Free Asp-Arg-Val-Tyr (pH)					Nickel(II) complex
		0.7	3.0	5.9	8.4	11.5	11.4
ν_A	Asp	2.98	2.81	2.68	2.54	2.38	2.40
	Tyr	2.91	2.95	2.85	2.86	2.85	2.32
ν_B	Asp	3.08	2.91	2.84	2.71	2.59	2.48
	Tyr	3.16	3.14	3.07	3.06	2.95	2.84
ν_C	Asp	4.38	4.33	4.28	3.96	3.70	3.38
	Tyr	4.61	4.53	4.39	4.40	4.38	4.38
J_{AB}	Asp	18.12	17.56	17.37	16.42	15.60	16.27
	Tyr	13.96	13.76	13.65	13.96	13.99	15.07
J_{AC}	Asp	7.76	8.16	8.72	8.13	8.52	6.08
	Tyr	9.48	9.12	8.92	8.80	7.38	7.26
J_{BC}	Asp	4.96	5.03	4.98	4.69	4.42	3.90
	Tyr	4.84	4.68	4.35	4.42	4.74	4.67
ν_D	Tyr	7.13	7.16	7.13	7.14	6.93	7.41
ν_E	Tyr	6.80	6.83	6.82	6.83	6.53	7.41
J_{DE}	Tyr	8.50	8.50	8.40	8.50	8.40	8.40
P_I	Asp	0.20	0.22	0.22	0.19	0.17	0.12
	Tyr	0.24	0.20	0.16	0.17	0.20	0.19
P_{II}	Asp	0.48	0.52	0.56	0.50	0.54	0.32
	Tyr	0.63	0.58	0.58	0.57	0.44	0.43
P_{III}	Asp	0.32	0.26	0.22	0.30	0.29	0.56
	Tyr	0.12	0.22	0.26	0.27	0.37	0.38

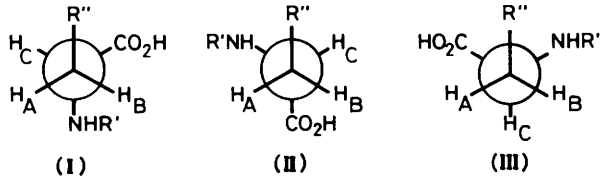

Figure 3. Notation for rotational isomers of Asp-Arg-Val-Tyr

was possible to obtain high-resolution n.m.r. spectra. The assignment of the metal-free tetrapeptide was achieved by selective irradiation (signal saturation) and by adjusting the pH. Table 3 summarises the proton n.m.r. parameters of the Asp and Tyr residues for the free and complexed peptide, including the rotational isomers of both residues (Figure 3). The most stable isomer of the unco-ordinated peptide is rotamer (**II**) (for both residues) in which the steric hindrance is minimised. At high pH the population of isomer (**III**) for the Tyr residue increases due, most probably, to the interaction of the negatively charged tyrosine side chain and the positive arginine residue. In a separate test it was found that gradual addition of Ni^{II} to peptide solutions resulted in variable shifts in the proton resonances as well as extensive changes throughout the whole of the n.m.r. spectrum. Since planar complexes of Ni^{II} are inert on the n.m.r. time-scale the spectra with excess of ligand consist of two sets of resonances, corresponding to free and complexed peptide (Figure 4).

The chemical shifts reported in Table 4 indicate that the largest variations on co-ordination to Ni^{II} occur for α-protons as a result of the involvement of the terminal amine and amide nitrogens in the metal binding. The α-proton resonance of Tyr is, however, not shifted although 4N co-ordination appears to be clear from the diamagnetic behaviour discussed above. In the free peptide, both δ-protons of the Arg residue have the same resonance but, in the complex, these are shifted downfield and are well separated (≈0.3 p.p.m.). In addition, the two CH_3 groups of the Val residue have the same chemical shifts in the free peptide but are separated in the co-ordinated species (≈0.2 p.p.m.), probably as a result of a more rigid structure. On co-ordination of the peptide the population of rotamer (**III**) for the Asp residue increases significantly. This could be a result of an interaction between the negative β-CO_2 of the Asp residue with the positive lateral side chain of the Arg residue, the Ni^{II} changing the conformation of the ligand to make this interaction possible. All n.m.r. spectra were recorded above pH 10 when all the Ni^{II} was co-ordinated as diamagnetic $[NiH_{-2}L]$ or $[NiH_{-3}L]$, and no bis complexes were present.

MeCO-Tyr-Ile-His:Ni^{II}.—Starting at low pH, the first complex to form is the [Ni(HL)] species [around pH 6, see Figure 1(*b*)] and spectroscopic studies confirm this to contain an octahedrally co-ordinated Ni^{II}. The Ni^{II} would be bound to the peptide through the pyridine-like nitrogen of the imidazole ring since the other potentially competitive primary binding site, the terminal amine group, is acetylated and as such is excluded from co-ordination in the low-pH region. This is confirmed by calculating the logarithmic stability constant for the reaction HL + Ni $\rightleftharpoons$ [Ni(HL)] (*i.e.* 12.53 − 9.78 = 2.75), which is close to that found for the nickel(II)–imidazole complex (3.0). As the pH is raised above 7 the octahedrally co-ordinated complexes $[NiHL_2]$ and $[NiL_2]$ could be detected. These have *d–d* transitions at 392 nm, typical of octahedral co-ordination, and would involve co-ordination of two nitrogens from the His residue, an imidazole N, and the peptide nitrogen. On raising the pH further, three protons dissociate with almost equal ease to form the 4N complex $[NiH_{-3}L]$. Deprotonation of the phenolic side group of the tyrosine residue overlaps with the metal-induced deprotonation of the two neighbouring peptide

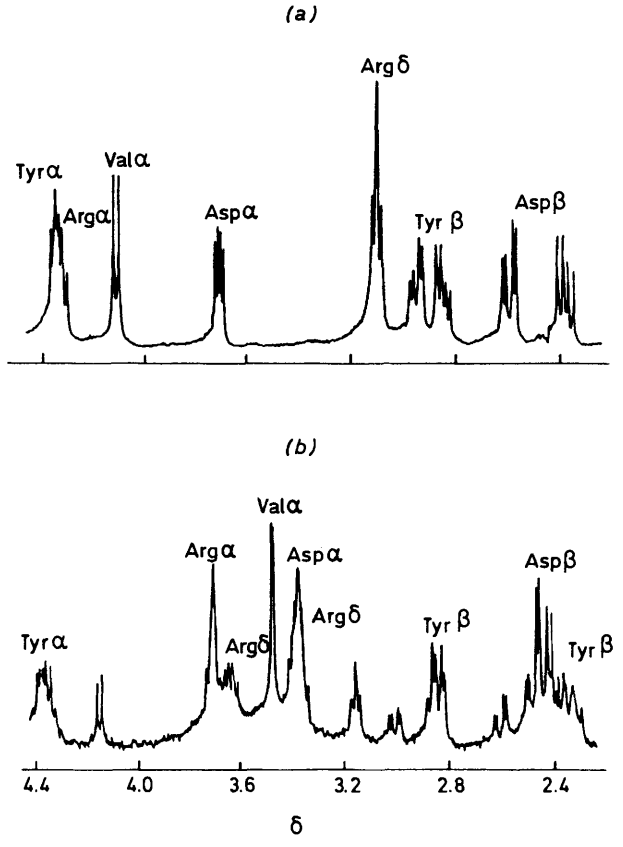

Figure 4. N.m.r. spectra of the free peptide Asp-Arg-Val-Tyr (*a*) and the complex with Ni^{II} at pH 11 (*b*)

Table 4. Proton n.m.r. chemical shifts of Asp-Arg-Val-Tyr and the complex with Ni^{II} at pH 11. Values are relative to $SiMe_4$ with HDO at 4.8 p.p.m.

		Free Asp-Arg-Val-Tyr	Nickel(II) complex
Asp	$C_\alpha H$	3.7	3.33
	$C_\beta H_2$	2.59, 2.38	2.48, 2.40
Arg	$C_\alpha H$	4.33	3.7
	$C_\delta H_2$	3.10	3.37, 3.64
	$C_\beta H_2$	1.77	1.79
	$C_\gamma H_2$	1.55	1.62
Val	$C_\alpha H$	4.11	3.47
	$C_\beta H$	2.05	2.02
	$C_\gamma H_3$	0.93	0.66, 0.87
Tyr	$C_\alpha H$	4.38	4.38
	$C_\beta H_2$	2.95, 2.85	2.84, 2.32
	$C_\varepsilon H$	6.54	6.77
	$C_\delta H$	6.94	7.4

nitrogens since this Tyr protonation constant (log K = 9.78) is lower than with Asp-Arg-Val-Tyr (10.27).

Spectroscopic data are given in Table 2. Surprisingly, high-resolution n.m.r. spectra at high pH could not be obtained as a result of paramagnetic species in the equilibrium mixture. This could also be seen in the absorption spectra in which the absorption coefficient of the 'planar' *d–d* transition observed at 420 nm is low (less than 20% of the value usually observed). The low amount of the planar species could be due to the presence of three bulky side groups in the ligand molecule which would destabilise the planar structure and allow the $[NiH_{-3}L]$ complex to exist as paramagnetic and diamagnetic isomers. The destabilising effect of these bulky side groups is also seen in the

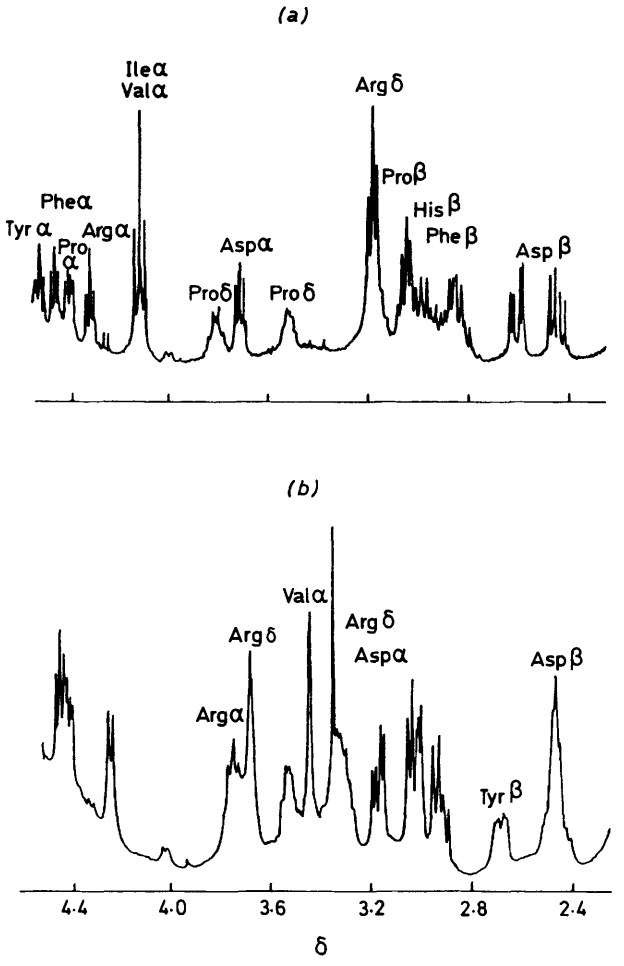

Figure 5. N.m.r. spectra of the free peptide angiotensin (*a*) and the complex with Ni^{II} at pH 11 (*b*)

case of the complexes of Ni^{II} and Cu^{II} with angiotensin (see below and ref. 8). Comparison of Figure 1(*a*) and (*b*) shows that Ni^{II} co-ordinates more readily with Asp-Arg-Val-Tyr than with MeCO-Tyr-Ile-His.

Angiotensin II:Ni^{II}.—In the pH range of 5—9 angiotensin forms relatively insoluble complexes with Ni^{II}. At above pH 9, potentiometric measurements were possible and indicated the formation of a planar $[NiH_{-2}L]$ complex, the Tyr side chain of which readily deprotonated to give the $[NiH_{-3}L]$ species. The absorption spectra show a transition at 412 nm, typical of planar complexes, and the c.d. spectra consist of a Cotton effect at 476 nm which is similar to that obtained for the corresponding complex of the Asp-Arg-Val-Tyr sub-unit (see above). This suggests that for the whole angiotensin molecule the 4N diamagnetic complex is bound to Ni^{II} through four nitrogens starting at the N-terminal of the hormone as was found with the copper(II) complex.[8] To confirm this the proton n.m.r. spectrum of the planar nickel(II) complex at pH 11 was recorded (see Figure 5) and the results are presented in Table 5. The n.m.r. spectrum of free angiotensin was in good accord with an earlier lower-resolution study (220 MHz).[12] The use of two-dimensional proton n.m.r. spectra and selective irradiation allowed assignment of the resonances of protons in residues of angiotensin co-ordinated to Ni^{II}. The availability of Asp-Arg-Val-Tyr for comparison purposes was very useful and results are summarised in Table 5. Changes in the chemical shift on co-ordination are clearly seen for the α-protons of four N-terminal

Table 5. Proton n.m.r. chemical shifts of angiotensin II and the complex with Ni^{II} at pH 11. Values are relative to $SiMe_4$ with HDO at 4.8 p.p.m.

		Free angiotensin	Nickel(II) complex
Asp	$C_\alpha H$	3.72	3.33
	$C_\beta H_2$	2.61, 2.45	2.46
Arg	$C_\alpha H$	4.33	3.74
	$C_\delta H_2$	3.18	3.68, 3.26
	$C_\beta H_2$	1.76	1.83
	$C_\gamma H_2$	1.58	1.59
Val	$C_\alpha H$	4.12	3.44
	$C_\beta H$	2.00	2.02
	$C_\gamma H_3$	0.87	0.68, 1.07
Tyr	$C_\alpha H$	4.54	Obscured by water
	$C_\beta H_2$	2.89, 2.83	2.68
	$C_\epsilon H$	6.60	6.66
	$C_\delta H$	6.95	7.2
Ile	$C_\alpha H$	4.12	4.24
	$C_\beta H$	1.89	1.93
	$C_\gamma H_2$	1.37, 1.11	1.15, 1.44
	$C_\gamma H_3$	0.93, 0.83	1.03, 0.87
	$C_\delta H_3$		
His	$C_\alpha H$	Obscured by water	Obscured by water
	$C_\beta H_2$	2.98, 3.05	2.92, 3.02
	$C_2 H$	7.70	7.62
	$C_4 H$	6.98	6.89
Pro	$C_\alpha H$	4.42	4.42
	$C_\beta H_2$	2.17, 1.94	2.16, 1.18
	$C_\gamma H_2$	1.94	1.83
	$C_\delta H_2$	3.81, 3.53	3.53, 3.68
Phe	$C_\alpha H$	4.48	4.46
	$C_\beta H_2$	3.04, 3.19	3.17, 3.02
	CH(arom.)	7.28, 7.37	7.27, 7.36

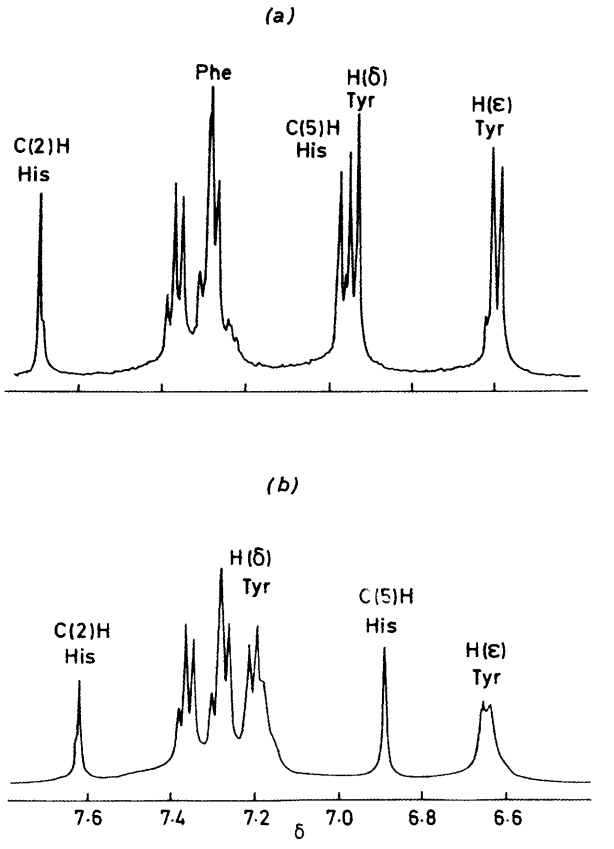

Figure 6. N.m.r. spectra of the aromatic region of the free peptide angiotensin (*a*) and the complex with Ni^{II} at pH 11 (*b*)

residues confirming that the Ni^{II} is bound to four nitrogens starting with the amine terminal nitrogen as was suggested for the 4N complex of Asp-Arg-Val-Tyr. From the n.m.r. spectrum of the aromatic region, only very small variation in the chemical shifts of the aromatic histidine protons (Figure 6) is apparent. What is more, the variations in the chemical shifts of the protons of the arginine and valine residues on metal complexation are comparable with those of Asp-Arg-Val-Tyr, suggesting similar conformation of this peptide fragment in the nickel(II) complexes of both angiotensin and its N-terminal sub-unit.

Conclusions

The studies performed in this work show Ni^{II} at high pH to interact with the 4 N-terminal amino-acid residues of angiotensin II, giving complexes closely similar to those formed with Asp-Arg-Val-Tyr itself. There may be a role for the imidazole N of the His residue at a lower pH, but comparison of nickel(II) complexes of MeCO-Tyr-Ile-His with those of Asp-Arg-Val-Tyr shows the latter peptide to form complexes more readily. These results suggest that, with Ni^{II}, the terminal amino N of the Asp residue is a more competitive centre to initiate co-ordination than is the imidazole N.

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