Interactions of a Vitamin B_{12} Model Complex with Amino-acids and Oligopeptides. A Visible and Nuclear Magnetic Resonance Spectroscopic Study

By G. Pellizer,* G. R. Tauszik, and G. Costa, Institute of Chemistry, University of Trieste, Trieste, Italy

Substitution of the water molecule of $[MeCo(tn)H_2O]ClO_4$ by some amino-acids and simple oligopeptides has been studied by ^1H n.m.r. and visible absorption spectroscopy. Amino-acids bind to cobalt through $-NH_2$, imidazole, nitrogen and $-S^-$; formation constants are higher for the sulphur ligands while no $Co-CH_3$ bond breaking is observed. Amino-acid protons nearest to cobalt undergo anomalous high fields shifts; for the *trans* methyl and the equatorial ligand proton shifts, the ring current effect of the entering ligand is the major factor. The existence of μ-amino-acido-complexes and of preferred orientation in solution of some ligands in the sixth co-ordination position is proposed.

In co-ordination compounds where cobalt is bound in the equatorial positions by a tetradentate ligand, such as corrin and those employed in vitamin B_{12} model molecules,[1,2] the axial positions can be occupied by a wide variety of ligands.

This is a particularly favourable situation for the understanding of the influence of a single ligand on the properties of the molecule and several interesting correlations have already been pointed out.[1,3,4]

Furthermore, when an organic ligand is present in one of the axial positions, the other axial position is the only one easily attacked by the usual ligands. This can be a useful system for the study of the behaviour of complex ligands when they are compelled to enter just one co-ordination site.

In this paper we report the study of solutions containing the vitamin B_{12} model complexes $[MeCo(tn)L]^x$, where L is an amino-acid or a di-, tri-, or tetra-peptide,

[1] H. A. O. Hill, 'Corrinoids in Inorganic Biochemistry,' ed. G. Eichorn, Elsevier, Amsterdam, 1972, and references therein.

[2] A. Bigotto, G. Costa, G. Mestroni, G. Pellizer, A. Puxeddu, E. Reisenhofer, L. Stefani, and G. Tauzher, *Inorg. Chim. Acta Rev.*, 1970, **4**, 41, and references therein.

[3] H. A. O. Hill, K. G. Morallee, G. Costa, G. Pellizer, and A. Loewenstein, 'Magnetic Resonances in Biological Research. An International Conference,' ed. C. Franconi, Gordon Breach, New York, 1971, 301.

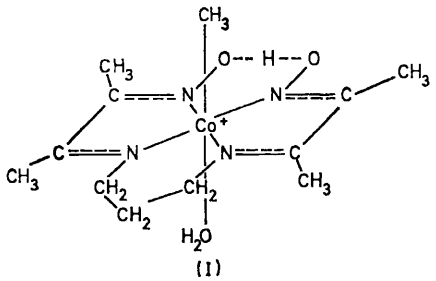
[4] G. Costa, G. Mestroni, G. Tauzher, D. M. Goodall, M. Green, and H. A. O. Hill, *Chem. Comm.*, 1970, 34.

and tn = 2,3,9,10-tetramethyl-1,4,8,11-tetra-azaundeca-1,3,8,10-tetraen-11-ol-1-olato anion.

Our results can also give some suggestions on the interactions between biologically active vitamin B_{12} compounds and the ligand groups of proteins. Moreover $[RCo(tn)L]^+$ (L = H_2O or *N*-methylimidazole) complexes inhibit competitively the vitamin B_{12} coenzyme-dependent propanedioldehydratase;[5] therefore it is likely that these complexes can bind to proteins through cobalt and our work can give some information about the possible binding groups.

RESULTS AND DISCUSSION

Visible Spectra.—The spectrum of the compound $[MeCo(tn)H_2O]^+$ [6] (I) (Figure 1) in the visible region is independent of pH in the range 1—10.



(I)

FIGURE 1

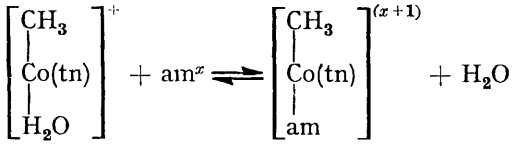
The addition of amino-acids at suitable pH values causes spectral changes (Figure 2A) which are always reversed on decreasing the pH. This is due to the substitution of the water molecule in the sixth co-ordination position by the amino-acid.

Amino-acids Binding via *Nitrogen.*—With glycine, L-alanine, L-phenylalanine, L-lysine, L-histidine, L-cystine, *S*-methyl-L-cysteine, glycylglycine, glycyl-L-alanine, L-alanylglycine, and tetraglycine the intense absorption region is shifted to shorter wavelengths with respect to (I), different amino-acids causing just minor differences in the spectra (Figure 2A).

With amines, pyridine, and imidazole the same spectral pattern is obtained (Figure 2B), suggesting that the above amino-acids are bonded to cobalt *via* the nitrogen.

Water substitution by these amino-acids is appreciable only when there is a relevant concentration of deprotonated ligand nitrogen, *i.e.* in alkaline solution, except for lysine and histidine which do not require alkaline conditions to bind cobalt, deprotonated basic nitrogens being present in the neutral molecules.

Omitting histidine, lysine, and cystine, the visible spectra obtained from solutions containing different amounts of the above amino-acids have one well defined isosbestic point *ca.* 444 nm. This is in agreement with the existence of only two cobalt species, *i.e.* of the equilibrium



(where am is the binding form of the amino-acid, $x = 0$ or -1). In these systems there is no evidence for amide nitrogen as a ligand for cobalt, that is all these amino-acids bind cobalt through the NH_2 groups.

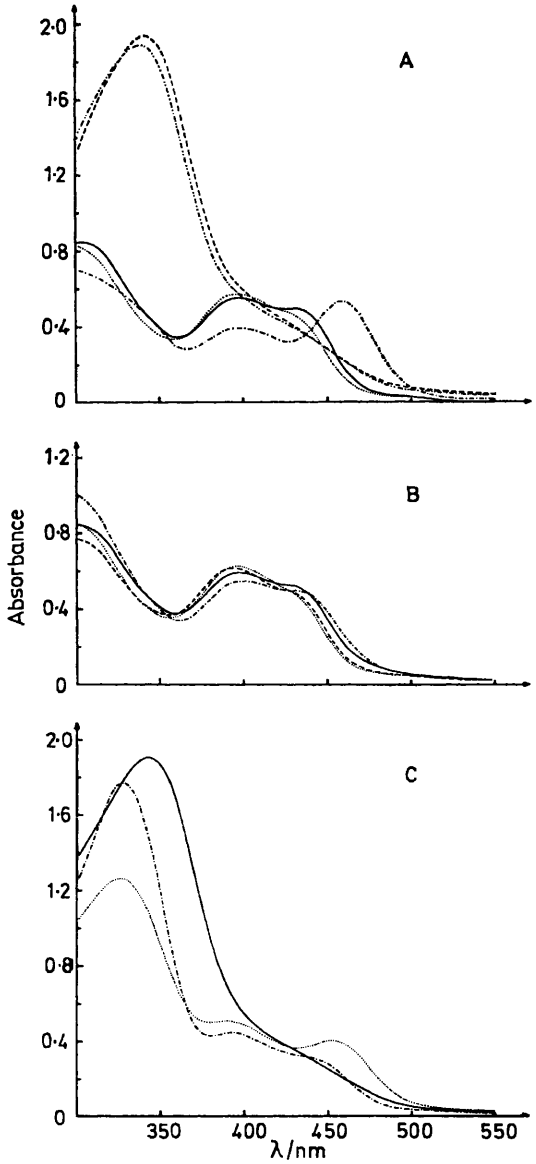


FIGURE 2 Absorption spectra of $[MeCo(tn)H_2O]ClO_4$ ($2{\cdot}32 \times 10^{-4}$M) in pH 10 buffered solutions after addition of large excesses of the following compounds; A, none: —·—·—; glycine: ——; L-histidine: · · · ·; L-cysteine: —··—; *N*-acetyl-L-cysteine: – – – –; B, ammonia: – – – –; methylamine: ——; imidazole: · · · ·; pyridine: —·—·—; C, sodium sulphide: · · · ·; 3-thiopropanoic acid: ——; sodium thiosulphate: —·—·—

Histidine, lysine, and cystine contain two nitrogen binding groups and therefore can give various complexes, including possibly bridged binuclear ones. Only the case of histidine, which contains different binding groups, was studied in detail. Addition of this amino-acid to

[5] N. Stagni, B. de Bernard, G. Costa, and G. Mestroni, *Nature*, 1970, **225**, 942.

[6] G. Costa, G. Mestroni, and E. de Savorgnani, *Inorg. Chim. Acta*, 1969, **3**, 323.

aqueous solutions of compound (I) gives three good isosbestic points, at 328, 355, and 440 nm; under these conditions only the imidazolic group behaves as a ligand. But, in buffered solutions of pH 10, the spectrum obtained in the presence of a large excess of histidine is appreciably different (Figure 3) in agreement with the presence of molecules with Co–NH_2 bond, besides those with Co–im bond, where im = imidazole group. At this pH histidine is mainly in the forms $C_3H_3N_2{\cdot}CH_2CH(COO^-)NH_2$ and $C_3H_3N_2{\cdot}CH_2CH(COO^-)NH_3^+$, their ratio being constant. Therefore here the concentration ratios between the complexes [MeCo(tn)–$C_3H_3N_2{\cdot}CH_2CH(COO^-)NH_2$], [MeCo(tn)–$C_3H_3N_2{\cdot}CH_2CH(COO^-)NH_3^+]^+$, and [MeCo(tn)–$NH_2CH(COO^-)CH_2{\cdot}C_3H_3N_2$] has to be constant, and so at least one isosbestic point near 440 nm should be observed if no other cobalt species were present. Actually no true isosbestic point can be observed and this

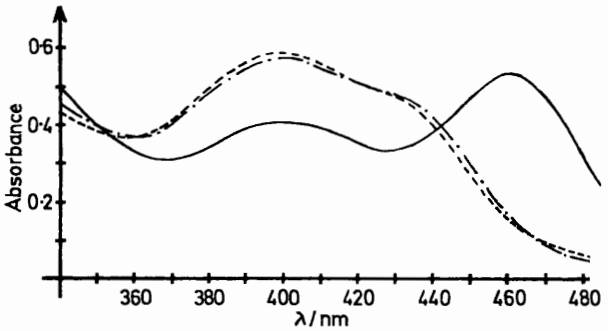


FIGURE 3 Absorption spectra of (I) ($2{\cdot}32 \times 10^{-4}$M) in the pH range 1—10: ——, of (I) ($2{\cdot}32 \times 10^{-4}$M) + L-histidine ($2{\cdot}32 \times 10^{-2}$M) in water: – – – –, and of (I) ($2{\cdot}32 \times 10^{-4}$M) + L-histidine ($2{\cdot}32 \times 10^{-4}$M) in pH 10 buffered solution: — · — · —

can be explained through the formation of a bridged complex: [MeCo(tn)–$C_3H_3N_2{\cdot}CH_2CH(COO^-)$–$NH_2$–Co(tn)–Me]$^+$, which should have an appreciable concentration only at low histidine : compound (I) ratios. The presence of bridged complexes was confirmed working in solutions of the maximum concentration suitable for visible spectra, to shift as far as possible the equilibrium toward the cobalt–amino-acid complexes. In these conditions visible spectra of solutions with various histidine : compound (I) ratios above 1 : 2 show only slight differences and retain the features typical of [MeCo(tn)$\leftarrow$N$\lessgtr$]^x spectra. Indeed the concentration of (I), as inferred from absorption at 460 nm (see Figure 2A), at a histidine : compound (I) ratio of 1 : 2, is such that one histidine molecule must bind through nitrogen atoms to more than one cobalt atom. An identical result, obtained from n.m.r. spectra, is reported below.

Amino-acids Binding via *Sulphur*.—On the addition of L-cysteine, *N*-acetyl-L-cysteine, glutathione (γ-L-glutamyl-L-cysteinylglycine), and of sulphur ligands such as SH^-, RS^-, and $HS_2O_3^-$ to compound (I), the spectra are different from those given by nitrogenous bases, having a strong absorption band in the region 320—350 nm (Figure 2A and C). This suggests that these amino-acids bind *via* sulphur.

It is noteworthy that cystine and *S*-methylcysteine bind *via* nitrogen, indicating that sulphur of RSSR′ and RSR′ does not bind cobalt to an appreciable extent in our solutions.

With cysteine, *N*-acetylcysteine, and glutathione water substitution occurs even at pH 7 and is strongly enhanced by increasing the pH, while acidification gives again the aquo-complex. Therefore these molecules bind in the form RS^- while there is no evidence of the formation of complexes of the kind RSH $\longrightarrow$ Co under our conditions. The possibility of complexation, at such pH values where the form RS^- has a low concentration, is due to the high value of the stability constant (see later) for the equilibrium:

$$[MeCo(tn)H_2O]^+ + RS^- \rightleftharpoons [MeCo(tn)RS] + H_2O$$

With *N*-acetylcysteine the spectral variation with concentration is identical at pH 7 and at pH 10, except of course for the rate, and two good isosbestic points are observed at 438 and 500 nm.

With cysteine and glutathione the spectral pattern is more complex depending on the pH. At pH 7, *i.e.* in conditions where only sulphur can bind, the two isosbestic points are observed again; at higher pH values, *i.e.* when the compounds can bind also through the amino-nitrogen, the spectral behaviour can be explained in the same way as for histidine. However, an excess of cysteine or glutathione gives also at pH 10 the same spectral pattern obtained at pH 7 indicating that the [Co–N] : [Co–S] ratio is under these conditions very small, in agreement with the much higher value of the formation constant for the latter.

The presence of bridged binuclear complexes at low amino-acid : compound (I) ratios was again investigated in conditions and with results analogous to those reported for histidine. The existence of binuclear complexes is also in agreement with results obtained from n.m.r. spectra (see below).

The complexes with these amino-acids binding *via* sulphur are stable for at least one day in the presence of an excess of ligand in the absence of air and acidification gives again $[MeCo(tn)H_2O]^+$, showing that in this case the cobalt–carbon bond is stable even when there is a *trans*-cobalt–sulphur bond and the presence of free thiols. In the presence of air and in alkaline solution, the oxidation of thiols can occur and in the case of cysteine, the NH_2-bonded MeCo(tn)–cystine is formed when the oxidation is almost complete.

Formation Constants.—Some approximate stoicheiometric constants were calculated for the equilibrium:

$$[MeCo(tn)H_2O]^+ + am^x \rightleftharpoons [MeCo(tn)(am)]^{x+1} + H_2O$$

by an iterative method, from the visible absorption data (Table 1).

^1H *N.M.R. Spectra*.—The reaction of complex (I) with amino-acids can be followed using the resonances of all the protons. Separate signals were observed for free and bound amino-acid molecules, and for the axial methyl

group and equational ligand of the aquo- and amino-acid-complexes. This is due to the low rate of exchange between the free and bound forms of the amino-acid and between water and amino-acid in the axial position.

TABLE 1

Stoicheiometric formation constants

$$K = \frac{\{[MeCo(tn)(am)]^{x+1}\}}{\{[MeCo(tn)H_2O]^{+}\}\{am^{x}\}}$$

	log K		pK_a [a]
Glycine	3·3 ± 0·2	*b*	9·78 [c]
L-Alanine	2·7 ± 0·1	*b*	9·87 [c]
L-Phenylalanine	3·2 ± 0·1	*b*	9·24 [c]
L-Histidine	2·8 ± 0·1	*d*	6·10 [c]
Glycylglycine	2·6 ± 0·1	*b*	8·17 [e]
N-Acetyl-L-cysteine	≥4·4	*b*, *f*	
Glutathione	≥6·6	*d*, *g*	9·12 [e]

[a] K_a is the free amino-acid acidic constant used in the determination of K. [b] From pH 10 buffered solutions (see Experimental section). [c] 'Handbook of Chemistry and Physics,' The Chemical Rubber Co., 1967—1968, p. C 703. [d] From pH 7 buffered solutions (see Experimental section). [e] J. P. Greenstein and M. Winitz, 'Chemistry of Amino-acids,' Wiley, New York, 1961, ch. 4. [f] The reported value is calculated on the basis of the total amino-acid concentration and not of its binding form. [g] It is not known which of the reported K_a values corresponds to SH dissociation; therefore the higher value was employed.

TABLE 2

Amino-acid proton δ values in p.p.m. from sodium 2,2-dimethyl-2-silapentane-5-sulphonate (dss), (I) when bonded to cobalt to form $[MeCo(tn)(am)]^{x}$, (II) for the free amino-acid as observed in the same solution, (III) for the free amino-acid in its binding form

		(I)	(II)	(III)	
Glycine	CH_2	2·78		3·22 [a]	*b*
Alanine	CH	2·85 [c]		3·32 [a]	*b*
	CH_3	1·16	1·44	1·22 [a]	*b*
Glycylglycine	CH_2(1) [d]	3·03	3·42	3·34 [e]	*b*
	CH_2(2)	3·68	3·78	3·78 [e]	*b*
Glycylalanine	CH_2	3·00		3·32 [f]	*b*
	CH_3	1·29	1·34		*b*
Alanylglycine	CH_2	3·77	3·77	3·77	*b*
	CH_3	1·27	1·35		*b*
Tetraglycine	CH_2(1) [d]	3·07	3·55	3·39 [e]	*b*
	CH_2(2)	3·98	4·02	4·01 [c]	*b*
	CH_2(3)	3·98	3·98	3·98 [e]	*b*
	CH_2(4)	3·77	3·77	3·77 [e]	*b*
Histidine	im C(2)H	7·18	7·70	7·68 [a]	*b*
	im C(4)H	6·27	6·97	6·93 [a]	*b*
	im C(2)H	7·22	7·78	7·75 [a]	*g*
	im C(4)H	6·33	7·07	7·05 [a]	*g*

[a] G. C. K. Roberts and O. Jardetzky, *Adv. Protein Chem.*, 1970, **24**, 451. [b] Chemical shifts of columns (I) and (II) are measured from D_2O pD 9·5 buffered solutions; shifts in column (III) are those of the anionic form. [c] Approximate value read on the external lock spectrum. [d] Carbon atoms in the amino-acids are ordered starting from the NH_2 group. [e] A. Nakamura and O. Jardetzky, *Biochemistry*, 1968, **7**, 1226. [f] A. Nakamura and O. Jardetzky, *Proc. Nat. Acad. Sci. U.S.A.*, 1967, **58**, 2212. [g] Chemical shifts of columns (I) and (II) are measured from D_2O non-buffered solutions; shifts in column (*III*) are those of the zwitterionic form.

Amino-acid Proton Resonances.—Seldom was it possible to obtain accurate values for the chemical shifts of all the bound amino-acid protons, as their resonances give complex low intensity signals, sometimes obscured by the equatorial ligand ones (Table 2; Figure 4B).

The signals due to hydrogens α to cobalt-bound nitrogen are shifted to high fields with respect to the free amino-acid at the same pH and also to its anionic form. The other amino-acid resonances are also affected by the complexation, the effect decreasing on increasing distance from cobalt.

The α proton high-field shifts are observed also for other ligands, *i.e.* pyridine, imidazole, *N*-methylimidazole, benzylamine, methylamine. Furthermore studying the interaction of amino-acids with $[Co(bae)(NH_3)_2]Cl$,[7] $[Co(salen)(H_2O)_2]Cl$,[2] and $[Co(tn)(H_2O)_2](ClO_4)_2$,[2] we found that again the α protons are shifted to high fields.

TABLE 3

Axial and equatorial methyl group δ values in Hz from dss for $[MeCo(tn)L]^{x}$

L	Axial methyl	Equatorial methyl				
Water	50	144	137			*a*
Glycine	46	143	137			*a*
L-Alanine	46	143	139	137	134	*a*
L-Phenylalanine	39	137	131	119		*a*
L-Lysine	46	143	140	137	134	*a*
L-Histidine (NH_2)	41	146	138	131	124	*a*, *b*
L-Histidine (im)	55	146	138			*c*
Glycylglycine	48	143	137			*a*
Glycyl-L-alanine	47	144	137			*a*
L-Alanylglycine	46	143	140	136	133	*a*
Tetraglycine	48	143	137			*a*
L-Cystine	48	145	140	138	135	*a*
S-Methyl-L-cysteine	47	144	140	137	134	*a*
N-Acetyl-L-cysteine	45	140	135			*a*
L-Cysteine	49	143	136			*a*
Glutathione	46	140	135			*a*
Imidazole	55	145	139			*c*
N-Methylimidazole	53	145	137			*a*
Pyridine	63	147	141			*c*
Methylamine	44	144	137			*a*

[a] From D_2O pD 9·5 buffered solutions. [b] This spectrum is obtained by subtracting the spectrum in D_2O from that in pD 9·5 buffered solution. [c] From D_2O non-buffered solutions.

It is possible that the magnetic anisotropy of the equatorial plane plays the dominant role in these shifts, in agreement with the interpretation of the XCo(dimethylglyoximato)$_2$py [8] and RCo(bae)L [9] spectra.

The high-field shift of the protons closest to the co-ordinating atom of the sixth position, always clearly stronger than those of the other protons, confirms the ligand atom. For example, in glycylalanine, the methylene protons are strongly shifted, while those in alanylglycine remain almost unaffected, showing that the amide nitrogen and the carboxylic group are not directly involved in binding cobalt.

Axial Methyl and Equatorial Ligand Resonances.—The spectrum of $[MeCo(tn)H_2O]ClO_4$ in the 4—10 pD range is shown in Figure 4A. The axial methyl gives the sharp singlet at high fields. The two strong signals at 2·40 and 2·28 p.p.m. from sodium 2,2-dimethyl-2-silapentane-5-sulphonate (dss) are due to the equatorial ligand methyls, but we could not decide to which of the two different kinds of methyl groups each signal has to be assigned. The

[7] G. Costa, G. Mestroni, G. Tauzher, and L. Stefani, *J. Organometallic Chem.*, 1966, **6**, 181.

[8] H. A. O. Hill and K. G. Morallee, *J. Chem. Soc.* (*A*), 1969, 554.

[9] H. A. O. Hill, K. G. Morallee, G. Pellizer, G. Mestroni, and G. Costa, *J. Organometallic Chem.*, 1968, **11**, 167.

propylene bridge signals are complex, the intermediate methylene resonance being partially obscured by the equatorial methyls and the nitrogen bonded methylenes giving the low-field signal.

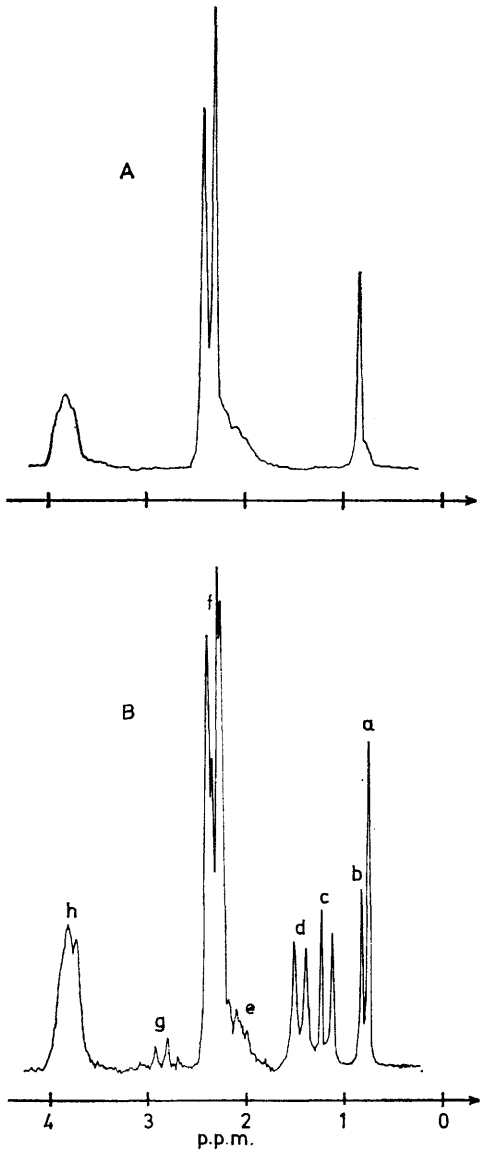


FIGURE 4 60 MHz ^1H n.m.r. accumulated spectra in pD 9·5 buffered solutions (shifts are from dss) of, A, $[MeCo(tn)H_2O]ClO_4$; B, $[MeCo(tn)H_2O]ClO_4$ + L-alanine; (a), axial methyl of [MeCo(tn)(Ala)]; (b), axial methyl of $[MeCo(tn)H_2O]^+$; (c), co-ordinated alanine methyl; (d), free alanine methyl; (e), $=N-CH_2-\mathit{CH}_2-CH_2-N=$; (f), equatorial ligand methyls; (g), co-ordinated alanine α hydrogen; (h), $=N-\mathit{CH}_2-CH_2-\mathit{CH}_2-N=$ (free alanine α hydrogen signals are obscured by this resonance)

In $[MeCo(tn)L]^x$, the MeCo(tn) moiety gives similar spectra (Table 3; Figure 4B).

Interaction with amino-acids causes small but real shifts to high field for the axial methyl, except when the amino-acid contains a conjugated ring. In the latter case the effect is more relevant, being to low fields when the ring is bound directly to cobalt (histidine bound through imidazole). As a similar effect is observed also using pyridine and imidazole instead of an amino-acid and is stronger for the former, we concluded that it is due mainly to the ring current deshielding effect. A roughly approximate evaluation of ring-current effect in $[MeCo(tn)(py)]^+$, using the Johnson–Bovey tables,[10] gives a value of *ca.* −0·15 p.p.m. for the axial methyl. The observed difference of −0·30 p.p.m., with respect to $[MeCo(tn)(NH_2CH_3)]^+$, probably reflects also the lower basicity of pyridine and the decreased electron density on cobalt through back donation.

On the other hand, for phenylalanine and NH_2-bonded histidine, the methyl resonance is shifted to high fields, with respect, for example, to alanine, this still being due to the ring current effect, now shielding because of the different geometrical situation.

With histidine, the simultaneous presence of various signals due to the axial methyl shows the existence of differently bound complexes. In D_2O, there is only one methyl signal, shifted to low fields with respect to that of the aquo-complex, as under these conditions histidine can bind only through imidazole, but at pD 9·5 another signal is observed besides the above one. This is shifted to high field with respect to the aquo-complex, and is due to NH_2-bonded molecules. The presence of the bridged form is shown by a (histidine-bound cobalt)/total histidine ratio higher than 1, for small amounts of histidine, but is not reflected by the presence of new methyl signals, probably because they overlap with those of the monomeric forms.

Such clear effects are not given by cysteine and glutathione, as *S*-bonded and NH_2-bonded complexes have axial methyl chemical shifts too near, but even in these cases the ratios (cysteine-bound cobalt)/total cysteine and (glutathione-bound cobalt)/total glutathione are in agreement with the existence of bridged complexes.

Substitution of a water molecule by amino-acids causes just small variations in equatorial proton resonances: therefore, only the shifts of equatorial methyl peaks are easily observable.

Glycine, glycylglycine, glycylalanine, and tetraglycine do not affect significantly any equatorial ligand resonance.

With alanine, phenylalanine, alanylglycine, lysine, NH_2-bound histidine, *S*-methylcysteine, and cystine, methyl signals move and are split, *i.e.* the remaining equivalence of equatorial methyls is lost. This suggests that in the presence of an asymmetrical carbon atom α to the binding nitrogen, that is when steric hindrance is increased with respect to a methylene, rotation around the Co–N bond is not free. In agreement with this interpretation, which explains non-equivalence by ' through space ' effects, phenylalanine and NH_2-bonded histidine, which have ring magnetic anisotropy, cause not only stronger variations in the methyl shifts, but also stronger splitting.

On the other hand, with imidazole-bound histidine, imidazole, and *N*-methylimidazole no splitting is observed for the two equatorial methyl signals, which slightly

[10] C. E. Johnson and F. A. Bovey, *J. Chem. Phys.*, 1959, **29**, 1012.

move downfield. With pyridine the downfield shift is stronger, while the central propylenic methylene signal becomes well separated, being itself shifted to high fields. This can be explained if we assume that the ring prefers to stay perpendicular to the symmetry plane of the equatorial ligand; only in this geometry can the ring current explain the signs of the observed shifts. This is in agreement with some π character in the Co–N bond, suggested also by the fluorine resonance results for *p*- and *m*-F-C_6H_4Co(tn)L.[11] Furthermore an analogous situation was proposed for the phenyl ring in PhCo(bae) [12] and probably it is also present for the pyridine ring of RCo(bae)(py).[9]

With cysteine, *N*-acetylcysteine, and glutathione the equatorial methyls undergo small high-field shifts, but their equivalence is not appreciably affected, indicating that here the rotation around the Co–S bond is free enough to give a pseudosymmetry. The small shielding observed in these cases is probably due to an increased electron density transmitted from sulphur *via* cobalt.

CONCLUSIONS

When amino-acids interact with metals they behave generally as chelating ligands. In the complexes $[RCo(tn)(H_2O)]^+$ however only the water molecule is easily displaced by Lewis bases. Thus amino-acids and simple oligopeptides in aqueous solutions bind the cobalt atom by only one of the $-NH_2$, imidazole, or $-S^-$ groups, the actual identity of ligand atom being inferred from visible spectroscopy. Moreover, even if the amino-acid contains two of these groups, it does not behave as a chelating ligand but under suitable conditions can form bridged binuclear complexes. N.m.r. spectra confirm these conclusions and also provide information on the existence in solution of preferred orientations of the axial ligand *trans* to the methyl group.

The highest thermodynamic stability is found for sulphur binding ligands. An analogous result was reported for cobalamins [13,14] and cobaloximes.[15] However, to our knowledge, this is the first report of stable complexes having an alkyl and a thiolate as the two axial ligands for vitamin B_{12} or its model complexes.*

Exchange rates of ligands in axial positions, inferred from n.m.r. spectra in $[MeCo(tn)(am)]^x$ and in MeCo(dimethylglyoximato)$_2$L [15] and calculated from kinetic measurements in $[MeCo(tn)L]^+$,[4] are much slower than for RCo(bae)L [9] where separate resonances cannot be observed. This correlates with the stability of five-coordinate complexes in agreement with a S_N1 mechanism.

* *Note added in proof:* Similar behaviour has recently been reported for methylcobaloxime (K. L. Brown and R. G. Kallen, *J. Amer. Chem. Soc.*, 1972, **94**, 1894).

EXPERIMENTAL

$[MeCo(tn)(H_2O)]ClO_4$ was prepared by reacting $Co(tn)Br_2$ with MeI in the presence of $NaBH_4$.[6]

L-Alanine, L-phenylalanine, L-lysine, glycylglycine, glycyl-L-alanine, L-alanylglycine, tetraglycine, and glutathione were Schuchartd products. *N*-Acetyl-L-cysteine was a B.D.H. product. L-Cysteine hydrochloride monohydrate and *S*-methyl-L-cysteine were Mann products. L-Cystine was a Fluka puriss product. Glycine was a Carlo Erba RS product.

Stohler I.C. D_2O (99·8% D), CIBA NaOD (99% D), and Merck, Sharp and Dohme sodium 2,2-dimethyl-2-silapentane-5-sulphonate (dss) were used for n.m.r. spectroscopy.

All these compounds were not further purified.

Deuterioboric acid, used for D_2O buffered solutions, was prepared by successive crystallizations of boric acid from D_2O, the extent of deuteriation being followed by n.m.r. and i.r. spectroscopy.

Other compounds were analytical or pure grade.

pH Values were measured with a Metrohm E 353 pH meter, with an EA 121 UX electrode, standardized with Carlo Erba Normex buffer solutions. pD Was measured with the same instrument, adding 0·4 pH units to the instrumental value.[16]

Visible and u.v. spectra were recorded by a Hitachi–Perkin–Elmer EPS 3 T spectrophotometer; 1 cm quartz cells were used.

For these measurements solutions were prepared by mixing fixed volumes of a solution of complex (I) with suitable volumes of solutions of amino-acids, and diluting until the cobalt concentration was $2{\cdot}32 \times 10^{-4}$M. For fixed pH phosphate pH 7 [KH_2PO_4, (0·5 mol), NaOH (0·35 mol), diluted to 1 l] and borate pH 10 [H_3BO_3 (0·5 mol), KCl (0·5 mol), NaOH (0·42 mol), diluted to 1 l] buffers were used as the solvent for the amino-acid and for dilution.

The approximate equilibrium constants were calculated from visible absorptions by an iterative method similar to that already employed in previous works for chemical shifts [9,12] and carried out by an IBM 7044 computer. Maximum amino-acid concentration for equilibrium constant determinations was 100 times higher than cobalt.

N.m.r. spectra were recorded by a JEOL C 60 HL spectrometer. Reported chemical shifts were measured working in internal lock (dss was used for locking signal). The audio-frequencies used to generate the locking and the measuring sidebands were measured on a Hewlett Packard 5216A 12·5 MHz electronic counter, accuracy being better than ± 1 Hz, and chemical shifts were calculated directly from their differences.

For fixed pD, the buffer was prepared by adding NaOD to a 0·5M deuterioboric acid solution in D_2O, till pD 9·5.

We thank Dr. H. A. O. Hill for helpful discussions, C.N.R. for financial support, and 'Fondazione G. Donegani' Accademia Nazionale dei Lincei for a Research Studentship to G. R. T.

[2/593 *Received*, 13*th March*, 1972]

[11] H. A. O. Hill, K. G. Morallee, G. Pellizer, and F. Cernivez, *J. Amer. Chem. Soc.*, 1972, **94**, 277.
[12] H. A. O. Hill, K. G. Morallee, and G. Pellizer, *J. Chem. Soc.* (*A*), 1969, 2096.
[13] J. M. Pratt and R. G. Thorp, *J. Chem. Soc.* (*A*), 1966, 187.
[14] H. A. O. Hill, J. M. Pratt, R. G. Thorp, B. Ward, and R. J. P. Williams, *Biochem. J.*, 1970, **120**, 263.
[15] L. M. Ludwick and T. L. Brown, *J. Amer. Chem. Soc.*, 1969, **91**, 5188.
[16] P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.