UDC 543.422

INFRARED SPECTRA OF AMINO ACIDS AND PEPTIDES

I. Determination of Ionization Constants

I. G. Orlov, V. S. Markin, Yu. V. Moiseev, and U. I. Khurgin

Khimiya Prirodnykh Soedinenii, Vol. 3, No. 2, pp. 197-201, 1967

As is well known, in aqueous solutions amino acids are present in the dipolar form. To determine the ionization constants of the carboxy and amine groups, potentiometric titration and IR spectroscopy are generally used [1, 2]. The second method has certain advantages over the first. Thus, if the molecule contains several groups capable of ionization, the IR spectra enable us to establish reliably just which group is associated with a given ionization constant, while when using the titration curve this can be done only by means of supplementary investigations. The IR spectroscopy method also makes it possible to investigate the mutual inductive influence of the amine and carboxy groups. The object of our work was to find the ionization constants of glycine, glycylglycine, DL-α-alanine, and β-alanine from their IR spectra and to study the inductive influence of the $-COO^-$ and $-NH_3^+$ groups on one another with an increase in the number of methylene groups between them.

Table 1

Molar Absorption Coefficients of some Bands in the IR Spectra of Aqueous Solutions of Glycine at Various pD, $\varepsilon \times 10^{-3}$ ($l \cdot mole^{-1} \cdot cm^{-1}$)

pD	1733 cm^{-1} (νC=O)	1620 cm^{-1} ($\nu_{as}COO^-$)	1580 cm^{-1} ($\nu_{as}COO^-$)	1418 cm^{-1} (ν_s COO^-)
1.22	0.40	0.10	—	0.20
2.11	0.30	0.30	—	0.20
2.71	0.20	0.80	—	0.40
3.11	0.11	1.10	—	0.50
5.11	—	1.50	—	0.60
8.31	—	1.50	—	0.60
8.99	—	1.40	0.10	0.55
9.31	—	1.20	0.20	0.50
9.42	—	1.10	0.40	0.50
9.90	—	0.90	0.60	0.50
10.10	—	0.60	0.90	0.50
10.20	—	0.50	0.80	0.40
10.60	—	0.35	0.90	0.40
13.70	—	—	1.10	0 40
14.20	—	—	1.20	0.30

Experimental

The IR absorption spectra of aqueous solutions of glycine, DL-α-alanine, β-alanine and glycylglycine were taken on a IKS-14 spectrometer in thermostated cells with germanium or fluorite windows at 25 ± 0.1° C. The solvents used were D_2O and solutions of KOD and D_2SO_4 prepared by a previously published method [3] (the use of D_2O, KOD, and D_2SO_4 excluded the influence of solvents on the spectra of the substances studied in the 1400-2000 cm^{-1} region). The concentrations of the substances in the solutions was 0.5-1.0 mole/l. The thickness of the absorbing layer was varied within the range 10-20 μ ± 0.25 μ. The positions of the absorption bands were determined with an accuracy of ±3 cm^{-1} and the peak intensities were used for calculating the ionization constants.

Data on the potentiometric titration and the pH of the solutions taken for investigating the IR spectra were obtained on a TTT-1A autotitrator at 25 ±0.1° C. Glass electrodes of type V were used. The values of pD were calculated from the equation pD = 0.4 + the measured value [5].

Table 1 gives values for the absorption of glycine and also their assignment to various types of vibrations [4]. In D_2O, glycine has strong absorption bands at 1620 and 1418 cm^{-1}, the first of which relates to the antisymmetric and the second to the symmetric stretching vibrations of the $-COO^-$ group in the dipolar ion. On passing to acid solutions, the intensities of these bands decrease while simultaneously a band appears at 1733 cm^{-1} corresponding to the absorption of the C=O bond of the unionized carboxy group −COOH. In alkaline solutions, the band at 1620 cm^{-1} disappears and in its place arises a band with a frequency of 1580 cm^{-1} corresponding to the absorption of the carboxylate group

$-COO^-$. An analogous picture was obtained for DL-α-alanine (Table 2).

Table 3 gives the results for β-alanine. The behavior of the bands in an acid medium is similar to the changes observed for glycine and DL-α-alanine but it differs substantially in an alkaline medium. On passing from a neutral to an alkaline medium, the band at 1570 cm^{-1}, relating to the ($-COO^-$) group, shifts in the low-frequency direction by 20 cm^{-1}, while its intensity changes only slightly.

Table 2

Molar Absorption Coefficients of some Bands in the IR Spectra of Aqueous Solutions of DL-α-alanine at Various pD, $\varepsilon \cdot 10^{-3}$ ($l \cdot mole^{-1} \cdot cm^{-1}$)

pD	1723 cm^{-1} (νC=O)	1610 cm^{-1} (ν_{as} COO^-)	1570 cm^{-1} (νCOO^-)	1418 cm^{-1} (ν_s COO^-)
0.75	0.77	0.06	—	0.32
1.80	0.67	0.18	—	0.27
2.40	0.58	0.49	—	0.49
2.70	0.48	0.95	—	0.44
3.30	0.16	1.50	—	0.53
7.50	—	1.90	—	0.66
8.00	—	2.00	—	0.63
9.00	—	1.98	0.20	0.63
9.50	—	1.80	0.42	0.65
9.80	—	1.71	0.59	0.68
10.20	—	1.48	0.79	0.61
10.60	—	1.10	1.21	0.71
13.00	—	—	2.00	0.60

The absorption bands of glycylglycine in D_2O and in solutions of KOD and D_2SO_4 at various pD values are given in Table 4. We have made a possible assignment of these bands based on literature data [2, 6] and on a comparison of the above spectra with those of compounds of similar structure: N-methylacetamide and glycylglycinamide. The change in the absorption bands of glycylglycine in the pD range from 0 to 14 is as follows: the band at 1597 cm^{-1} which we assign to the stretching vibrations of the ($-COO^-$) group in the dipolar ion does not change its position and intensity on passing from D_2O to alkaline solutions. Similarly, the band at 1677 cm^{-1}, relating to the absorption of the carbonyl group ($-C=O$) of the peptide linkage in glycylglycine remains unchanged on passing to acid solutions.

Table 3

Molar Absorption Coefficients of Some Bands in the IR Spectra of Aqueous Solutions of β-Alanine at Various pD, $\varepsilon \cdot 10^{-3}$ ($l \cdot mole^{-1} \cdot cm^{-1}$)

pD	1710 cm^{-1} (νC=O)	1583 cm^{-1} * (ν_{as} COO^-)	1410 cm^{-1} (ν_s COO^-)
0.30	0.51	—	0.37
1.00	0.56	—	0.37
1.25	0.50	—	0.37
2.30	0.54	0.10	0.40
3.00	0.40	0.26	0.47
3.80	0.03	0.88	0.60
5.20	0.06	1.48	0.55
6.80	—	1.63	0.55
9.00	—	1.61	0.72
9.50	—	1.60	0.73
10.20	—	1.57	0.85
10.40	—	1.62	0.58
14.00	—	1.60	0.70

*In the pH range from 9.00 to 14.00, the absorption band shifts from 1583 to 1563 cm^{-1}.

Tables 1–4 give information on the change in the extinction coefficients of the main absorption bands of glycine, DL-α-alanine, β-alanine, and glycylglycine. In the case of glycine and DL-α-alanine, the band assigned to the stretching vibrations of the ($-COO^-$)group in the dipolar ion changes its position and intensity on passing either to alkaline or to acid solutions. For β-alanine and glycylglycine, the analogous band changes with an increase in the acidity of the medium but remains practically unchanged in alkaline solutions. Such an effect can be explained as follows. On passing from the dipolar form to the anionic form, a proton is detached from the amine group which, however, has little effect on the absorption band of the carboxy group since, in the case of β-alanine, it is separated from the amine group by two methylene groups, which considerably weaken the mutual influence of the carboxy and amine groups. For γ-aminobutyric acid, the absorption band of the carboxylate group at 1555 cm^{-1} does not shift at all on passing to an alkaline solution. Consequently, the induction effect dies out completely along a chain of three methylene groups. For the same reason, in the case of glycylglycine the addition of a proton to the carboxy group in an acid medium has practically no effect on the position of the absorption band of the carbonyl of the peptide group. Conversely, the closeness of the carbonyl of the peptide group and the $-NH_3^+$ group leads to a shift of the absorption band at 1677 cm^{-1} in an alkaline medium. Thus, the mutual influence of an amine and a carboxy (carbonyl) group is considerably weakened with an increase in the chain of intermediate atoms.

Table 4

Molar Absorption Coefficients of Some Bands in the IR Spectra of Aqueous Solutions of Glycylglycine at Various Values of pD, $\varepsilon \times 10^{-3}$ ($l \cdot mole^{-1} \cdot cm^{-1}$)

pD	1730 cm^{-1} (νC=O)	1677 cm^{-1} (νC=O)	1635 cm^{-1} (νC=O)	1597 cm^{-1} (ν_{as} COO^-)
0.00	0.35	0.60	—	0.20
1.27	0.35	0.60	—	0.40
2.77	0.25	0.60	—	0.50
3.11	0.20	0.60	—	0.90
3.41	0.10	0.50	—	1.20
3.81	0.05	0.70	—	1.20
6.02	—	0.70	—	1.20
7 30	—	0.70	—	1.20
7.84	—	0.50	—	1.20
8.00	—	0.45	—	1.20
8.32	—	0.40	0.20	1.20
8.40	—	0.35	0.20	1.20
8.91	—	0.15	0.30	1.20
10.00	—	—	0.50	1.20
13.60	—	—	0.50	1.20
14.20	—	—	0.45	1.20

From the results obtained it has proved possible to determine the pK value of the compounds considered. We have assumed that the change in the absorption band with a change in the medium is due to a displacement of the protolytic equilibrium in the direction of the cationic or the anionic form. The ionization constants have been calculated from the spectral data using the equation

$$pK = pD - \log \frac{\varepsilon_i}{\varepsilon_0 - \varepsilon_i},$$

where ε_0 and ε_i are the final and instantaneous values of the extinction coefficient of the band.

The values of pK_1 and pK_2 obtained for the amine and carboxy groups, respectively, of glycine and glycylglycine agree satisfactorily with the corresponding values found by potentiometric titration:

Substance	From IR spectra		Potentiometric titration	
	pK_1	pK_2	pK_1	pK_2
Glycine	2.7	10.0	2.74	10.00
Glycylglycine	3.5	8.5	3.46	8.53
DL-α-alanine	2.6	10.4	-	-
β-Alanine	4.0	-*	-	-

* The value of pK_2 for β-alanine cannot be obtained since on passing to an alkaline medium there is only a small shift of the 1583 cm^{-1} band and no new band appears. Potentiometric measurements were not carried out for DL-α-alanine and β-alanine.

The values given show that the investigation of IR spectra over a wide range of concentrations of acids and alkalies may be a convenient method for measuring the protolytic equilibria of dipolar structures, especially of amino acids and peptides.

Summary

The ionization constants of glycine, glycylglycine, DL-α-alanine, and β-alanine have been determined from their IR spectra.

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1 February 1962

Institute of Chemical Physics, AS USSR