Previous results[4] indicate that the phenol group in the sulfonyl enzyme IV participates in the desulfonylation of this species since α-toluenesulfonyl-α-chymotrypsin does not desulfonate at an appreciable rate in the neutral pH range. We had anticipated therefore that the sulfonated enzyme VIII might also undergo desulfonation at a significant rate with participation of the *ortho* phenolic group in this reaction. However, as indicated before, we have found that VIII does not desulfonate appreciably to give the monosulfate IX near the neutral pH range over a time period of more than 12 hr. At present we do not know the reasons for the difference in the stability of the modified enzymes IV and VIII. Also, we mention again here that while our spectrophotometric titration results show that the spectral behavior of the nitrophenol chromophore in VIII can be understood in terms of reaction scheme 6, as is the case with IV, the p*K* values obtained for the various ionizations shown in the scheme are quite different for the two modified enzymes.

Further studies on the modification of enzymes and other proteins with highly reactive cyclic esters are currently under way in our laboratory.[19]

Acknowledgments. This research was supported by Agriculture Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-9145(71), administered by the Northern Utilization Research and Development Division, Peoria, Ill. We would like to thank Professor F. J. Kezdy and Mr. John H. Heidema for many helpful discussions.

(19) The nitrosultone II has recently been discovered to be an excellent reagent for probing the action of the sulfhydryl enzyme, papain (M. Iwatsuru, unpublished results).

Ultraviolet Circular Dichroism of Cupric and Nickel Ion Complexes of Amino Acids and Peptides[1]

John M. Tsangaris, Joyce Wen Chang, and R. Bruce Martin

Contribution from the Chemistry Department, University of Virginia, Charlottesville, Virginia 22901. *Received March 22, 1968*

Abstract: Nickel ion complexes of L-amino acids exhibit negative circular dichroism near 210 mμ in agreement with the prediction of the octant rule for an n → π* carbonyl group transition. This transition is evidently obscured by a charge transfer transition in cupric complexes. Optically active charge transfer transitions also occur at 230–280 mμ in the peptide complexes of both transition metal ions. Cupric complexes of di- and tripeptides exhibit circular dichroism from 300 to 315 mμ where no discernible shoulders or maxima appear in the absorption spectra. It is suggested that this low-absorption intensity optically active transition might be due to an n → π* transition moved to longer wavelengths in the cupric chelates of peptides with ionized amide hydrogens. The CD of aromatic transitions is enhanced by incorporation of the amino acid into a dipeptide, and a lesser augmentation in CD intensity occurs on chelation by cupric ion.

Amino acids and peptides bearing aliphatic side chains exhibit absorption maxima only at less than 200 mμ, but a weaker n → π* transition gives rise to measurable optical activity at longer wavelengths. Aromatic side chains introduce further absorption at less than 300 mμ. It is part of the purpose of this paper to describe and analyze the changes wrought in the circular dichroism (CD) through these absorption bands by cupric and nickel ion chelation of these ligands. Urry and Eyring have already shown us how to apply the octant rule to the n → π* carbonyl group transition in metal ion complexes of amino acids.[2] In addition, new ultraviolet absorption bands are introduced by cupric and nickel ion chelation.

Upon addition of base to solutions containing peptides and cupric or nickel ions, amide hydrogen ionization is promoted even in neutral solutions.[3] All the peptide complexes referred to in this paper have undergone peptide hydrogen ionizations. Since earlier conclusions from solution studies regarding the geometry of metal ion complexes of amino acids and peptides have been confirmed by X-ray diffraction,[4] interpretation of CD results of these complexes may be predicated on well understood and often relatively rigid structures. CD involving d–d transitions on the metal ions in the visible region of the spectrum have already been reported[5] for the cupric[6] and planar nickel[7] ion complexes of the amino acids and peptides presented in this paper. Since the transitions reported here involve primarily the ligands, the ultraviolet CD results for the free ligands and chelates with both transition metal ions are discussed together.

(1) This paper is abstracted from the Ph.D. dissertations of Drs. J. M. Tsangaris and J. W. Chang (both 1967), and the research was supported by a grant from the National Science Foundation.

(2) D. W. Urry and H. Eyring, *J. Am. Chem. Soc.*, 86, 4574 (1964).

(3) R. B. Martin, M. Chamberlin, and J. T. Edsall, *ibid.*, 82, 495 (1960).

(4) H. C. Freeman, *Advan. Protein Chem.*, 22, 257 (1967).

(5) R. B. Martin, J. M. Tsangaris, and J. W. Chang, *J. Am. Chem. Soc.*, 90, 821 (1968).

(6) J. M. Tsangaris and R. B. Martin, submitted for publication.

(7) J. W. Chang and R. B. Martin, submitted for publication.

Experimental Section

Most experimental details have been supplied elsewhere.[7] Absorptions of solutions for CD measurements did not exceed 1.5. If the CD curve at wavelengths shorter than 220 mμ is noisy because of high absorptivities, an average of two or three repetitions is reported. Since the transitions reported in this paper are associated primarily with the ligand, all molar absorptivities, ϵ, in absorption spectra and differential molar absorptivities between left and right circularly polarized light, $\Delta\epsilon$, in circular dichroism are reported in reference to molar concentration of ligand rather than in terms of metal ion or complex concentration. Occasionally ligands bearing one asymmetric center derived from D-amino acids were employed; for consistency the results have been reported as if the ligand were derived from L-amino acids as in the majority of cases. All experiments were conducted at room temperature, near 23°.

Results

Free L-amino acids exhibit positive CD extrema near 210 mμ in general agreement with an earlier tabulation.[8] Absorption in this region exhibits a molar absorptivity of about 10^2. The nickel ion complexes of L-amino acid also exhibit CD extrema at 210 mμ, but generally with negative sign. The differential molar absorptivities of the nickel ion complexes shown in Table I are based on the molar concentration of ligand. If each L-amino acid ligand contributes independently to the CD, values for the 1:1 and 2:1 nickel ion complexes of Table I should be identical. The lack of magnitude identity for the weaker complexes suggests that the 2:1 complexes are less fully formed than the 1:1 complexes at the ligand concentrations (10^{-2} *M*) employed. Absorption in the nickel ion complexes increases to $\epsilon \sim 10^3$ at 210 mμ. The CD extremum of the histidine complexes in Table I is at a slightly longer wavelength of 218 mμ while at this wavelength $\epsilon \simeq 4 \times 10^3$. In neither the free amino acids nor any of the complexes does the absorption spectrum exhibit a maximum at the CD extremum as the absorption is increasing toward a maximum further into the ultraviolet region.

Table I. Circular Dichroism of Nickel Ion Complexes of L-Amino Acids at Extrema Near 210 mμ

L-Amino acid	Ligand to nickel ion ratio 1:1	2:1
Aspartic acid	+0.3	−2.3
Alanine	−0.4	−0.3
Serine	−0.8	−0.5
Asparagine	−0.3	
Glutamic acid	−0.7	−0.4
Arginine	−0.7	
Threonine	−1.7	−1.0
Proline	−0.9	−0.8
Histidine	−6.4	−6.4

The ultraviolet CD of cupric ion complexes containing two amino acid ligands are tabulated in the second column of Table II. Typically two CD extrema appear, the longer wavelength one from 245 to 260 mμ and a shorter wavelength one from 215 to 225 mμ. An absorption maximum with $\epsilon \sim 3 \times 10^3$ appears in the longer wavelength region while no maximum appears in the shorter wavelength region of somewhat lesser absorption. For the phenylalanine and tyrosine cupric complexes the absorption intensity of the 240- to 260-mμ transition is unaffected by the presence of aromatic chromophores but now $\epsilon \sim 10^4$ at 220 mμ and an additional CD peak appears at 278 mμ with $\Delta\epsilon = -0.2$ for the 2:1 phenylalanine–cupric ion complex and at 290 mμ with $\Delta\epsilon = -0.1$ for the 2:1 tyrosine complex. The 2:1 or 1:1 histidine–cupric ion complexes exhibit negative CD peaks at 242 and 215 mμ with the magnitudes given in Table II. For both complexes absorption maxima appear at 240 mμ with $\epsilon = 1800$ and at 210 mμ with $\epsilon = 7 \times 10^3$. An absorption band with a maximum $\epsilon \simeq 5 \times 10^3$ also appears in the spectrum of basic, protonated, and cupric ion complexed imidazole at 205 mμ.

Table II. Circular Dichroism of Cupric Complexes of L-Amino Acids (2:1) and Glycyl Dipeptides (1:1)

L-Amino acid, X	X (mμ)		Gly-X (mμ)		X-Gly (mμ)	
	255	220	315	270	310	235
Aspartic acid	−0.4	+0.5	−0.4	+1.0	−0.1	−1.1
Alanine	−0.4	+0.3	+0.2		+0.3	
Serine	−0.3	+0.3	+0.3	−1.2		
Asparagine			+0.1	−1.2		
Leucine	−0.8	+	+0.3	−1.5	+0.2	−2.1
Glutamic acid	−0.7		+0.4	−1.8		
Arginine	−0.2				+0.2	−0.7
Threonone	−0.8	+0.1	+0.6	−2.0	+	−2.0
Valine	−0.7	+0.4	+0.5	−2.2	+0.1	
Phenylalanine	+0.8	−2.4	+0.1	−3.1	+0.5	−7
Tyrosine	+0.6	−3.2		−1.9	+0.5	−10
Histidine	−2.1	−7.0	+0.2	+0.4	+1.3	−7.5

Circular dichroism of other 2:1 complexes of L-amino acids with cupric ion not reported in Table II are proline, $\Delta\epsilon = -0.2$ at 275 mμ and $\Delta\epsilon = +1.4$ at 230 mμ; N-methylproline, $\Delta\epsilon = +0.2$ near 280 mμ; and phenylglycine, $\Delta\epsilon = +0.5$ at 285 mμ and $\Delta\epsilon = -0.5$ at 232 mμ. For the 2:1 complexes of L-amino acid amides which have undergone amide hydrogen ionization, the following CD were obtained: alaninamide, $\Delta\epsilon = +0.3$ at 260 mμ and $\Delta\epsilon = -0.5$ at 222 mμ; prolinamide, $\Delta\epsilon = +0.9$ at 275 mμ and $\Delta\epsilon = -0.6$ at 236 mμ.

A noncomplexed dipeptide such as glycyl-L-leucine exhibits a CD peak with $\Delta\epsilon \simeq +1.0$ at 220 mμ in the dipolar ion and anionic forms while protonation of the leucyl carboxylic acid group yields $\Delta\epsilon = 1.6$ at 220 mμ. In this region of low absorption no absorption maxima appear in any of these forms and large negative CD appear in all cases near 200 mμ. The dipeptide L-leucylglycine exhibits nearly zero CD at 220 mμ in cationic and dipolar ion forms while ionization of the leucyl ammonium group to give the anionic form yields $\Delta\epsilon = -1.0$ at this wavelength. All three ionic forms exhibit larger positive CD near 200 mμ. Similar CD curves have been reported for the dipolar ion forms.[9] Glycyl-L-tyrosine exhibits two positive CD extrema, $\Delta\epsilon = +7$ and +19 at 222 and 200 mμ, respectively.[9] Both dipolar ion L-histidylglycine and glycyl-L-histidine show positive CD peaks at 205 and 215 mμ, respectively. The CD of glycylhistidine is not much affected by addition of base and zinc ion, lending no support to an earlier suggestion[10] that amide hydrogen ionization has

(8) M. Legrand and R. Viennet, *Bull. Soc. Chim. France*, 679 (1965); 2798 (1966).

(9) M. Legrand and R. Viennet, *Compt. Rend., Ser. C*, **262**, 943 (1966).

(10) R. B. Martin and J. T. Edsall, *J. Am. Chem. Soc.*, **82**, 1107 (1960).

been promoted by this metal ion and indicating instead that ionization of a coordinated water molecule takes place. Upon addition of 1 equiv of nickel ion and 1 or 2 equiv of base to histidylglycine, a negative CD peak with $\Delta\epsilon = -3$ appears at 220 mμ similar to the results reported above for histidine. The addition of 1 equiv of nickel ion and 1 equiv of base to dipolar ion glycylhistidine also yields a negative CD extremum at 217 mμ with $\Delta\epsilon = -4.8$. Addition of a second equivalent of base produces an additional CD peak with $\Delta\epsilon = +3.0$ at 245 mμ where no absorption maximum appears and $\epsilon = 175$. Occurrence of this last CD peak coincides with ionization of the peptide hydrogen.[10]

Circular dichroism for 1:1 cupric complexes of glycyl–amino acid dipeptides are recorded in the third column of Table II. A CD extremum appears from 310 to 320 mμ where there is no peak in the absorption spectrum and molar absorptivities read off the spectra are only 100–300. For CD extrema from 260 to 280 mμ the absorption spectra exhibit shoulders with ϵ greater than 10^3. At still shorter wavelengths larger positive CD extrema appear. CD and absorption spectra for the aminoacylglycine dipeptides reported in the last column of Table II are similar but appear from 300 to 320 mμ and from 230 to 240 mμ. The cupric complex of glycyl-L-asparagine in excess base where a biuret color appears yields $\Delta\epsilon = -0.1$ at 305 mμ and $\Delta\epsilon = +3.1$ at 256 mμ, the sign changes possibly reflecting hydrogen ionization and chelation of the side-chain amide function. No sign changes occur upon addition of two equivalents of excess base to the glycylserine and glycylthreonine complexes consistent with the conclusion from visible CD that their alcoholate functions are not chelated to cupric ion.[6] In addition to the peaks reported in Table II, the glycylphenylalanine complex exhibits $\Delta\epsilon = +26$ at 220 mμ and the glycyltyrosine complex $\Delta\epsilon = +19$ at 210 mμ where from absorption spectra $\epsilon > 10^4$. For the cupric–glycylhistidine complex of net zero charge there is an additional CD peak with $\Delta\epsilon = -7.8$ at 217 mμ where $\epsilon \sim 10^4$. Except for tyrosylglycinamide which yields $\Delta\epsilon = -0.2$ at 325 and $\Delta\epsilon = +0.6$ at 285 mμ, the cupric complexes of several dipeptide amides[6] give ultraviolet CD sign patterns identical with those of the corresponding dipeptides in Table II.

Considerations similar to those of the previous paragraph also apply to the CD of cupric–dipeptide complexes tabulated in Table III. At shorter wavelengths for the cupric complex of valylphenylalanine we obtain $\Delta\epsilon = +31$ near 215 mμ. For the free ligand Val-Phe in 0.1 *N* base we measure $\Delta\epsilon = +22$ near 215 mμ and $\Delta\epsilon = -0.7$ at 259 mμ.

In addition to the CD extremum at 230–250 mμ reported in the second column of Table IV for the nickel ion complexes of tripeptides, a longer wavelength $\Delta\epsilon = -0.4$ at 265 mμ is observed for the Gly-Gly-Ala complex and a shorter wavelength extremum at 220–230 mμ of negative sign is apparent in the nickel ion complexes of Gly-Ala-Gly, Ala-Ala-Ala, Leu-Leu-Leu, and Gly-Phe-Gly. In addition to the CD maximum at 250 mμ reported in Table IV, the Phe-Phe-Phe complex also exhibits a positive CD shoulder at 240 mμ. Comparison of the magnitudes and signs of CD as well as absorption spectra with aliphatic tripeptides suggests that aromatic groups are not major contributors to CD at greater than 220 mμ in the nickel ion complexes. These observations suggest that two transitions not involving any aromatic rings appear in the 220–250-mμ region, and their presence may be obscured in those complexes where they occur at nearly identical wavelengths or possess the same CD sign. In absorption spectra shoulders on curves ascending to peaks further into the ultraviolet are apparent in the complexes of nickel ion and tripeptides, and the molar absorptivities display values of several thousand. The nickel–glycyl-L-alaninamide complex gave $\Delta\epsilon = +0.4$ at 250 mμ.

Table IV also lists CD peaks for cupric complexes of tripeptides. Once again the CD extremum from 290 to 310 mμ is characterized by low ϵ of 100 to 400 without any maximum or shoulder in absorption spectra. The CD peak from 260 to 280 mμ occurs where the absorption spectra usually exhibit shoulders with $\epsilon > 10^3$. At still shorter wavelengths, <220 mμ, most of the complexes display a positive CD extremum with $\Delta\epsilon$ from +3 to +12 except for Pro-Gly-Gly, which exhibits a negative CD peak. The CD curves are somewhat more complicated than Table IV indicates as shoulders apparently occur on some of the larger magnitude low-wavelength peaks. Cupric complexes bearing aro-

Table III. Circular Dichroism of Cupric Complexes of Dipeptides Composed of L-Amino Acid Residues

Dipeptide	315 mμ	265 mμ
Ala-Ala	+0.3	−1.0
L-Ala-D-Ala	−0.1	+1.1
Ala-Leu	+0.2	−0.8
Leu-Ala	+0.4	−1.3
Leu-Leu	+0.5	
Arg-Glu	+0.5	−1.6
Val-Phe	+0.1	−2.6
Phe-Val	+0.5	−2.5
Leu-Tyr		−2.4
Tyr-Leu	+0.3	−1.0

Table IV. Circular Dichroism of Nickel and Cupric Ion Complexes of Tripeptides Composed of L-Amino Acids

Tripeptide	Nickel 240 mμ	Cupric 300 mμ	Cupric 270 mμ
Gly-Gly-Ala	+1.9		
Gly-Ala-Gly	+3.3	+0.6	−0.5
Ala-Gly-Gly	+0.5		
Ala-Ala-Ala	+5.5		
Gly-Ala-Leu	+3.5	+0.3	−0.5
Gly-Gly-Leu-NH_2	+1.4	−0.6	
Gly-Gly-Leu	+1.0	−0.6	
Gly-Leu-Gly	+2.9	+0.6	−0.4
Leu-Gly-Gly	0	+0.4	−0.1
Leu-Leu-Leu	+3.3	+0.4	−0.4
Val-Gly-Gly	0	+0.3	−1.1
Pro-Gly-Gly	−0.4	+0.3	−0.6
Gly-Gly-Phe-NH_2	+8	−0.5	
Gly-Gly-Phe	+6	−0.7	
Gly-Phe-Gly	+5.4	+0.6	−2.8
Gly-Phe-Phe	+11		
Phe-Gly-Gly	−2.7	−0.2	+0.6
Phe-Phe-Phe	+10	−0.8	
Lysine-vasopressin	+3.8	+0.2	−0.7

matic side chains in the second or third positions yield $\Delta\epsilon = +20$ to $+30$ at about 210 mμ while the cupric complex of Phe-Gly-Gly exhibits $\Delta\epsilon = -16$ at 215 mμ. The cupric complex of lysine-vasopressin at high pH also displays $\Delta\epsilon = +9$ and $+16$ at 240 and 220 mμ, respectively.

Discussion

The positive CD exhibited by typical free L-amino acids near 210 mμ where no absorption maximum appears and $\epsilon \simeq 10^2$ is consistent with assignment to an electric dipole forbidden $n \rightarrow \pi^*$ transition in the carbonyl group. Upon complexation of L-amino acids with nickel ions the CD sign in the 210-mμ region changes to negative and while no absorption maximum appears, ϵ increases to about 10^3. Thus in the nickel ion complexes also the CD near 210 mμ appears to be due to an $n \rightarrow \pi^*$ transition on the carbonyl group. The residual absorption in this region is evidently due to an electric dipole allowed $\pi \rightarrow \pi^*$ transition occurring further into the ultraviolet. The magnetic dipole allowed $n \rightarrow \pi^*$ transition should dominate the CD near 210 mμ in both the free amino acids and the nickel ion complexes.

Application of an octant rule[2,11] to the $n \rightarrow \pi^*$ transition of the carbonyl group in the carboxylate function of nearly planar nickel ion chelates of L-amino acid ligands reveals that all atoms other than hydrogen are in or close to a nodal plane while the side chain of an L-amino acid is in a negative octant consistent with the signs of the CD in Table I. In the tridentate L-aspartic acid–nickel ion complex, chelation of the second carboxylate group distorts the planarity of the five-membered chelate ring and introduces a second $n \rightarrow \pi^*$ transition so that the CD magnitude is the sum of contributions from two carboxylate groups. For the conformation adopted in the crystal,[4] application of the octant rule to the $n \rightarrow \pi^*$ transition of the β-carboxylate group reveals that the metal ion is the main perturbant and appears in a positive octant. The small positive value for CD of the 1:1 L-aspartic acid–nickel ion complex in Table I may be accommodated by a greater positive contribution from the β-carboxylate group than a negative one from the α-carboxylate function.

Because of high intensity imidazole ring absorption in the 210–220-mμ region where the weaker $n \rightarrow \pi^*$ transition occurs, application of the octant rule to histidine complexes depends upon the $n \rightarrow \pi^*$ transition dominating the optical activity in spite of the aromatic transition of 10^2 times greater intensity dominating the absorption. By making this assumption, Urry and Eyring[2] applied the octant rule to transition metal ion complexes of histidine and its methyl ester, accounting for negative and positive Cotton effects, respectively, in the 210–220-mμ region. Their argument for the 210–220-mμ Cotton effect being controlled by the carbonyl $n \rightarrow \pi^*$ transition is based on a comparison of the ORD curves of free histidine with alanine and lactic acid rather than of their metal ion complexes. A negative octant sign is consistent, however, with the crystal structure[12] of the nickel ion–histidine complex and simply represents another example of the argument employed above in accounting for the negative signs of the other nickel ion complexes in Table I. In order to account for positive signs in the 210-mμ Cotton effect of metal ion complexes of histidine methyl ester, Urry and Eyring assumed a bias in the orientation of the ester group due to its chelation with the metal ion. Comparisons of acid ionization and formation constants of histidine methyl ester[13] and histidine[14] lend no support to such additional chelation. Studies of transition metal ion promoted hydrolyses of histidine methyl ester indicate only little or no super acid catalysis, suggesting that no significant biasing of the ester group by chelation to the metal ion takes place in solution.[13] Hence, the prediction of the octant sign for metal ion complexes of histidine methyl ester is evidently only accidentally correct. Indeed, the identity of sign and similarity in magnitude of the positive 210-mμ Cotton effect in metal ion complexes of histidine methyl ester and free histidine suggests freely rotating ester groups in the complexes.

Interpretation of the results for cupric ion complexes of L-amino acids listed in Table II is relatively complicated. The high intensity absorption maximum associated with the CD peak near 255 mμ suggests that this is a ligand to cupric ion charge transfer transition. The shorter wavelength CD peak near 220 mμ in the amino acids with aliphatic side chains appears at a wavelength where the $n \rightarrow \pi^*$ transition might be expected, but failure to observe the predicted negative octant sign suggests that contributions from a $\pi \rightarrow \pi^*$ transition might be dominant. Thus the $n \rightarrow \pi^*$ transition evidently is obscured in both absorption and CD of the cupric ion–amino acid complexes and it may well be lost in the 255-mμ charge-transfer band.

For the L-phenylalanine and L-tyrosine cupric complexes CD at 240–260 mμ in the charge transfer and aromatic absorption regions is now of positive sign. For these aromatic amino acids and phenylglycine the CD and absorption at 278–290 mμ, assigned to the secondary band (L_b) of the aromatic chromophore, has changed sign upon chelation with cupric ion. The CD near 220 mμ is accompanied by a high absorption indicating that contributions from the primary band (L_a) of the aromatic chromophore contribute to this region in addition to the contributions discussed above for aliphatic side chains.

In view of the difficulties with octant signs of the cupric ion complexes of amino acids with aliphatic side chains, the negative sign for the L-histidine complex does not seem to be significant for application of the octant rule. Urry and Eyring also applied an octant rule to the 245-mμ peak of the cupric–histidine complex by assigning it to a charge transfer transition from imidazole to cupric ion.[2] Since all the other amino acids of Table II also exhibit CD peaks and charge transfer absorption maxima of similar high intensity near this wavelength where a cupric–imidazole complex does not display such intense absorption, their identification of the origin of the charge transfer transition seems incorrect. In all these cupric complexes the charge transfer absorption bands arise from transitions from amino and carboxylate groups.

(11) W. Moffitt, R. B. Woodward, A. Moscowitz, W. Klyne, and C. Djerassi, *J. Am. Chem. Soc.*, 83, 4013 (1961).
(12) K. A. Fraser and M. M. Harding, *J. Chem. Soc., A*, 415 (1967).
(13) H. L. Conley, Jr., and R. B. Martin, *J. Phys. Chem.*, 69, 2923 (1965).
(14) R. Leberman and B. R. Rabin, *Trans. Faraday Soc.*, 55, 1660 (1959).

In the pair of glycyl and leucyl containing dipeptides the CD peaks appearing in the absence of any metal ions at 220 mμ in a region of low-absorption intensity may be identified as due to the $n \rightarrow \pi^*$ transition of the amide bond. This transition exhibits a change in sign as the optically active center moves from one side of the amide chromophore to the other. Ionization of the group attached to the leucyl residue appears to have the greatest effect on the CD magnitude; carboxylic acid group in glycylleucine and ammonium group in leucylglycine. The octant rule may be applied to the $n \rightarrow \pi^*$ transition of the amide group by considering the probable conformations of the dipeptides as deduced from dipeptide maps.[15] On this basis a positive octant sign is predicted for glycyl-L-leucine with the amino acid side chain as the main perturber, while the amino group must be considered as the main perturber in order to accommodate the negative sign for anionic L-leucylglycine. This conclusion is consistent with the disappearance of sensible CD upon protonation to yield a more symmetrical ammonium ion.

In the nickel ion complexes of tripeptides at least two transitions give rise to CD extrema in the 250-mμ spectral region. These two transitions could be of two different kinds such as $n \rightarrow \pi^*$ and charge transfer from ionized peptide nitrogen to nickel ion or one kind of transition may be associated with two peptide groups. We suggest that both situations are occurring so that though octant signs may be predicted in these planar nickel ion chelates, the identification with unresolved peaks is difficult. The occurrence of positive CD peaks near 240 mμ in the nickel ion complexes of the set of three alanyl tripeptides at the top of Table IV suggests that a transition other than $n \rightarrow \pi^*$ contributes significantly to the CD in this region. A fair additivity is attained in these nickel ion complexes as demonstrated by the sum of the $\Delta\epsilon$ values for first three alanyl complexes (+5.7) being nearly equal to $\Delta\epsilon = +5.5$ for the fourth. Lower CD magnitudes observed when the optically active amino acid residue is N-terminal may be due to the greater amount of freedom provided to the side chain when held to the metal ion *via* tetrahedral rather than planar trigonal nitrogens.[7]

In the cupric complexes of dipeptides and tripeptides the high absorption intensity band yielding CD extrema from 235 to 270 mμ may be identified as consisting of at least a charge transfer transition. Charge transfer from ionized peptide nitrogens to cupric ion may also occur. More difficult to assign is the low absorption intensity band which exhibits no discernible shoulders or maxima in absorption spectra and gives CD peaks from 300 to 315 mμ in cupric complexes of dipeptides and tripeptides. Only two possiblities apparently exist for this low absorption intensity transition, a d–d transition on the cupric ion or an $n \rightarrow \pi^*$ transition on the ligand. In order for the 310-mμ band to be due to a d–d transition, it would evidently have to represent a split out component of a transition from a degenerate e_g level (in D_{4h}), the other component of which appears near 500 mμ.[6] This amount of splitting seems unreasonable. In addition four d–d transitions in cupric complexes of amino acids and peptides have already been counted in the visible and near-infrared regions.[6] Finally the relative insensitivity of the position of the 310-mμ CD peak to the number of nitrogen donors and the lack of such a peak in other cupric complexes where the nitrogen donors are not in amide bonds suggest that the 310-mμ band is a feature of the amide group.

It has already been suggested above that the $n \rightarrow \pi^*$ transition which occurs near 220 mμ in uncomplexed amides is shifted to 240 mμ in the nickel ion complexes. Therefore, the $n \rightarrow \pi^*$ transition may be shifted to even longer wavelengths near 310 mμ in the cupric ion complexes. An objection to this assignment is the positive signs for both Gly-X and X-Gly cupric complexes in Table II, whereas on the basis of the octant rule, a positive sign is expected only for the former class of dipeptide complexes. The side chain in the Gly-X dipeptide complexes is held in a relatively rigid conformation by the hydrogen-ionized trigonal peptide nitrogen while the tetrahedral nitrogen permits the side chain in X-Gly dipeptide complexes a greater flexibility. This conformational variability has been invoked to account also for unusual features of the d–d transitions in X-Gly complexes.[6] If it should turn out from X-ray work that the metal ions are displaced from the chelate rings of peptide complexes composed of L-amino acid residues to the same side as the side chains, then positive octant signs for the $n \rightarrow \pi^*$ carbonyl transitions are predicted for the metal ion as perturbant. Negative CD peaks for the 300-mμ transition in cupric–tripeptide complexes recorded in Table IV containing leucyl or phenylalanyl groups in the C-terminal position may reflect extension of these large side chains into negative front octants of the second amide carbonyl groups. Comparisons of values in Tables II and III reveal that contributions to the 310-mμ band are fairly independent, but not as independent as they are in visible CD.[6] For instance, the sum of the $\Delta\epsilon$ values at 310–315 mμ for glycylalanine and leucylglycine in Table II is equal to the $\Delta\epsilon$ value for leucylalanine in Table II. Other comparisons are possible within Table IV.

The aromatic transitions in amino acid side chains show enhancement of CD magnitude upon incorporation of aromatic amino acids into peptides and upon chelate formation. For instance, the secondary (L_b) band near 258 mμ exhibits $\Delta\epsilon = +0.02$ in cationic phenylalanine,[8] −0.2 in the 2:1 cupric chelate, −0.7 in valylphenylalanine, and −2.6 in the cupric chelate of the dipeptide. For the same four substances CD at the primary (L_a) band near 210 mμ gives $\Delta\epsilon = -2.5$, −2.4, +22, and +31, respectively. The primary band of tyrosine near 220 mμ shows $\Delta\epsilon = +2.5$ in the protonated amino acid,[8] −3.3 in the 2:1 cupric chelate, +7 in glycyltyrosine,[9] and +19 in the cupric chelate of the dipeptide. From these and other examples it appears that the greater enhancement occurs on incorporation of the aromatic amino acid residue into a dipeptide, particularly if incorporation is into a non-N-terminal position, and that chelation by cupric ion yields a lesser augmentation. Greater enhancements of CD magnitudes are expected upon incorporation of aromatic amino acids into dipeptides than into the random form of homopolymers because the differential molar residue absorptivity in the homopolymer represents a weighted average value over many aromatic side-chain dispositions. Due to the low values of $\Delta\epsilon$ in the amino acid,

(15) S. J. Leach, G. Némethy, and H. A. Scheraga, *Biopolymers*, 4, 369 (1966).

large enhancement factors frequently occur in the secondary band of phenylalanine and derivatives. Little change in absorption intensities occurs in either primary or secondary bands upon incorporation into peptides or chelation by metal ions. In either free or complexed ligands the dissymmetry factors[16] of $g = \Delta\epsilon/\epsilon$ remain less than 0.01, conforming to the magnetic dipole forbidden nature of the primary and secondary band transitions in benzene. Results of an optical rotatory dispersion study have been interpreted as a major enhancement of optical activity of primary band aromatic transitions upon cupric ion chelation of ligands such as phenylalanine, tyrosine, and glycyltyrosine.[17] This measurement, however, exaggerates optical activity when Cotton effects of opposite sign appear appropriately spaced on the wavelength scale and has missed Cotton effects at longer wavelengths. The augmentations of CD magnitudes observed in aromatic transitions upon incorporation of aromatic amino acid residues into peptides or chelation by cupric ions seems consistent with more restricted rotation in these systems, and no special interactions need be invoked.

(16) S. F. Mason, *Proc. Chem. Soc.*, 137 (1962).

(17) J. E. Coleman, *Biochem. Biophys. Res. Commun.*, **24**, 208 (1966)

Photooxygenation of Phylloquinone and Menaquinones[1]

Clinton D. Snyder[2] and Henry Rapoport

Contribution from the Department of Chemistry, University of California, Berkeley, California 94720. *Received July 29, 1968*

Abstract: Phylloquinone was photolyzed aerobically in cyclohexane to yield a ketonic side-chain fragment, 6,10,14-trimethylpentadecan-2-one, *via* an intermediary hydroperoxide, *trans*-2-methyl-3-(3-hydroperoxy-3,7,11,15-tetramethyl-1-hexadecenyl)-1,4-naphthoquinone. The analogous hydroperoxide from the menaquinone MK-1, *trans*-2-methyl-3-(3-methyl-2-butenyl)-1,4-naphthoquinone, was also studied. Its structure was established by reduction of the hydroperoxide to *trans*-2-methyl-3-(3-hydroxy-3-methyl-1-butenyl)-1,4-naphthoquinone which was synthesized independently. The general photooxygenation reaction proceeded by attack on the quinone of singlet oxygen, which could be generated *via* methylene blue sensitized photolysis, to give the 3′-hydroperoxide. The hydroperoxide then photolytically rearranged to ketone. In the menaquinone series, this reaction is useful as a side-chain degradation procedure, particularly for those alkenyl naphthoquinones in which the second isoprenoid side-chain unit is saturated. When applied to the natural quinone of *Mycobacterium pheli*, MK-9 (II-H), photolysis in cyclohexane yielded a side-chain fragment which confirmed the structure previously assigned to this quinone.

Primary evidence for the involvement of quinone in oxidative phosphorylation has come from experiments in which the native quinone of crude bacterial extracts has been depleted thus permitting reconstitution of these extracts with structurally modified quinone analogs.[3] In this manner, the structural aspects of the naphthoquinone necessary for activity in oxidative phosphorylation have been deduced. In some cases depletion of the quinone has been effected by solvent extraction but for the most relevant studies the method of choice has been ultraviolet irradiation. Restoration of oxidative phosphorylation activity to near original levels by the addition only of exogenous quinone is illustrative of the selectivity of the irradiation method, the other components of the electron transport particle being little affected.

Although the photolability of quinones has been long recognized[4] and exploited, not until recently[5] has the reaction been studied in any way other than in a very qualitative manner. This is somewhat surprising in view of the biological significance of the photoreaction; for example, the light-induced structural modifications which cause the quinone to become inactive could have significance in determining its role in oxidative phosphorylation. Or perhaps the photoproducts could act during oxidative phosphorylation as inhibitors which have to be displaced by the addition of exogenous quinone. Questions like these have moved us to investigate the photolysis of some compounds of the vitamin K series.

Previous work[5a] has shown that exhaustive aerobic photolysis of phylloquinone in benzene solution gave phthiocol in unspecified yield. The presence of this photoproduct is interesting since phthiocol is incapable of restoring oxidative phosphorylation activity as determined with the *M. pheli* system,[3] but perhaps may

(1) Supported in part by Grant AI-04888 from the National Institutes of Health, U. S. Public Health Service.

(2) National Institutes of Health Predoctoral Fellow.

(3) A. F. Brodie, *Federation Proc.*, 20, 995 (1961), and references therein. For reviews concerning the postulated role of naphthoquinone in oxidative phosphorylation see E. Lederer and M. Vilkas, *Vitamins Hormones*, **24**, 409 (1966), and A. F. Brodie in "Biochemistry of Quinones," R. A. Morton, Ed., Academic Press, New York, N. Y., 1965, Chapter 11.

(4) (a) H. J. Almquist, *J. Biol. Chem.*, **117**, 517 (1937); (b) D. T. Ewing, F. S. Tomkins, and O. Kamm, *ibid.*, **147**, 233 (1943); (c) J. P. Green and H. Dam, *Acta Chem. Scand.*, **8**, 1341 (1954).

(5) (a) G. Katsui and M. Ohmae, *J. Vitaminol.*, **12**, 117 (1966); (b) M. Ohmae and G. Katsui, *Vitamins* (Kyoto), **35**, 116 (1967); (c) S. Fugisawa, S. Kawabata, and R. Kamamoto, *J. Pharm. Soc. Japan*, 87, 1451 (1967).