The Infra-Red Absorption Spectra of Some Amino Acid-Metal Chelates at Liquid Air Temperature

ANDREAS ROSENBERG

Institute of Biochemistry, Uppsala, Sweden

A method for obtaining infra-red absorption spectra at low temperatures is described. The infra-red spectra of some amino acid-metal chelates are examined and evidence presented in support of the concept that the carboxyl groups and the metal ions in certain chelates tend to form bonds of different, probably more covalent, character than the usual ionic carboxyl-metal ion linkage.

The use of infra-red (IR) absorption measurements for the investigation of coordination compounds has been scarce. Recently, however, a number of investigations on metal chelate compounds have been published. In these studies changes in IR absorption have been used in order to gain information about the behaviour of chelate forming groups in the molecule and thus even about the nature of the bond with the metal ion. Such spectral changes have also been used as a means of distinguishing between different geometrical isomers of the same chelate. Thus, for example, Bryant, Parriaud and Fernelius [1] have studied the effect of metal ions on the position of the carbonyl frequency of tropolone and its derivatives. They have observed shifts of about 20 cm^{-1} towards lower wavenumbers for the carbonyl frequency after chelate formation. On the other hand differences between shifts caused by various metal ions were negligible. This seems to confirm the conclusion arrived at by Bellamy and Branch [2] from investigations on the metal chelates of diketones. They found that the values for the carbonyl frequency of acetylacetone chelates are essentially the same for all the metals investigated. The shift in carbonyl frequency in this case is probably mostly influenced by an increase in the resonance effects of the p-diketone molecule and not by any difference in the type of linkage with the metal ion. That this kind of behaviour is not general is shown by the same authors with salicylaldehyde. The chelates of this compound with different metal ions do show significant differences in the position of the carbonyl frequency. A slightly different way of applying IR absorption was used by Merrit and Wiberley [3] who showed that differences exist in IR absorption between *cis* and *trans* isomers of Co^{+++} chelates.
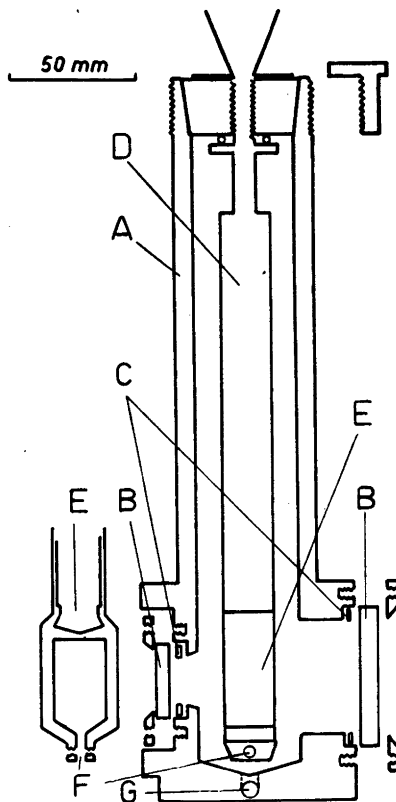
*Fig. 1. Low-temperature cell. For expla-
nations, see the text.*

The aim of the present study was to examine the possibilities of using the IR methods for investigating biochemically interesting metal chelates. The investigation of complicated compounds can only be successful provided that the behaviour of the important chelate forming groups, such as NH_2, has been studied in simple molecules with established configurations. Previously Lenormant [4] has published some IR curves for such compounds and recently Sen et al.[5] have studied Cu^{++} and Ni^{++} chelates of glycine. The present investigation of amino acid-metal chelates was carried out as a necessary introduction to further studies of metal-ion peptide complexes. Pt^{++}, Cu^{++} and Ni^{++} were used since these ions form chelates of the same square-planar type [6].

<center>EXPERIMENTAL</center>

A Perkin-Elmer model 21 spectrophotometer equipped with a CaF_2 prism was used for the IR measurements. The substances, all in the solid state, were mixed with powdered KBr and pressed to transparent discs according to the method developed by Schiedt and Reinwein [7]. Earlier investigations [5] and preliminary experiments made here showed that the changes in the spectra were such that the NaCl prism was not able to give the required resolution. The important regions around 3 and 6 μ showed broad absorption

bands strongly influenced by the presence of the water of crystallization. Even with the use of a CaF$_2$ prism the resolution was not always sufficient. In search for a method giving still better resolution, the low-temperature approach seemed to be most promising. In recent years low-temperature IR spectroscopy has been the subject of some controversy. It is generally accepted that cooling a substance so that it changes its state of aggregation, causes changes in its IR absorption. On the other hand the effects produced by continued cooling of a given crystalline phase are open to discussion. King, Hainer and Mc Mahon [8], for example, have investigated a number of high-molecular-weight substances and conclude that very little is gained by the use of low temperatures. However, work reviewed by Hornig [9] indicate that the low-temperature method is very effective when used for low-molecular-weight substances. The main difficulty encountered in the present work was to obtain definite bands from regions with broad non-specific absorption. Thus even if the results obtained by low-temperature measurements were only caused by an increased transparency giving new peaks in the region of non-specific absorption, the use of low temperatures would be justified. The next step was the construction of a simple low-temperature cell suitable for work with crystalline substances. The main obstacle in low-temperature work with crystals is the heat transfer from the substance to the refrigerant. For this reason the KBr-disc method was utilized. The crystals investigated were mixed with powdered KBr and pressed to rectangular plates which were firmly attached to metal frames. The only problem of heat transport was thus the transfer from the metal frame to the metal reservoir containing liquid air and this was easily solved. Fig. 1 shows a schematic drawing of the cell. The outer case (A) is made of Perspex. The windows (B) are standard Perkin Elmer cell windows made of NaCl which are tightened with rubber washers (C). The metal container (D) for the liquid air is made of brass. Its lower part (E) is formed as a holder for the metal frame of the KBr disc. Good contact between the frame and the holder is obtained by tightening with a screw at (F). The cell is connected to a vacuum pump *via* (G). When the pressure was maintained below 0.5 mm Hg, no fogging of the windows could be detected after approximately 100 runs. The following procedure was used to control the effectiveness of the cell. A copper-constantan thermocouple was embedded in powdered KBr and pressed into the disc. When liquid air was introduced in the container by means of a siphon the temperature sank rapidly reaching an equilibrium value of −170° after approx. 8 minutes and it remained constant as long as the reservoir contained liquid air.

Cu^{++} and Ni^{++} chelates were prepared by mixing aqueous solutions of Na salts of amino acids with solutions of CuCl$_2$ and NiCl$_2$, respectively. The water-soluble chelates were recrystallized from water, the insoluble chelates washed extensively with water and alcohol. The Pt^{++} chelates were prepared from K$_2$PtCl$_4$ and glycine according to Refs [11,12]. The *cis* and *trans* forms were separated by fractional crystallization.

Table 1. Analyses.

Chelate	% N		% Metal	
	Calc.	Found	Calc.	Found
Cu(NH$_2$CH$_2$COO)$_2$H$_2$O	12.20	12.27		
Cu(CH$_3$CHNH$_2$COO)$_2$H$_2$O	10.87	10.95		
Ni(NH$_2$CH$_2$COO)$_2$2H$_2$O	11.54	11.67		
Ni(CH$_3$CHNH$_2$COO)$_2$2H$_2$O	10.34	10.20		
Cu((CH$_3$)$_2$CHCH$_2$CH(NH$_2$)COO)$_2$	8.65	8.50	Cu 19.62	Cu 19.85
Ni((CH$_3$)$_2$CHCH$_2$CH(NH$_2$)COO)$_2$	8.78	8.43	Ni 18.40	Ni 18.76
Pt(NH$_2$CH$_2$COO)$_2$ *cis*	8.16	8.06		
Pt(NH$_2$CH$_2$COO)$_2$ *trans*	8.16	8.20		
KPtCl$_3$(NH$_2$CH$_2$COO)	3.69	3.84		
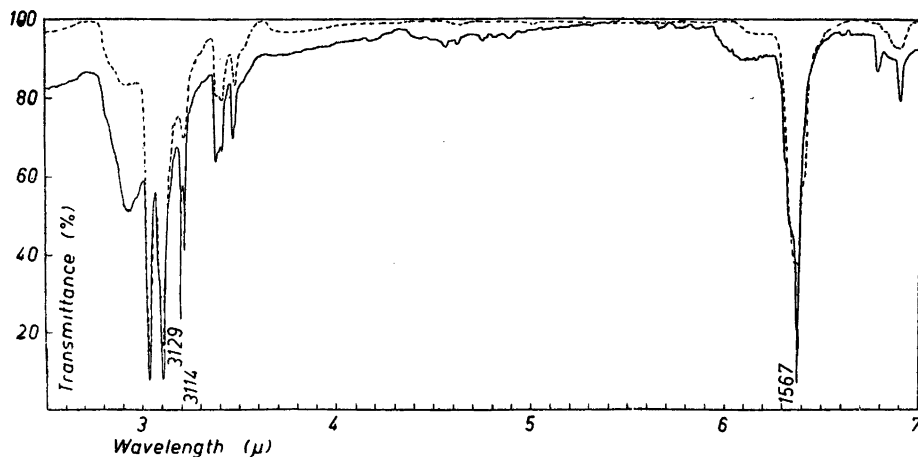
Fig. 2. IR absorption of ethylenediamine Cu^{++} $CuCl_4$.

.... at 25°
——————— at —170°

RESULTS OF THE ABSORPTION MEASUREMENTS

In order to illustrate the effect of low temperature on the IR absorption, the IR spectra of a group of substances were recorded both at room temperature and at —170°. The substances were deliberately chosen among those closely related to the compounds which were to be investigated. The effects obtained vary with different substances. Figs. 2 and 3 show two possibilities
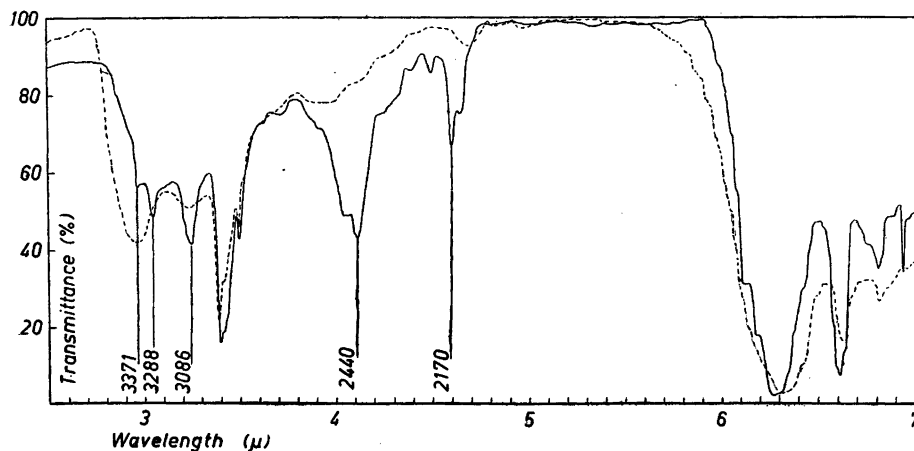

Fig. 3. IR absorption of Na^+ leucine.

.... at 25°
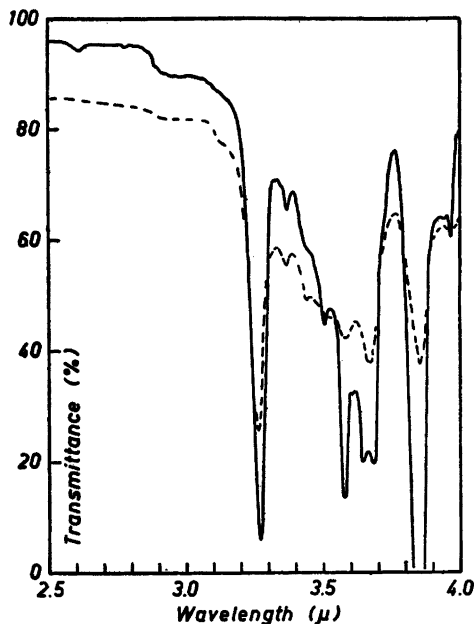——————— at —170°

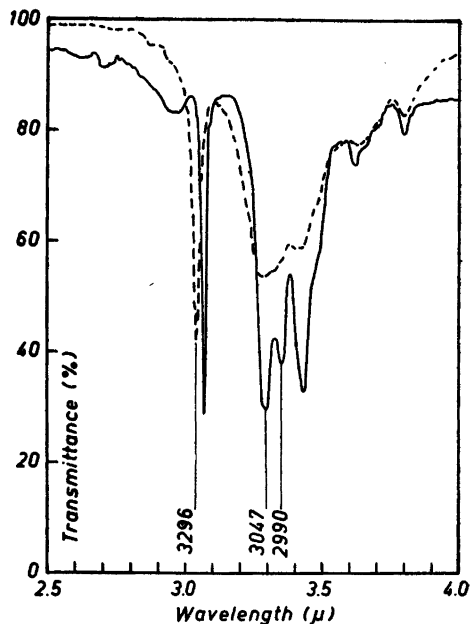Fig. 4. IR absorption of DL-alanine.
·············· at 25°
——— at −170°


Fig. 5. IR absorption of glycylglycine.
·············· at 25°
——— at −170°

of an extreme nature. $EDA_2Cu \cdot CuCl_4$ (Fig. 2), prepared according to Chatta-way and Drew [10], shows a simple and well-defined spectrum already at normal temperatures. The only effects obtained by the low temperature were the splitting of one band into two at 3 129 cm^{-1} (3.196 μ) and 3 114 cm^{-1} (3.211 μ) besides the narrowing of the 1 567 cm^{-1} (6.380 μ) band. In sharp contrast stands the behaviour of Na DL-leucine (Fig. 3) where the effects are consider-able. The NH_2 bands at 3 371 cm^{-1} (2.966 μ) and 3 288 cm^{-1} (3.041 μ) emerge from a broad band of continuous absorption. The bands at 3 086, 2 440 and 2 170 cm^{-1} (3.240, 4.10 and 4.61 μ) are most likely due to water molecules present in the crystals as these bands vanish completely after heating to 120° over P_2O_5. The NH_2 bands remain unperturbed with the exception of a slight shift. That the effects obtained by the low temperature are not entirely due to the presence of water molecules is shown by Figs. 4 and 5, displaying the IR absorption spectra of DL-alanine and glycylglycine from 2.5 μ to 4 μ. In this connection it is of interest to note that while the absorption bands due to the NH_3^+ group of glycylglycine at 3 047 cm^{-1} and 2 990 cm^{-1} (3.282 and 3.344 μ) scarcely show any shifts due to the temperature change, the band at 3 296 cm^{-1} (3.034 μ) ascribed to the NH of the amid linkage shows a shift of approxi-mately 25 cm^{-1} towards lower wavenumbers.

Before further discussion some words ought to be said about the effects due to moisture. As it is difficult to keep KBr powder absolutely dry and as
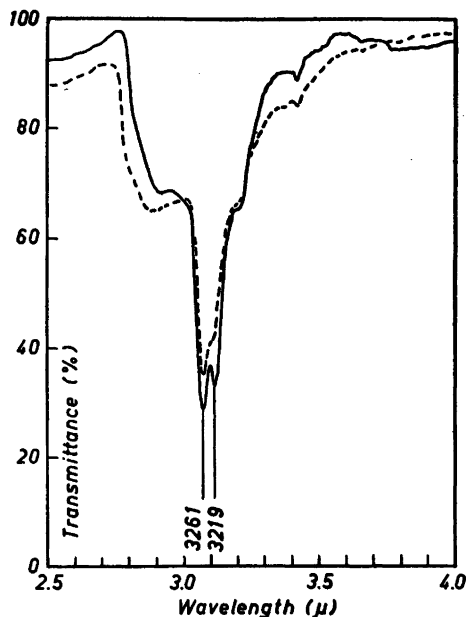
Fig. 6. IR absorption of $KPt^{++}Cl_2$ glycine.

.... at 25°
——————— at −170°
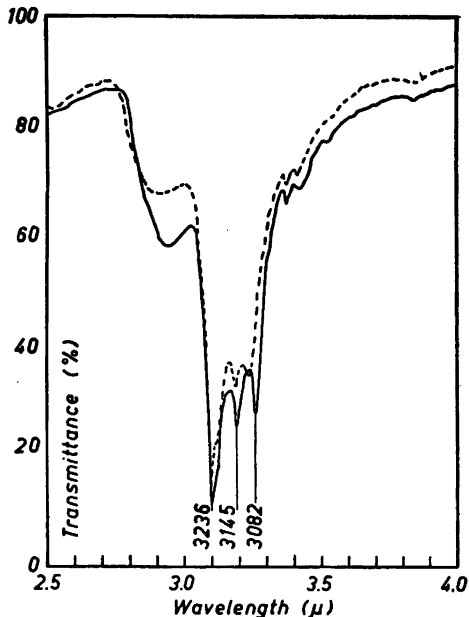
Fig. 7. IR absorption of Pt^{++} glycine$_2$(cis).

.... at 25°
——————— at −170°

during the low-temperature run a certain amount of water vapour condenses on the KBr disc, a relatively weak and broad absorption appears at 2.95 μ (3 400 cm^{-1}). This kind of absorption has very little effect on the sharp and well defined bands of NH_2 and no special effort was made to compensate for it.

In general all the metal-amino acid chelates investigated showed different and highly individual absorptions in the "fingerprint region" (8—15 μ). As the purpose of this investigation was to study the behaviour of NH_2 and COOH groups the discussion is limited to the regions around 3 and 6 μ where well-defined absorption bands of these groups appear. The first question to be answered was if the difference between geometric isomers of the same chelate has any influence on the IR absorption of NH_2 and COOH vibrations. For this purpose the three possible forms of Pt^{++} glycine chelates were prepared according to Grünberg and Pitzyn [11] and Ficken and Ley [12]. Figs. 6—8 show the IR absorption in the 3 μ region of Pt^{++} glycine$_2$cis (I), Pt^{++} glycine$_2$trans (II) and $KPt^{++}Cl_2$-glycine (III).
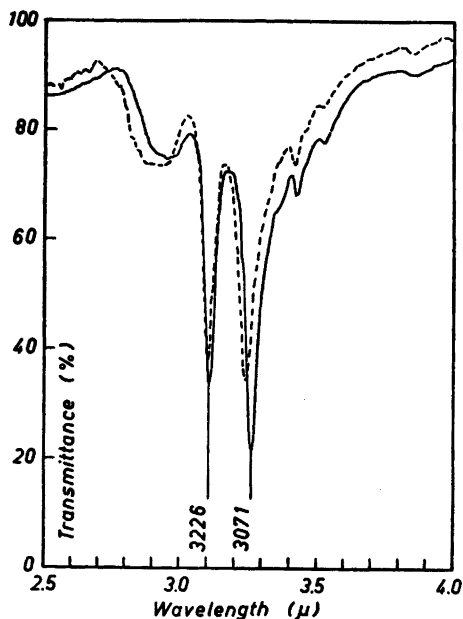

I   II   III
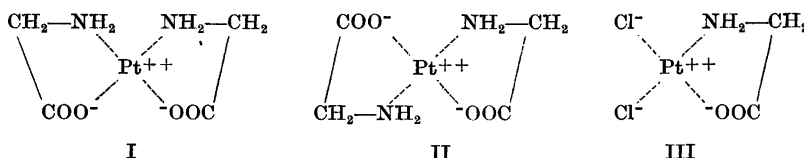
Fig. 8. IR absorption of Pt++ glycine₂
(trans).
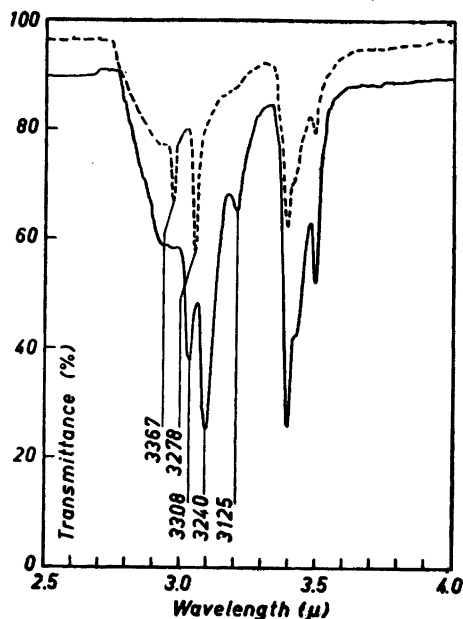............... at 25°
————— at −170°

Fig. 9. IR absorption at −170° of
.... Ni++ leucine₂
————— Cu++ leucine₂

Low-temperature values are given in Table 2 as the absorption curve of monoglycine shows inadequate resolution at normal temperatures.

Table 2.

NH₂ frequencies in wavenumbers (cm⁻¹)

KPt++Cl₂-glycine	3 261	3 219	
Pt++-diglycine *cis*	3 236	3 145	3 082
Pt++-diglycine *trans*	3 226	3 071	

The same kind of differences are present in the 6 μ region. Here both the *mono* and *trans* chelates display a well-defined absorption band of great intensity at 1 655 cm⁻¹ (6.04 μ) and 1 644 cm⁻¹ (6.08 μ), respectively. These bands are naturally not as sharp as those in the NH region so that an uncertainty of ± 5 cm⁻¹ must be taken into account. They can with certainty be ascribed to the carboxyl group. The NH bending mode is found at 1 582 cm⁻¹ (6.321 μ) for the *mono*chelate and at 1 608 cm⁻¹ (6.220 μ) for the *trans* isomer of the dichelate. The *cis* form displays a broad band of overlapping carbonyl frequencies with distinct maxima at 1 681 (5.95 μ) 1 661 cm⁻¹ (6.02 μ) and 1 639 cm⁻¹ (6.10 μ). The region of NH bendings also shows two peaks at 1 610 cm⁻¹ (6.208 μ) and 1 570 cm⁻¹ (6.370 μ), respectively. These perturbations in the

Table 3.

	NH$_2$ frequencies in wavenumbers (cm^{-1})		
Na$^+$ leucine	3 378.	3 289	
Ni^{++} leucine$_2$	3 367	3 278	
Cu^{++} leucine$_2$	3 308	3 240	3 125

spectrum are not due to the presence of water molecules since the spectrum remains unchanged after the substance is dried at 120° over P$_2$O$_5$. The same conclusion can be drawn from the analytical data. The next step was to investigate the influence of different metal ions on the NH$_2$ and COOH frequencies. The presence of water molecules in most of the Cu^{++} and Ni^{++} chelates greatly complicates the spectra. Although these effects can be reduced by drying it seemed advisable to avoid such complications in the beginning. For this reason the salts of DL-leucine with Na$^+$, Ni^{++} and Cu^{++} were compared. Both the Cu^{++} and Ni^{++} chelates of leucine contain no water, the spectrum remaining unchanged after heating over P$_2$O$_5$. Fig. 9 presents the IR absorption of Ni^{++} and Cu^{++} chelates of DL-leucine. The NH$_2$ frequencies of Ni^{++}, Cu^{++} and a dried sample of Na$^+$ leucine are presented in Table 3.
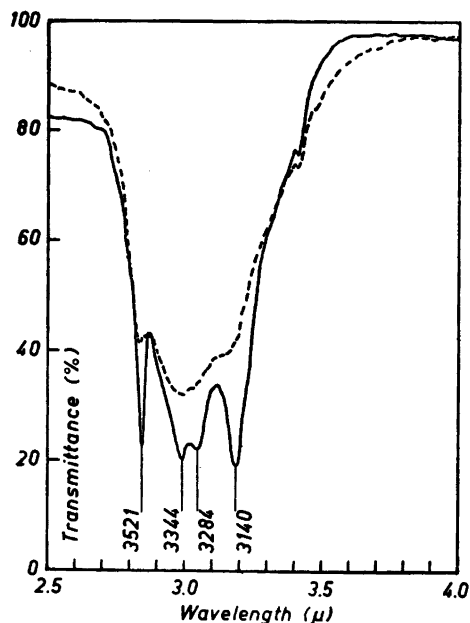
Fig. 10. IR absorption of KMg^{++} nitrilotriacetic acid.

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................. at 25°
——————— at −170°
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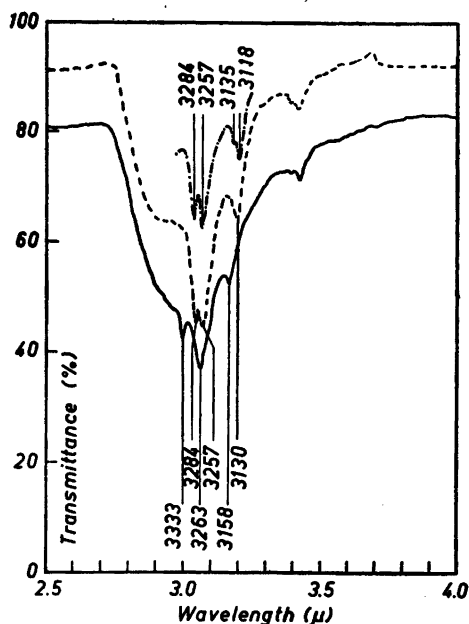

Fig. 11. IR absorption of Cu^{++} glycine$_2$ · H$_2$O ——————— at −170° and Cu^{++} glycine$_2$

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................. at     25°
—·—·—·— · at −170°
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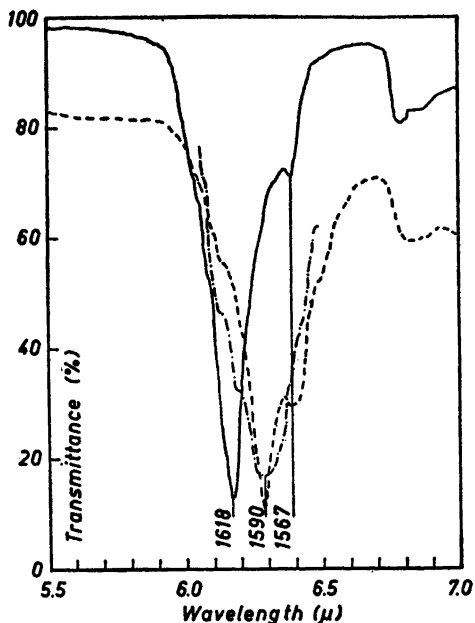
Fig. 12. IR absorption at −170° of

················· Cu++ leucine₂
——————— Ni++ leucine₂
—·——·——· Na+ leucine
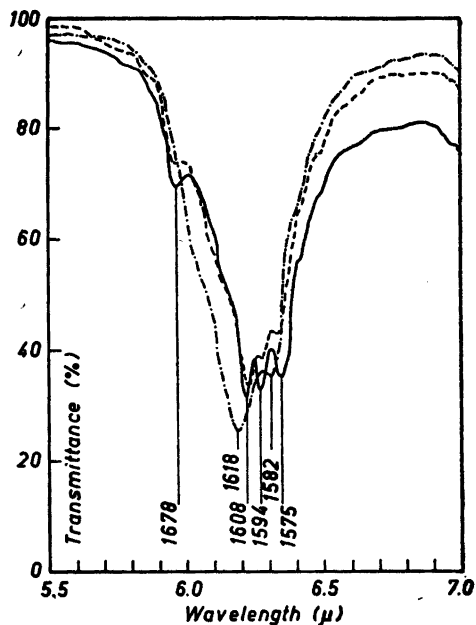
Fig. 13. IR absorption of Cu++ glycine₂
H₂O.

················· at   25°
——————— at −170°
Cu++ glycine₂ —·——·——· at −170°

All the values in Table 3 are low-temperature values. As the low temperature curves of Ni^{++} and Cu^{++} chelates showed no shifts of absorption bands when compared to the curves at normal temperature, Fig. 9 shows only one, the low-temperature curve of each substance. The IR spectra of glycine and alanine chelates in this region are rather complicated due to hydration. That water molecules itself may possess, well defined and sharp bands in the neighbour-hood of 3 μ can conveniently be demonstrated by the IR absorption spectrum of KMg-nitrilotriacetic acid prepared according to Schwarzenbach [13]. This salt of a substituted amino acid has no NH or OH groups of its own. But as it crystallizes with several molecules of water the IR spectrum shown in Fig. 10 has several sharp absorption peaks reminding of the frequencies of NH_2. The different absorption curves of Cu^{++} glycine₂ are shown in Fig. 11. It is dif-ficult to decide without further investigations if the absorption maximum at 3 333 cm⁻¹ (3.000 μ) being NH frequency is shifted to 3 248 cm⁻¹ (3.045 μ) after drying or if this frequency belongs to the water of hydration. The be-haviour of Cu^{++}DL-alanine₂ is analoguous. The spectra of dried substances, however, show the same kind of differences with varying metal ions as leucine chelates. For exampel Ni^{++} alanine₂ has its NH_2 frequencies at 3 361 cm⁻¹ (2.975 μ) and 3 284 cm⁻¹ (3.045 μ) whereas the Cu^{++} chelate of alanine shows
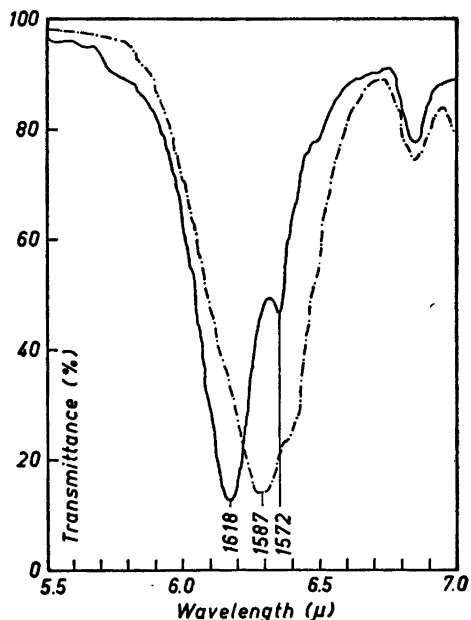
Fig. 14. IR absorption at −170° of Cu⁺⁺ alanine₂ ——— Ni⁺⁺ alanine₂ —·—·—·

two strong bands at 3 263 and 3 231 cm⁻¹ (3.065 and 3.095 μ) with an additional weak band at 3 145 cm⁻¹ (3.180 μ).

The behaviour of the carboxyl frequencies at about 6 μ of different salts of DL-leucine is demonstrated by Fig. 12. The general picture resembles that of NH₂ stretchings. The Ni⁺⁺ chelate shows a frequency practically identical with the frequency of the Na⁺ salt whereas the carboxyl absorption band of the Cu⁺⁺ chelate is shifted towards higher wavenumbers. The water of crystallization in Ni⁺⁺ and Cu⁺⁺ chelates has an effect, even more pronounced than in the 3 μ region. Fig. 13 shows the absorption bands of Cu⁺⁺ glycine₂ before and after drying. The four distinct peaks present yield when the chelate is dried a single intense band at 1 618 cm⁻¹ (6.18 μ) with a shoulder at 1 582 cm⁻¹ (6.32 μ) probably due to the NH bending. These changes are reversible and the original spectrum can be restored through recrystallization from water. These water effects were probably the reason why Sen et al.[5] only observed one broad band between 1 639 and 1 563 cm⁻¹ (6.10 and 6.40 μ) for Cu⁺⁺ and Ni⁺⁺ chelates of glycine. The differences between Ni⁺⁺ and Cu⁺⁺ chelates are further illustrated by Fig. 14 which shows the spectra of dried chelates of DL-alanine. The behaviour of the carboxyl frequencies of Na salts and water-free chelates is summarized in Table 4.

Table 4. The carboxyl frequency in wavenumbers (cm⁻¹).

Na⁺ leucine	1 590	Na⁺ glycine	1 588	Na⁺ alanine	1 590
Ni⁺⁺ leucine₂	1 590	Ni⁺⁺ glycine₂	1 587	Ni⁺⁺ alanine₂	1 587
Cu⁺⁺ leucine₂	1 618	Cu⁺⁺ glycine₂	1 618	Cu⁺⁺ alanine₂	1 618

The Cu^{++} chelates of valine, sarcosine, proline and serine showed the same position of the carboxyl frequency which confirms the conclusion that the shift to 1 618 cm^{-1} seems to be characteristic of Cu^{++} chelates.

The frequencies of NH_2 and COOH were unchanged when L-amino acids were used instead of DL-acids.
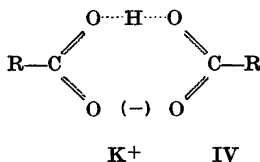
DISCUSSION

When compared to the NH_2 frequencies of Na glycine at 3 400 and 3 360 cm^{-1} (2.941 and 2.975 μ) all the chelates of Pt^{++} show a definite shift towards lower wavenumbers. The slight trend of NH_2 frequencies from *mono* to *trans* form can be explained by the assumption that the *trans* form is most favourable for the formation of a symmetric bond structure. Another feature of interest here is the fact that the *cis* form shows a far more complicated spectrum than the *trans* form. There is more than one plausible explanation for this. In the first place the stretching vibrations of NH_2 groups may be coupled due to a rigid structure and as the *trans* form is more symmetrical some of its vibrations may be IR-inactive. Another possibility is that due to the unsymmetrical position of the chelate forming groups the bonds formed by the two NH_2 groups with the metal ion are not identical. Even a change in the system of hydrogen bonds between NH_2 and the oxygen of the carboxyl group between different molecules of the chelate may render a possible explanation. The existence of such a system of hydrogen bonds has been demonstrated by Stosick[14]. The NH_2 groups of Cu^{++} and Ni^{++} chelates show differences in position of frequencies resembling those of the different forms of Pt^{++} glycine chelates. The NH_2 frequencies of Cu^{++} chelates are found at lower wavenumbers than the frequencies of Ni^{++} chelates. This, together with the fact that Pt^{++} chelates have their NH_2 frequencies at still lower wavenumbers, may indicate that the magnitude of the shift towards lower wavenumbers is dependent on the strength of the covalent linkage between nitrogen and metal ion. The pattern of NH_2 frequencies together with the single and well-defined carboxyl group frequency when compared with the different forms of Pt^{++} glycine, support the conclusion from the X-ray work of Stosick[14] that the Cu^{++} and Ni^{++} chelates with amino acids are the *trans* forms of the coplanar structure. This conclusion is naturally valid only for the dehydrated forms of glycine and alanine chelates. The behaviour of the carboxyl frequency is of special interest. Previous investigators[5] came to the conclusion that the carboxyl group retains its ionic character and does not partake in bond formation with the central metal ion. If this were the case the carboxyl frequency should lie unchanged at approx. 1 590 cm^{-1} as in salts with alkali metals. This is the case with Ni^{++} chelates. Cu^{++} and Pt^{++} chelates, on the other hand, do show a definite shift. The behaviour of the carboxyl frequency with different metals can be summarized for glycine chelates.

Na^+ glycine 1 590 cm^{-1}, Ni^{++} glycine$_2$ 1 590 cm^{-1}, Cu^{++} glycine$_2$ 1 618 cm^{-1}, Pt^{++} glycine$_2$ (*trans*) 1 644 cm^{-1}. This gives a shift of 28 cm^{-1} for the Cu^{++} chelate and of 54 cm^{-1} for the Pt^{++} chelate.

It is suggested that a shift of this magnitude and direction can be correlated with covalent bond formation. A parallel case is that of K acetate and

acetic acid. K acetate and acetic acid form, when present in equimolar amounts a wellknown addition compound. For this investigation the addition compound between chloroacetic acid and K chloroacetate was used as the acid in this case is crystalline and the separation of the addition compound is simple. Pure chloroacetic acid shows a carboxyl frequency at 1 735 cm^{-1} and the K-salt the normal frequency for ionized carboxyl at 1 590 cm^{-1}. The addition compound showed no trace of either of these bands. The only band present in this region was an intense band at 1 645 cm^{-1}. It is doubtful that the structure in question may be represented by a configuration involving a symmetrical H-bond (IV),

$$\text{R—C} \underset{\displaystyle O}{\overset{\displaystyle O\cdots H\cdots O}{\big\langle}} \underset{\displaystyle O}{\overset{\displaystyle}{\big\rangle}} \text{C—R}$$

O···H···O

R—C   C—R

O (−) O

K+   IV

but that the carboxyl groups in this case are connected through covalent bonds with a H atom is very plausible. Thus it seems probable that the shift of the carboxyl group frequency observed with Cu^{++} and Pt^{++} chelates really indicates that the carbonyl group partakes in bond formation with the central metal ion, while in the Ni^{++} chelates it shows no such tendency. This is naturally restricted to the crystalline state as the situation in aqueous solutions may be different due to the fact that the water molecules can to a certain extent replace the carboxyl groups in bond formation with the central ion. Likewise it is clear that the results are valid only for the waterfree chelates as the role played by water molecules is not sufficiently elucidated.

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