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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, ILLINOIS INSTITUTE OF TECHNOLOGY, CHICAGO 16, ILL.]

Infrared Spectra of Aqueous Solutions. IV. Glycine and Glycine Peptides[1]

BY MYO K. KIM AND ARTHUR E. MARTELL

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Infrared spectra of glycine and glycine peptides have been measured at various pD values in aqueous solutions. On the basis of the antisymmetric stretching frequencies of the ionized and un-ionized carboxyl groups, and the frequency changes of the peptide carbonyl groups as a function of solution acidity, the structures of the free ligands are inferred. Ionization constants are determined from the plots of the absorbancy *vs.* $-\log [D^+]$ and are compared with the results of potentiometric studies. All optical measurements have been made at 20° and at an ionic strength adjusted to 1.0 *M* with KCl. Potentiometric titrations have been made at ionic strengths of 1.0 *M* (KCl) and 0.1 *M* (KNO_3).

Introduction

Before undertaking an infrared study of the structures of metal chelates formed from peptides in solution, it is necessary to determine the infrared spectra of the various free ligand species present in solution. As has been shown by previous workers,[2-4] it is possible to detect the cationic, neutral, dipolar, and anionic species of amino acids in aqueous solutions by measurement of the infrared carboxyl group absorption as a function of pD. In this paper the infrared technique is extended to a determination of the structures of glycine peptides in aqueous solution. The dissociation constants obtained from spectral (infrared) measurements will be compared to the dissociation constants determined potentiometrically under the same conditions, so that the microscopic information obtained from infrared spectra can be applied to the extensive potentiometric investigations previously reported in the literature.[5] Values of dissociation constants in D_2O will also be available for comparison with the corresponding values previously obtained in H_2O.

Experimental

Spectral Measurements.—Infrared spectra were obtained with a Perkin–Elmer Model 21 spectrophotometer fitted with sodium chloride optics. Silver chloride absorption cells of 0.0165 mm. thickness were employed. The concentrations of ligand solutions were 0.2–0.6 *M* in 99.8% D_2O as solvent, purchased from Bio-Rad Lab., Richmond, Calif. The wave numbers reported are accurate to ±3 $cm.^{-1}$. The ionic strengths of all the solutions were adjusted to 1 *M* by adding reagent grade potassium chloride which was shown to have no absorption in the range of frequencies investigated. Deuterium ion concentrations were measured with a Beckman model G pH meter fitted with extension glass and calomel electrodes of the special "one-drop" type and calibrated at 20° in the same way as is described below for potentiometric measurements. Calibration of the D_2O–pH meter electrode system was made by titration of a strong acid (DCl) and a strong base (NaOD), as well as a weak acid (acetic acid, $DC_2H_3O_2$) under the conditions employed for the infrared measurements. The DCl solution employed to adjust pD in D_2O was obtained from concentrated hydrochloric acid by dilution with D_2O, and the sodium deuteroxide (NaOD) solution was prepared by dissolving sodium metal in D_2O. Two sample solutions of the same ligand concentration and the same ionic strength, but of different pD (one very high, the other very low), were mixed to prepare samples having the desired pD values.

Potentiometric Measurements.—The method employed is the same as reported by others.[6,7] Measurements were made under three different reaction conditions: a, 0.1 *M* ionic strength adjusted with KNO_3 at 25°; b, 1.0 *M* ionic strength adjusted with KCl at 25°; and c, 1.0 *M* ionic strength adjusted with KCl at 20° in D_2O solvent. The results obtained under these conditions were compared with the results of the spectral studies. The concentration of the ligands were 0.001–0.0015 *M*.

Compounds.—Glycine was purchased from the Fisher Scientific Co., Fair Lawn, N. J.; glycylglycine was obtained from California Biochemical Research, Los Angeles, Calif.; triglycine and pentaglycine were purchased from Mann Research Lab., New York, N. Y.; and tetraglycine was obtained from the Nutritional Biochemicals Corp., Cleveland, Ohio. Glycine, glycylglycine, and tetraglycine were recrystallized two times from aqueous alcohol solutions. Tetraglycine was analyzed by the Micro-Tech Lab., Skokie, Ill. Other compounds were used without recrystallization. Before the solutions were prepared, the compounds were dried carefully at 110° and cooled in a desiccator over P_2O_5. The triglycine employed was specified to be chromatographically pure grade.

Results

Glycine.—The infrared spectra of aqueous glycine at four deuterium ion concentrations are given in Fig. 1. The frequencies of the absorption maxima correspond-

TABLE I

ANTISYMMETRIC CARBOXYL AND PEPTIDE CARBONYL BANDS OF GLYCINE AND GLYCINE PEPTIDES AS A FUNCTION OF pD

Ligand		pD	Observed bands, $cm.^{-1}$				Probable species
			COOH	(N^+)–COO^{-a}	COO^{-a}		
G	A	1.54	1732vs	1617w	....		I and IIa
	B	4.84	....	1617vs	....		IIa
	C	9.74	....	1618vs	1580m		IIa and III
	D	10.61	....	1616m	1580vs		IIa and III
			COOH	(N^+)–CO^b	(N)–CO^b	COO^-	
GG	A	1.75	1720m	1675vs	....	....	I
	B	4.31	1720vw	1675vs	....	1595vs	I and IIa
	C	8.77	....	1665m	1630w	1595vs	IIa and III
	D	10.29	....	....	1632m	1595vs	III
GGG	A	1.31	1723m	1678vs	1656s	....	I
	B	3.77	1724w	1678vs	1653s	1597s	I and IIa
	C	5.67	....	1678vs	1648s	1597vs	IIa and III
	D	10.16	....	....	1644vs	1597vs	III
GGGG	A	1.20	1722m	1675[c]	1658vs[c]	....	I
	B	3.68	1725w	1670[c]	1650vs[c]	1597m	I and IIa
	C	6.70	....	1673[c]	1648vs[c]	1596vs	IIa and III
	D	12.74	....	....	1643vs	1597vs	III

[a] (N^+)–COO^- indicates the band due to dipolar structure while COO^- indicates the band due to anionic structure. [b] Peptide carbonyl group; (N^+)–CO represents carbonyl band with positive α-nitrogen atom, (N)–CO represents carbonyl band with neutral α-nitrogen atom. [c] Broad band observed since constituent bands not resolvable.

(1) This investigation was supported by research grant AM-06019-0152 from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

(2) K. Nakamoto, Y. Morimoto, and A. E. Martell, *J. Am. Chem. Soc.*, **83**, 4528 (1961).

(3) K. Nakamoto, Y. Morimoto, and A. E. Martell, *ibid.*, **84**, 2081 (1962).

(4) Y. Morimoto, A. E. Martell, and K. Nakamoto, *ibid.*, **85**, 309 (1963).

(5) See "Stability Constants, Part I, Organic Ligands," J. Bjerrum, G. Schwarzenbach, and L. G. Sillén, Ed., The Chemical Society, London, 1957.

(6) M. M. Taqui Khan and A. E. Martell, *J. Phys. Chem.*, **66**, 10 (1962).

(7) C. Richard, R. L. Gustafson, and A. E. Martell, *J. Am. Chem. Soc.*, **81**, 1033 (1959).

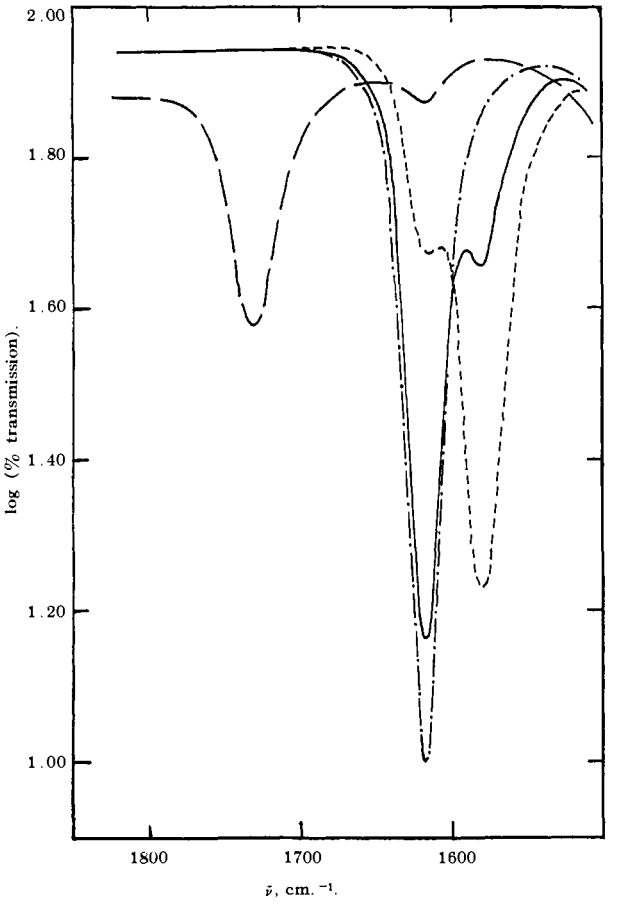


Fig. 1.—Infrared absorption spectra of glycine in D_2O solution at 0.657 *M* concentration and ionic strength 1.0, adjusted with KCl: — — —, pD 1.54; .—.—.—, pD 4.84; ———, pD 9.74; - - - - - - - - - -, pD 10.61.

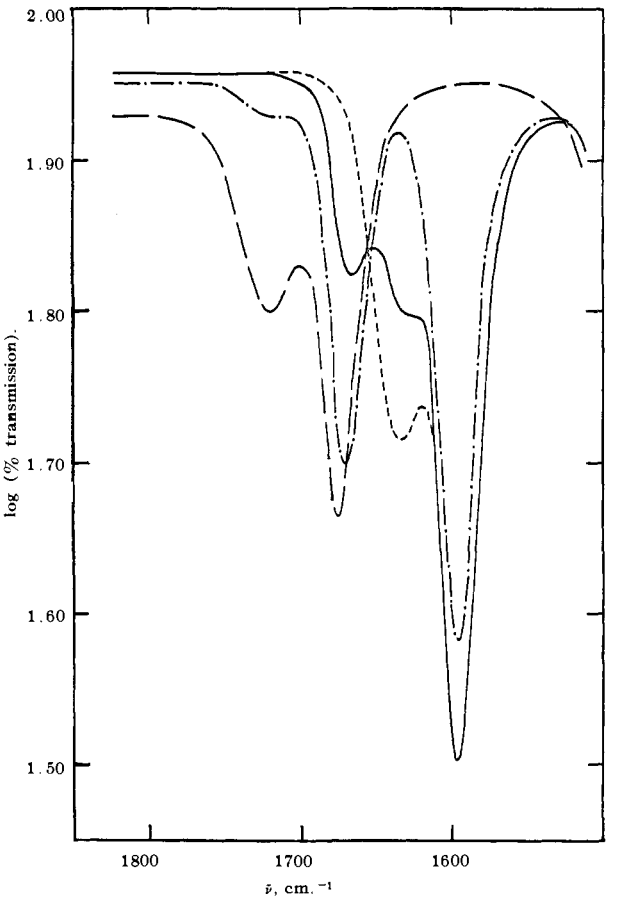


Fig. 2.—Infrared absorption spectra of glycylglycine in D_2O solution at 0.288 *M* concentration and ionic strength 1.0, adjusted with KCl; — — —, pD 1.75; .—.—.—, pD 4.31; ———, pD 8.77; - - - - - - - -, pD 10.29.

ing to the various forms of glycine present in solution are listed in Table I. At low pD the only important absorption band is found at 1732 cm.$^{-1}$, corresponding to the fully protonated structure $H_3N^+CH_2COOH$, although a small amount of dipolar ion is seen to be present, as indicated by the weak band at 1617 cm.$^{-1}$. When the pD is increased to 4.84 this band becomes very strong while the higher frequency band disappears completely. As the pD is further increased, the 1617 cm.$^{-1}$ band decreases in intensity, while a new band at 1580 cm.$^{-1}$ corresponding to the completely dissociated ligand appears and becomes very strong at very high pD. Since glycine has a low pK_1 value and a high value of pK_2 compared to the peptides, the 1617 cm.$^{-1}$ band appears in a rather low pD region, while the 1580 cm.$^{-1}$ band does not begin to appear until high pD is attained.

The dissociation constants obtained from a plot of absorbancies of these bands *vs.* $-\log [D^+]$ are listed in Table II.

Polyglycines.—The aqueous infrared spectra of di-, tri-, and tetraglycine are shown in Fig. 2–4, respectively, and the frequencies of the absorption maxima are given in Table I. Good aqueous infrared spectra were not obtained for pentaglycine because of its relative insolubility in water. Similarly, the potentiometric data (Table II) obtained for pentaglycine was not as reliable as that taken from the lo er analogs, since the solution prepared for potentiometric titration, obtained by dissolving the ligand in excess base, was not absolutely clear, probably because of the presence of trace high molecular weight impurities. The infrared spectrum of pentaglycine, measured in KBr medium, was found to be very similar to the spectrum of tetraglycine taken under similar conditions.

For all of the polyglycines illustrated in Fig. 2–4 the low pD band characteristic of the un-ionized carboxyl group, ~1720 cm.$^{-1}$, is readily apparent. Also,

TABLE II

DISSOCIATION CONSTANTS OF GLYCINE AND GLYCINE PEPTIDES

Ligand	Method	Temp., °C.	Ionic strength	Constants pK_1	Constants pK_2
G	gl[d]	20–30	0.09 KCl	2.33[a]	9.44[b]
	gl	20	1.0 (KCl)(D_2O)	2.81	10.14
	IR[e]	20	1.0 (KCl)(D_2O)	2.8	10.2
GG	gl	25.6	0.1 KNO_3	3.18	8.04
	gl	24.9	1.0 KCl	3.21	8.12
	gl	20	1.0 (KCl)(D_2O)	3.55	8.72
	IR	20	1.0 (KCl)(D_2O)	3.6	8.8
GGG	gl	25.6	0.1 KNO_3	3.38	7.97
	gl	24.9	1.0 KCl	3.27	8.00
	gl	20	1.0 (KCl)(D_2O)	3.48	8.56
	IR	20	1.0 (KCl)(D_2O)	3.8	8.7
GGGG	gl	25.6	0.1 KNO_3	3.39	7.81
	gl	24.9	1.0 KCl	3.26	7.84
	gl	20	1.0 (KCl)(D_2O)	3.71	8.51
	IR	20	1.0 (KCl)(D_2O)	3.8	8.5
GGGGG	gl	24.9	1.0 KCl	(3.05)[c]	(7.86)[c]

[a] S. Glasstone and E. F. Hammel, Jr., *J. Am. Chem. Soc.*, **63**, 243 (1941), at 20°; ionic strength not given. [b] C. B. Murphy and A. E. Martell, *J. Biol Chem.*, **37**, 226 (1957), 30°, μ = 0.09 (KCl). [c] Pentaglycine is not soluble in water. Solution in excess sodium hydroxide, slightly turbid, used for titration. [d] gl = potentiometric method with glass electrode. [e] IR = aqueous infrared measurements.

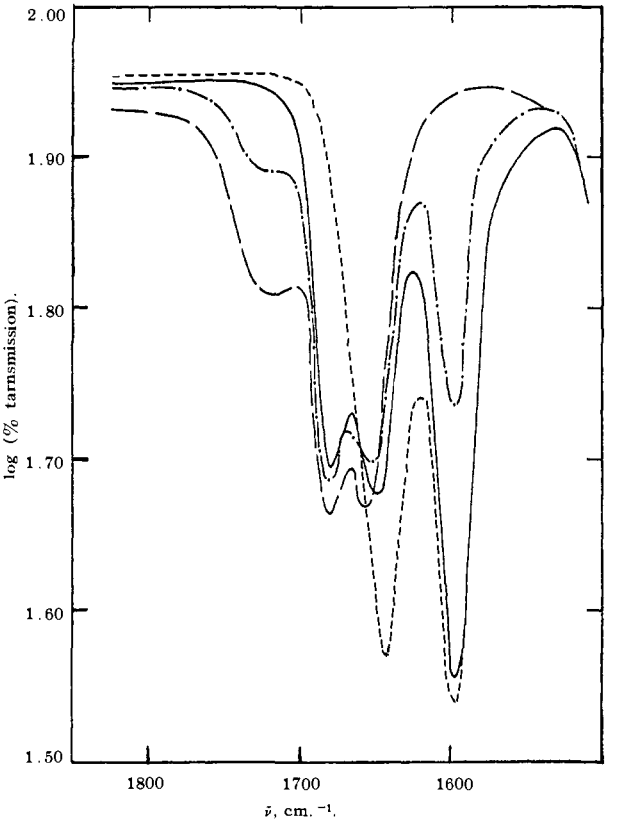


Fig. 3.—Infrared spectra of glycylglycylglycine in D_2O solution at 0.265 M concentration and ionic strength 1.0 adjusted with KCl: — — —, pD 1.31; .—.—.—, pD 3.77; ———, pD 5.67; - - - - - -, pD 10.16.

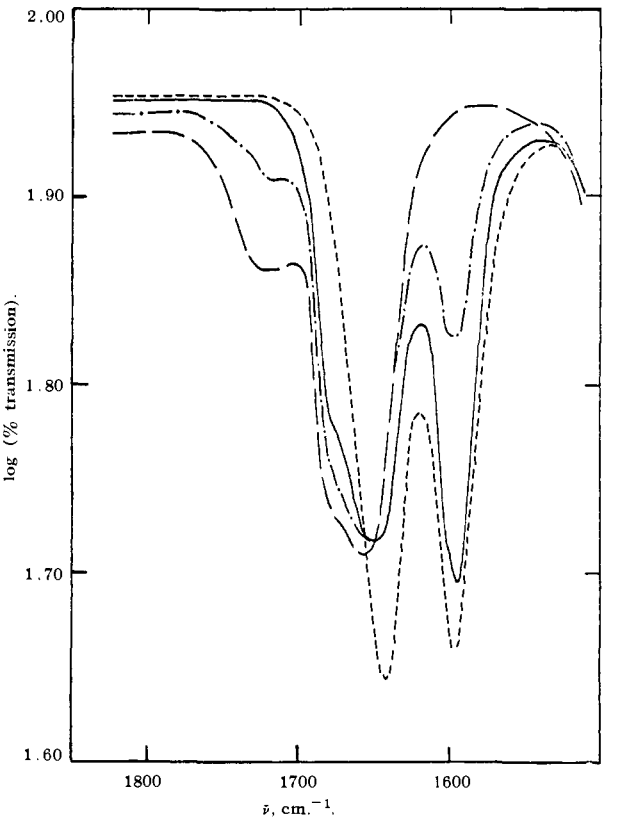


Fig. 4.—Infrared spectra of glycylglycylglycylglycine in D_2O solution at 0.157 M concentration and ionic strength 1.0, adjusted with KCl: — — —, pD 1.20; .—.—.—, pD 3.68; ———, pD 6.70; - - - - - -, pD 12.74.

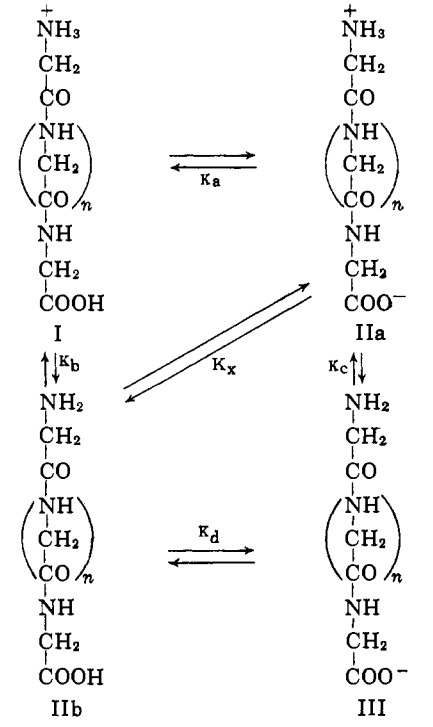
at high pD, the intense absorption of the free carboxyl group at ~1596 cm.$^{-1}$ also appears for all three peptides. However, instead of the absorption of the carboxylate group specific for the dipolar ion, which was found for glycine and other α-amino acids, amide carbonyl bands are found for the peptides.

As is indicated in Table I, the higher frequency peptide carbonyl band at ~1675 cm.$^{-1}$ is assigned to the carbonyl group adjacent to the terminal ammonium group. The inductive effect of this group is responsible for the shift of the carbonyl absorption to higher frequency.

The amide carbonyl absorptions of tri- and tetraglycine differ from that of diglycine at low pD in that the higher polyglycines have both peptide carbonyl absorptions at 1670–1678 and 1650–1658 cm.$^{-1}$, whereas only the higher frequency band appears in the diglycine spectrum. This is what one would expect if an inductive effect is responsible for the observed band shifts, since triglycine and higher peptides have additional peptide carbonyl groups farther removed from the terminal positive ammonium group than is the peptide carbonyl of diglycine. It is interesting to note, however, that the lower frequency band in the tri- and tetrapeptide at low pD is still considerably higher than the high pD peptide carbonyl absorption of diglycine. It appears, therefore, that the inductive influence of the remote positive ammonium groups of tri- and tetraglycine is still considerable, two peptide linkages away. This conclusion is strengthened by the fact that at high pD where the ammonium group of triglycine is converted to a neutral amino group the frequency of the peptide carbonyl group decreased by about 10 cm.$^{-1}$. This effect is also seen in the spectrum of tetraglycine as the pD changes from slightly acid to strongly alkaline. A comparison of the low pD spectra of the three peptides indicates increasing complexity of bands as the number of peptide carbonyl groups increases. The higher frequency peptide carbonyl bands at 1670–1678 cm.$^{-1}$ disappear and the lower

PLATE I

SOLUTION EQUILIBIRA OF GLYCINE PEPTIDES



n = 0, glycylglycine (GG); n = 1, triglycine (GGG)
n = 2, tetraglycine (GGGG); n = 3, pentaglycine (GGGGG)

frequency peptide carbonyl bands at 1640–1650 cm.$^{-1}$ further increase in intensity as pD is increased to very high values. At the highest measured pD, however, only a single peptide carbonyl band is observed regardless of the number of peptide groups present. The observed complexity, therefore, is due to the fact that each peptide group is a different distance from the positive ammonium group and is influenced by its inductive effect to different degrees, thus giving rise to absorption bands of different frequency. It is therefore seen that the greater the number of peptide linkages, the larger is the number of low pD peptide carbonyl bands, resulting in an increasingly complex spectrum in this region.

The pK values for the over-all dissociation reactions were determined from plots of the type shown in Fig. 5 for triglycine. Similar plots were obtained for the other peptides. It is noted that the values obtained from two different absorption bands agree quite well for each dissociation step. The dissociation constants obtained from both spectral data and potentiometric measurement are listed in Table II.

Discussion

It is interesting to observe that since the nature of the terminal amino group in the peptide species indicated in Plate I is reflected by the frequency of the peptide carbonyl, it is possible to identify each of the individual peptide species present in solution. At low and neutral pH the positive charge of the terminal amino group may be detected by the frequency of the adjacent peptide carbonyl group. This type of evidence, coupled with the solution conditions, would be useful in determining if a metal ion is coordinated to the terminal amino group. Similarly, the displacement of a proton from the peptide nitrogen atom by a metal ion would be expected to produce a large shift of the peptide carbonyl absorption to lower frequency. Effects of this type should be useful in the determination of the specific sites on the peptide molecule to which the metal ion becomes coordinated.

The variation of the relative intensities of ionized and un-ionized carboxyl absorptions with pH provide an interesting comparison of the zwitterion formation tendencies of the polyglycines. When one goes from glycine to tetraglycine, the carboxyl group is seen to persist to an increasing extent as the pH is increased in the acid range, whereas the carboxylate bands become less intense in the pH range just before they acquire maximum intensity. An analysis of this effect will be discussed in a future publication.

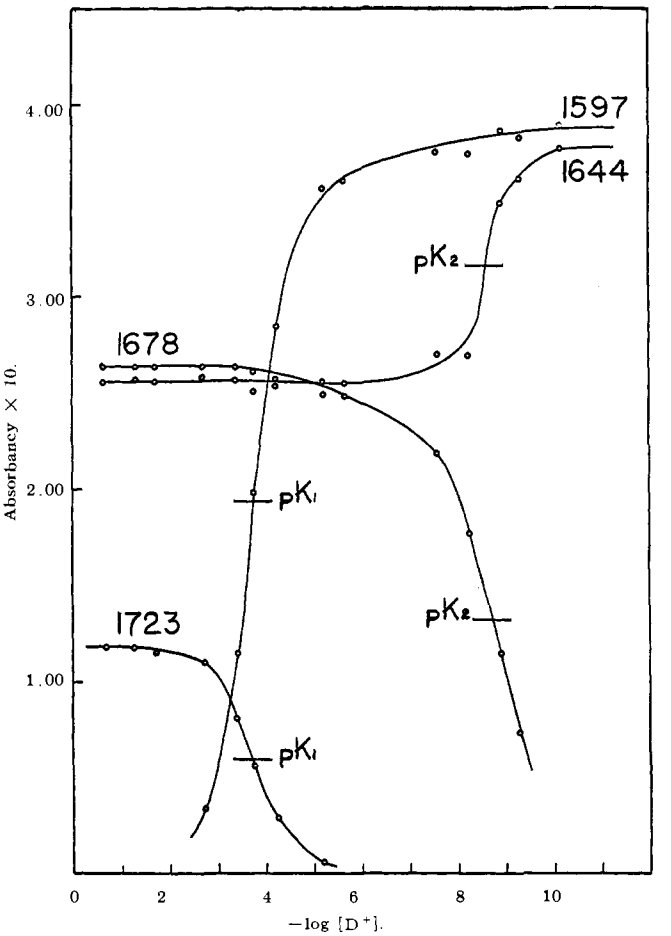


Fig. 5.—Variation of absorbancy of glycylglycylglycine in D_2O as a function of $-\log [D^+]$; frequency of absorption maxima indicated on curves.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, AND THE INORGANIC MATERIALS RESEARCH DIVISION OF THE LAWRENCE RADIATION LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY 4, CALIF.]

The Symmetrical Deformation Frequencies of Methyl, Silyl, and Germyl Groups

BY WILLIAM L. JOLLY

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The symmetrical deformation frequencies of the MH_3 groups in compounds of the type H_3M–X (where M = Si or Ge, and where X may be bound to other atoms) increase with increasing electronegativity of the atom X. These correlations and the similar correlation for methyl groups may be explained in terms of the repulsions between the M–H bonding electrons and the electrons in the valence orbitals of the X atom (in particular, the M–X bonding electrons and any nonbonding electrons on the X atom).

Introduction

It has been pointed out[1,2] that the symmetrical deformation frequency of a CH_3–X group may be correlated with the electronegativity of the atom X (which may be bound to other atoms). A plot of the symmetrical deformation frequency against the electronegativity of X yields a series of parallel straight lines, each line corresponding to X atoms from a particular horizontal row of the periodic table.[1] In order to test the generality of this type of correlation and to aid in the identification of substituted silanes and germanes, we have examined the existing infrared spectral data on silyl and germyl compounds.

Correlations with Electronegativity

The symmetrical deformation frequencies for silyl compounds and germyl compounds are presented in Tables I and II, respectively. Most of the frequency assignments are taken from the literature; those few cases in which we have made the assignments are marked in the tables. In making assignments, we

(1) N. Sheppard, *Trans. Faraday Soc.*, **51**, 1465 (1955).
(2) L. J. Bellamy and R. L. Williams, *J. Chem. Soc.*, 2753 (1956).